Division of Intramural Research

NAEHS Council Update

September 2012

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 been identified.

Chief of the Comparative Medicine Branch, Attending Veterinarian, Animal Program Director

The National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health is searching for a Chief of the Comparative Medicine Branch (CMB), Attending Veterinarian, and Animal Program Director. CMB provides a broad range of services and collaborative support for NIEHS intramural research programs. The incumbent will be responsible for an AAALAC accredited animal care and use program and for support of NIEHS animal research programs that study the effects of environmental agents in order to develop methods of disease prevention and treatment. The incumbent will represent NIEHS on the NIH Animal Program Directors Committee and will serve as attending veterinarian on the NIEHS ACUC. The incumbent will be expected to actively support the NIEHS mission, participate in ongoing planning and management discussions to successfully resolve pertinent issues and challenges, participate in long range strategic planning processes to develop and implement effective goals and directions for the animal care program and provide information and recommendations to the Scientific Director. Minimum qualifications include a DVM/VMD from an AVMA-accredited or approved college, a current license to practice veterinary medicine in any state in the United States and board certification by the American College of Laboratory

Animal Medicine. In addition, applicants must demonstrate a proven record of management and operation of an AAALAC accredited animal care program of similar magnitude and complexity, and demonstrated experience meeting all regulations and policies pertaining to animal care and use. Applicants must possess demonstrable experience and skills in management and supervision, budget oversight, resource allocation, facility design, rodent colony management and disease control. In addition, applicants must possess the ability to analyze, prioritize and delegate resources while managing multiple projects and programs. Expertise and experience should include a positive and collegial interaction and cooperation with staff at all levels and with scientific staff to promote and facilitate their research. Dr. David Miller, Chief, Laboratory of Toxicology and Pharmacology, is chair of the search committee. Candidates are being interviewed.

DIR NEW HIRES

Office of Fellows Career Development
Dr. Tammy Collins has been named to carry out the duties associated with the Office of Fellows Career Development (OFCD). Dr. Collins received her B.S. in chemistry from Appalachian State University, and the Ph.D. in biochemistry from Duke University in 2008. She did postdoctoral research for two years in the laboratory of T.S. Hsieh at Duke University, and then joined the laboratory of Dr. Bill Copeland, Chief, Laboratory of Molecular Genetics, NIEHS. She has extensive experience in research, teaching, publications, oral presentations, and professional development. For the past few years she has also been heavily involved in the activities of the NIEHS Trainees' Assembly (NTA) of which she served as President and as a participating member of the Steering Committee. She will be coordinating and arranging for all career-related workshops and other requirements of the NTA as her first priority.

BSC REVIEW OF LABORATORY OF STRUCTURAL BIOLOGY AND DR. MARILYN DIAZ

The DIR Board of Scientific Counselors reviewed the Laboratory of Structural Biology and Dr. Marilyn Diaz June 3-5, 2012.

Members of the Board of Scientific Counselors that Attended:

- Steven A. Belinsky, Ph.D., Acting Chair, Director, Lung Cancer Program, Lovelace Respiratory Research Institute, Albuquerque, NM
- Jay I. Goodman, Ph.D., Professor, Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI
- Serrine S. Lau, Ph.D., Professor, Department of Pharmacology & Toxicology, University of Arizona College of Pharmacy, Tucson, AZ
- Thomas A. Louis, Ph.D., Professor, Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
- José E. Manautou, Ph.D., Associate Professor, Department of Pharmaceutical Sciences, University of Connecticut School of Pharmacy, Storrs, CT
- Roland A. Owens, Ph.D., Ex-Officio BSC Member, Assistant Director, Office of Intramural Research, NIH, Bethesda, MD

Ad Hoc Reviewers that Attended:

- Kenneth Bruce Adler, Ph.D., Professor, Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC
- Juan C. Celedón M.D., Dr.PH., Neil K. Jerne Professor, Department of Pediatrics, Children's Hospital of Pittsburgh of University of Pittsburgh Medical Center, Pittsburgh, PA
- Stephen H. Clarke, Ph.D., Professor, Dept. of Microbiology and Immunology, University of North Carolina School of Medicine, Chapel Hill, NC
- Margaret O. James, Ph.D., Professor and Chair, Department of Medicinal Chemistry, College of Pharmacy, University of Florida, Gainesville, FL
- Thomas L. James, Ph.D., Professor, Department of Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, CA
- Michael S. Krangel, Ph.D., Mary Bernheim Professor and Chair, Department of Immunology, Duke University Medical Center, Durham NC
- Leona D. Samson, Ph.D., Director, Center for Environmental Health Sciences, Professor of Toxicology, and Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA
- Sankar Mitra, Ph.D., Professor, Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX
- Gabriele Varani, Ph.D., Professor, Departments of Chemistry and Biochemistry, University of Washington, Seattle, WA,
- Gregory L. Verdine, Ph.D., Harvard College Professor, Erving Professor of Chemistry, Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA

- Michael R. Waterman, Ph.D., Natalie Overall Warren Distinguished Professor, Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt School of Medicine, Nashville, TN
- James R. Williamson, Ph.D., Professor and Dean, Graduate and Postdoctoral Studies, Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA

Agenda:

Sunday, June 3, 2012: Doubletree Guest Suites, Closed Session

7:00-8:00 p.m.	Welcome and Discussion of Past Board Reviews, Drs. Linda Birnbaum, Thomas A. Kunkel and Darryl Zeldin
8:00-10:00	BSC Discussion of Review, Steven Belinsky, Ph.D.

Monday, June 4, 2012: NIEHS Conference Rooms 101 ABC

8:30-8:45 a.m.	Welcome, Drs. Linda Birnbaum and Darryl Zeldin
8:45-9:05	Overview--Laboratory of Structural Biology, Thomas A. Kunkel, Ph.D.
9:05-9:55	DNA Replication Fidelity Group, Thomas A. Kunkel, Ph.D.
9:55-10:45	Collaborative Crystallography Group, Lars C. Pedersen, Ph.D.
10:45-11:00	Break
11:00-11:50	Genome Stability Structural Biology, R. Scott Williams, Ph.D.
11:50-12:35	Closed Session with Investigators: Drs. Kunkel, Pedersen and Williams
12:35-1:30	Lunch
1:30-3:00	Poster Session, Postdoctoral Fellows and other presenters
3:00-3:15	Break
3:15-3:45	Closed Session with Fellows
3:45-4:35	Macromolecular Structure Group, Traci M. T. Hall, Ph.D.
4:45-5:25	DNA Repair and Nucleic Acid Enzymology Group, Samuel H. Wilson, M.D.
5:30-6:00	Closed Session with Investigators: Drs. Hall & Wilson
6:00-6:15	Return to Doubletree Hotel
6:15-8:00	Dinner
8:00-10:00	Closed Session, BSC Discussion of review: Dr. Belinsky and all review team members

Tuesday, June 5, 2012: NIEHS Conference Rooms 101 ABC

8:30-9:20 a.m.	Nuclear Magnetic Resonance Group, Robert E. London, Ph.D.
9:20-10:10	Somatic Hypermutation Group, Laboratory of Molecular Genetics, Marilyn Diaz, Ph.D.
10:10-10-25	Break
10:25-10:45	Closed Session with Investigators: Drs. London and Diaz

10:55-12:00	Closed – BSC Executive Session
12:00-1:00	Closed Session – Debriefing to NIEHS/DIR Leadership: Dr. Belinsky and all review team members; Drs. Zeldin, Schrader and Birnbaum
1:00	Adjournment: Depart for Airport

DIR RESEARCH ACCOMPLISHMENTS

Cellular Damage from Normal Metabolism May Cause Cancer

DNA mutations are thought to be rare events that occur randomly and over time, but NIEHS researchers have identified DNA regions in yeast and in three different types of cancers that have a disproportionately high number of mutations that arose simultaneously. In yeast, the clusters are produced by exposure to environmental toxins; in cancers, they are produced through biochemical process normally involved in innate immunity. The DNA sequence surrounding clustered mutations suggested that specific proteins called apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) cytosine deaminases, which inactivate viruses attacking the body, are also damaging DNA and causing mutations.

Roberts SA, Sterling J, Thompson C, Harris S, Mav D, Shah R, Klimczak LJ, Kryukov GV, Malc E, Mieczkowski PA, Resnick MA, Gordenin DA. Clustered mutations in yeast and in human cancers can arise from damaged long single-strand DNA regions. *Mol. Cell*, 46: 424–435, 2012.

EETs and Tumor Metastasis

Epoxyeicosatrienoic acids (EETs) are small molecules produced by cytochrome P450 epoxygenases. They are lipid mediators that act as autocrine or paracrine factors to regulate inflammation and vascular tone. As a result, drugs that raise EET levels are in clinical trials for the treatment of hypertension and many other diseases. However, despite pleiotropic effects on cells, little is known about the role of these epoxyeicosanoids in cancer. Here, using genetic and pharmacological manipulation of endogenous EET levels, we demonstrate that EETs are critical for primary tumor growth and metastasis in a variety of mouse models of cancer. Remarkably, we found that EETs stimulated extensive multi-organ metastasis and escape from tumor dormancy in several tumor models. This systemic metastasis was not caused by excessive primary tumor growth but depended on endothelial-derived EETs at the site of metastasis. Administration of synthetic EETs recapitulated these results while EET antagonists suppressed tumor growth and metastasis, demonstrating in vivo that pharmacological modulation of EETs can affect cancer growth. Furthermore, inhibitors of soluble epoxide hydrolase (sEH), the enzyme that metabolizes EETs, elevated endogenous EET levels and promoted primary tumor growth and metastasis. These data indicate a central role for EETs in tumorigenesis, offering a mechanistic link between lipid signaling and cancer and emphasizing the critical importance of considering possible effects of EET-modulating drugs on cancer.

Panigrahy D, Edin ML, Lee CR, Huang S, Bielenberg DR, Butterfield CE, Barnés CM, Mammoto A, Mammoto T, Luria A, Benny O, Chaponis DM, Dudley AC, Greene ER, Vergilio JA, Pietramaggiori G, Scherer-Pietramaggiori SS, Short SM, Seth M, Lih FB, Tomer KB, Yang J, Schwendener RA, Hammock BD, Falck JR, Manthati VL, Ingber DE, Kaipainen A, D'Amore PA, Kieran MW, Zeldin DC. Epoxyeicosanoids stimulate multiorgan metastasis and tumor dormancy escape in mice. *J. Clin. Invest.*, 122: 178-191, 2012.

Fertility drugs and young-onset breast cancer

Fertility drugs have hormonal effects that have raised concern about breast cancer. Researchers at NIEHS used a sister-controlled study to investigate possible effects of prior use of ovulation-stimulating drugs on risk of young-onset (under age 50) breast cancer. Women who had used either Clomid or follicle-stimulating hormone (FSH) to stimulate hyperovulation had slightly reduced risk compared to the general population. The fertility drug exposure was then subclassified according to whether any such treatment had produced a pregnancy lasting at least 10 weeks. Women who had conceived under treatment had significantly elevated risk compared to similarly treated women who had not conceived. The abnormally high levels of hormones experienced in the first trimester of a stimulated pregnancy may alter pregnancy-associated remodeling of breast tissue**.**

Fei C, Deroo LA, Sandler DP, Weinberg CR. Fertility drugs and young-onset breast cancer: results from the two sister study. *J. Natl. Cancer Inst*., 104: 1021-1027, 2012

High-resolution visualization of the roots of a heritable neurodegenerative disease

The cellular DNA repair machinery defends our genetic material against a continuous assault from stresses including environmental exposures to chemical toxicants, mutagens, and DNA-damaging radiation. The final critical step in repairing damaged DNA is a process called DNA ligation, which involves the chemical joining of broken DNA strands together. Like many biological processes ligation can fail, and this failure produces additional DNA damage ("DNA-adenylates") that in turn must be repaired by a dedicated DNA ligation proofreader, the Aprataxin protein. To shed light onto how Aprataxin acts to maintain the integrity of our genomes, NIEHS scientist used a high-resolution molecular imaging technique (X-ray crystallography) to directly visualize the Aprataxin in the process of repairing DNA. This work provides key insights into the chemistry of DNA-adenylate repair, and explains how inherited mutations in the Aprataxin gene (APTX) result in small, but devastating changes to the proteins' shape that underlie progression of a crippling neurodegeneration syndrome – Ataxia with Oculomotor Apraxia type1 (AOA1).

Tumbale P, Appel CD, Kraehenbuehl R, Robertson PD, Williams JS, Krahn J, Ahel I, Williams RS. Structure of an Aprataxin-DNA complex with insights into AOA1 neurodegenerative disease. *Nat. Struct. Mol. Biol.,* 18: 1189-1195, 2011.

Allergy may reduce the risk for heart attack

Studies in animals suggest that atherosclerosis is driven by ‘type 1’ immunity (i.e., inflammation) and reduced by ‘type 2’, or allergic/atopic immunity. Whether this applies to humans remains unclear, although it is a critical issue as vaccination strategies are under development for the treatment and prevention of human atherosclerosis. In an analysis of U.S. national survey data (National Health and Nutrition Examination Survey 2005-2006), NIEHS investigators determined that allergen-specific immunoglobulin E (IgE), a specific and objective blood test for allergy, is inversely related to myocardial infarction (i.e., ‘heart attack’) in a manner that is independent of a long list of established coronary risk factors (e.g., smoking, cholesterol, diabetes, etc.). While this was a cross-sectional analysis, precluding confident conclusions from being drawn on causality, it raises the intriguing possibility that allergy may be protective against heart attack 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*., in press.

Over 32 million Americans have autoantibodies that target their own tissues

More than 32 million people in the United States have autoantibodies, which are proteins made by the immune system that target the body's tissues and define a condition known as autoimmunity. The first nationally representative sample looking at the prevalence of the most common type of autoantibody, known as antinuclear antibodies (ANA), found that the frequency of ANA is highest among women, older individuals, and African-Americans. These findings should serve as a useful baseline for future studies looking at changes in ANA prevalence over time and the factors associated with ANA development. The paper is the first in a series analyzing these data from the National Health and Nutrition Examination Survey (NHANES) dataset, and exploring possible environmental associations with ANA.

Satoh M, Chan EK, Ho LA, Rose KM, Parks CG, Cohn RD, Jusko TA, Walker NJ, Germolec DR, Whitt IZ, Crockett PW, Pauley BA, Chan JY, Ross SJ, Birnbaum LS, Zeldin DC, Miller FW. Prevalence and sociodemographic correlates of antinuclear antibodies in the United States. *Arthritis Rheum.*, 64: 2319-2327, 2012.

Caffeine strengthens connections between neurons in a little-known area of the brain

Previous research had determined that caffeine's effects were due to its ability to block the inhibitory effects of adenosine on cyclic AMP production in the brain, but this study represents the first investigation of the effects of caffeine in the area with the highest expression of adenosine A1 receptors in the rodent brain: hippocampal area CA2. The study reported that caffeine dramatically increased the synaptic effectiveness in CA2 and was the first demonstration of long-lasting synaptic plasticity induced solely by in vivo exposure to caffeine. The role of hippocampal CA2 in brain function is unknown, but the robust potentiation induced by caffeine exposure both in vivo and in vitro strongly suggests that synaptic potentiation in CA2 is the physiological substrate for the cognitive enhancement provided by caffeine consumption.

Simons SB, Caruana DA, Zhao M, Dudek SM. Caffeine-induced synaptic potentiation in hippocampal CA2 neurons. *Nat. Neurosci*., 15: 23-25, 2011.

Understanding cellular communication through high-energy pyrophosphate bond formation

The catalysis of phosphate transfer between molecules is the basis of intracellular communication, and it is essential to life. This study demonstrates the unique and unexpected molecular adaptations that a particular phosphoryltransferase, PPIP5K2, that helps cells communicate by synthesizing high-energy pyrophosphate bonds in a group of cell signaling molecules. NIEHS scientists obtain this information by producing and analyzing atomic-level structures of PPIP5K2, immobilized at key stages of the reaction process. This work provides insight into the conservation and specialization of diverse phosphoryltransferases, it facilitates the rational design of therapeutically-relevant drugs to target this enzyme, and it furthers our understanding of this enzyme's interactions with environmental toxicants.

Wang H, Falck JR, Hall TM, Shears SB. Structural basis for an inositol pyrophosphate kinase surmounting phosphate crowding. *Nat. Chem. Biol*., 8: 111-116, 2011.

Stimulus-responsive networks are tuned at the level of transcription elongation
Different cells in the human body display highly specialized and variable responses to environmental exposures: for example, cells of the innate immune system are exquisitely sensitive to harmful and pathogenic stimuli that do not elicit significant responses in other cell types or tissues. A critical mechanism for tuning the activity of particular stimulus responsive networks in differing cell types involves modulating the expression levels of the receptors, kinases and transcription factors that serve as 'hubs' in these networks. However, how these factors might be co-regulated in a given cell type remains poorly understood. NIEHS scientists discovered that genes expressing most network hubs are regulated- not by the level of Pol II recruitment to their promoters- but instead by the rate of release of this Pol II into productive elongation. These findings extend from immune-responsive networks to those involved in early mammalian development and broadly suggest that signaling network activity can be regulated in a coordinate fashion by factors that control the release of Pol II from the promoter region into productive RNA synthesis.

Gilchrist DA, Fromm G, dos Santos G, Pham L, McDaniel I, Burkholder A, Fargo DC, Adelman K. Regulating the regulators: the pervasive effects of Pol II pausing on stimulus-responsive gene networks. *Genes Dev*., 26: 933-944, 2012

Prenatal and early life factors influence adult health
Two separate analyses using data collected at baseline from participants in the Sister Study, a nationwide cohort of 50,844 women age 35-74 who had a sister diagnosed with breast cancer, provide evidence that early life factors influence health later in life. Uterine leiomyomata (fibroids) are hormonally responsive tumors that are the leading cause of hysterectomies in US women, but little is known about risk factors. Using baseline data on self-reported exposures and physician diagnosed fibroids by 30 years of age (n = 561) from 3,534 black women, 35-59 years of age we found that early onset fibroids were associated with in utero diethylstilbestrol (DES) (RR = 2.02; 95% CI, 1.28-3.18), maternal pre-pregnancy or gestational diabetes (RR = 1.54; 95% CI, 0.95-2.49), and having been born from a multiple birth (monozygotic) (RR = 1.94; 95% CI, 1.26-2.99). Other factors such as maternal hypertensive disorder, preterm birth, and having been fed soy formula were also associated with later risk. The greater frequency of some of these factors in blacks may contribute to the higher prevalence of fibroids in black women.

Rheumatoid arthritis (RA) has been associated with lower socioeconomic status (SES), but the reasons for this are not known. Using data from the Sister Study, investigators examined childhood SES measures, SES trajectory, and other perinatal factors in relation to treated RA diagnosed after age 16 (n=424 cases). RA risk was associated with several measures of childhood adversity including lower household education (OR=1.7; 95%CI 1.1, 2.5), food insecurity (OR=1.5, 95%CI 1.1, 2.0), and young maternal age (OR=1.7, 95%CI 1.2, 2.5), with increased risk with increasing numbers of adverse factors and evidence that risk was greatest

among those with lower SES continuing into adulthood. Low birthweight (<2500 gm) and pre-conception paternal smoking were independently associated with RA.

D'Aloisio, AA, Baird DD, DeRoo LA, Sandler DP. Early-life exposures and early onset uterine leiomyomata in black women in the Sister Study. *Environ. Health Perspect.*, 120: 406-412, 2011.

Parks, CG, D'Aloisio AA, DeRoo LA. Huber K. Rider LG, Miller FW, Sandler DP. Childhood socioeconomic factors and perinatal characteristics influence development of rheumatoid arthritis (RA) in adulthood. *Ann. Rheum. Dis.*, in press, epub ahead of print doi: 10.1136/annrheumdis-2011-201083.

Calcium influx is a critical component of embryonic development

When mammalian sperm and egg first interact, a sperm-specific phospholipase C zeta contributes to the release of calcium from endoplasmic reticulum stores. Using a technique known as calcium insulation, the investigators induced persistent calcium oscillations in the egg cell while preventing both influx and efflux of calcium. In the absence of calcium influx, the fertilized eggs failed to undergo spindle rotation and emit the second polar body, thus resulting in the formation of three pronuclei, a fertilization abnormality. These studies have important implications for clinically assisted reproduction and fertility preservation technologies.

Miao Y-L, Stein P, Jefferson WN, Padilla-Banks E, Williams CJ. Calcium influx-mediated signaling is required for complete mouse egg activation. *Proc. Natl. Acad. Sci. USA.*, 109: 4169-4174, 2012.

Second trimester miscarriage risk is higher for African Americans than whites

African American women are at higher risk than white women for several adverse pregnancy outcomes such as preterm birth, but careful study of disparity in miscarriage risk has been lacking. Women recruited from several communities in the South Eastern US (N= 932 blacks and 3,138 whites) enrolled early in pregnancy in Right From The Start and were followed prospectively through their pregnancy. Using life-table analyses to account for variation in gestational age at enrollment and adjusting for confounders, gestational-week-specific risk of miscarriage was similar for blacks and whites through gestational week 9, but thereafter blacks had nearly twice the risk of miscarriage compared to whites. The causes of miscarriage are not understood, but these findings support the need to consider miscarriages at different developmental stages as different outcomes. In contrast to "embryonic loss" (gestational weeks 6-9), "fetal loss" (weeks 10-19) may share etiologies with early preterm birth and stillbirth.

Mukherjee S, Velez Edwards DR, Baird DD, Savitz DA, Hartmann KE. Risk of miscarriage among black and white women in a U.S. prospective cohort study. *Am. J. Epidemiol.,* in press.

The cytokine interleukin 9 (IL-9) suppresses melanoma tumor growth.

The transcription factor RORγ plays an important role in several immune responses. Now NIEHS investigators showed that melanoma tumor growth was significantly suppressed in RORγ KO mice. Antibodies against IL-9 reversed this suppression suggesting that IL-9 plays a critical

role in the suppression of tumor development in RORγ KO mice. The study further indicates the potential for therapeutic strategies for cancer using IL-9.

Purwar R, Schlapbach C, Xiao S, Kang HS, Elyaman W, Jiang X, Jetten AM, Khoury, SJ, Fuhlbrigge RC, Kuchroo VK, Clark RA, Kupper TS. Robust tumor immunity to melanoma mediated by interleukin 9. *Nat. Med.,* in press, epub ahead of print doi: 10.1038/nm.2856.

A novel system for detecting single- and double-strand breaks and repair in human DNA and the impact of PARP inhibitors

DNA strand breaks are an important source of genome instability that can lead to severe biological consequences including tumorigenesis and cell death. Although much is known about breaks induced directly by ionizing radiation and radiomimetic cancer drugs, there is a relative dearth of direct information about the formation of single or double-strand breaks (SSB or DSB) and direct repair in human cells. In this work NIEHS investigators developed a novel in vivo repair assay for the simultaneous detection of SSBs and DSBs based on changes in the large endogenous Epstein Barr virus (EBV) circular episome in human lymphoblastoid cells which has many chromatin features comparable to chromosomes. Utilizing this system, the investigators establish the impact of Poly(ADP-ribose) polymerases (PARP) and PARP inhibitors on repair of random, radiation breaks in non-replicating cells. Given the dearth of mechanistic studies on PARP inhibitors, the wide use of them in research on DNA repair and especially their promise in cancer therapy, this study has broad implications.

Ma W, Halweg CJ, Menendez D, Resnick MA. Differential effects of PARP inhibition and depletion on single- and double-strand break repair in human cells are revealed by changes in EBV minichromosomes. *Proc. Natl. Acad. Sci. USA*., 109: 6590-6595, 2012.

Molecular interactions within the DNA polymerase β active site that enable pro-mutagenic DNA synthesis with oxidized substrates containing 8-oxoguanine discovered

Oxidation of genomic DNA forms the guanine lesion 7,8-dihydro-8-oxoguanine (8-oxoG). When in the template base position during DNA synthesis the 8-oxoG lesion has dual coding potential by virtue of its anti- and syn-conformations, base pairing with cytosine and adenine, respectively. This impacts mutagenesis, since insertion of adenine opposite template 8-oxoG can result in a G to T transversion. DNA polymerases vary by orders of magnitude in their preferences for mutagenic vs. error-free 8-oxoG lesion bypass, yet the structural basis for lesion bypass specificity is not well understood. The DNA base excision repair enzyme DNA polymerase (pol) β is presented with gap filling synthesis opposite 8-oxoG during repair and has similar insertion efficiencies for dCTP and dATP. NIEHS researchers report the structure of pol β in binary complex with template 8-oxoG in a base excision repair substrate. The structure reveals both the syn- and anti-conformations of template 8-oxoG in the confines of the polymerase active site, consistent with the dual coding observed kinetically for this enzyme. A ternary complex structure of pol β with the syn-8-oxoG:anti-A Hoogsteen base pair in the closed fully assembled pre-insertion active site is also reported. The syn-conformation of 8-oxoG is stabilized by minor groove hydrogen bonding between the side chain of Arg283 and O8 of 8-oxoG. An adjustment in

the position of the phosphodiester backbone 5′-phosphate enables 8-oxoG to adopt the syn-conformation.

Batra VK, Shock DD, Beard WA, McKenna CE, Wilson SH. Binary complex crystal structure of DNA polymerase β reveals multiple conformations of the templating 8-oxoguanine lesion. *Proc. Natl. Acad. Sci. USA.*, 109: 113-118, 2012.

Dissecting the substrate recognition of 3-O-sulfotransferase for the biosynthesis of therapeutic anticoagulant heparin

The 3-O-sulfotransferase isoform 1 catalyzes the critical sulfation of heparan sulfate/heparin to produce anticoagulant heparin. Current technology is focusing on using enzymes to produce heparan sulfate/heparin for therapeutic purposes. NIEHS investigators have solved the crystal structure of the 3-O-sulfotransferase with bound heparan sulfate substrate which provides atomic details of substrate binding. Information gained from this study is allowing researchers to manipulate the enzyme to improve its use as a tool in anticoagulant drug production.

Moon AF, Xu Y, Woody SM, Krahn JM, Linhardt RJ, Liu J, Pedersen LC. Dissecting the substrate recognition of 3-O-sulfotransferase for the biosynthesis of anticoagulant heparin. *Proc. Natl. Acad. Sci. USA.*, 109: 5265-5270, 2012.

Teasing out genetic differences in sensitivity to pain.

Genetic variants of the ion channel receptor P2X7 influence sensitivity to pain. Carriers of a variant that encodes a larger ion channel pore have a higher sensitivity to pain. This finding was established by examining mouse strains and extended to two human pain conditions by combining results of observational studies, using a recently developed statistical approach. The findings may help development of personalized drugs for management of pain.

Sorge RE, Trang T, Dorfman R, Smith SB, Beggs S, Ritchie J, Austin JS, Zaykin DV, Vander Meulen H, Costigan M, Herbert TA, Yarkoni-Abitbul M, Tichauer D, Livneh J, Gershon E, Zheng M, Tan K, John SL, Slade GD, Jordan J, Woolf CJ, Peltz G, Maixner W, Diatchenko L, Seltzer Z, Salter MW, Mogil JS. Genetically determined P2X7 receptor pore formation regulates variability in chronic pain sensitivity. *Nat. Med*. 18: 595-599, 2012

Maternal smoking leads to epigenetic changes at specific genes in newborns.

Maternal smoking during pregnancy leads to numerous health outcomes in children include reduced birthweight and respiratory problems but mechanisms remain uncertain. It is increasingly being appreciated that epigenetic changes to DNA may underlie effects of in utero exposures. NIEHS researchers analyzed DNA samples from newborns from a birth cohort in Norway to examine methylation using a newly available platform that gives good coverage across the genome. The researchers found difference in methylation at 26 different sites localized to 10 genes. This replicated their earlier findings in a birth cohort from the US. The genes include two previously implicated in response to tobacco smoke and novel genes that may help uncover new understanding of how maternal smoking in pregnancy impacts childhood health.

Joubert BR, Håberg SE, Nilsen RM, Wang X, Vollset SE, Murphy SK, Huang Z, Hoyo C, Midttun Ø, Cupul-Uicab LA, Ueland PM, Wu MC, Nystad W, Bell DA, Peddada SD, London JS. 450K Epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. *Environ. Health Perspect*., in press, epub ahead of print, doi: 10.1289/ehp.1205412

Early-life soy exposure and gender-role play behavior in children

According to scientists from NIEHS and the University of North Carolina Gillings School of Global Public Health, early-life soy exposure is associated with play behavior that is less typically female in girls at 42 months of age. The team did not observe similar changes in boys. The work represents an important issue in children's health, because soy-based infant formula contains isoflavones, estrogen-like compounds that can induce changes in gender-typical behavior in experimental animals if they are exposed to high doses at a young age. In this study, scientists examined parents' reports of gender-typical play behavior in 3,664 boys and 3,412 girls at 30, 42 and 57 months of age, in relation to their exposure to soy-based and non-soy-based infant feeding methods, categorized as primarily breast-fed, early formula-fed, early soy-fed, and late soy-fed.

Adgent MA, Daniels JL, Edwards LJ, Siega-Riz AM, Rogan WJ. Early-life soy exposure and gender-role play behavior in children. *Environ. Health Perspect*., 119: 1811-1816, 2011.

Keeping cell division tidy

When cells divide, they need to push most of their contents aside to allow the critical sorting of chromosomes (genes) to occur unimpeded. How this rearrangement of cell contents occurs has been a mystery for cell biologists for some time. In a recent collaboration between scientists at the NIEHS and NHLBI, a mechanism responsible for at least a part of this sorting mechanism has been discovered that involves the attachment of phosphate groups to a key intracellular signaling protein.

Smyth JT, Beg AM, Wu S, Putney JW Jr, Rusan NM. Phosphoregulation of STIM1 leads to exclusion of the endoplasmic reticulum from the mitotic spindle. *Curr. Biol.*, in press, epub ahead of print doi: 10.1016/j.cub.2012.05.057.

The ubiquitin interaction motif (UIM)-containing protein RAP80 deficiency promotes genomic instability and causes an increase in cancer risk.

RAP80 plays an important role in DNA damage response through its interaction with the tumor suppressor BRCA1. Using mice deficient in the RAP80, it was shown that the loss of RAP80 expression increases genomic stability and cancer development in RAP80-/- mice using several tumorigenesis models, including IR-induced lymphoma and 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary cancer. The results demonstrated that RAP80 protects against genomic instability and reduces cancer risk supporting the hypothesis that RAP80 functions as a tumor suppressor.

Yin Z, Menedez D, Janardhan K, Resnick M, French J, Jetten AM. RAP80 plays a critical role in the maintenance of genomic stability and tumor suppression. *Cancer Res.*, in press

Link between DNA damage, tumor suppressor and immune genes in cancer cells.
Following up on previous work with primary cells from human subjects, NIEHS investigators now provide the first evidence that DNA damage can lead to a general regulation of inflammatory responses in cancer cells. Specifically, damage to chromosomes alters the expression of a family of genes known as Toll-like receptors (TLRs). TLRs are proteins that play a role in the immune system by defending the body from infection and are being exploited in cancer therapies. Following damage, the tumor suppressor p53 is greatly increased and interacts with TLR genes to regulate the amount of inflammatory response in a variety of cancer cell lines. The cells were exposed to anti-cancer agents to activate p53 and expression of TLR genes was determined. Studies with several human cancer cell lines establish that agents commonly used in cancer treatment such as doxorubicin, 5-fluorouracil and ionizing radiation can elicit changes in TLR expression that are cell line- and damage-specific. In addition, several p53 cancer-associated mutants dramatically alter the pattern of TLR gene expression. Since each of the 10 members of the innate immune TLR gene family tested was differentially inducible, these findings demonstrate that the matrix of p53 status, chromosome stress, and responsiveness of individual TLRs should be considered in TLR-based cancer therapies.

Shatz M, Menendez D, Resnick MA. The human TLR innate immune gene family is differentially influenced by DNA stress and p53 status in cancer cells. *Cancer Res.*, in press, epub ahead of print doi: 10.1158/0008-5472.CAN-11-4134

Offspring of women who smoked while pregnant are more likely to develop gestational diabetes
If a woman smokes while pregnant, the effect on the offspring, when they become adults, is increased risk of obesity and related disorders. To investigate this relationship further, women were studied in Norway who reported whether their mothers had smoked when they were pregnant with the subject. Women with such an exposure were not only more likely to be obese, as expected, but they were also more likely to develop gestational diabetes, even after accounting for the effect on weight. This is the first report of such an association.

Cupul-Uicab LA, Skjaerven R, Haug K, Melve KK, Engel SM, Longnecker MP. In utero exposure to maternal tobacco smoke and subsequent obesity, hypertension, and gestational diabetes among women in the MoBa cohort. *Environ. Health Perspect*., 23: 355-360, 2012.

Rapid Mutation in an RNA virus
Riboviruses make up the majority of pathogens for all life forms and are known to mutate at high rates but have never been well characterized for patterns and precise rates of mutations. Using a classical model phage system, we have confirmed their suspected mode of genome replication (the "stamping machine" mechanism), carefully measured their mutation rate, and found an extreme bias against indel mutations and in favor of transition mutations, information that may assist in the design of antivirals. Human pathogenic riboviruses seem to have higher mutations

rates than do phages, perhaps to more efficiently circumvent the immune response and/or to assist their transmission between individuals.

García-Villada L, Drake JW. The three faces of riboviral spontaneous mutation: spectrum, mode of genome replication, and mutation rate. *PLoS Genet.,* 8: e1002832, 2012.

Fine tuning gene regulation by mRNA-binding proteins

A family of proteins called PUF proteins regulates expression of their target genes by recognizing sequences in mRNA. Recent studies provide a deeper understanding of how the specificity of mRNA recognition can be narrowed or broadened to allow correct control. Narrowed specificity of a PUF protein allows coordination of control of genes with related biological function. Broadened specificity may allow a PUF protein to be used to control more than one biological process.

Qiu C, Kershner A, Wang Y, Holley CP, Wilinski D, Keles S, Kimble J, Wickens M, Hall TM. Divergence of Pumilio/fem-3 mRNA binding factor (PUF) protein specificity through variations in an RNA-binding pocket. *J. Biol. Chem.*, 287: 6949-6957, 2012.

Valley CT, Porter DF, Qiu C, Campbell ZT, Hall TM, Wickens M. Patterns and plasticity in RNA-protein interactions enable recruitment of multiple proteins through a single site. *Proc. Natl. Acad. Sci. USA.*, 109: 6054-6059, 2012.

Metal-induced DNA translocation in pol β

Cell DNA is subject to damage by chemical and physical agents of environmental concern, and consequently in need of continual repair. Several different damage-dependent repair systems are present in the cell to deal with this need. It is estimated that more than 20,000 DNA lesions/cell/day are repaired by the base excision repair system. DNA polymerase β plays a central role in this process. During the past year, we found that the substrate for this enzyme – double stranded DNA containing a single nucleotide gap – can translocate along the active DNA-binding track in the presence of divalent metal ions. This translocation is accompanied by conformational activation of the enzyme and can facilitate the reverse, "depolymerization" reaction. This depolymerization pathway may enhancethe ability of this enzyme to deal with various types of DNA damage.

Kirby TW, DeRose EF, Cavanaugh NA, Beard WA, Mueller GA, Wilson SH, London RE. Metal-induced DNA translocation leads to DNA polymerase conformational activation. *Nucl. Acids Res.*, 40: 2974-2983, 2012.

Cytochrome P450 and the Heart

Cytochrome P450 (CYP) epoxygenases CYP2C8 and CYP2J2 generate epoxyeicosatrienoic acids (EETs) from arachidonic acid. Mice with expression of CYP2J2 in cardiomyocytes (αMHC-CYP2J2 Tr) or treated with synthetic EETs have increased functional recovery after ischemia/reperfusion (I/R); however, no studies have examined the role of cardiomyocyte- vs. endothelial-derived EETs or compared the effects of different CYP epoxygenase isoforms in the ischemic heart. In this study, we generated transgenic mice with increased endothelial EET

biosynthesis (Tie2-CYP2C8 Tr and Tie2-CYP2J2 Tr) or EET hydrolysis (Tie2-sEH Tr). Compared to wild-type (WT), αMHC-CYP2J2 Tr hearts showed increased recovery of left ventricular developed pressure and decreased infarct size after I/R. In contrast, recovery and infarct size were unchanged in Tie2-CYP2J2 Tr and Tie2-sEH Tr hearts. Surprisingly, Tie2-CYP2C8 Tr hearts had significantly reduced recovery and increased infarct size after I/R. Tie2-CYP2C8 Tr hearts also exhibited increased reactive oxygen species (ROS) generation, dihydroxyoctadecenoic acid (DiHOME) formation and coronary resistance after I/R. ROS scavengers and CYP2C8 inhibition reversed the detrimental effects of CYP2C8 expression in Tie2-CYP2C8 Tr hearts. Treatment of WT hearts with 9,10-DiHOME decreased recovery and increased coronary resistance after I/R. These data demonstrate that increased ROS generation and enhanced DiHOME synthesis by endothelial CYP2C8 impair functional recovery and mask the beneficial effects of increased EET production following I/R.

Edin ML, Wang Z, Bradbury JA, Graves JP, Lih FB, DeGraff LM, Foley JF, Torphy R, Ronnekleiv OK, Tomer KB, Lee CR, Zeldin DC. Endothelial expression of human cytochrome P450 epoxygenase CYP2C8 increases susceptibility to ischemia-reperfusion injury in isolated mouse heart. *FASEB J*., 25: 3436-3447, 2011.

Parkinson's disease risk increased by head injury

Parkinson's disease, a movement disorder, is the second most common neurodegenerative disease. Its causes are not well understood, but likely include both genetic and environmental factors. For example, either genetic changes in alpha-synuclein or head injury may contribute to Parkinson's disease risk. In a study of 476 Parkinson's disease cases and 488 matched controls, it was found that by themselves either head injury or a genetic change in alpha-synuclein leading to increased expression had little effect on Parkinson's disease risk, but together they increased risk 3.5-fold and decreased age at disease onset by nearly five years. Thus the combination of a genetic change with an environmental insult has a much greater effect on disease risk than either alone.

Goldman SM, Kamel F, Ross GW, Jewell SA, Bhudhikanok GS, Umbach DM, Marras C, Hauser RA, Jankovic J, Factor SA, Bressman S, Lyons KE, Meng C, Korell M, Roucoux DF, Hoppin JA, Sandler DP, Langston JW, Tanner CM. Head injury, alpha-synuclein Rep1, and Parkinson's disease. *Ann .Neurol*., 71: 40-48, 2012.

Mechanism for generating high affinity antibodies

To test the hypothesis that DNA polymerase ζ participates in Ig hypermutation, NIEHS investigators generated two mouse models of Pol ζ function: a B cell-specific conditional knockout and a knock-in strain with a Pol ζ mutagenesis-enhancing mutation. Pol ζ-deficient B cells had a reduction in mutation frequency at Ig loci in the spleen and in Peyer's patches, whereas knock-in mice with a mutagenic Pol ζ displayed a marked increase in mutation frequency in Peyer's patches, revealing a pattern that was similar to mutations in yeast strains with a homologous mutation in the gene encoding the catalytic subunit of Pol ζ. Combined, these data are best explained by a direct role for DNA polymerase ζ in Ig hypermutation.

Daly J, Bebenek K, Watt DL, Richter K, Jiang C, Zhao ML, Ray M, McGregor WG, Kunkel TA, Diaz M. Altered Ig hypermutation pattern and frequency in complementary mouse models of DNA polymerase ζ activity. *J. Immunol*., 188: 5528-5537, 2012.

Novel molecular mechanism of ribonucleotide discrimination employed within the DNA polymerase β active site discovered

DNA polymerases can misinsert ribonucleotides that lead to genomic instability. DNA polymerase β discourages ribonucleotide insertion with the backbone carbonyl of Tyr-271; alanine substitution of Tyr-271 resulted in a >10-fold loss in discrimination. Crystallographic structures of Y271A- and wild type-substrate complexes indicate that rCTP is well accommodated in the active site, but that O2′ of rCTP and the carbonyl oxygen of Tyr-271 or Ala-271 are unusually close (~2.5 and 2.6 Å, respectively). Structure-based modeling indicates that the local energetic cost of positioning these closely spaced oxygens is ~2.2 kcal/mol for the wild-type enzyme. Since the side chain of Tyr-271 also hydrogen bonds with the primer terminus, loss of this interaction affects its catalytic positioning. The results support a model where DNA polymerase β utilizes two strategies, steric and geometric, with a single protein residue to deter ribonucleotide insertion.

Cavanaugh N, Beard WA, Batra VK, Perera L, Pedersen LG, Wilson SH. Molecular insights into DNA polymerase deterrents for ribonucleotide insertion. *J. Biol. Chem*., 286: 31650-31660, 2011.

Source of ribonucleotides in human mitochondrial DNA revealed

It has been known for decades that the DNA in human mitochondria contains a portion of ribonucleotides instead of the canonical deoxynucleotides. Human mitochondrial DNA contains, on average, about 30 ribonucleotides per genome copy. The source of these ribonucleotides has eluded researches but now, for the first time, researchers at the NIEHS report that the DNA polymerase gamma, which replicates human mitochondrial DNA, can incorporate ribonucleotides into mitochondrial DNA. They find that normal DNA replication by the DNA polymerase gamma has to potential to incorporate 1-10% ribonucleotides, depending on the nucleotide base, into mitochondrial DNA. The implication helps to explain the source of ribonucleotides in mtDNA. Furthermore, they also studied the ability of the polymerase to copy RNA, called reverse transcription. They found that while the DNA polymerase γ is proficient in performing single-nucleotide reverse transcription reactions, its bypass efficiency is significantly diminished with increasing stretches of ribonucleotides in template DNA suggesting that reverse transcription in vivo is unlikely.

Kasiviswanathan R, Copeland WC. Ribonucleotide discrimination and reverse transcription by the human mitochondrial DNA polymerase. *J. Biol. Chem*., 286: 31490-31500, 2011.

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 researchers 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). 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. The important role of the 5'-deoxynucleoside-triphosphates (dNTPs) was also demonstrated, the building blocks used by the polymerases for synthesizing DNA, and how cells must control the dNTP levels to keep replication accurate.

Gawel D, Jonczyk P, Fijalkowska IJ, Schaaper RM. The *dnaX36* mutator of *Escherichia coli*: effects of the DNA Polymerase III holoenzyme τ subunit on chromosomal DNA replication fidelity. *J. Bacteriol*., 193: 296-300, 2011.

Makiela-Dzbenska K, Jonczyk P., Schaaper RM, Fijalkowska IJ. Proofreading deficiency of Pol I increases the levels of spontaneous *rpoB* mutations in *E. coli. Mutat. Res.*, 712: 28-32, 2011.

Fijalkowska IJ, Schaaper RM, Jonczyk P. DNA replication fidelity in *Escherichia coli*: a multi-DNA polymerase affair. *FEMS Microbiol. Rev*., in press, epub ahead of print doi: 10.1111/j.1574-6976.2012.00338.x.

Conte E, Vincelli G, Schaaper RM, Bressanin D, Stefan A, Dal Piaz F, Hochkoeppler A. Stabilization of the *E. 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.

Ahluwalia D, Bienstock RJ, Schaaper RM. Mutator mutants of NrdAB ribonucleotide reductase provide insight into allosteric regulatory sites and control of mutation rates. *DNA Repair*, 11: 480-487, 2012.

SIRT1 regulates systemic energy homeostasis and steroid hormone metabolism

SIRT1 is a highly-conserved NAD^+-dependent protein deacetylase that plays essential roles in the regulation of energy metabolism, genomic stability, and stress response. Although the functions of SIRT1 in many organs have been extensively studied in tissue-specific knockout mouse models, the systemic role of SIRT1 is still largely unknown due to the severe developmental defects that result from whole-body knockout in mice. In the past four years, NIEHS scientists have investigated the systemic functions of SIRT1 in metabolic homeostasis by utilizing a whole-body SIRT1 heterozygous mouse model. These mice are phenotypically normal under standard feeding conditions. However, when chronically challenged with a 40% fat diet, they become obese and insulin resistant, display increased serum cytokine levels, and develop hepatomegaly. Hepatic metabolomic analyses revealed that SIRT1 heterozygous mice have elevated gluconeogenesis and oxidative stress. Surprisingly, they are depleted of glycerolipid metabolites and free fatty acids, yet accumulate lysolipids compared to wild type control mice. Moreover, high-fat feeding induces elevation of serum testosterone levels and enlargement of seminal vesicles in SIRT1 heterozygous males. Microarray analysis of liver mRNA indicates that they have altered expression of genes involved in steroid metabolism and glycerolipid metabolism. Taken together, our findings indicate that SIRT1 plays a vital role in the regulation of systemic energy and steroid hormone homeostasis.

Purushotham A, Xu Q, Li X. Systemic SIRT1 insufficiency results in disruption of energy homeostasis and steroid hormone metabolism upon high-fat-diet feeding. *FASEB J*., 26:656-667, 2012.

RORγ regulates interleukin 17a gene directly.

IL-17a plays a critical role in inflammation and autoimmune disease. It was shown that RORγ regulates the expression if Il-17a. The nuclear receptors RORγt and RORα regulate *Il17a* by binding directly to the regulatory region CNS2 within the *Il17a* gene. This study provides evidence for the transcriptional mechanism underlying *Il17a* expression by RORs.

Wang X, Zhang Y, Yang XO, Nurieva RI, Chang SH, Ojeda SS, Kang HS, Schluns KS, Gui J, Jetten AM, Dong C. Transcription of *Il17* and *Il17f* is controlled by conserved non-coding sequence 2. *Immunity,* 36: 23-31, 2012.

A white cell protein is critical in conferring resistance to septic shock

One consequence of overwhelming bacterial infections is the cardiovascular collapse that occurs due to release of powerful mediators from white cells, in an often lethal event called a "cytokine storm". NIEHS researchers found that specific removal of an anti-inflammatory protein, tristetraprolin or TTP, from white cells in mice, makes the mice more susceptible to this effect of bacterial toxins. Thus, TTP is a naturally occurring "resistance element" that prevents bacterial and possibly viral infections from causing lethal septic shock.

Qiu LQ, Stumpo DJ, Blackshear PJ. Myeloid-specific tristetraprolin deficiency in mice results in extreme lipopolysaccharide sensitivity in an otherwise minimal phenotype. *J. Immunol.*, 188: 5150-5159, 2012

RORγ provides a link between the regulation of the clock machinery and its regulation of metabolism.

Circadian regulation is critical in the regulation of metabolism and disruption of circadian rhythm has been linked to increased risk for cancer and diabetes. RORα and RORγ regulate a number of clock genes as well as several metabolic genes. This study indicates that ROR transcription factors provide a link between the regulation of metabolism and circadian rhythm.

Takeda Y, Birault V, Jetten AM. The Retinoic acid-related Orphan Receptor γ (RORγ) directly regulates the circadian expression of clock genes and downstream targets in vivo. *Nucleic Acids Res.,* in press, epub ahead of print doi: 10.1093/nar/gks630

NRF2 binding sites in human genome links oxidative stress response with development of fat cells

NIEHS scientists for the first time surveyed potential binding sites of NRF2, the master regulator of antioxidant response pathway in human genome, using chromatin immunoprecipitation coupled with next-generation sequencing technology. This study has discovered more than 800 novel NRF2 binding sites in lymphoid cells treated with the dietary antioxidant, sulforaphane. Among the most promising candidates, a confirmed target gene named "retinoid X receptor alpha (RXRA)" plays central roles in fat metabolism and adipocyte differentiation. NRF2

regulation of RXRA in fat cell development suggests the potential for NRF2 activators to therapeutically manipulate RXRA in numerous retinoid-mediated pathways.

Chorley BN, Campbell MR, Wang X, Karaca M, Sambandan D, Bangura F, Xue P, Pi J, Kleeberger SR, Bell DA. Identification of novel NRF2-regulated genes by ChIP-Seq: influence on retinoid X receptor alpha. *Nucleic Acids Res*., in press, epub ahead of print doi: 10.1093/nar/gks409

Impaired development and response to oxidative stress in neonatal mice is linked to the transcription factor Nrf2.

Nrf2 is an essential transcription factor for protection against oxidant disorders. However, its role in organ development and neonatal disease has received little attention. NIEHS investigators found that Nrf2 is essential for cell cycle and DNA repair, immune function, and antioxidant defense during post-natal normal lung maturation in mice. They also found a beneficial role for Nrf2 in hyperoxia-induced injury of undeveloped lung. The work has important implications for a wide range of oxidative stress-associated neonatal disorders, including bronchopulmonary dysplasia (BPD), a chronic lung disorder found in roughly 20 percent of low birth weight infants born each year in the United States. The results also suggest a possible therapeutic role for Nrf2 in the protection of human BPD.

Cho HY, van Houten B, Wang X, Miller-Degraff L, Fostel J, Gladwell W, Perrow L, Panduri V, Kobzik L, Yamamoto M, Bell DA, Kleeberger SR. Targeted deletion of Nrf2 impairs lung development and oxidant injury in neonatal mice. *Antioxid. Redox. Signal*., in press, epub ahead of print, doi: 10.1089/ars.2011.4288.

Nano-sized diesel fuel additive is toxic for human immune cells

Engineering of nano-sized particles (i.e. particles that are smaller than viruses and as big as human proteins or DNA fragments) has led to significant advances in electronics, optics and medicine. However, the health risks of engineered nanoparticles are not well understood. Nano-sized cerium dioxide is used in many parts of the world as a diesel fuel additive, because it improves fuel combustion and decreases soot emissions. However, nano-sized cerium dioxide is also emitted in the environment by the diesel engines, and could have its own adverse effects. NIEHS investigators tested whether nano-sized cerium dioxide can be toxic for human cells. The researchers show that this is indeed the case, and identify the mechanisms of toxicity. This work provided impetus for more studies that are currently underway and raise concerns about potential toxic effects of nano-sized cerium dioxide on human tissues.

Hussain S, Al-Nsour F, Rice AB, Marshburn J, Yingling B, Ji Z, Zink JI, Walker NJ, Garantziotis S. Cerium dioxide nanoparticles induce apoptosis and autophagy in human peripheral blood monocytes. *ACS Nano.*, 6: 5820-5829, 2012.

Can cardiac responses predict lung injury induced by oxidative stress?

While damaging effects of oxidative stress on the lung are well studied, little is known about cardiovascular system involvement in oxidative lung injury. NIEHS investigators characterized cardiac responses (from ECG recordings) in a well-established mouse model for inducing oxidative lung injury (hyperoxia) and to identify genetic determinants of susceptibility to

observed responses. They found consistent and highly significant hyperoxia-induced reductions in heart rate (HR) and minute ventilation, and more subtle changes in heart rate variability (HRV; an index for autonomic nervous system regulation of the myocardium). Moreover, heart rate responses preceded pulmonary function changes and lung injury, suggesting these responses may act as useful predictors of impending lung responses or injury induced by oxidative stress in the lung. Diversity in the potential roles of the candidate genes that were identified illustrates the genetic complexity of cardiopulmonary responses to hyperoxia. It may be possible to use measures such as HR and HRV, especially in susceptible patients, to predict adverse outcomes in pulmonary function.

Howden R, Cho HY, Miller-DeGraff L, Walker C, Clark JA, Myers PH, Rouse DC, Kleeberger SR. Cardiac physiologic and genetic predictors of hyperoxia-induced acute lung injury in mice. *Am. J. Respir. Cell. Mol. Biol.*, 46: 470-478, 2012.

Mechanism for endocrine disrupting chemicals is dose and cell type specific

NIEHS researchers have uncovered the mechanisms by which endocrine disrupting chemicals (EDCs) initiate adverse effects on human cells. They found that bisphenol A (BPA) and bisphenol AF (BPAF), two synthetic chemicals found in polycarbonate plastics and electronic materials, and Zearalenone, an estrogenic mycotoxin found in cereal crops and bread, can function as both agonists and antagonist EDCs. Since EDCs are widely present in the environment, this study may help scientists understand the impact of environmental exposure on human health and wildlife populations. Previous experimental studies have shown that the antagonistic effects of BPA at low concentrations inhibit key adipokines, which are thought to protect humans from complex diseases, such as the induction of metabolic syndrome. The researchers used three different human cell lines with low endogenous expression of estrogen receptor (ER) alpha to assess the estrogenic actions of the three EDCs. The results showed that both BPA and BPAF act as antagonists for ER alpha and ER beta at low concentrations, less than 10 nanomolar, but act as agonists at higher concentrations, greater than 10 nanomolar, in a cell specific manner. The findings may help explain the tissue selective actions. Moreover, these EDCs not only activate endogenous ER alpha target genes, but can also mediate rapid action responses, such as the activation of p44/p42 mitogen-activated protein kinase (MAPK) pathway, which indicates their mechanisms of action may also involve not only gene responses, but also extranuclear cell signaling activities.

Li Y, Burns KA, Arao Y, Luh CJ, Korach KS. Differential estrogenic actions of endocrine-disrupting chemicals bisphenol A, bisphenol AF, and Zearalenone through estrogen receptor alpha and beta in vitro. *Environ. Health Perspect.*, 120: 1029-1035, 2012.

Persistent pesticides used in the past not related to male birth defects

A type of persistent pesticide, chlordane, frequently used in the U.S. in the past, has recently been implicated in the etiology of testicular germ cell tumors, indicating that a search for other effects of this agent on male reproductive was merited. NIEHS researchers addressed this question using data and specimens from a study that had been conducted by NIH in the 1960s, and found that male birth defects were unrelated to maternal serum levels of chlordanes.

Trabert B, Longnecker MP, Brock JW, Klebanoff MA, McGlynn KA. Maternal pregnancy levels of trans-nonachlor and oxychlordane and prevalence of cryptorchidism and hypospadias in male offspring. *Environ. Health Perspect*., 120: 478-482, 2012.

High pesticide exposure events have long term chronic cognitive health effects

High pesticide exposure events, not related to pesticide poisoning events, were associated with poorer performance on two tests on neurobehavioral function in a study of 693 farmer pesticide applicators in the Agricultural Health Study. Individuals with a history of high pesticide exposure events did worse on both the Digit Symbol test, a test of visual scanning and processing, and on the Sequences A test, a test of visual scanning and motor speed. The effect of high pesticide exposure events on cognitive function was equivalent to being 3.9 years older. This study was the first study to suggest that high level exposures which do not result in poisoning, have long term impacts on health.

Starks SE, Gerr F, Kamel F, Lynch CF, Alavanja MC, Sandler DP, Hoppin JA. High pesticide exposure events and central nervous system function among pesticide applicators in the Agricultural Health Study. *Int. Arch. Occ. Environ. Health.*, 85: 505-515, 2012.

Hepatic SIRT1 prevents formation of cholesterol gallstones in mice

SIRT1, a highly conserved NAD^+-dependent protein deacetylase, is a key metabolic sensor that directly links nutrient signals to animal metabolic homeostasis. Although SIRT1 has been implicated in a number of hepatic metabolic processes, the mechanisms by which hepatic SIRT1 modulates bile acid metabolism are still not well understood. NIEHS scientists recently reported that deletion of hepatic SIRT1 reduces efflux of bile acids from hepatocyte to bile duct, resulting in a unbalanced lipid profile in bile. As a result, mice lacking hepatic SIRT1 have an increased incidence of development of cholesterol gallstones on a lithogenic diet. It was discovered that SIRT1 regulates bile acid metabolism through modulating the expression of a key transcription factor involved in bile acid homeostasis. Taken together, these findings indicate that SIRT1 plays a vital role in the regulation of hepatic bile acid homeostasis and suggest that activation of SIRT1 activity may be beneficial for the treatment of human cholesterol gallstone disease.

Purushotham A, Xu Q, Lu J, Foley JF, Yan X, Kim DH, Kemper JK, Li X. Hepatic deletion of SIRT1 decreases hepatocyte nuclear factor 1α/farnesoid X receptor signaling and induces formation of cholesterol gallstones in mice. *Mol. Cell. Biol*., 32: 1226-1236, 2012.

Increasing the potential anti-inflammatory effect of a protein by association with a second cellular protein

Tristetraprolin (TTP) is a powerful anti-inflammatory protein that acts by binding directly to the messenger RNA (mRNA) for tumor necrosis factor alpha, the most powerful pro-inflammatory cytokine that is responsible for the cardiovascular collapse that occurs with overwhelming infections. NIEHS investigators found that another cellular protein, AUF1, can bind directly to TTP, at the same site used in TTP's RNA binding. Surprisingly, this protein-protein interaction

led to an increase in TTP's affinity for RNA. This normal cellular interaction suggests that small molecules could be identified that use a similar mechanism to increase the binding of TTP to mRNA, resulting in a beneficial increase in its anti-inflammatory properties.

Kedar VP, Zucconi BE, Wilson GM, Blackshear PJ. Direct binding of specific AUF1 isoforms to tandem zinc finger domains of tristetraprolin (TTP) family proteins. *J. Biol. Chem.*, 287: 5459-5471, 2012.

SPINK2 activity is required to prevent germ cell death in the testis

NIEHS investigators identified the SPINK2 protein and determined it is present in the germ cells in the mouse testis and has trypsin-inhibitory activity. Disruption of the *Spink2* gene significantly impaired fertility in male mice, disrupted testis integrity, increased germ cell apoptosis, and resulted in a reduction in sperm numbers. These studies demonstrated that SPINK2 is required for maintaining normal spermatogenesis and potentially regulates serine protease-mediated apoptosis in male germ cells.

Lee B, Park I, Jin S, Choi H, Kwan JT, Kim J, Jeong J, Eddy EM. Impaired spermatogenesis and fertility in mice carrying a mutation in the Spink2 gene expressed predominantly in the testes. *J. Biol. Chem.*, 286: 29108-29117, 2011.

A new mechanism for the sensitivity and responsiveness to sulfite.

Sulfur dioxide is a commonly encountered urban air pollutant that significantly contributes to increased morbidity in human populations since it is formed during the combustion of fossil fuels near large cities. In addition, its two forms in aqueous solution, (bi)sulfite and sulfite, are widely used as preservatives and antioxidants to prevent food and beverage spoilage. It is known that sulfite can cause allergic reactions in humans. Accordingly, the response to sulfite in individuals with asthma is more intense than in healthy subjects which may lead to severe aggravation of asthma symptoms. This investigation provided a new free radical mediated mechanism for the adverse effects of sulfite. It was found that myeloperoxidase (MPO), an abundant heme protein secreted from activated neutrophils that catalyzes the formation of cytotoxic oxidants implicated in asthma and allergic inflammatory disorders, is able to use (bi)sulfite as a substrate and oxidize it to a free radical metabolite. In summary, this study has identified sulfite radicals that contribute to oxidative protein damage in sulfite or sulfur dioxide-exacerbated allergic reactions suggesting a new mechanism for the response to widely used sulfite.

Ranguelova K, Rice AB, Khajo A, Triquigneaux M, Garantziotis S, Magliozzo RS, Mason RP. Formation of reactive sulfite-derived free radicals by the activation of human neutrophils: An ESR study. *Free Radic. Biol. Med.*, 52: 1264-1271, 2012.

Subtelomeric proteins found

Proteomics of isolated chromatin segments (PICh) was used to identify proteins that interact with the Drosophila subtelomeric DNA repeats, also known as telomere-associated sequence (TAS). This procedure identified several previously known chromatin proteins as being enriched at subtelomeric sequences. Members of the Brm protein complex were also shown to play a role in mediating the formation of heterochromatin at telomeres and silencing neighboring genes.

Antao, JM, Mason JM, Dejardin J, Kingston RE. The protein landscape at Drosophila telomere-associated sequence repeats. *Mol. Cell. Biol.*, 32: 2170-2182, 2012.

Expression of factors controlling cancer metastasis leads to dramatic changes in cellular signaling

This study utilized exogenous expression of transcription factors that induce metastasis in breast cancer cells that are non-metastatic. The cells acquired many of the properties of metastatic cells. The predominant signaling pathways operative in this system changed to resemble those characteristic of metastatic tumors. The study demonstrates that these transcription factors contribute to the metastatic phenotype through 2 distinct mechanisms – downregulation of the epithelial program and upregulation of the metastatic program.

Dhasarathy A, Phadke D, Mav D, Shah RR, Wade PA. The transcription factors Snail and Slug activate the transforming growth factor-beta signaling pathway in breast cancer. *PLoS One*, 6: e26514, 2011.

Estrogen and Estrogen Receptor are Critical in the Development of Endometriosis

Endometriosis is a gynecological disease where aberrant growth of uterine tissue is found in the pelvic cavity. These endometriosis lesions affect 5.5 million women in the US with symptoms that include painful periods, chronic pain, pain with intercourse, and infertility. Endometriosis has many facets and no cure exists. This study using estrogen receptor (ER) knockout mice as donor uterine tissue and as the host peritoneal environment demonstrates ER is critical in disease development. Uterine tissue without ER does not form endometriosis lesions, and lesions established in a peritoneal cavity without ER do not fully respond to estrogen. These studies begin to clarify the important role ER plays in endometriosis and will play a role in elucidating this enigmatic disease.

Burns KA, Rodriguez KF, Hewitt SC, Janardhan KS, Young SL, Korach KS. Role of Estrogen Receptor Signaling Required for Endometriosis-Like Lesion Establishment in a Mouse Model. *Endocrinology*, in press, epub ahead of print doi:10.1210/en.2012-1294

Early life plant estrogen exposure causes embryo development abnormalities in pregnant mice

When female mice are treated just after birth with plant estrogens, they are completely infertile as adults. Part of the explanation for their infertility is that the environment within the oviduct, where mouse embryos grow for the first 4 days of pregnancy, is not normal. These abnormalities in the oviduct environment cause the developing embryos to have alterations in the numbers of cells that will eventually form the embryo and placenta.

Jefferson WN, Padilla-Banks E, Phelps JY, Cantor AM, Williams CJ: Neonatal phytoestrogen exposure alters oviduct mucosal immune response to pregnancy and affects preimplantation embryo development in the mouse. *Biol. Reprod.*, 87:1-10, 2012.

Division in male germ cells is regulated by RHOX13

Meiosis in female germ cells begins in the mouse embryo, but does not begin until after birth in males. NIEHS investigators identified the Rhox13 gene and determined it is turned on at the same time in germ cells in male and female embryos. However, RHOX13 protein is made in germ cells in female embryos, but is not in male germ cells until after birth. The investigators found that the NANOS2 protein is present in male germ cells until after birth, but then disappears when male germ cells enter meiosis, strongly suggesting that NANOS2 suppresses production of RHOX13 protein.

Geyer CB, Saba R, Kato Y, Anderson AJ, Chappell VK, Saga Y, Eddy EM. Rhox13 is translated in premeiotic germ cells in male and female mice and is regulated by NANOS2 in the male. *Biol. Reprod.*, 86: 127, 1-9, 2012.

Persistent fluorinated chemical exposure such as that occurring in the general population does not affect fertility

A recent report of a population of women in Denmark suggested that at current levels of exposure to a class of ubiquitous environmental pollutants, perfluorinated alkyl acids, women with higher blood levels of these compounds were less fertile. This study addressed this question in a population of Norwegian women, and the results showed that the findings in the previous study were probably due to deficiencies in the method of data analysis, and not to an effect on fertility.

Whitworth KW, Haug LS, Baird DD, Becher G, Hoppin JA, Skjaerven R, Thomsen C, Eggesbo M, Travlos G, Wilson, R, Longnecker MP. Perfluorinated compounds and subfecundity in pregnant women. *Epidemiology*, 23: 257-263, 2012.

Exposure to persistent fluorinated chemicals, such as that occurring in the general population, in pregnant women is associated with giving birth to smaller babies.

Several studies have suggested that at current levels of exposure to a class of ubiquitous environmental pollutants, perfluorinated alkyl acids, pregnant women with higher blood levels of these compounds tend to deliver smaller babies. This relationship was investigated in a population of pregnant women from Norway, and verified the association. However, it appears that the association is due to less excretion of the compound during pregnancies resulting in smaller babies, and that the association is unlikely to be casual.

Whitworth KW, Haug LS, Baird DD, Becher G, Hoppin JA, Skjærven R, Thomsen C, Eggesbo E, Travlos G, Wilson R, Cupul-Uicab LA, Brantsaeter AL, Longnecker MP. Perfluorinated compounds in relation to birth weight in the Norwegian Mother and Child Cohort Study (MoBa). *Am. J. Epidemiol*., 175: 1209-1216, 2012.

Exposure to DDT early in life not related to subsequent IQ.

Several recent studies have reported that early-life exposure to the persistent pesticide, DDT, decreases children's IQ; this is an important issue because DDT is still used for malaria control in a dozen countries. The previous studies, however, were conducted in populations with relatively low levels of exposure. This question was addressed using data and specimens from a

study that had been conducted by NIH in the 1960s, when DDT exposure in the U.S. was high. No evidence of an adverse effect on IQ within the range of exposure studied.

Jusko TA, Klebanoff MA, Brock JW, Longnecker MP. In utero exposure to DDT and cognitive development among infants and school-aged children. *Epidemiology*, in press, epub ahead of print doi 10.1097/EDE.0b013e31825fb61d

Juvenile Myositis Clinical Phenotypes Defined

As part of the Childhood Myositis Heterogeneity Collaborative Study Group, NIEHS researchers enrolled more than 400 patients with juvenile idiopathic myopathies in a nationwide registry study, with the aims of comparing demographics, clinical features, laboratory measures, including myositis autoantibodies, and outcomes, among these clinical subgroups, as well as with adult myositis patients, using a novel statistical method called Random forest classification analysis. While juvenile dermatomyositis was characterized by a number of photosensitive and vasculopoathic rashes, juvenile polymyositis, the subset without rashes, was characterized by more severe weakness and more frequent cardiac disease. Patients with juvenile overlap myositis had more frequent interstitial lung disease, Raynaud's phenomenon, and a higher mortality. Several demographic and clinical features were shared between juvenile and adult myositis subgroups, but some features differed. We conclude that juvenile myositis is a heterogeneous group of illnesses with distinct clinical subgroups, defined by varying clinical and demographic characteristics, laboratory features and outcomes. A better understanding of the phenotypes of juvenile myositis should enhance studies of genetic and environmental factors.

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*. 2012, in press.

Studies focus on the role of the blood-spinal cord barrier in drug resistance in amyotrophic lateral sclerosis (ALS)

The blood-spinal cord barrier, which resides within spinal cord capillaries, regulates the movement of water and solutes into and out of the spinal cord and thus critically contributes to homeostasis and to neuroprotection. Together, this work demonstrates that multiple ATP-driven drug efflux transporters, like P-glycoprotein, are major contributors to barrier selectivity. Transporter expression and activity increases following exposure to xenobiotics, such as, dioxins. In an animal model of ALS P-glycoprotein expression and activity at the blood-spinal cord barrier are upregulated. Similar upregualtion is seen in tissue samples from ALS patients. Preclinical drug trials in the mouse model of ALS have failed to decisively slow or arrest disease progression; pharmacoresistance imparted by ABC transporters is one possible explanation for these failures. These observations have implications for ALS therapeutics in humans and suggest that the obstacle provided by these transporters to drug treatments must be overcome to develop effective ALS pharmacotherapies.

Campos CR, Schroeter C, Wang, X, Miller DS. ABC transporter function and regulation at the blood-spinal cord barrier. *J. Cereb. Blood Flow Metab.*, in press, epub ahead of print doi: 10.1038/jcbfm.2012.47.

Jablonski MR, Jacob DA, Campos CR, Miller DS, Maragakis NJ, Pasinelli P, Trotti D. Selective increase of two ABC transporters at the blood-spinal cord barrier suggests disease-driven pharmacoresistance in ALS. *Neurobiol. Dis.*, 47: 194-200, 2012.

MicroRNAs regulate genes involved in metabolism of clinical and over the counter drugs
MicroRNAs (miRNAs) (a new group of noncoding RNAs) have been recently identified which downregulate large groups of genes by preventing synthesis of the protein from RNA. Two microRNAs (103 and 107) have been found to down-regulate a group of human drug metabolizing genes in liver (the cytochrome P450 2Cs) which metabolize 20% of prescription and over-the counter drugs. These include important drugs such as the drug paclitaxel (used to treat breast cancer , coumadin and clopidogrel (both used in individuals at risk of fatal clots), statins, type II diabetes drugs, valium, antidepressants and anti-inflammatory drugs such as celebrex and ibuprofen, and the popular proton pump type antiulcer drugs (e.g. omeprazole).

Zhang SY, Surapureddi S, Coulter S, Ferguson SS, Goldstein JA. Human CYP2C8 is post-transcriptionally regulated by microRNAs 103 and 107 in human liver. *Mol. Pharmacol*., in press, epub ahead of print doi: 10.1124/mol.112.078386.

The tumor suppressor CYLD inhibits melanoma growth and progression
In collaboration with investigators at Duke University, NIEHS investigators have shown that exogenous expression of the tumor supressor CYLD markedly inhibits melanoma cell proliferation and migration in vitro and subcutaneous tumor growth in vivo. In addition, the melanoma cells expressing exogenous CYLD were unable to form pulmonary tumor nodules following tail-vein injection. At the molecular level, CYLD decreased b1-integrin expression and inhibited pJNK induction by TNFa or cell-attachment to collagen IV. Moreover, CYLD induced an array of other molecular changes associated with modulation of the 'malignant' phenotype, including a decreased expression of cyclin D1, N-cadherin and nuclear Bcl3, and an increased expression of p53 and E-cadherin. Most interestingly, co-expression of the constitutively active MKK7 or c-Jun mutants with CYLD prevented the above molecular changes, and fully restored melanoma growth and metastatic potential in vivo. These findings demonstrate that JNK/AP-1 signaling pathway underlies the melanoma growth and metastasis that is associated with CYLD loss-of-function. Thus, restoration of CYLD and inhibition of JNK and b1-integrin function represent potential therapeutic strategies for treatment of malignant melanoma.

Ke H, Augustine CK, Gandham VD, Jin JY, Tyler DS, Akiyama SK, Hall RP, Zhang JY. CYLD inhibits melanoma growth and progression through suppression of the JNK/AP-1 and β1-integrin signaling pathways. *J. Invest. Dermatol*., in press, epub ahead of print, doi: 10.1038/jid.2012.253

Importance of the 5'-dRP blocking group in BER intermediates toward making cells sensitive to poly(ADP-ribose) polymerase inhibition recognized
Treatment of base excision repair–proficient mouse fibroblasts with the DNA alkylating agent methyl methanesulfonate (MMS) and a small molecule inhibitor of PARP-1 results in a striking cell killing phenotype, as previously reported. Earlier studies showed that the mechanism of cell death is apoptosis and requires DNA replication, expression of PARP-1, and an intact S-phase

checkpoint cell signaling system. It is proposed that activity-inhibited PARP-1 becomes immobilized at DNA repair intermediates, and that this blocks DNA repair and interferes with DNA replication, eventually promoting an S-phase checkpoint and G2-M block. Here NIEHS scientists report studies designed to evaluate the prediction that inhibited PARP-1 remains DNA associated in cells undergoing repair of alkylation-induced damage. Using chromatin immunoprecipitation with anti–PARP-1antibody and qPCR for DNA quantification, a higher level of DNA was found associated with PARP-1 in cells treated with MMS plus PARP inhibitor than in cells without inhibitor treatment. These results have implications for explaining the extreme hypersensitivity phenotype after combination treatment with MMS and a PARP inhibitor.

Kedar PS, Stefanick DF, Horton JK, Wilson SH. DNA associated with inhibited PARP-1 in mouse fibroblasts. *Mol. Cancer Res*., 10: 360-368, 2012.

Inhibition of a specific cell receptor-associated enzyme blocks cancer metastasis in an animal model

Human breast cancers frequently metastasize to the lungs, causing chest pain, shortness of breath, weakness and weight loss. A specific cell receptor-associated kinase, called TAK1, was identified as important for cancer cells to adhere to surfaces in vitro, and was then knocked out using genetic techniques. Cancer cells in which the kinase was eliminated no longer efficiently attached to surfaces in vitro, and when these cells were placed back into mice to grow into tumors, they were no longer able to metastasis to the lungs, and their growth was limited to the original site of implantation in the mouse. These results suggest that strategies to inhibit this enzyme in human patients might greatly reduce the spread of breast cancer to the lungs and other organs, and may be a useful approach for improving the quality of life of breast cancer patients.

Ray DM, Myers PH, Painter JT, Hoenerhoff MJ, Olden K, Roberts JD. Inhibition of transforming growth factor-β-activated kinase-1 blocks cancer cell adhesion, invasion, and metastasis. *Br. J. Cancer*, 107: 129-136, 2012.

Pitfalls in Measuring Mutation Rates

Mutation rates are key indices for heritable disease, the adaptation of pathogens to new individuals and new species, the rate of evolution, and the slow decay of genomes. The rapidly falling costs of DNA sequencing have facilitated direct sequencing of whole genomes in order to count and characterize newly arisen mutations. Some such results disagree with results using more classical methods. Sometimes, the results obtained with incautious sequencing experiments can be ascertained.

Drake JW. Contrasting mutation rates from specific-locus and long-term mutation-accumulation procedures. *G3: Genes, Genomes, Genetics,* 2: 483-485, 2012.

UV induced photolesions in mitochondrial DNA have the potential to cause mutations

It was long been known that mitochondria lacked the ability to repair DNA damage from UV irradiation. UV light produces photolesions in DNA that represent blocks to normal DNA replication. However, the fate of UV photolesion in mtDNA is unknown. Researchers at the NIEHS and Duke University studied how the mitochondrial DNA polymerase deals with these

lesions and whether it could bypass UV photolesion. Their results suggest that UV lesions usually stalls mitochondrial DNA replication but some bypass replication did occur and some of this was mutagenic. This study suggests a mechanism for the introduction of point mutations and deletions in the mitochondrial genomes of chronically UV-exposed cells.

Kasiviswanathan R, Gustafson M S, Copeland W C, Meyer JN. Human mitochondrial DNA polymerase γ exhibits potential for bypass and mutagenesis at UV-induced cyclobutane thymine dimers. *J. Biol. Chem*., 287: 9222-9229, 2012.

Conformational effects of ribonucleotide contamination of DNA

NIEHS investigators have recently demonstrated that the incorporation of ribonucleotides represents the most common mutation present in newly-synthesized DNA. This ribonucleotide-contaminated DNA will in general be more labile and can interact differently with the many enzymes that participate in the maintenance and metabolism of the cellular DNA. Previous crystallographic analyses of ribonucleotide-contaminated DNA have indicated that the presence of a single ribonucleotide will convert an entire double-stranded DNA decamer from a "B" to an "A" conformation. In contrast, NMR analysis of the behavior of ribonucleotide-contaminated DNA in solution indicates that the conformational perturbation produced by the ribonucleotide is much more localized, resulting in a more A-like conformation only near the ribonucleotide-substitution site.

DeRose, EF, Perera L, Murray MS, Kunkel TA, London RE. Solution structure of the dickerson DNA dodecamer containing a single ribonucleotide. *Biochemistry*, 51: 2407-2416, 2012.

Activation-induced deaminase is a major player in the development of autoimmune antibodies

B cells contribute to autoimmunity both as secretors of pathogenic antibodies and through the activation of autoreactive T cells. B cells and antibodies acquire higher affinity to self-antigen through a process known as immunoglobulin hypermutation or SHM. The contribution of SHM to pathogenic antibody development in lupus has been established in various autoimmune mouse models and by examining antibodies from patients. However, its role in the antibody-independent contribution of B cells to autoimmunity has not been examined. NIEHS investigators generated a lupus-prone MRL/lpr mice with a limited IgM-only B cell repertoire, no secreted antibodies and no SHM. This enabled them to isolate the role of somatic hypermutation in B cell-mediated autoimmunity. They found that SHM-deficiency correlated with a reduction in autoreactive B cells, a decrease in T cell activation and a decrease in kidney lymphocytic infiltration. These data establish AID as an important contributor to the antibody-independent role of B cells in autoimmunity.

Jiang C, Zhao ML, Waters KM, Diaz M. Activation-induced deaminase contributes to the antibody-independent role of B cells in the development of autoimmunity. *Autoimmunity*, in press, epub ahead of print doi:10.3109/08916934.2012.682668.

Identification of an optimal anti-AIDS drug with little host toxicity

AIDS is still a major health problem in the world as a result of HIV infection. The most effective therapy to thwart infection makes use of nucleoside reverse transcriptase inhibitors (NRTIs). Many NRTIs also cause a devastating side effect by induction of mitochondrial dysfunction leading to muscle weakness, lipodystrophy and peripheral neuropathy. This induced mitochondrial disorder is caused by the NRTI-inhibition of the human DNA polymerase gamma, the enzyme responsible for copying our mitochondrial DNA. In this study, Two novel thymidine analogs, 3'-fluoro-3'-deoxythymidine (FLT) and 2',3'-didehydrot3'-deoxy-4'-ethynylthymidine (Ed4T), have been investigated as NRTIs for treatment of HIV infection by a collaborative project involving researchers from Yale, Emory, Japan, the NCI and the NIEHS. These scientists found that Ed4T-triphosphate (TP) is the first analog to be preferred over native nucleotides by the HIV reverse transcriptase. They also showed minimal incorporation by the human DNA pol γ, which demonstrates an ideal balance between high antiretroviral efficacy and minimal host toxicity. These molecular mechanisms of analog incorporation, which are critical for understanding pol γ-related toxicity, shed light on the unique toxicity profiles observed during clinical trials.

Sohl CD, Kasiviswanathan R, Kim J, Pradere U, Schinazi RF, Copeland WC, Mitsuya H, Baba M, Anderson KS. Balancing antiviral potency and host toxicity: identifying a nucleotide inhibitor with an optimal kinetic phenotype for HIV-1 reverse transcriptase *Mol. Pharmacol.,* 82: 125-133, 2012.

Post-transcriptional regulation of cell-cell interaction protein-encoding genes

Zfs1p is a member of the tristetraprolin (TTP) family of CCCH tandem zinc finger proteins that bind directly to AU-rich elements in mRNAs and promote transcript deadenylation and decay. This study identified the targets of Zfs1p using both deep sequencing and microarray approaches, including the gene Cbf12p that is known to increase cell-cell adhesion and flocculation when over-expressed. The study suggested that Zfs1p can both directly and indirectly regulate the levels of transcripts involved in cell-cell adhesion in *S. pombe*.

Wells M, Huang W, Li L, Gerrish K, Fargo D, Ozsolak F. Post-transcriptional regulation of cell-cell interaction protein-encoding transcripts by Zfs1p in S. pombe. *Mol. Cell. Biol.*, in press.

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 wider role in the general detoxification of N-hydroxylated compounds.

Itsko M, Schaaper RM. The *dgt* gene of *E. coli* facilitates thymine utilization in thymine-requiring strains. *Mol. Microbiol*., 81: 1221-1232, 2011.

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.

Odet F, Gabel SA, Williams J, London RE, Goldberg E, Eddy EM. Lactate dehydrogenase C and energy metabolism in mouse sperm. *Biol. Reprod*., 85: 556-564, 2011.

Early life plant estrogen exposure causes genital defects in mice

In female mice, the urethra normally exits from the end of the clitoris. Urethral development is incomplete at birth, but finishes by 5-7 days of age. As was previously shown in female mice treated with the potent estrogenic compound diethylstilbestrol, female mice treated just after birth with plant estrogens form an abnormal opening of the urethra on the lower side of the clitoris.

Padilla-Banks E, Jefferson WN, Myers PH, Goulding DR, Williams CJ. Neonatal phytoestrogen exposure causes hypospadias in female mice. *Mol. Reprod. Dev.,* 79:3, 2012.

Making activity calls in quantitative high throughput screening data

Quantitative high-throughput screening (qHTS) assays are multiple-concentration experiments that can simultaneously assay thousands of chemicals over a wide chemical space with reduced cost per substance. A three-stage algorithm classifies substances from qHTS data into statistically supported activity categories relevant to toxicological evaluation. The first stage finds active substances with a robust concentration-response profile within the tested concentration range. The second stage finds relatively potent substances with substantial activity at the lowest tested concentration not captured in the first stage. The third and final stage separates statistically significant (but toxicologically suspect) profiles from responses that lack statistically compelling support, or "inactives".

Shockley KR. A three-stage algorithm to make toxicologically relevant activity calls from quantitative high throughput screening data. *Environ. Health Perspect.,* in press, epub ahead of print doi:10.1289/ehp.1104688

A next-generation sequencing simulator

NIEHS investigators described a method and produced a software package for simulating the error profiles of the widely used next-generation sequencing machines.

Huang W, Li L, Myers JR, Marth GT. ART: a next-generation sequencing simulator. *Bioinformatics*, 28: 593-594, 2012.

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
Kin Chan, Ph.D.	Dmitry Gordenin	A reporter system for identifying mutagens acting preferentially on single-strand DNA
Georgette M. Charles, Ph.D.	Guang Hu	Regulation of APA site choice in the maintenance of ES cells
Tracy M. Clement, Ph.D.	Mitch Eddy	Testis Expressed Actin-like 7b (Actl7b) is Required for Mouse Spermatid Morphogenesis and Male Fertility
Jacqueline de Marchena Powell, Ph.D.	Patricia Jensen	A novel approach to isolate the function of the galanergic subpopulation of the locus coeruleus
Swati Ghosh, Ph.D.	Raja Jothi	An integrated approach reveals that Tet1 maintains mouse embryonic stem cell identity partly by regulating LIF dependent Stat3-mediated gene activation
Zhenglin Gu, Ph.D.	Jerrel Yakel	Cholinergic coordination of pre- and postsynaptic activity induces timing-dependent hippocampal synaptic plasticity
Brant Hamel, Ph.D.	John Cidlowski	The N-terminus of the glucocorticoid receptor regulates its nucleocytoplasmic localization
Bonnie R. Joubert, Ph.D.	Stephanie London	Epigenome-wide association study identifies DNA methylation differences in cord blood related to in utero tobacco smoke exposure
Fumin Lin, Ph.D.	Anton Jetten	Role of GLIS3 in the generation of pancreatic beta cells from ES and iPS cells
Julie M. Lowe, Ph.D.	Michael Resnick	An unexpected role for p53 in NF-kappaB-mediated inflammatory responses

Steven A. Roberts, Ph.D.	Dmitry Gordenin	A permanent record of transient hyper-mutation associated with single-strand DNA in human cancers
Maria Shatz, Ph.D.	Michael Resnick	p53 cooperates with MAP kinase and NFkB signal transduction pathways to potentiate human immune/inflammatory response
Lindsay K. Smith, Ph.D.	David Miller	Glucocorticoid Receptor (GR) Regulation of P-glycoprotein (Pgp) at the Blood-Brain Barrier (BBB)
Dan Su, Ph.D.	Douglas Bell	Chromatin state primes stress specific p53-regulated gene responses
Darshini Trivedi, Ph.D.	Robert Langenbach	The deficiency of beta-arrestin2 attenuates abdominal aortic aneurysm formation in mice.
Kirsten C. Verhein, Ph.D.	Steven Kleeberger	Differential susceptibility to ozone-induced lung inflammation maps to mouse chromosome 17: role of Notch receptors
Staton L. Wade, Ph.D.	Trevor Archer	MicroRNA-mediated regulation of the BRG1 chromatin remodeling complex underlies the balance between pluripotency and differentiation in human embryonic stem cells
Qingshan Wang, M.D.	Jau-Shyong Hong	Substance P exacerbates neurotoxins-induced nigral dopaminergic neurodegeneration through activation of microglial NADPH oxidase
Xiaofeng Zheng, Ph.D.	Guang Hu	Identification of a novel component of the self-renewal circuitry conserved in mouse and human ES cells

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, college undergraduate and graduate 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 26, and awards were presented for Best Poster in three categories, High School Interns, Undergraduate Interns, and Graduate Student/Professional Interns. At the Awards Ceremony the following awards were presented:

Best Poster by High School Interns
Diana Dayal attends William G. Enloe High School, Raleigh NC, and worked in the Laboratory of Neurobiology under the direction of Dr. David Armstrong. Poster: Dayal, D., Ezequiel, M. and Armstrong, D. Testing for Xenobiotic Disruption of Calcium Oscillations in Rat Pituitary Cells.

Best Poster by Undergraduate Interns
Kaushik Annam attends the University of Pennsylvania, Philadelphia, PA, and worked in the Biostatistics Branch under the direction of Dr. Clarice Weinberg. Poster: Annam, K., Dinse, G., Jusko, T., Ho, L. and Weinberg, C. Association between Serum Levels of Environmental Contaminants in the US Population and Antinuclear Antibodies: A Novel Application of Cox Regression.

Best Poster by Graduate Interns
Bridget Mayer attends North Carolina State University, Raleigh, NC, and worked in the Comparative Medicine Branch under the Direction of Dr. Terry Blankenship-Paris. Poster: Mayer, B., Myers, P., Goulding, D., Gray, R., Clark, J. and Blankenship-Paris, T. A Comparison of Sustained Release Tramadol and Buprenorphine as Analgesics in Rats.