Division of Intramural Research

NAEHS Council Update

September 2011

DIR RECRUITMENTS

Director Division of Intramural Research
The NIEHS is seeking an exceptional scientific leader interested in being a part of a dynamic management team to fill the position of Director, Division of Intramural Research. In addition, the selectee will also serve as the Scientific Director. The incumbent of this position will direct laboratory and clinical research. The Director, DIR, also serves as a principal advisor to the Institute Director on intramural scientific activities involving interdisciplinary biomedical research programs; develops and recommends policies for the execution of research programs; determines effectiveness of current programs and recommends policies for new programs; and develops new and revised programs to meet national environmental health needs. The Division is organized into five scientific programs, including the Clinical Research Program, which highlight the areas of research excellence of NIEHS. These programs are highly interrelated, interactive and synergistic. Using the interdisciplinary biomedical research approach, the mission of the DIR is to contribute to the basic understanding of biological and chemical processes, understanding of the contributions of environmental agents to human disease and dysfunction and to the underlying mechanisms of environmentally associated diseases. Dr. Patricia Grady, Director, National Institute of Nursing Research, is chair of the search committee. A candidate has been identified.

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.

Staff Scientist for Oil Spill Study, Biostatistics Branch

The Biostatistics Branch is seeking a Staff Scientist with a strong background in biostatistics and a lively interest in methods and epidemiologic applications to participate in team-oriented research on a large prospective study of clean-up workers exposed to petroleum products, chemicals and other environmental hazards following the BP/Deepwater Horizon oil spill in the Gulf of Mexico. The position is ideal for a person with proven experience in applications to environmental epidemiology and interest in working collaboratively to assess potential human health effects of this recent environmental disaster. The position offers opportunities for collaboration with other ongoing projects in biostatistics and epidemiology and will include time and support for carrying out investigator-initiated methodology research related to inference in epidemiologic studies. Minimum qualifications include a doctoral degree (Ph.D. or equivalent) with a strong background in biostatistics and documented interest in epidemiology. Familiarity with methods for exposure assessment and modeling in the context of longitudinal studies is desirable. Dr. Freya Kamel, Epidemiology Branch, is chair of the search committee. A candidate has been identified.

NEW APPOINTMENTS IN THE DIVISION OF INTRAMURAL RESEARCH

Dr. David Kurtz, Comparative Medicine Branch

Dr. David Kurtz recently joined the Comparative Medicine Branch at NIEHS as a Staff Veterinary Scientist. Dr. Kurtz received his Doctor of Veterinary Medicine in 1989 from the University of Tennessee. After spending a few years in private, small animal practice, Dr. Kurtz entered a residency in laboratory animal medicine at the University of Alabama at Birmingham (UAB). At UAB, Dr. Kurtz went on to complete his Ph.D., in Molecular Pathology in 1998. His research focused on inborn errors of lipid metabolism, and he developed both transgenic and knockout mouse models used in these studies. Dr. Kurtz completed a post-doctoral fellowship and became faculty at Washington University School of Medicine in St. Louis (WUSTL) where he studied the role of nuclear hormone receptors in the regulation of cardiac and hepatic lipid metabolism. While at WUSTL, Dr. Kurtz also served as a clinical veterinarian for the Division of Comparative Medicine. From 2003 until his arrival at NIEHS in May 2011, Dr. Kurtz served as the Attending Veterinarian for the U.S Environmental Protection Agency, National Health and Environmental Effects Research Laboratory (NHEERL). During this period, he also served as the Attending Veterinarian for the Hamner Institutes for Health Sciences and Integrated Laboratory Services, Inc. both located in Research Triangle Park. Dr. Kurtz is board certified in laboratory animal medicine through the American College of Laboratory Animal Medicine (ACLAM).

At the NIEHS, Dr. Kurtz supports the service responsibilities and research goals of the Comparative Medicine Branch. His clinical responsibilities include participation in the NIEHS veterinary medical program for all animals and oversight of the NIEHS aquatic species. He will assist the Quality Assurance Laboratory in the NIEHS animal health monitoring plan including animal import/export. Dr. Kurtz will also assist the NIEHS Animal Care and Use Committee (ACUC) office in implementation of an electronic animal study proposal submission database. Dr. Kurtz's research interests include the development and implementation of molecular diagnostics for diseases of laboratory animal species as well as the identification of valid physiological biomarkers used for the assessment of the health and well-being of aging laboratory rodents.

Selected Publications:

Phillips PM, Jarema KA, Kurtz DM, MacPhail RC. An observational assessment method for aging laboratory rats. J. Am. Assoc. Lab. Anim. Sci., 49: 792-799, 2010.

Wood PA, Kurtz DM, Cox KB, Nyman LR, Elgavish A, Hamm DA, Gower BA, Nagy TR. Role of genetic deficiency of fatty acid oxidation in metabolic syndrome/obesity. *Prog. Obesity Res.*, 9: 293-296, 2003.

Kurtz DM, Tian L, Gower BA, Nagy TR, Pinkert CA, Wood PA. Transgenic studies of fatty acid oxidation gene expression in nonobese diabetic mice. *J. Lipid Res.*, 41: 2063-2070, 2000.

Kurtz DM, Rinaldo P, Rhead WJ, Tian L, Millington D, Vockley J, Hamm DA, Brix AE, Lindsey JR, Pinkert CA, O'Brien WE, Wood PA. Targeted disruption of mouse long-chain acyl-CoA dehydrogenase gene reveals crucial roles for fatty acid oxidation. *Proc. Natl. Acad. Sci. U.S.A.*, 95: 15592-15597, 1998.

SPECIAL RECOGNITION FOR DIR PRINCIPAL INVESTIGATOR

NIH honors Kunkel as distinguished investigator

NIH announced in early June its approval for the promotion of NIEHS Principal Investigator Thomas A. Kunkel, Ph.D., to the rank of NIH Distinguished Investigator, one of the highest honors NIH awards to its scientists. The promotion is achieved by an estimated two to three percent of NIH scientists. This title is reserved for tenured intramural senior investigators who are at the highest level of accomplishment in their respective fields.

Dr. Kunkel serves as Director of the NIEHS Experimental Biology Program, head of the Laboratory of Molecular Genetics DNA Replication Fidelity Group and chief of the Laboratory of Structural Biology.

DIR RESEARCH UPDATE

Nicotinic Acetylcholine Receptor Function in the Brain: Role in Synaptic Excitability, Plasticity, and Disease

Jerrel L. Yakel, Ph.D., Principal Investigator

Ion Channel Physiology Group
Laboratory of Neurobiology, DIR, NIEHS

Nicotine is one of the most potent and prevalent neurotoxins in the environment; infant deaths have been linked to exposure *in utero*, and there are cognitive and behavioral disabilities in children exposed *in utero*, or to second hand smoke. However nicotine can enhance cognition in adults, and also appears to be neuroprotective in Parkinson's and perhaps Alzheimer's disease. Nicotine exerts its effect in the nervous system by acting on the nicotinic acetylcholine receptor channel (nAChR). The nAChRs are ligand-gated ion channels and are widely expressed throughout the brain and nervous system in general, where they are involved in a variety of brain functions including (but not limited to) development, learning and memory formation, and reward. Furthermore deficits in nAChR signaling are associated with neurodegenerative diseases, such as Alzheimer's (AD) and Parkinson's disease (PD). In the Ion Channel Physiology Group, we focus on understanding structure/function aspects of native and expressed nAChRs, and what role these receptors play in neural circuits, including the hippocampus, an important region for learning and memory. Using a multitude of electrophysiological and light and laser-based imaging techniques, we have recently shown the remarkable temporal precision of cholinergic functions in the hippocampus that induces synaptic plasticity, a cellular model of learning and memory. Our recent work provides a novel mechanism for information processing in cholinergic-dependent higher cognitive functions.

DIR SCIENTIFIC ACCOMPLISHMENTS 2011

GuLF Study Initiated
NIEHS investigators launched the GuLF STUDY, a study of as many as 55,000 oil spill clean-up workers, in response to a national public health disaster directly affecting residents in the Gulf states as well as workers dispatched from all over the US to help clean up after the explosion on the Deep Water Horizon drilling rig.

Preventing a genetic identity crisis.
Ribonucleotides are the building blocks for RNA but are undesirable in DNA because they sensitizes DNA to cleavage, potentially destabilizing the genome. Thus the integrity of DNA-based genomes depends on the ability of replication enzymes to prevent incorporation of ribonucleotides into DNA. Given this, NIEHS investigators were surprised to discover that ribonucleotides are frequently incorporated into DNA in cells. The researchers found that these ribonucleotides are normally removed by a dedicated DNA repair system, but if this repair system is defective, cells accumulate unusual mutations that inactivate genes, with the potential to initiate human diseases. Two subsequent studies have provided a likely, but unexpected explanation for how these unusual mutations are generated.

Nick McElhinny SA, Kumar D, Clark AB, Watt DL, Watts BE, Lundström E-B, Johansson E, Chabes A, Kunkel TA. Genome instability due to ribonucleotide incorporation into DNA. *Nat. Chem. Biol.* 6: 774-781, 2010.

Clark AB, Lujan SA, Kissling GE, Kunkel TA. Mismatch repair-independent tandem repeat sequence instability resulting from ribonucleotide incorporation by DNA polymerase e. *DNA Repair* 10: 476-482, 2011.

Kim N, Huang S, Williams JS, Li YC, Clark AB, Cho J-E, Kunkel TA, Pommier Y, Jinks-Robertson S. Mutagenic processing of ribonucleotides in DNA by yeast topoisomerase 1. *Science* 332: 1561-1564, 2011

Mutations resulting from mimicry
When Watson and Crick first described the structure of the DNA helix in 1953, they proposed that mutations might result from mismatches whose geometry mimics that of correct base pairs. This year NIEHS scientists provided direct evidence for this idea, in the form of a crystal structure of a polymerase that has a common catalytic pathway for inserting both a correct and an incorrect nucleotide. This study reveals how the chemical complexity of the genetic information can sometimes trick even a normal, healthy cell into making a mistake, for good (evolution) or bad (disease). In other words, mutations underlying diseases do not necessarily require that a person have a genetic defect or be exposed to radiation or chemicals in the environment.

Bebenek K, Pedersen LC, Kunkel TA. Replication infidelity via a mismatch with Watson-Crick geometry. *Proc. Natl. Acad. Sci. U.S.A.* **108**: 1862-1867, 2011.

Launch of SELF Study
NIEHS investigators have successfully launched the Study of Environment, Lifestyle & Fibroids (SELF) in Detroit, Michigan. Investigators have enrolled 400 of the 1600 African American women ages 23-34 who will participate. Each participant has completed two self-administered

questionnaires, a detailed telephone interview, and a clinic visit for ultrasound, blood and urine collection, and measurement of height, weight, blood pressure, and skin reflectance. The video describing the study won a Plain Language award, and the website (detroitself.org) and brochure have attracted much acclaim. The objective of the study is to identify factors that influence the risk of developing fibroids or increase growth of existing fibroids. Investigators are especially interested in evaluating the potential protective role of vitamin D. This could provide marked public health gains against this condition that remains the leading indication for hysterectomy in the United States.

Parkinson patients may have double the risk for melanoma

In a meta-analysis, NIEHS scientists found that patients with Parkinson's disease were twice more likely to have melanoma than individuals without Parkinson's disease. Non-melanoma skin cancer was however not associated with Parkinson's disease. Further studies are needed to understand why and how these two conditions are linked. One possibility is that they may have shared genetic or environmental risk factors.

Liu R, Gao X, Lu Y, Chen H. Meta-analysis of the relationship between Parkinson disease and melanoma. *Neurology*, 76: 2002-2009, 2011

Diet may protect against mutagens in fried meat

Investigators from NIEHS, along with collaborators from the University of North Carolina at Chapel Hill, and the Environmental Protection Agency, have found that dietary factors can reduce DNA damage caused by heterocylic amines (HCAs), carcinogenic compounds formed in meat cooked at high temperatures. The researchers determined that eating cruciferous vegetables, such as broccoli and cauliflower, chlorophyll-derived chlorophyllin (CHL) tablets, and yogurt reduced the amount of DNA damage found in colon cells obtained by biopsy, compared to the tissue of other volunteers on a control diet. The study is the first to show that eating these foods can measurably reduce DNA damage in human colon cells.

Shaughnessy DT, Gangarosa LM, Schliebe B, Umbach DM, Xu Z, MacIntosh B, Knize MG, Matthews PP, Swank AE, Sandler RS, DeMarini DM, Taylor JA. Inhibition of fried meat-induced colorectal DNA damage and altered systemic genotoxicity in humans by crucifera, chlorophyllin, and yogurt. *PLoS One*, 6: e18707, 2011.

New mechanism that may be important for learning and memory

Neurons in the hippocampus, one of the parts of the brain that is thought to have a critical function in learning and memory, communicate with each other at synapses by releasing various neurotransmitters, including acetylcholine and glutamate, which stimulate electrical signals in the adjacent neurons. New findings by NIEHS scientists in mice suggest that the timing when the neurotransmitter acetylcholine is released in the hippocampus may play a key role in regulating the strength of nerve cell synapses. Understanding the complex nature of neuronal signaling at synapses could lead to better understanding of learning and memory, and novel treatments for relevant disorders, such as Alzheimer's disease and schizophrenia.

Gu Z, Yakel JL. Timing-dependent septal cholinergic induction of dynamic hippocampal synaptic plasticity. *Neuron,* 71: 155-165, 2011.

Impact of poised RNA polymerase II on neuronal gene transcription.
NIEHS scientists found strong evidence that the rapid induction of neuronal immediate early genes (IEGs) requires poised RNA polymerase II (Pol II), thus suggesting a role for this mechanism in a wide range of processes including learning and memory. The study made use of genome-wide sequencing to find that this particular enzyme is enriched in the proximity of neuronal IEGs with rapid response kinetics. The scientists further found that Negative Elongation Factor (NELF) proteins were required for both Pol II stalling and the rapid kinetics of the 'rapid IEGs'. Finally, IEGs with slower responses were found to lack Pol II stalling in most instances and, as such, they remained unaffected by reducing levels of NELF proteins. The data derived from this study support the idea that rapidly induced IEGs are a specialized subset of genes poised for an immediate response mediated by Pol II stalling, though the investigators are quick to note that further testing is required to determine the role of this process in brain function and behavior.

Saha RN, Wissink EM, Bailey ER, Zhao M, Fargo DC, Hwang JY, Daigle KR, Fenn JD, Adelman K, Dudek SM. Rapid activity-induced transcription of Arc and other IEGs relies on poised RNA polymerase II. *Nat. Neurosci.*, 14: 848-856, 2011.

A dynamic competition between the transcription machinery and chromatin regulates stimulus-responsive gene expression
Genes in stimulus-responsive networks are precisely regulated to ensure that environmental cues and signals elicit the appropriate transcriptional responses. Recent work by NIEHS investigators has revealed that many rapidly induced genes are pre-loaded with RNA polymerase II prior to full gene activation: this polymerase is transcriptionally engaged but paused just within the gene, where it awaits an activating signal. These investigators now report that pausing serves a critical role in establishing an open, accessible chromatin architecture around target promoters, to enable further and future gene activation. Notably, stimulus-responsive promoters became occluded by nucleosomes in the absence of paused polymerase, in part because their promoter DNA sequence favors local nucleosome assembly. In this way, competition for promoter occupancy between chromatin and paused RNA polymerase can regulate gene accessibility and activity, to facilitate rapid yet balanced responses to environmental stimuli.

Gilchrist DA, dos Santos G, Fargo DC, Xie B, Gao Y, Li L, Adelman K. Pausing of RNA polymerase II disrupts DNA-specified nucleosome organization to enable precise gene regulation. *Cell*, 143: 540-551, 2010.

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.,* in press, 2011.

High-resolution crystal structure of pro-mutagenic 8-oxodGTP in the confines of a DNA polymerase active site

A major product of oxidative base damage is 8-oxo-7,8-dihydro-2'-deoxyguanine (8odG). The oxidation of the guanosine base occurs both in DNA and the nucleotide pool used in the cell for DNA synthesis. The coding potential of this lesion is modulated by its glycosidic torsion angle that controls whether its Watson-Crick or Hoogsteen edge is utilized for base pairing. The 2.0 Å structure of DNA polymerase (pol) β bound with 8odGTP opposite the template base adenine indicated that the modified nucleotide assumes the pro-mutagenic *syn*-conformation and that the non-mutagenic *anti*-conformation would be incompatible with efficient DNA synthesis.

Batra VK, Beard WA, Hou EW, Pedersen LC, Prasad R, Wilson SH. Mutagenic conformation of 8-oxo-7,8-dihydro-2'-dGTP in the confines of a DNA polymerase active site. *Nat. Struct. Mol. Biol.*, 17: 889-890, 2010.

Genome-wide analysis of the methylome

NIEHS scientists describe a novel methodology to assess the methylation status of the mammalian genome at a single GpG resolution. It is able to interrogate the methylation status of 1/3 of all CpGs in a mammalian genome and ½ of the CpGs found in CpG islands. This method will be used to address effects of environmental exposures on this epigenetic mark in an easy and cost-effective manner that has not been possible to do before.

Colaneri A, Staffa N, Fargo DC, Gao Y, Wang T, Peddada SD, Birnbaumer L. Expanded methyl-sensitive cut counting reveals hypomethylation as an epigenetic state that highlights functional sequences of the genome. *Proc. Natl. Acad. Sci. U.S.A.,*108: 9715-9720, 2011.

Identification of 16 new genetic loci regulating pulmonary function

The collaborative group within the CHARGE consortium to do GWAS meta-analysis of pulmonary function headed up by NIEHS investigators worked with the SpiroMeta consortium in the UK to team-up to provide more than twice the sample size. This joint effort has led to the discovery of an additional 16 novel loci for pulmonary function.

Soler Artigas M‡, Loth D‡, Wain LV‡, Gharib SA‡, Obeidat M‡, Tang W‡, Zhai G, Zhao JH, Smith AV, Huffman JE, Albrecht E, Jackson CM, Evans DM, Cadby G, Fornage M, Manichaikul A, Lopez JM, Johnson T, Aldrich MC, Aspelund T, Barroso I, Campbell H, Cassano PA, Couper DJ, Eiriksdottir G, Franceschini N, Garcia M, Gieger C, Gislason GK, Grkovic I, Hammond CJ, Hancock DB, Harris TB,

Ramasamy A, Heckbert SR, Heliövaara M, Homuth G, Hysi PG, James AL, Jankovic S, Joubert BR, Karrasch S, Klopp N, Kritchevsky SB, Koch B, Kritchevsky SB, Launer LJ, Liu Y, Loehr LR, Lohman K, Loos RJP, Lumley T, Balushi A, Ang WQ, Barr GR, Beilby J, Beilin LJ, Blakey JD, Boban M, Boraska V, Brisman J, Britton JR, Brusselle GG, Cooper C, Curjuric I, Dahgam S, Deary IJ, Ebrahim S, Eijgelsheim M, Francks C, Gaysina D, Granell R, Gu X, Hankinson JL, Hardy R, Harris SE, Henderson J, Henry A, Hingorani AD, Hofman A, Holt PG, Hui J, Hunter ML, Imboden M, Jameson KA, Kerr SM, Kolcic I, Kronenberg F, Liu JZ, Marchini J, McKeever T, Morris AD, Olin A-C, Porteus D, Postma DS, Rich SS, Ring SM, Rivadeneira F, Rochat T, Sayer AA, Sayers I, Sly PD, Smith GD, Sood A, Starr JM, Uitterlinden AG, Vonk JM, Wannamethee SG, Whincup PH, Wijmenga C, Williams OD, Wong A, Mangino M, Marciante KD, McArdle WL, Meibohm B, Morrison AC, North KE, Omenaas E, Palmer LJ, Pietiläinen KH, Pin I, Polašek O, Pouta A, Psaty BM, Hartikainen A-L, Rantanen T, Ripatti S, Rotter JI, Rudan I, Rudnicka AR, Schulz H, Shin S-Y, Spector TD, Surakka I, Vitart V, Völzke H, Wareham NJ, Warrington NM, Wichmann H-E, Wild SH, Wilk JB, Wjst M, Wright AF, Zgaga L, Zemunik T, Pennell CE, Nyberg F, Kuh D, Holloway JW, Boezen HM, Lawlor DA, Morris RW, Probst-Hensch N, The International Lung Cancer Consortium, GIANT Consortium, Kaprio J, Wilson JF, Hayward C, Kähönen M, Heinrich J, Musk AW, Jarvis DL, Gläser S, Järvelin M-R, Stricker BHC‡, Elliott P‡, O'Connor GT‡, Strachan DP‡, London SJ‡*, Hall IP‡, Gudnason V‡, Tobin MD‡*. Genome wide association and scale follow-up identifies 16 novel loci for lung function. *Nat. Genet.*, in press, 2011.

A genetic locus associated with asthma in African-Americans

NIEHS investigators joined in a consortium of all NIH-funded investigators with asthma GWAS data. The consortium reanalyzed their data after doing a procedure called imputation to enable combining across datasets with different GWAS platforms. They identified a novel locus associated with asthma in African-Americans.

Torgerson DG, Ampleford EJ, Chiu GY, Gauderman WJ, Gignoux CR, Graves PE, Himes BE, Levin AM, Mathias RA, Hancock DB, Baurley J, Eng C, Stern DA, Celedón JC, Rafaels N, Capurso D, Conti DV, Roth LA, Soto-Quiros M, Togias A, Li X, Myers RA, Romieu I, Van den Berg DJ, Hu D, Hansel NN, Hernandez RD, Israel E, Salam MT, Galanter J, Avila PC, Avila L, Rodriquez-Santana JR, Chapela R, Rodriguez-Cintron W, Diette GB, Adkinson NF, Abel RA, Ross KD, Shi M, Faruque WMU, Dunston GM, Watson HR, Mantese VJ, Ezurum SC, Liang L, Ruczinski I, Ford JG, Huntsman S, Chung KF, Vora H, Li X, Calhoun WJ, Castro M, Sierra-Monge JJ, del Rio-Navarro B, Deichmann KA, Heinzmann A, Wenzel SE, Busse WW, Gern JE, Lemanske Jr. RF, Beaty TH, Bleecker ER, Raby BA, Meyers DA, London SJ, Gilliland FD, Burchard EG, Martinez FD, Weiss ST, Williams LK, Barnes KD, Ober C*, Nicolae DL*. Meta-analysis of Genome-wide Association Studies of Asthma in Ethnically Diverse North American Populations. *Nat. Genet.*, in press, 2011.

Coffee consumption interacts with the GRIN2A genotype in reducing the risk for Parkinson's disease

Using genome-wide screening and follow-up confirmation, NIEHS investigators in a collaborative study found a potential interaction between coffee drinking and the gene GRIN2A. Coffee drinking was much more associated with lower risk of Parkinson's disease among carriers of this polymorphism than among wild-type carriers. This is the first gene-environment interaction that was identified using a genome-wide approach. This finding may not only have significant implications in understanding Parkinson etiology, but also in its prevention and treatment.

Hamza TH, Chen H, Hill-Burns EM, Rhodes SL, Montimurro J, Kay DM, Tenesa A, Kusel VI, Sheehan P, Eaaswarkhanth M, Yearout D, Roberts J, Agarwal P, Bordelon Y, Park Y, Wang L, Gao J, Vance JM, Kendler KS, Scott W, Ritz B, Nutt J, Factor SA, Zabetian CP, Payami H. Genome-Wide Gene-Environment Study Identifies Glutamate Receptor Gene GRIN2A as a Parkinson's Disease Modifier Gene via Interaction with Coffee. *PLoS Genet.,* in press, 2011.

Environmental factors preceding illness onset vary in different forms of autoimmune muscle diseases in children

Previous studies have suggested that respiratory infections commonly occur prior to the onset of a rare autoimmune muscle disease in children, which is defined by chronic muscle inflammation, called juvenile myositis. To understand if other exposures might be important in the development of this heterogeneous disease, NIEHS researchers studied 285 juvenile myositis patients from across the US. Sixty percent of these had a reported exposure previously associated with autoimmune diseases within six months before illness onset. Patients older than the median age at diagnosis, those with a longer delay to diagnosis and those with anti-signal recognition particle autoantibody had a higher frequency of documented exposures. Exposures varied by age at diagnosis, delay to diagnosis, race, disease course, and the presence of certain myositis autoantibodies. Children younger than the median age at diagnosis, those with ≤ 4 months delay to diagnosis, or with a polycyclic illness course were more likely to have an infection within six months of diagnosis, whereas older children had a higher frequency of stressful life events prior to illness onset. Caucasian patients and those without a myositis autoantibody had a higher frequency of infections in the six months prior to illness onset. While infections were the most common exposure temporally associated with the onset of autoimmune muscle disease in children, supporting prior findings, many other exposures, including non-infectious agents, were also documented within six months before disease onset. Autoimmune muscle diseases in children may be related to multiple exposures, and these exposures appear to vary among different forms of these illnesses.

Rider LG, Wu L, Mamyrova G, Targoff IN, Miller FW, for the Childhood Myositis Heterogeneity Collaborative Study Group. Environmental Factors Preceding Illness Onset Differ in Phenotypes of the Juvenile Idiopathic Inflammatory Myopathies. *Rheumatology,* 49: 2381-2390, 2010.

Critical roles of ROR nuclear receptor in Th17 cell differentiation, immunity, and obesity.

Previously NIEHS investigators reported that the nuclear orphan receptors RORα and RORγt play a critical role in the generation of proinflammatory TH17 T cells and Il17a expression, which play an important role in autoimmune diseases. They now show that in cooperation with

RORγt and RORα, IκBζ enhances Il17a expression by binding directly to the regulatory region of the Il17a gene. In contrast vitamin D inhibits RORγ and Il17a expression. This study provides evidence for the transcriptional mechanisms underlying TH17 development and points to a molecular basis for a novel therapeutic strategy against autoimmune disease. In addition, roles for vitamin D receptor (VDR) and IkBζ in Th17 differentiation were identified. They further showed that RORα-null mice are resistant to high fat diet-induced obesity, hepatic steatosis and inflammation, and do not develop insulin resistance. RORα might be a new target in the management of obesity and insulin resistance.

Okamoto K, Iwai Y, Oh-Hora M, Yamamoto M, Morio T, Aoki K, Ohya K, Jetten AM, Akira S, Muta T, Takayanagi H. IkappaBzeta regulates T(H)17 development by cooperating with ROR nuclear receptors. *Nature*, 464: 1381-1385, 2010.

Palmer MT, Lee YK, Maynard CL, Oliver JR, Bikle DD, Jetten AM, Weaver CT. Lineage-specific effects of 1,25-dihydroxyvitamin D(3) on the development of effector CD4 T cells. *J. Biol. Chem.*, 286: 997-1004, 2011.

Kang HS, Okamoto K, Takeda Y, Beak JY, Gerrish K, Bortner CD, Degraff LM, Wada T, Xie W, Jetten AM. Transcriptional profiling reveals a role for RORα in regulating gene expression in obesity-associated inflammation and hepatic steatosis. *Physiol. Genomics*, 43: 818-828, 2011.

DNA-bound PARP-1 is poised for 5'-dRP/AP lyase activity in base excision repair

NIEHS investigators discovered that poly(ADP-ribose) polymerase-1 (PARP-1) is able to recognize and bind to the initial intermediate in base excision repair (BER). Using purified human PARP-1, the specificity of binding to apurinic/apyrimidinic (AP) site-containing DNA was demonstrated, and we also observed that PARP-1 has 5'-dRP/AP lyase activity against this BER intermediate. Yet, PARP-1 was only weakly activated to conduct poly(ADP-ribose) synthesis upon binding to AP site-containing DNA, but was strongly activated upon strand incision by AP endonuclease 1 (APE1). By virtue of its binding to AP sites, PARP-1 is poised for its role in base excision repair, pending DNA strand incision by either APE1 or the 5'-dRP/AP lyase activity we described here for the first time in PARP-1.

Khodyreva SN, Prasad R, Ilina ES, Sukhanova MV, Kutuzov MM, Liu Y, Hou EW, Wilson SH, Lavrik OI. Apurinic/apyrimidinic (AP) site recognition by the 5'-dRP/AP lyase in poly(ADP-ribose) polymerase-1 (PARP-1). *Proc. Natl. Acad. Sci. U.S.A.*, 107: 22090-22095, 2010.

New pINDUCER toolkit enhances disease gene studies

A new system called lentiviral pINDUCER enables researchers to turn a gene on or off whenever they want in cell culture models and experimental animals. This method for interfering with disease genes will enhance drug discovery for cancer and other disorders, and will allows investigators to precisely and conveniently test any gene in the genome for its role in human diseases.

Meerbrey KL, Hu G, Kessler JD, Roarty K, Li MZ, Fang JE, Herschkowitz JI, Burrows AE, Ciccia A, Sun T, Schmitt EM, Bernardi RJ, Fu X, Bland CS, Cooper TA, Schiff R, Rosen JM, Westbrook TF, Elledge SJ. The pINDUCER lentiviral toolkit for

inducible RNA interference in vitro and in vivo. *Proc. Natl. Acad. Sci. U.S.A.,* 108: 3665-3670, 2011.

The G protein Goα2 regulates insulin release

NIEHS researchers had previously showed that insulin release from pancreatic islets is tonically repressed by Go's action to decrease the available number of releasable insulin secretory vesicles. Loss of Go, lead to increased insulin secretion due to augmented number of docked pre-secretory vesicles. This constituted a new cellular function regulated by G proteins. These researchers now show that it is the Go2 splice variant that is responsible for this action by developing splice variant specific knock-out mice. Further, Go protein is shown by immuno-histochemical means to be expressed only in the endocrine compartment of the pancreas.

Wang Y, Park S, Bajpayee NS, Nagaoka Y, Boulay G, Birnbaumer L, Jiang M. Augmented glucose-induced insulin release in mice lacking G(o2), but not G(o1) or G(i) proteins. *Proc. Natl. Acad. Sci. U.S.A.,* 108: 1693-1698, 2011.

Diabetes is associated with higher risk of Parkinson's disease

In a large prospective study of 288,662 participants, NIEHS investigators found that diabetes mellitus was associated with a moderate higher risk of having Parkinson disease in the future. The higher risk was primarily limited to individuals with diabetes mellitus for more than 10 years. This study suggests that diabetes may be a risk factor for Parkinson's disease or these two conditions have shared risk factors.

Xu Q, Park Y, Huang X, Hollenbeck A, Blair A, Schatzkin A, Chen H**:** Diabetes and risk of Parkinson's disease. *Diabetes Care,* 34: 910-915, 2011

Link between DNA damage, tumor suppressor and immune response

This work provides the first evidence that DNA damage can lead to a general regulation of inflammatory responses, the body's reaction to injury. 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. Following damage, the tumor suppressor p53 is greatly increased and interacts with TLRs genes to regulate the amount of inflammation. This study is one of the first to come out of the recently established NIEHS Clinical Research Unit (CRU). White blood cells were isolated from samples obtained from healthy volunteers. These cells were exposed to anti-cancer agents to activate p53 and expression of TLR genes was determined. While there were large variations among individuals, the p53 generally led to the activation of several TLR genes in patient cells. TLR activation could be prevented by adding the p53 inhibitor pifithrin. Because the immune system plays a role not only in all inflammatory diseases that afflict humans, but also in cancer, the new connections provide a novel means to manipulate responses that affect those diseases. Surprisingly, the integration of p53 and inflammation only occurs in primates.

Menendez D, Shatz M, Azzam K, Garantziotis S, Fessler MB, Resnick MA. The Toll-like receptor gene family is integrated into human DNA damage and p53 networks. *PLos Genet.,* 7: e1001360, 2011.

Interplay between DNA glycosylases and DNA polymerases beta and lambda could be important in coordinating base excision repair

Base excision repair (BER) is a DNA repair pathway designed to correct small base lesions in genomic DNA. While DNA polymerase beta (pol β) is known to be the main polymerase in the BER pathway, various studies have implicated other DNA polymerases in back-up roles. One such polymerase, DNA polymerase lambda (pol λ), was shown here to be important in BER of oxidative DNA damage in mouse cells in culture. In addition, using co-immunoprecipitation experiments with purified enzymes and cell extracts, we found that both pol λ and pol β interact with the upstream DNA glycosylases for repair of alkylated and oxidized DNA bases. Such interactions could be important in coordinating roles of these polymerases during BER. Development of a mouse cell line with double knock-out of these polymerases was described.

Braithwaite EK, Kedar PS, Stumpo DJ, Bertocci B, Freedman JH, Samson LD, Wilson SH. DNA polymerases beta and lambda mediate overlapping and independent roles in base excision repair in mouse embryonic fibroblasts. *PLoS One*, 5: e12229, 2010.

Exposure to tobacco smoke while in womb has detrimental effect on fertility in adulthood

Environmental factors influencing the developmental origins of health and disease need to be identified and investigated. Researchers found that early-life exposure to tobacco smoked was related to fertility problems in adulthood. This large study, conducted in collaboration with investigators at the Norwegian Institute of Public Health, documents that women whose mothers smoked while pregnant with them took longer to become pregnant as adults.

Ye X, Skjaerven R, Basso O, Baird D, Eggesbo M, Cupul Uicab LA, Haug K, Longnecker MP. In Utero Exposure to Tobacco Smoke and Subsequent Reduced Fertility in Females. *Hum. Reprod.,* 25: 2901-2906, 2010.

Succimer ineffective for mercury chelation

Succimer, a chelating drug that lowers blood lead levels by 40% in children, has little or no effect on blood mercury.

Cao Y, Chen A, Jones RL, Radcliffe J, Dietrich KN, Caldwell KL, Peddada S, Rogan WJ**.** Efficacy of succimer chelation of mercury at background exposures in toddlers: a randomized trial. *J. Pediatr.,* 158: 480-485.e1, 2011.

Using biochemistry to elucidate genetic defects in mitochondrial disease

In a cohort of 112 patients with mitochondrial disease and with signs of POLG related disorders but absent of POLG mutations, researchers at the NIEHS in collaboration with Baylor College of Medicine identified eight heterozygous mutations in the POLG2 gene, the gene for the accessory subunit of the mitochondrial DNA polymerase. Using biochemistry, researchers at the NIEHS ascertained that three of these eight mutations caused a defective function of the protein where one of the mutations displayed a severe effect. This biochemical analysis helps explain the pathogenesis of POLG2 mutations in mitochondrial disease and emphasizes the need to quantitatively characterize the biochemical consequences of newly discovered mutations before classifying them as pathogenic.

Young MJ, Longley MJ, Li F, Kasiviswanathan R, Wong L-J, Copeland WC. Biochemical analysis of human POLG2 variants associated with mitochondrial disease. *Hum. Mol. Genet.*, 20: 3052-3066, 2011.

Critical role for the G protein Goα in male aggressive behavior

In a collaborative study NIEHS investigators provide a mechanistic explanation for the neuronal circuitry that underlies male aggressive behavior in response to pheromones interacting with Go-coupled, MHC- and β2 microglobulin-associated type 2 vomeronsal receptors (V2Rs).

Chamero P, Katsoulidou V, Hendrix P, Bufe B, Roberts R, Matsunami H, Abramowitz J, Birnbaumer L, Zufall F, Leinders-Zufall T. G protein Gαo is essential for vomeronasal function and aggressive behavior in mice. *Proc. Natl. Acad. Sci. U.S.A.*, 108: 12898-12903, 2011.

Women with diabetes found to have decreased fertility

Due to the reproductive abnormalities experienced by women with diabetes, a reduction in fecundability (the ability to conceive a pregnancy) is expected. Yet data that directly address the effects of diabetes on women's fecundability or fertility are scarce. To address this deficit, NIEHS investigators examined the fertility of a large number of women with diabetes and found that they took longer to become pregnant than women without diabetes.

Whitworth KW, Baird DD, Stene LC, Skjaerven R, Longnecker MP. Time to Pregnancy among women with Type 1 and Type 2 Diabetes in the Norwegian Mother and Child Cohort Study. *Diabetologia*, 54: 516-522, 2011.

New paradigm for the field of environmental toxicology

The blood-brain barrier regulates the movement of water and solutes into and out of the CNS and thus critically contributes to brain homeostasis and to neuroprotection. Major components of this barrier are ATP-driven drug efflux transporters, like P-glycoprotein, that prevent many therapeutic drugs from crossing the barrier and thus complicate treatment of brain diseases, including, cancer, neuroAIDS and elilepsy. This work identifies the blood-brain barrier as a target for widespread environmental pollutants, such as dioxins, that activate the aryl hydrocarbon receptor (AhR). Exposing rats to low doses of dioxin increased expression and activity of three efflux transport proteins and severely reduced drug delivery to the brain. Thus, we would expect exposure to certain pollutants to make it even more difficult to use drugs to treat brain diseases.

Wang X, Hawkins BT, Miller DS. Aryl hydrocabon receptor-mediated upregulation of ATP-driven xenobiotic efflux transporters at the blood-brain barrier. *FASEB J.*, 25: 644-52, 2011.

Nuclear orphan receptor NR2C2 (TAK1/TR4) plays critical role in the regulation of cerebellar development and metabolic syndrome.

Using mice deficient in the nuclear orphan receptor TAK1, NIEHS investigators showed that TAK1 is an important transcriptional modulator of cerebellar development and neurodevelopmentally-regulated behavior. The study supports the concept that TAK1 has a

pivotal role in brain development and suggest that the developmental alterations in the cerebellum of the TAK1-/- mice provide an excellent model to enhance our understanding of developmental neuronal-glial interactions and its impact on neurobehavioral functions. In addition, it was demonstrated that TAK1 knockout mice are resistant to the development of age- and high fat diet-induced obesity, hepatic steatosis, insulin resistance, and adipose tissue-associated inflammation. TAK1 may provide a new therapeutic target in the management of obesity, diabetes, and liver steatosis.

Kang HS, Okamoto K, Kim YS, Takeda Y, Bortner CD, Dang H, Wada T, Xie W, Yang XP, Liao G, Jetten AM. Nuclear orphan receptor TAK1/TR4-deficient mice are protected against obesity-linked inflammation, hepatic steatosis, and insulin resistance. *Diabetes*, 60: 177-188, 2011.

Phosphoglycerate kinase 2 (PGK2) is essential for sperm function and male fertility
PGK2 catalyzes the first ATP-generating step in the glycolytic pathway and is encoded by an autosomal retrogene that is expressed only during spermatogenesis. It replaces the ubiquitously expressed PGK1 following repression of Pgk1 transcription by X chromosome inactivation during meiotic prophase. The targeted disruption of Pgk2 by homologous recombination eliminates PGK activity in sperm and severely impairs male fertility, but does not block spermatogenesis, showing that PGK2 is not required for the completion of spermatogenesis, but is essential for sperm motility and male fertility.

Danshina PV, Geyer CB, Dai Q, Goulding EH, Willis WD, Kitto GB, McCarrey JR, Eddy EM, O'Brien DA. Phosphoglycerate kinase 2 (PGK2) is essential for sperm function and male fertility in mice. *Biol. Reprod.,* 82: 136-145, 2010.

Lactate dehydrogenase C is essential for sperm function and metabolism
Glycolysis is the primary source of energy required for sperm motility. NIEHS scientists demonstrated previously that disruption of the germ cell-specific lactate dehydrogenase C (LDHC) gene leads to defects in sperm function. In these studies the scientist determined that the metabolic disorders induced by treatment with an LDH inhibitor were different from those induced by the lack of LDHC, suggesting that LDHC has non-catalytic functions that are essential for maintenance of glycolysis in sperm.

Odet F, Gabel SA, Williams J, London RE, Goldberg E, Eddy EM. Lactate dehydrogenase C (LDHC) and energy metabolism in mouse sperm. *Biol. Reprod.*, in press 2011,

Food allergy and asthma in the U.S.
Food allergy is a large public health problem in the United States and the prevalence is growing. Awareness of the problem continues to grow and child day care, schools, and public institutions are developing protective policies and health programs to deal with the issue. A representative nationwide sample is needed to get an accurate estimate of the prevalence of food allergies in the entire U.S. population to help public health policy makers and care providers in planning and allocating resources for the recognition and treatment of food allergy. NIEHS scientists used data collected in the National Health and Nutrition Examination Survey (NHANES) 2005-2006 survey on food sensitivity to derive estimates of clinically confirmed food allergy, identify its

high-risk populations, and explore links with other immune-related conditions in the U.S. population. The investigators studied the results of blood tests, medical histories, and household demographics from both children and adults participating in the study. Four specific food allergens (peanut, milk, egg, and shrimp) were tested. From these findings, it was estimated that the incidence of food allergy in the U.S. is 2.5%. Risk for positive or likely food allergy (determined by tests of serum IgE) is increased in non-Hispanic blacks, males, and children. Study participants with doctor-diagnosed asthma showed an increased risk for food sensitizations, and those with likely food allergy had a notably increased chance of current asthma and emergency room visits for asthma within the prior year. Food allergy may contribute to episodes of problematic asthma.

Liu AH, Jaramillo R, Sicherer SH, Wood RA, Bock SA, Burks AW, Massing M, Cohn RD, Zeldin DC. National Prevalence and Risk Factors for Food Allergy and Relationship to Asthma: Results from the National Health and Nutrition Examination Survey 2005-2006. *J. Allergy Clin. Immunol.,* 126: 798-806, 2010.

Similar Gene Pathways are Shared in Multiple Autoimmune Diseases

Autoimmune diseases share some genetic and clinical features. To understand if they also share similar activity in gene expression that could provide insights into pathogenic mechanisms or molecular pathways common to these disorders, we studied blood gene expression via RNA microarrays in 20 identical (monozygotic (MZ)) twin pairs discordant for autoimmune diseases. Six affected probands with systemic lupus erythematosus (SLE), six with rheumatoid arthritis (RA), eight with idiopathic inflammatory myopathies (IIM), and their same-gender unaffected twins, were enrolled in this study. Comparisons were made between discordant twin pairs and these were also each compared to 40 unrelated control subjects using statistical and molecular pathway analyses. Probands and unrelated, matched controls differed significantly in gene expression for 104 probes corresponding to 92 identifiable genes. Differentially expressed genes involved several overlapping pathways including immune responses (18%), signaling pathways (22%), transcription/translation regulators (26%), and metabolic functions (17%). Interferon (IFN)-response genes (*IFI27*, *OASF*, *PLSCR1*, *EIF2AK2*, *TNFAIP6*, and *TNFSF10*) were up-regulated in probands compared to unrelated controls. No significant differences were found in gene expression when comparing probands to unaffected twins or unaffected twins to unrelated controls. Gene expression levels for unaffected twins appeared intermediate between that of probands and unrelated controls. Alterations in gene expression may influence the dysregulation of numerous, integrated immune response, cell signaling and regulatory pathways that are common to a number of autoimmune diseases. Gene expression profiles in peripheral blood suggest that for genes in these critical pathways, unaffected twins may be in a transitional or intermediate state of immune dysregulation between twins with autoimmune diseases and unrelated controls, perhaps predisposing them to the development of autoimmune disease given the necessary and sufficient environmental exposures.

O'Hanlon TP, Rider LG, Gan L, Fannin R, Paules RS, Umbach DM, Weinberg CR, Shah RR, Mav D, Gourley MF, Miller FW. Gene expression profiles from discordant monozygotic twins suggest that molecular pathways are shared among multiple systemic autoimmune diseases. *Arthritis Res. Ther*., 13:R69, 2011.

Perchlorate exposure highest in breast-fed children

Perchlorate is the most recent water pollutant proposed for regulation by EPA. It interferes with thyroid function, and NIEHS scientist showed in a companion paper to this one that it appears to do so at background levels of exposure in US children. The current shows that breast-fed kids excrete more perchlorate in urine than formula fed kids, and are thus at highest risk of impaired thyroid function. At these levels of exposure, though, such impairment is preventable by adequate maternal intake of iodine.

Valentín-Blasini L, Blount BC, Otero-Santos S, Cao Y, Bernbaum JC, Rogan WJ. Perchlorate exposure and dose estimates in infants. *Environ. Sci. Technol.,* 45: 4127-4132, 2011.

Cononical transient potential receptor channel 3 (TRPC3)-mediated Ca^{2+}-dependent cytotoxicity

Excessive Ca^{2+} influx mediates many cytotoxic processes, including those associated with autoimmune inflammatory diseases such as acute pancreatitis and Sjögren syndrome. In a collaborative study NIEHS investigators demonstrate that the TRPC3 member of the seven-membered family of TRPCs is responsible for Ca^{2+}-mediated cellular toxicity in epithelial cells.

Kim MS, Lee KP, Yang D, Shin DM, Abramowitz J, Kiyonaka S, Birnbaumer L, Mori Y, Muallem S. Genetic and pharmacologic inhibition of the Ca^{2+} influx channel TRPC3 protects secretory epithelia from Ca^{2+}-dependent toxicity. *Gastroenterology,* 140: 2107-2115, 2011.

Toll-like receptor 4 (TLR4) contributes to lung inflammation caused by air pollution

Ozone (O_3) is a major component of air pollution, and exposure may lead to premature death, shortness of breath, wheezing and coughing, increased susceptibility to lung infection, and increased risk of asthma and asthma attacks. Approximately 131 million United States residents live in cities out of attainment, or cities in which the O_3 levels are higher than federal regulations. However, the causes of these effects are not completely understood. NIEHS investigators have found that TLR4 is involved in O_3-induced pulmonary edema and inflammation, and that heat shock protein 70 is an effector molecule downstream of TLR4, and is involved in the regulation of O_3-induced lung inflammation by triggering similar pathways to TLR4. These novel findings may have therapeutic and preventive implications for inflammatory diseases resulting from environmental exposures.

Bauer AK, Rondini EA, Hummel KA, Degraff LM, Walker C, Jedlicka AE, Kleeberger SR. Identification of Candidate Genes Downstream of TLR4 Signaling after Ozone Exposure in Mice: A Role for Heat Shock Protein 70. *Environ. Health Perspect.,* in press, 2011

Exposure to tobacco smoke while in womb unrelated to subsequent risk of miscarriage and stillbirth

Previous evidence suggested that girls who were exposed to second-hand smoke, when they became pregnant as adults, experienced increased risk of miscarriage. Therefore, NIEHS researchers examined the relationship of exposure to tobacco smoke in the womb and subsequent

risk of miscarriage and stillbirth in adulthood in a large study of Norwegian women, and found no indication of an increased risk.

Cupul-Uicab LA, Baird DD, Skjaerven R, Saha-Chaudhui P, Haug K, Longnecker MP. In utero exposure to maternal tobacco smoke and women's risk of pregnancy loss later in life in the Norwegian Mother and Child Cohort (MoBa). *Hum. Reprod.,* 26:458-465, 2011.

Parkinson's disease related to agricultural use of two pesticides

Parkinson's disease (PD), a movement disorder characterized by tremor and slow movements, was found to be related to agricultural use of two pesticides, rotenone and paraquat. Researchers studied 110 individuals with PD and 358 age- and sex-matched controls without PD, all of whom were participants in a large study of licensed pesticide applicators and their spouses. The scientists found that ever having used either pesticide was associated with a more than two-fold increase in risk of developing PD. These findings may suggest approaches to prevent or slow progression of PD.

Tanner CM, Kamel F, Ross GW, Hoppin JA, Goldman SM, Korell M, Marras C, Bhudhikanok GS, Kasten M, Chade AR, Comyns K, Richards MB, Meng C, Priestley B, Fernandez HH, Cambi F, Umbach DM, Blair A, Sandler DP, Langston JW. Rotenone, paraquat, and Parkinson's disease. *Environ. Health Perspect.,* 119: 866-872, 2011.

A novel pathway for chromosome assembly

A prerequisite for normal cell division is the complete duplication of all chromosomes to ensure passage of genetic material from generation to generation. In human cells, the DNA is packaged by chromosomal proteins in a manner that provides protection of the DNA molecule and establishes regulatory information governing DNA function. NIEHS scientists have identified a role for a large protein complex, the NuRD complex, in the assembly of normal structures at several human chromosomes in lymphocytes. The results provide novel insights into the process of genome protection and regulation**.**

Sims JK, Wade PA. Mi-2/NuRD complex function is required for normal S phase progression and assembly of pericentric heterochromatin. Mol. Biol. Cell., in press, 2011.

Deriving chromosome double-strand breaks

DNA double-strand breaks (DSBs) are an important source of genome instability that can lead to severe biological consequences including tumorigenesis and cell death. Although much is known about DSBs induced directly by ionizing radiation and radiomimetic cancer drugs, there is a relative dearth of information about the formation of derived DSBs that arise from processing of single-strand lesions. Since as many as 10,000-200,000 single-strand lesions have been estimated to occur each day in mammalian cells, conversion of even a small percentage of such lesions to DSBs during normal or altered base excision repair (BER) could dramatically affect genome stability. Using a yeast-based model eukaryote system in which results are applicable to other organisms, NIEHS investigators have addressed the mechanism of formation and repair of

derived DSBs in vivo during the processing of DNA methylation damage in cells that are defective in BER due to a lack of AP endonucleases (Apn1 and Apn2). Armed with a technique recently developed by the investigators that detects resection at DSBs, a first step in repair, they demonstrated formation of DSBs in G2 arrested cells and the role of recombinational repair in subsequent chromosome restitution. Furthermore, they have identified a novel repair intermediate that can be generated if abasic sites are nicked by AP lyases, providing additional insights into the processing of 3'-blocked groups at single-strand breaks.

Ma W, Westmoreland JW, Gordenin DA, Resnick MA. Alkylation base damage is converted into repairable double-strand breaks and complex intermediates in G2 cells lacking AP endonuclease. *PLoS Genet.,* 7: e1002059, 2011.

Crystal structures reveal how human Pumilio proteins bind their target mRNAs
Researchers at the NIEHS have determined three-dimensional atomic structures of human RNA-binding proteins that show how they can be targeted to regulate specific genes. The proteins called Pumilio 1 and Pumilio 2 are important components to regulate the activity of MAP kinase enzymes at proper levels in cells. These studies show the molecular details of how the Pumilio proteins interact with the MAP kinase messenger RNAs. MAP kinase enzymes and the cellular pathways that they control are important for inflammatory response to the environment and in development of cancer.

Lu G, Hall TM. Alternate modes of cognate RNA recognition by human PUMILIO proteins. *Structure,* 19: 361-367, 2011.

Synthesis and biological evaluation of fluorinated deoxynucleotide analogs based on bis-(difluoromethylene)triphosphoric acid
It is difficult to overestimate the importance of nucleoside triphosphates in cellular chemistry: they are the building blocks for DNA and RNA and important sources of energy. Generation of nucleotide analogs that are both 'bioisosteric' and isopolar to nucleoside triphosphates is of great importance in studying structure and function of enzymes. Modifications of biologically important organic molecules with fluorine are of great interest to chemists and biologists since the size and electronegativity of the fluorine atom can be used to make defined structural alterations to biologically important models. Significant progress has been achieved in the area of modified triphosphates to date. NIEHS investigators report the preparation of hitherto unknown bis(difluoromethylene)triphosphoric acid **1**. The analog **1** compared to triphosphoric acid is enzymatically non-hydrolyzable due to substitution of two bridging oxygen atoms with CF2 groups, maintaining minimal perturbations in steric bulkiness and overall polarity of the triphosphate anion. Further, the fluorinated triphosphoric acid was used for the preparation of the corresponding fluorinated deoxynucleotides (dNTPs). The investigators also tested these dNTP analogs in single-turnover gap filling assay and obtained a crystal structure of a ternary complex with DNA polymerase beta and one of the deoxynucleotide analogs.

Surya Prakash GK, Zibinsky M, Upton TG, Kashemirov BA, McKenna CE, Oertell K, Goodman MF, Batra VK, Pedersen LC, Beard WA, Shock DD, Wilson SH, Olah GA. Synthesis and biological evaluation of fluorinated deoxynucleotide analogs

based on bis-(difluoromethylene)triphosphoric acid. *Proc. Natl. Acad. Sci. U.S.A.,* 107: 15693-15698, 2010.

Double-strand DNA break along with activation-induced deaminase expression causes mutations that are reminiscent of somatic hypermutation in human B-cells

NIEHS researchers examined the mutagenic consequences of introducing a double-strand DNA break (DSB) on mutagenesis mediated by activation-induced deaminase (AID). They made use of an elegant yeast system for study of mutagenesis that was introduced by the Resnick group here at NIEHS.They engineered a yeast strain that can express the homing endonuclease, I-SecI, to create a DSB, and this strain also can be induced to express the human AID enzyme. The results showed that simultaneous formation of a DSB along with induction of AID expression strongly increased the frequency of mutations in a marker gene, and these mutations were strand biased and base specific as expected for AID-mediated mutagenesis. In addition, the results showed that the mutagenesis required both DNA polymerase d and exonuclease I. These results are consistent with the idea that replication of single-stranded DNA produced secondary to a double-strand break can participate in AID-mediated mutagenesis. The information will be useful toward understanding the role of AID in somatic hypermutation during B-cell maturation.

Poltoratsky V, Heacock M, Kissling GE, Prasad R, Wilson SH. Mutagenesis dependent upon the combination of activation-induced deaminase expression and a double-strand break. *Mol. Immunol.,* 48: 164-170, 2010.

Natural anitbodies protect against lupus nephritis

NIEHS investigators demonstrated that IgM anti-dsDNA protected MRL/lpr mice from lupus nephritis, likely by stopping the inflammatory cascade leading to kidney damage. These results suggest that natural IgM autoantibodies provide a novel therapy against lupus neprhitis.

Jiang C, Zhao ML, Scearce RM, Diaz M. Activation-induced deaminase-deficient MRL/lpr mice secrete high levels of protective antibodies against lupus nephritis. *Arthritis Rheum.,* 63: 1086-1096, 2011.

Transient receptor potential channel 1 (TRPC1) potentiation of catecholamine release

NIEHS investigators described the existence of a signaling pathway whereby neurotransmitter release triggered by membrane depolarization and that triggered by activation of a Gq-coupled GPCR (H1 histamine, B1 bradykinin), potentiate each other affording responsiveness that bypasses activation of voltage gated Ca channels. This Potentiation of neuroTransmitter Release (PTR) was shown to depend on TRPC1 plus either TRPC4 or TRPC5 channels. The PTR phenomenon, discovered in chromaffin cells, was shown to exist in cortical granule cells and offers new avenues to modulate neurotransmitter release that may vary with the neuronal circuit under study.

Marom M, Birnbaumer L, Atlas D. Membrane depolarization combined with Gq-activated G-protein-coupled receptors induce transient receptor potential channel 1-dependent potentiation of catecholamine release. *Neuroscience,* in press, 2011.

Importance of mechanical stretch in lung epithelial injury

Investigators from NIEHS have uncovered the mechanism by which the mechanical stretch of the lung epithelia can lead to lung scarring. Mechanical stretch usually occurs during mechanical ventilation of patients with acute or chronic lung conditions. This study is the first to demonstrate that stretch injury of the alveolar epithelia can induce epithelial to mesenchymal transition (EMT), responsible for scarring, via the activation of innate immunity.

Heise RL, Stober V, Cheluvaraju C, Hollingsworth JW, Garantziotis S. Mechanical stretch induces epithelial-mesenchymal transition in alveolar epithelia via hyaluronan activation of innate immunity. *J. Biol. Chem.,* 286: 17435-17444, 2011.

Interleukin 10 (IL-10) protects the lung against injury caused by air pollution.
Ozone (O_3) remains a prevalent air pollutant and a major public health concern because of its adverse effects on the lung, including shortness of breath, wheezing and coughing, increased susceptibility to lung infection, and increased risk of asthma and asthma attacks. NIEHS scientists have found that the cytokine IL-10 protects the lung against O_3-induced lung injury and inflammation. Furthermore, gene expression analyses identified three response pathways and several genetic targets through which IL-10 may modulate the innate and adaptive immune response. These novel mechanisms of protection against the pathogenesis of O_3-induced pulmonary inflammation may also provide potential therapeutic targets to protect susceptible individuals.

Backus GS, Howden R, Fostel J, Bauer AK, Cho HY, Marzec J, Peden DB, Kleeberger SR. Protective role of interleukin-10 in ozone-induced pulmonary inflammation. *Environ. Health Perspect.,* 118: 1721-1727, 2010.

Understanding how Dicer enzymes produce the correct small RNA gene regulators
Small RNAs called siRNAs and miRNAs control the expression of numerous genes in cells. Enzymes called Dicers make these small RNAs from different precursor RNAs. Researchers at the University of Massachusetts Medical School and NIEHS have shown that if the Dicers try to process the wrong precursor RNA, the incorrect small RNA can be made and the wrong genes would be regulated. They further showed that the Dicer enzymes are directed to the correct precursor RNAs by partner proteins and the molecule phosphate, and the enzyme called Dicer-2 produces siRNAs from longer precursor RNAs and requires ATP to cut multiple siRNAs in succession from one long RNA.

Cenik ES, Fukunaga R, Lu G, Dutcher R, Wang Y, Hall TMT, Zamore PD. Phosphate and R2D2 restrict the substrate specificity of Dicer-2, an ATP-driven ribonuclease. *Mol. Cell*, 42: 172-184, 2011.

Cytochrome P450 and blood pressure
Renal cytochrome P450-derived epoxyeicosatrienoic acids (EETs) regulate sodium transport and blood pressure. Although endothelial CYP-derived EETs are potent vasodilators, their contribution to the regulation of blood pressure remains unclear. NIEHS scientists developed transgenic mice with endothelial expression of the human CYP2J2 and CYP2C8 epoxygenases to increase endothelial EET biosynthesis. Compared to wild-type littermate controls, an attenuated afferent arteriole constrictor response to endothelin-1 and enhanced dilator response

to acetylcholine was observed in CYP2J2 and CYP2C8 transgenic mice. Mean arterial pressure was significantly lower in both CYP2J2 and CYP2C8 transgenic mice during co-administration of N-nitro-L-arginine methyl ester and indomethacin. In addition, angiotensin/high-salt-induced increase in systolic blood pressure, proteinuria and glomerular injury was significantly attenuated in CYP2J2 and CYP2C8 transgenic mice compared to wild-type controls. Collectively, these data demonstrate that increased endothelial CYP epoxygenase expression attenuates afferent arteriolar constrictor reactivity and hypertension-induced increases in blood pressure and renal injury in mice. It was concluded that endothelial CYP epoxygenase function contributes to the regulation of blood pressure.

Lee CR, Imig JD, Edin ML, Foley J, DeGraff LM, Bradbury JA, Graves JP, Lih FB, Clark J, Myers P, Perrow L, Lepp AN, Kannon A, Ronnekleiv OK, Alkayed NJ, Falck JR, Tomer KB, Zeldin DC. Endothelial expression of human cytochrome P450 epoxygenases lowers blood pressure and attenuates hypertension-induced renal injury in mice. *FASEB J.*, 24: 3770-3781, 2010.

Cytochrome P450 and inflammation

Cytochrome P450 (CYP)-derived epoxyeicosatrienoic acids (EETs) possess potent anti-inflammatory effects in vitro. However, the impact of increased CYP-mediated EET biosynthesis and decreased soluble epoxide hydrolase (sEH)-mediated EET hydrolysis on vascular inflammation in vivo has not been rigorously investigated. In this study, NIEHS investigators characterized acute vascular inflammatory responses to endotoxin in transgenic mice with endothelial expression of the human CYP2J2 and CYP2C8 epoxygenases and mice with targeted disruption of sEH. Compared to wild-type controls, CYP2J2 transgenic, CYP2C8 transgenic and sEH-/- mice each exhibited a significant attenuation of endotoxin-induced activation of NF-κB signaling, cellular adhesion molecule, chemokine and cytokine expression, and neutrophil infiltration in lung in vivo. Furthermore, attenuation of endotoxin-induced NF-κB activation and cellular adhesion molecule and chemokine expression was observed in primary pulmonary endothelial cells isolated from CYP2J2 and CYP2C8 transgenic mice. This attenuation was significantly inhibited by a putative EET receptor antagonist and CYP epoxygenase inhibitor, directly implicating CYP epoxygenase-derived EETs with the observed anti-inflammatory phenotype. Collectively, these data demonstrate that potentiation of the CYP epoxygenase pathway by either increased endothelial EET biosynthesis or globally decreased EET hydrolysis attenuates NF-κB-dependent vascular inflammatory responses in vivo, and may serve as a viable anti-inflammatory therapeutic strategy.

Deng Y, Edin ML, Theken KN, Schuck RN, Flake GP, Kannon MA, DeGraff LM, Lih FB, Foley J, Bradbury JA, Graves JP, Tomer KB, Falck JR, Zeldin DC, Lee CR. Endothelial CYP Epoxygenase Overexpression and Soluble Epoxide Hydrolase Disruption Attenuate Acute Vascular Inflammatory Responses in Mice. *FASEB J.*, 25: 703-713, 2011.

Cyclooxygenases and Th17 cells

Cyclooxygenase (COX) enzymes are known to be important regulators of Th1-Th2 balance in allergic lung disease; however, it is not known whether COX-1 or COX-2-derived eicosanoids regulate Th17 cell differentiation or function, or the mechanisms involved. NIEHS investigators

identified COX-2 as a key regulator of Th17 cell differentiation and function in allergic lung inflammation via an autocrine loop that involves PGI2, PGF2α and their respective cell surface receptors.

Li H, Bradbury JA, Dackor RT, Edin ML, Graves JP, DeGraff LM, Wang PM, Bortner CD, Maruoka S, Lih FB, Cook DN, Tomer K, Jetten AM, Zeldin DC. Cyclooxygenase-2 (COX-2) Regulates Th17 Cell Differentiation During Allergic Lung Inflammation. *Am. J. Respir. Crit. Care Med.*, In Press, 2011.

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. NIEHS scientists 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 ZJ, Bradbury JA, Graves JP, Lih FB, DeGraff LM, Foley JF, Torphy R, Ronnekleiv OK, Tomer KB, Lee CR, Zeldin DC. Endothelial Expression of Human CYP2C8 Increases Susceptibility to Ischemia-Reperfusion Injury in Isolated Mouse Heart. *FASEB J.*, in press, 2011.

Med25 helps direct HNF4α-mediated gene expression

NIEHS scientist demonstrated that Med 25 is an HNF4α interacting protein which brings the mediator complex to HNF4 sites in the genes of certain promoters and recruits RNA polymerase II to initiate transcription. Ingenuity pathway analysis shows that certain HNF4α regulated pathways are under the control of Med 25. Silencing Med25 inhibits many but not all HNF4α regulated pathways. Those under control of Med 25 include certain CYP (cytochrome P450 pathways) including both those regulating drug metabolism as well as those involved in metabolism of endogenous compounds, as well as lipid metabolic pathways.

Rana R, Surapureddi S, Kam W, Ferguson SS, Goldstein JA. Med25 is required for RNA Pol II recruitment to specific promoter's thus regulating xenobiotic and lipid metabolism in human liver. *Mol. Cell Biol.* 31: 466-461, 2011.

Glis3 is critical for maintaining normal renal functions and is required for the generation of pancreatic β-cells.

Study of mice deficient in the transcriptional regulator Glis3, a Krüppel-like zinc finger protein, were generated and characterized. NIEHS investigators demonstrated that Glis3-deficient mice develop polycystic kidney disease that ultimately results in renal failure. In addition, they further showed that Glis3-deficient mice develop neonatal diabetes demonstrated that Glis3 plays a key role in cell lineage specification, particularly in the development of mature pancreatic β-cells. In addition, evidence was provided that Glis3 regulates insulin gene expression through two Glis-binding sites in its proximal promoter indicating that Glis3 also regulates β-cell function. These findings suggest that Glis3 might provide a new therapeutic target to intervene in diabetes.

Zeruth GT, Yang XP, Jetten AM. Modulation of the transactivation function and stability of Kruppel-like zinc finger protein Gli-similar 3 (Glis3) by suppressor of fused. *J. Biol. Chem.*, 286: 22077-22089, 2011.

Kang HS, ZeRuth G, Lichti-Kaiser K, Vasanth S, Yin Z, Kim YS, Jetten AM. Gli-similar (Glis) Krüppel-like zinc finger proteins: insights into their physiological functions and critical roles in neonatal diabetes and cystic renal disease. *Histol. Histopathol.*, 25: 1481-1496, 2010.

Activating signaling at the blood-brain barrier reverses induction of efflux transporter expression and restores drug delivery to the CNS

The blood-brain barrier regulates the movement of water and solutes into and out of the CNS and thus critically contributes to brain homeostasis and to neuroprotection. Major components of this barrier are ATP-driven drug efflux transporters, like P-glycoprotein, that prevent many therapeutic drugs from crossing the barrier and thus complicate treatment of brain diseases, including, cancer, neuroAIDS and elilepsy. Upregulation of blood-brain barrier P-glycoprotein expression by the environmental pollutant, dioxin, causes central nervous system pharmacoresistance. However, activation of barrier protein kinase C-β1 rapidly reduces basal P-glycoprotein transport activity. This work shows that PKC-β1 activation at the blood-brain barrier reverses CNS drug resistance caused by dioxin acting through aryl hydrocarbon receptor. Thus, targeting PKC-β1 may be an effective strategy to improve drug delivery to the brain, even in drug-resistant individuals.

Wang X, Hawkins BT, Miller DS. Activating PKCβI at the blood-brain barrier reverses induction of P-glycoprotein activity and restores drug delivery to the CNS. *J. Cerebral Blood Flow Metab.*, 31: 1371-1375, 2011.

Crystal structure of the major peanut allergen Ara h 2 reveals insight into patient epitope diversity

The crystal structure of the peanut allergen Ara h 2 was determined by NIEHS scientists using a maltose binding protein (MBP) fusion system to aid in the crystallization. Serendipitously, it was discovered that the MBP protein likely blocked interactions with Ara h 2 by a subpopulation

of patients' antibodies. This result combined with the structure itself provides information for the future development of hypoallergenic Ara h 2 proteins as therapeutic agents to protect sensitive patients against anaphylactic reactions to peanuts.

Mueller GA, Gosavi RA, Pomés A, Wünschmann S, Moon AF, London RE, Pedersen LC. Ara h 2: crystal structure and IgE binding distinguish two subpopulations of peanut allergic patients by epitope diversity. *Allergy*, 66: 878-885, 2011.

Speriolin is a Novel Human and Mouse Sperm Centrosome Protein

Speriolin as a spermatogenic cell-specific protein that localizes to centrioles in mouse and human sperm. NIEHS researchers found that speriolin is carried into the mouse egg during fertilization, remains associated with the decondensing sperm head in zygotes, and appears to undergo duplication during the first interphase and remains detectable in some two-cell embryos. Mouse eggs lack centrosomes and speriolin present in the connecting piece region of mouse and human sperm may contribute to regeneration of centrosomes in the zygote.

Goto M, O'Brien DA, Eddy EM. Speriolin is a novel human and mouse sperm centrosome protein. *Hum. Reprod.,* 25: 1884-1894, 2010.

Role for Gαo splice variants in regulating visual signals

In a collaborative study NIEHS investigators finished the description of what each of two splice variants of Go does in visual signaling in the bipolar cells of the retina: the data showed that Go1α and Go2α both mediate a depolarizing light response in rod bipolar ON cells without occluding each other's actions, suggesting they act independently on a common effector. Thus, Go2α plays a role in improving the sensitivity of rod bipolar cells through its action with Go1α. The coordinated action of two splice variants of a single Gα represents a novel mechanism for the fine control of G-protein activity.

Okawa H, Pahlberg J, Rieke F, Birnbaumer L, Sampath AP. Coordinated control of sensitivity by two splice variants of Gα(o) in retinal ON bipolar cells. *J. Gen. Physiol.,* 136: 443-454, 2010.

Requirement for NBS1 in the S phase checkpoint response to DNA methylation combined with PARP inhibition

Treatment of PARP-1-expressing cells with the combination of a DNA methylating agent (MMS) and the PARP inhibitor 4-amino-1,8-naphthalimide (4-AN) leads to an ATR/Chk1-dependent S phase checkpoint and cell death by apoptosis. Activation of ATM/Chk2 is involved in sustaining the S phase checkpoint, and double strand break (DSB) accumulation was demonstrated. NBS1, part of the MRN complex that responds to DSBs, is known to modulate ATR- and ATM-dependent checkpoint responses to UV and IR, but a role in the response to PARP inhibition has not been addressed. NIEHS investigators show that the S phase checkpoint observed 4 to 8 h after MMS + 4-AN treatment was absent in cells deficient in NBS1, but was present in NBS1-complemented (i.e., functionally wild-type) cells, indicating a critical role for NBS1 in this checkpoint response. NBS1 was phosphorylated in response to MMS + 4-AN treatment, and this was partially ATR- and ATM-dependent, suggesting involvement of both upstream kinases. NBS1 expression had little effect on ATR-mediated phosphorylation of Chk1

and ATM-mediated phosphorylation of Chk2 in response to MMS + 4-AN. Phosphorylation of SMC1 was also observed in response to MMS + 4-AN treatment. In the absence of ATM and NBS1, phosphorylation of SMC1 was weak, especially at early times after MMS + 4-AN treatment. In the absence of ATR activation, reduced SMC1 phosphorylation was seen over a 24 h time course. These results suggested that both ATR and ATM phosphorylate SMC1 in response to MMS + 4-AN and that this phosphorylation is enhanced by phospho-NBS1. The loss of the MMS + 4-AN-induced S phase checkpoint in NBS1-deficient cells may be due to a reduced cellular level of the critical downstream effector, phospho-SMC1.

Horton JK, Stefanick DF, Zeng JY, Carrozza MJ, Wilson SH. Requirement for NBS1 in the S phase checkpoint response to DNA methylation combined with PARP inhibition. *DNA Repair (Amst).,* 10: 225-234, 2011.

Localized mutagen attacks

Genome instability is an important source of cancer, genetic disease and death. However, it also provides genome plasticity which is essential for development and evolution. This study utilized genome-wide, high-throughput sequencing technology to address genome instability in small regions of DNA, while the rest of the genome remains intact. It was found that the budding yeast, a eukaryotic model organism, can tolerate artificially created single-strand DNA regions of over 20 kilobase that contain multiple UV-damaged nucleotides and that these regions can be converted into double-strand DNA with multiple mutations. Importantly, the number of changes in these small hypermutation patches was comparable to the total mutations in the rest of the genome which was nearly1000-fold larger than the patches. This novel form of genome instability that involves high multiplicity and density of mutations may play significant roles in generating new alleles for evolutionary selection as well as in the appearance of cancer and genetic disease.

Burch LH, Yang Y, Sterling JF, Roberts SA, Chao FG, Xu H, Zhang L, Walsh J, Resnick MA, Mieczkowski PA, Gordenin DA. Damage-Induced Localized Hypermutability, *Cell Cycle,* 10: 1073-1085, 2011

SNPs modulate cellular stress response

Researchers from the NIEHS Environmental Genomics Group have identified human single-nucleotide polymorphisms (SNPs) that change the expression of a nearby gene in response to cellular stress. Binding of the p53 tumor suppressor to a specific sequence of DNA, which activates transcription of the target genes, was altered by the SNPs. Most of 14.5 million human SNPs likely include non-coding SNPs that affect gene expression by altering DNA binding by transcription factors. This study used a bioinformatic approach to narrow the 6538 SNPs in suggested p53 binding sites to 32 likely candidates and validated this approach with a global search using chromatin immunoprecipitation. Binding of p53 to sequences containing these SNPs was directly tested using a microsphere DNA binding assay. Finally, the researchers reported changes in gene expression in human cell lines treated with a known agonist of the p53 response, doxorubicin. Activation of the cascade of genes under p53 control in response to DNA damaging agents is important for suppression of tumors. Changes in the intensity of the p53 response changes depending on genetic sequence, as seen in this study, may explain different susceptibilities of environmentally induced diseases such as cancer.

Bandele OJ, Wang X, Campbell MR, Pittman GS, Bell DA. Human single-nucleotide polymorphisms alter p53 sequence-specific binding at gene regulatory elements. *Nucleic Acids Res.*, 39: 178-189, 2011.

Finding the origin of RNA in mitochondrial DNA

The presence of RNA in mitochondrial DNA has been known for several decades but the origin has been a topic of controversy. The existence of RNA in the mitochondrial genome provides opportunities for genomic instability, specifically mediating breaks in DNA, which can lead to deletions. Deletions in mitochondrial DNA are associated with several mitochondrial diseases as well as aging. Researchers at the NIEHS have explored the origin of RNA by asking how the DNA polymerase that copies the mitochondrial DNA, DNA polymerase gamma, can discriminate against RNA. They found that DNA polymerase gamma has the potential to incorporate RNA precursor at a frequency of one in seven events as compared to DNA precursors. These findings help to explain the origin of RNA in the mitochondrial genome and provide a mechanism for deletions in this genome.

Kasiviswanathan R, Copeland WC. Ribonucleotide discrimination and reverse transcription by the human mitochondrial DNA polymerase. *J. Biol. Chem.*, in press, 2011.

Completing the code to design custom RNA-binding proteins

Researchers at the University of North Carolina and NIEHS identified the missing code for cytosine that expands the ability to design custom RNA-binding proteins. With this new information, proteins can be designed to recognize more than 64,000 RNA sequences vs ~9,000 sequences that could be recognized previously. These custom proteins have the potential to be used to change gene expression in cells and aid the study of biological processes that respond to environmental exposures and the development of therapies for human disease.

Dong S, Wang Y, Cassidy-Amstutz C, Lu G, Bigler R, Jezyk MR, Li C, Hall TM, Wang Z. A specific and modular binding code for cytosine recognition in Pumilio/FBF (PUF) RNA-binding domains. *J. Biol. Chem.*, in press, 2011.

Heterotrimeric Gi2 proteins are activated by endotoxin

NIEHS scientists report the surprising finding that endotoxin (lipopolysaccharide, LPS) causes a rapid but transient activation of the Gi2 G protein and that mice lacking Gi2 (KO or pertussis toxin treatment) are hyper sensitive to LPS and septic shock, whilst activation of Gi proteins with mastoparan had a protective effect. This is the first evidence of a connection between Toll-Like Receptor and G protein signaling.

Fan H, Li P, Zingarelli B, Borg K, Halushka PV, Birnbaumer L, Cook JA. Heterotrimeric Gα(i) proteins are regulated by lipopolysaccharide and are anti-inflammatory in endotoxemia and polymicrobial sepsis. *Biochim. Biophys. Acta*, 1813: 466-472, 2011.

Advancements towards predictive genomics

Bioinformaticians at NIEHS led a team of investigators within the MicroArray Quality Control Consortium in the use of gene expression data from the blood of rats exposed to acetaminophen (the active agent in Tylenol) and chemical toxicants to predict injury in the liver with a high degree of accuracy. The results support the hypothesis that genomic indicators can be acquired in a non-evasive manner to serve as biomarkers of drug-induced liver injury and that toxicogenomics can potentially be used to explore personalized medicine as well as improve public health decisions based on genomic profiles. Continued research in toxicogenomics to develop predictive biomarkers, particularly with emerging genomic technologies and extrapolations from animal models to humans, will advance efforts to reduce the burden of human illness and disability from exposure to environmental stressors, incipient toxicity and adverse drug reactions.

Huang J, Shi W, Zhang J, Chou JW, Paules RS, Gerrish K, Li J, Luo J, Wolfinger RD, Bao W, Chu TM, Nikolsky Y, Nikolskaya T, Dosymbekov D, Tsyganova MO, Shi L, Fan X, Corton JC, Chen M, Cheng Y, Tong W, Fang H, Bushel PR. Genomic indicators in the blood predict drug-induced liver injury. *Pharmacogenom. J.,* 10: 267-277, 2010.

Shi L, et al. MicroArray Quality Control Consortium. The MicroArray Quality Control (MAQC)-II study of common practices for the development and validation of microarray-based predictive models. *Nat. Biotechnol.,* 28: 827-838, 2010.

Improved method for studying gene-by-environment interaction

Most disease is not purely genetic or purely environmental, but arises through joint effects of lifestyle, bad luck, and environmental factors acting in a context of genetic susceptibility. Variants of genes can, for example, disrupt metabolic pathways that we depend on for protection, mechanisms nature has designed to help us detoxify our teratogenic and harmful exposures. A powerful approach for identifying genes that interact with environmental factors (proposed by NIEHS investigators in 1994) involves recruiting only cases and analyzing correlations between exposures and genetic variants, correlations that can reveal super-multiplicative synergistic effects on risk. However, such correlations can sometimes be noncausal, because cultural factors and genes tend to be inherited together. NIEHS investigators developed a novel method for identifying gene-by-environment interaction (and also estimating effects of the environmental factor) by incorporating exposure data (but not genotype data) from random unaffected siblings of individuals with the disease under study. When used for young-onset disease, the new method can be powerful while protecting against the biases that can distort a case-only study.

Weinberg CR, Shi M, Umbach DM. A sibling-augmented case-only design for assessing multiplicative gene-environment interaction. *Am. J. Epidemiol.,* in press, 2011.

Accessory DNA polymerases in chromosomal replication fidelity

The accuracy of DNA replication is a crucial factor for the processes by which organisms undergo mutation. To gain understanding in this area we are studying the accuracy (fidelity) of DNA replication in the bacterium *Escherichia coli*, which is a simplified but useful model system for these questions. The bacterial chromosome is replicated by the DNA polymerase III holoenzyme (HE), whose accuracy we have studies in detail. But in addition, other DNA

polymerases play a role (*E. coli* has five such accessory DNA polymerases) and they can affect the overall error rate. These studies have shown that two accessory DNA polymerases (Pol II and Pol IV) directly contribute to the chromosomal error rate, reducing or increasing replication errors, respectively, while DNA Pol I fulfills an indirect role through the error-free filling of the Okazaki fragment gaps.

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.

Structure of DNA repair complex determined

Cell DNA is subject to damage by chemical and physical agents of environmental concern. However, the integrity of cellular DNA must be maintained in order for cells to function normally. Since repair of the DNA is a complex, multi-step process, it involves multi-protein complexes that maintain contact with the DNA and transfer it from one step to the next. The final step in several DNA repair processes called, "ligation" and involves a ligase enzyme that reconnects the phosphate backbone to restore the double stranded DNA structure. The active form of one of the important ligase enzymes, L3alpha, forms a complex with a DNA repair scaffold protein: XRCC1. NIEHS scientists have determined the structure of this complex, and found that it involves a specialized interaction domain, the "BRCT" domain, in combination with linker residues that precede the BRCT domains.

Cuneo MJ, Gabel SA, Krahn JM, Ricker MA, and London RE. The structural basis for partitioning of the XRCC1/DNA ligase III-alpha BRCT-mediated dimer complexes. *Nucl. Acids Res.,* in press, 2011.

Molecular insights into ribonucleotide discrimination

DNApolymerase 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 NA, Beard WA, Batra VK, Perera L, Pedersen LG, Wilson SH. Molecular insights into DNA polymerase deterrents for ribonucleotide insertion. *J. Biol. Chem.,* in press 2011.

Deletion of DNA-binding Domain of Estrogen Receptor Alpha Results in Male Infertility

Disruption of the gene for estrogen receptor alpha (ERα) previously was shown to result in infertility in male mice. However, alternative splicing resulted in the expression at a low level of a truncated form of the receptor and the effect of this on male fertility was unknown. This study examined male mice lacking the truncated form of the receptor. NIEHS investigators found that the effect on male fertility was nearly identical to what was seen previously, indicating that full-length ERα is required for maintenance of male fertility.

Goulding EH, Hewitt SC, Nakamura N, Hamilton K, Korach KS, Eddy EM. Ex3αERKO male infertility phenotype recapitulates the αERKO male phenotype. *J. Endocrinol.*, 207: 281-288, 2010.

New statistical methods and software tools for identifying genome-wide changes in DNA sequences from the next-generation sequencing data

NIEHS investigators in the Biostatics Branch developed computational/statistical methods and software tools for identifying genome-wide changes in epigenetic marks or transcript levels and for identifying a transcription factor and its co-regulator binding sites in DNA sequences from the next-generation sequencing data. In collaboration with Karen Adelman's group at NIEHS, they identified intrinsic sequence elements that may contribute to the pausing of RNA polymerase II when transcribing DNA to RNA. In collaboration with Gary Johnson's group at the University of North Carolina at Chapel Hill, they identified an epigenetic switch that maintains the epithelial phenotype in trophoblast stem cells and reveals previously unrecognized genes potentially contributing to breast cancer.

Gilchrist DA, Dos Santos G, Fargo DC, Xie B, Gao Y, Li L, Adelman K. Pausing of RNA polymerase II disrupts DNA-specified nucleosome organization to enable precise gene regulation. *Cell*, 143: 540-551, 2010.

Abell AN, Jordan NV, Huang W, Prat A, Midland AA, Johnson NL, Granger DA, Mieczkowski PA, Perou CM, Gomez SM, Li L, Johnson GL. MAP3K4/CBP-regulated H2B acetylation controls epithelial-mesenchymal transition in trophoblast stem cells. *Cell Stem Cell*, 8: 525-537, 2011.

Huang W, Umbach DM, Jordon NV, Abell AN, Johnson GL, Li L. Efficiently identifying genome-wide differential changes using Next-Gen sequencing data. *Nucleic Acids Res.*, in press, 2011.

Xu M, Weinberg CR, Umbach DM,Li L. coMOTIF: A Mixture Framework for Identifying Transcription Factor and a Co-regulator Motif in ChIP-seq Data. *Bioinformatics,* in press, 2011.

Data harmonization tools developed

Through a collaboration between RTI International, NHGRI, NLM, and leading academics across the country, the PhenX Toolkit was developed to provide researchers with a set of high priority measures for use in Genome-wide Association Studies (GWAS) and other large-scale research efforts. This Toolkit will enhance collaborative opportunities for future researchers by facilitating cross-study analyses of these data collection efforts.

Hamilton CM, Strader LC, Pratt JG, Maiese D, Hendershot T, Kwok RK, Hammond JA, Huggins W, Jackman D, Pan H, Nettles DS, Beaty TH, Farrer LA, Kraft P, Marazita

ML, Ordovas JM, Pato CN, Spitz MR, Wagener D, Williams M, Junkins HA, Harlan WR, Ramos EM, Haines J. The PhenX Toolkit: Get the Most From Your Measures. *Am. J. Epidemiol.,* 174: 253-260, 2011.

Hamilton CM, Strader LC, Pratt JG, Maiese D, Hendershot T, Kwok RK, Hammond JA, Huggins W, Jackman D, Pan H, Nettles DS, Beaty TH, Farrer LA, Kraft P, Marazita ML, Ordovas JM, Pato CN, Spitz MR, Wagener D, Williams M, Junkins HA, Harlan WR, Ramos EM, Haines J. Hamilton et al. Respond to "Consolidating Data Harmonization". *Am. J. Epidemiol.*, in press, 2011.

Polychlorinated biphenyl exposure found to have adverse effects on child development only at high doses

NIEHS scientists developed a new statistical analysis method that allowed an improved examination of the dose-response relationship between early-life exposure to polychlorinated biphenyls (PCBs) and child IQ. Polychlorinated biphenyls are a ubiquitous environmental contaminant. Using the new method, it was demonstrated that PCBs did have an adverse effect on IQ, but only at doses that are much higher than what is typically found in developed countries.

Zhou H, Qin G, Longnecker MP. A partial linear model in the outcome dependent sampling setting to evaluate the effect of prenatal PCB exposure on cognitive function in children. *Biometrics,* in press, 2011.

Low levels of exposure to polybrominated diphenyl ethers in Europe found not to affect thyroid hormone levels

Unlike North America, the level of exposure to polybrominated diphenyl ethers (used as flame retardants), in Europe is relatively low. Nonetheless, concerns exist about potential health effects even at the low levels of exposure found in Europe. NIEHS investigators conducted a study among women in Norway and found that within the range of exposure observed, no association with thyroid hormone levels was present.

Eggesbø M, Thomsen C, Jørgensen JV, Becher G, Odland JO, Longnecker MP. Associations between brominated flame retardants in human milk and Thyroid-Stimulating Hormone (TSH) in neonates. *Environ. Res.,* in press, 2011.

Mother's age at menarche predicts the pattern of growth in her children

The height and weight of children is determined in part by the height and weight of their parents. In this new study, it was found that additional predictive information about child height and weight is provided by knowledge of the mother's age at menarche, suggesting that the tempo of growth is also an inherited trait.

Basso O, Pennell ML, Chen A, Longnecker MP. Mother's age at menarche and offspring size. *Int. J. Obes.,* 34:1766-1771, 2010.

Magnetic resonance imaging and acute lung injury

The objective of this study was to characterize hypointense regions observed by ^{3}He MRI in a mouse model of acute lung injury. Lipopolysaccharide (LPS) was intratracheally administered to

mice under anesthesia. Four hours following exposure, mice were imaged via hyperpolarized ^{3}He MRI. All images were evaluated to identify regions of hypointense signal. Lungs were then characterized by conventional histology or used to obtain tissue samples from regions of normal and hypointense ^{3}He signal and analyzed for cytokine content. Characterization of the ^{3}He MRI images identified three distinct types of hypointense patterns: persistent defects, atelectatic defects, and dorsal lucencies. Persistent defects were associated with LPS administration; the number of persistent defects depended on LPS dose. Atelectatic defects predominated in LPS-dosed mice under conditions of low-volume ventilation and could be reversed with deep inspiration. Dorsal lucencies were present in nearly all mice studied regardless of the experimental conditions. Comparison of ^{3}He MRI with histopathology did not identify tissue abnormalities in regions of low ^{3}He signal. Furthermore, there were no differences in levels of IL-1β, IL-6, MIP-1α, MIP-2, KC, TNFα and MCP-1 between hypointense and normally ventilated lung regions in LPS-dosed mice.

Thomas AC, Driehuys B, Voltz JW, Fubara B, Bradbury JA, Zeldin DC. Ventilation Defects Observed with Hyperpolarized ^{3}He Magnetic Resonance Imaging in a Mouse Model of Acute Lung Injury. *Am. J. Respir. Cell Mol. Biol.*, 44: 648-654, 2010.

Mass spectrometry characterization of antibody glycosylation distinguishes between patients with the autoimmune disease myositis.

NIEHS scientists investigated the glycosylation pattern of immunoglobulin of patients with myositis, a debilitating autoimmune disease that leads to increasing muscle weakness, their asymptomatic siblings, and age and gender matched controls and found that there is a decrease in terminal galactosylation of the N-linked glycans in affected individuals, as observed in several other autoimmune diseases. The unaffected siblings have galactosylation levels intermediate between the affected sibling and the appropriate control, indicating a genetic component to the disease. Importantly, a difference in galatosylation levels was observed between an affected patient and their unaffected monozygotic twin, indicating the possible environmental aspect to the disease. Changes in galatosylation level may also prove predictive of the progression of the disease in individuals with a family history of autoimmune disorders.

Perdivara I, Peddada SD, Miller FW, Tomer KB, Deterding LJ. Mass Spectrometric Determination of IgG Subclass-Specific Glycosylation Profiles in Siblings Discordant for Myositis Syndromes. *J. Proteome Res.*, 10: 2969–2978, 2011.

Specific IgE and allergy in the U.S.

In this study, NIEHS investigators estimated prevalence of allergy-related outcomes and examined relationships between serum IgE levels and these outcomes in a representative sample of the US population. Study subjects aged 6 years and older enrolled in NHANES 2005-2006 had blood taken for measurement of total IgE and 19 specific IgEs against common aeroallergens (Alternaria alternata, Aspergillus fumigatus, Bermuda grass, birch, oak, ragweed, Russian thistle, rye grass, cat dander, cockroach, dog dander, dust mite, mouse and rat urine proteins) and selected foods (egg white, cow's milk, peanut, and shrimp). Serum samples were analyzed for total and allergen-specific IgEs using the Pharmacia CAP System. Information on allergy-related outcomes and demographics was collected by questionnaire. Overall, 6.6% reported current hay fever and 23.5% suffered from current allergies. Allergy-related outcomes increased with

increasing total IgE. Elevated levels of plant, pet, and mold-specific IgEs contributed independently to allergy-related symptoms. The greatest increase in odds was observed for hay fever and plant-specific IgEs (OR=4.75, 95% CI:3.83-5.88). Thus, in the US population, self-reported allergy symptoms are most consistently associated with elevated levels of plant-, pet-, and mold-specific IgEs.

Salo PM, Calatroni A, Gergen PJ, Hoppin JA, Sever ML, Jaramillo R, Arbes SJ, Zeldin, D.C. Allergy-Related Outcomes in Relation to Serum IgE: Results from the National Health and Nutritional Examination Survey 2005-2006 *J. Allergy Clin. Immunol.*, 127: 1226-1235, 2011.

Structural characterization of the endonuclease (EndA) from *Streptococcus pneumoniae*

Streptococcus pneumoniae commonly colonizes the human upper respiratory tract, but can produce invasive pneumococcal disease (IPD) in young children, the elderly and in individuals immunocompromised due to cancer, diabetes or human immunodeficiency virus (HIV) infection. The role of EndA as a virulence factor in pneumococcal infection makes it an attractive target for antimicrobial therapeutics. NIEHS investigators determined the three dimensional structure of the EndA nuclease, and evaluated the role of various residues in the catalytic mechanism. This information will provide a basis for future targeting of this nuclease by antibiotics.

Midon M, Schaefer P, Pingoud A, Ghosh M, Moon AF, Cuneo MJ, London RE, Meiss G. Molecular cloning and biochemical characterization of the DNA-entry nuclease EndA from Streptococcus pneumoniae. *Nucl. Acids Res.*, 39: 623-634, 2011.

Moon AF, Midon M, Meiss G, Pingoud A, London RE, Pedersen LC. Structural insights into catalytic and substrate binding mechanisms of the strategic EndA nuclease from Streptococcus pneumoniae. *Nucl. Acids Res.*, 39: 2943-2953, 2011.

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. We have discovered a novel detoxification system for this compound in the bacterium *E. coli*, and have shown that this system requires the Molybdenum Cofactor. NIEHS scientists 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.

Kozmin SG, Wang J, Schaaper RM. Role for CysJ flavin reductase in molybdenum cofactor-dependent resistance of *Escherichia coli* to 6-*N*-hydroxylaminopurine. *J. Bacteriol.*, 192: 2026-2033, 2010.

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

Design and follow-up of large-scale genetic association studies

In recent years, genome-wide association studies have uncovered a large number of susceptibility variants. Initially, large-scale studies provide only tentative evidence of association, and replication efforts focusing on top hits are required to establish their validity.

The number of top hits to carry forward into the replication step is often determined ad hoc. NIEHS researchers developed a novel statistical approach based on controlling the proportion of genuine associations among a specified number of top hits. This approach is useful for designing large-scale studies and for selection of promising results for following up.

Kuo C-L, Zaykin DV. Novel rank-based approaches for discovery and replication in genome wide association studies. *Genetics*, in press, 2011.

Understanding mechanisms of cellular communication within discrete sub-domains of single cells.

Even though many cellular "messenger" molecules are small and freely-mobile, their actions are frequently restricted to dedicated sub-domains within single cells. Unraveling the molecular basis for this compartmentalization is part of an increasing research focus upon understanding, at a microscopic level, normal cell function and its perturbation by environmental insults. A discovery in this area concerns an enzyme (PPIP5K1) that synthesizes so-called "inositol pyrophosphates" that help cells adapt to stress and aging. NIEHS investigators found that hormones such as insulin control movement of PPIP5K1 from the cell's interior to its plasma membrane, leading to highly-localized production of inositol pyrophosphates. The region within PPIP5K1 that mediates membrane association is a hitherto unrecognized (cryptic) stretch of amino-acids endowed with unique regulatory properties; this new understanding of the control over synthesis and actions of inositol pyrophosphates offers new mechanisms of intracellular communication.

Gokhale NA, Zaremba A, Shears SB. Receptor-dependent compartmentalization of PPIP5K1, a kinase with a cryptic polyphosphoinositide binding domain. *Biochem. J.*, 434: 415-426, 2011.

Women who work full time have shorter telomeres

Perceived and chronic stress have been associated with shorter DNA telomeres, a marker of cellular aging and risk for aging-related diseases. Work and work schedule may be a source of chronic stress. In a study of a subset of women in the Sister Study, a prospective study of 50,000 women, NIEHS investigators found that women who worked full time had shorter telomeres than women who did not work outside of the home. In addition, full-time workers had shorter telomeres than those who worked part-time. While these differences could not be explained by differences in health status or behaviors, investigators did find that the association between shorter telomeres and full-time work was more pronounced among women who reported higher perceived stress and those who had higher urinary levels of the stress hormone epinephrine. Data on changes in telomere length changes over time in relation to work schedule are needed to confirm these findings.

Parks CG, DeRoo LA, Miller DB, McCanlies EM, Cawthon RM, Sandler DP. Employment and work schedule are related to telomere length in women. *Occup. Environ. Med.*, in press, 2011.

New signaling system identified in cancer cells

Researchers at the NIEHS have identified a new signaling system in breast cancer cells that appears to be important for these tumor cells to metastasize. The researchers discovered that a signaling molecule that controls cell structure forms a complex with a protein normally found in the nucleus of cells. The inhibition of this novel complex blocks the cancer cells from attaching to a surface coated with proteins normally found in between organs in the body, suggesting that drugs targeting this signaling system might be useful in slowing or stopping these cancer cells from moving away from their initial location.

Garcia MC, Williams J, Johnson K, Olden K, Roberts JD. Arachidonic acid stimulates formation of a novel complex containing nucleolin and RhoA. *FEBS Lett.,* 585: 618-622, 2011.

Women can accurately report whether or not they were exposed to tobacco smoke when they were in the womb

Over 10,000 women reported whether or not their mothers smoked when they were pregnant with the woman, 14-47 years previously. When the same question was asked several years later, women's ability to give the same answer as previously was extremely high, suggesting this exposure is well reported and useful in epidemiologic studies of health effects. It was also found that the women who reported that their mothers smoked while pregnant with them weighed about 150 grams less at birth, additionally attesting to the validity of the information on this exposure.

Cupul-Uicab LA, Ye X, Skjaerven R, Haug K, Longnecker MP. Reproducibility of reported in utero exposure to tobacco smoke. *Ann. Epidemiol.,* 21: 48-52, 2011.

Easing the mathematical complexity of how molecules interact

Molecules react because of collisional interactions. A measure of the degree to which molecules will interact is their polarizability, a property that depends on how easily a molecule's charge density can distort. This work eases the mathematical and computational complexity of working with polarizability of molecules.

Elking DM, Perera L, Duke R, Darden T, Pedersen LG. A finite field method for calculating molecular polarizability tensors for arbitrary multipole rank. *J. Comput. Chem.,* in press, 2011.

Generalized methods for assessing chemical relative potency

Relaxing the usual assumption that the potency of one chemical relative to another is constant, NIEHS investigators viewed relative potency as a function of the dose of either chemical, the level of a specific response, or the percentage of the range of possible response levels. These relative potency functions are constructed from dose-response curves for test and reference chemicals, and they all provide equivalent information if the chemicals have the same lower and upper limits of response. The function of the response range percentage provides distinct information, however, if the response limits differ. The preferred relative potency function depends on the application (e.g., chemical ranking or dose conversion) and whether one views differences in response limits as intrinsic to the chemicals or as extrinsic, arising from idiosyncrasies of data sources.

Dinse GE, Umbach DM. Characterizing non-constant relative potency. *Regu. Toxicol. Pharmacol.,* 60: 342-353, 2011.

Placental characteristics as a proxy measure of in utero hormone exposure

Exposure to natural hormones early in life may affect subsequent risk of cancer, but measurement of such exposure is difficult. However, measurements such as placental weight are easily collected and may serve as a more accessible proxy measure of early-life hormone exposure. Investigators documented that placental weight is related to hormone levels during pregnancy, thus providing a new approach to studies on early-life hormone exposure and subsequent cancer risk.

Trabert B, Longnecker MP, Graubard BI, Klebanoff MA, Stanczyk FZ, McGlynn KA. Placental characteristics as a proxy measure of in utero hormone exposure. *Cancer Causes Control,* 22: 689-695, 2011.

Simple ultrasound techniques can follow the effects of estrogen in infants

Using simple ultrasound techniques from the body surface, we followed the normal shrinking of infants' uterus and breast tissue and the rise and fall of ovarian volume over the first few months of life. These techniques would be useful for assessing the effects of estrogen-like "endocrine disrupters" on infants.

Nguyen RH, Umbach DM, Parad RB, Stroehla B, Rogan WJ, Estroff JA. US assessment of estrogen-responsive organ growth among healthy term infants: piloting methods for assessing estrogenic activity. *Pediatr. Radiol.,* 41: 633-642, 2011.

Analgesic studies in laboratory animals

NIEHS investigators in the Comparative Medicine Branch evaluate new anesthesia and analgesia regimes in laboratory rodents with the goal of improving our anesthetic and analgesic protocols. This results in better animal well-being, resulting not only in refinement of a procedure, but improved research model.

Cannon CZ, Kissling GE, Goulding DR, King-Herbert AP, Blankenship-Paris T. Analgesic effects of tramadol, carprofen or multimodal analgesia in rats undergoing ventral laparotomy. *Lab. Anim. (NY),* 40: 85-93, 2011.

Tubbs JT, Kissling GE, Travlos GS, Goulding DR, Clark JA, King-Herbert AP, Blankenship-Paris TL. Effects of buprenorphine, meloxicam, and flunixin meglumine as postoperative analgesia in mice. *J. Am. Assoc. Lab. Anim. Sci.,* 50: 185-191, 2011.

Cannon CZ, Kissling GE, Hoenerhoff MJ, King-Herbert AP, Blankenship-Paris T. Evaluation of dosages and routes of administration of tramadol analgesia in rats using hot-plate and tail-flick tests. *Lab. Anim. (NY),* 39: 342-351, 2010.

Goulding DR, Myers PH, Goulding EH, Blankenship TL, Grant MF, Forsythe DB. The effects of perioperative analgesia on litter size in Crl:CD1(ICR) mice undergoing embryo transfer. *J. Am. Assoc. Lab. Anim. Sci.,* 49: 423-426, 2010.

DNA mutations in rare primates and its correlation to humans and other primate species

Investigators have determined the DNA consensus sequence of H-ras, a gene that causes genetic disorders in humans including cancer, from captive lemurs and lorises and evaluated samples of nonneoplastic liver and hepatocellular carcinomas (HCC) from affected animals for mutations in this gene. Given the limited number of these species in captivity, little is known about their propensity to develop neoplastic disease in general and, more specifically, the pathogenesis of hepatic tumor formation. The investigators have determined that the DNA consensus sequence of H-ras is identical to that of human H-ras and differs only slightly from the chimpanzee sequence. Point mutations were identified in 6 of the 9 HCC samples examined. Two carcinomas had double mutations, and one tumor had triple mutations. One HCC had a mutation in codon recognized as an H-ras "hot spot" in rodent neoplasia that has also been reported in human tumors.

Cullen JM, Williams C, Zadrozny L, Otstot JT, Solomon GG, Sills RC, Hong HH. H-ras Consensus Sequence and Mutations in Primary Hepatocellular Carcinomas of Lemurs and Lorises. *Vet. Pathol.*, 48: 868-874, 2011.

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 awards are funded 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 21 winners of FARE awards:

FARE Awardee	Mentor	Fare Abstract Title
Joseph E. Burgents, Ph.D.	Donald Cook	CD103+ and CD11b+ pulmonary dendritic cells display distinct migratory properties during steady-state and following allergic sensitization
Nisha A. Cavanaugh, Ph.D.	Samuel Wilson	New Insights into Ribonucleotide Discrimination by DNA Polymerase Beta
Xiaoqing Chang, Ph.D.	Ray Tice	A Physiologically Based Pharmacokinetic Model of Micro and Nano Sized Fluorescent Polystyrene Spheres in Rats
Saurabh Chatterjee, Ph.D.	Ronald Mason	Leptin signaling synergizes environmental bromodichloromethane exposure-induced post translational protein oxidations, antigen presentation and exacerbation of steatohepatitis of obesity
Saiful M. Chowdhury, Ph.D.	Michael Fessler	Proteomic analysis of lipid rafts from ATP Binding Cassette Transporter A1-deficient macrophages reveals novel regulatory events in the innate immune response
Johannes M. Freudenberg, Ph.D.	Raja Jothi	A meta-analysis reveals novel regulators required for mouse embryonic stem cell self-renewal
Huiming Gao, M.D., Ph.D.	Jau-Shyong Hong	HMGB1 (high-mobility group box 1) acts on microglia Mac1 (macrophage antigen complex 1) to mediate chronic neuroinflammation that drives progressive neurodegeneration
Jill E. Hesse, Ph.D.	Richard Paules	Identification and Discovery of DNA Damage-Induced microRNA Expression Changes by Microarray and Next-Generation Sequencing in Wild-Type and ATM-Deficient Human Mammary Epithelial Cells

Anne Y. Lai, Ph.D	Paul Wade	DNA methylation primes the memory B cell epigenome for plasma cell differentiation
Hong Li, M.D.	Darryl Zeldin	Cyclooxygenase-2 (COX-2) Negatively Regulates IL-9+/CD4+ T cells (Th9) Differentiation during Allergic Lung Inflammation through down-regulation of IL-17RB
Kristin N. Lichti-Kaiser, Ph.D.	Anton Jetten	The Role of Glis3 in the Development of Functional Pancreatic Beta-cells and Diabetes
Leelavati Narlikar, Ph.D.	Raja Jothi	Genome-wide characterization of CTCF's role as an enhancer-blocker
Kosuke Saito, Ph.D.	Masa Negishi	The role of the potassium channel KCNK1 in the sexual dimorphic centrilobular hypertrophy induced by phenobarbital in mouse liver
Yukimasa Takeda, Ph.D	Anton Jetten	Retinoic acid-related orphan receptor gamma, RORgamma, coordinates the circadian regulation of energy homeostasis through the control of hepatic lipid and glucose metabolism
Percy Tumbale, Ph.D.	Scott Williams	Structural Basis of DNA Ligase Proofreading by Aprataxin with insights into AOA1 Neurodegenerative Disease
Kirsten C. Verhein, Ph.D.	Steven Kleeberger	Candidate susceptibility genes in a murine model of RSV-induced bronchiolitis
Xueqian Wang, Ph.D.	David Miller	Nuclear Factor E2-Related Factor-2 (Nrf2) Regulates P-glycoprotein Expression at the Blood-Brain Barrier (BBB) by Acting Through p38 MAP Kinase
Huanchen Wang, Ph.D.	Stephen Shears	Substrate Specificity and Catalysis Mechanism of Inositol Pyrophosphate Kinase
Wipawee Winuthayanon, Ph.D.	Kenneth Korach	Role of epithelial estrogen receptor alpha in the oviduct during fertilization and embryo development
Mengyuan Xu, Ph.D.	Leping Li	coMOTIF: A Mixture Framework for Identifying Transcription Factor and a Co-regulator Motifs in ChIP-seq Data
Zhengyu Yin, Ph.D.	Anton Jetten	RAP80 Plays a Critical Role in Maintaining Genomic Stability and Tumor Suppressing

2011 NIH WSA Scholar Award

One FARE award winner, Anne Y. Lai, Ph.D, from the Laboratory of Molecular Carcinogenesis, was selected to receive one of two 2011 NIH WSA (Women Scientist Advisors Committee) Scholar awards, for scientific excellence for her FARE research project titled "DNA methylation primes the memory B cell epigenome for plasma cell differentiation". Her mentor is Paul Wade, Ph.D., Laboratory of Molecular Carcinogenesis.

Endocrine Society Presidential Poster Award
Javier Revollo, Ph.D, a visiting fellow in the Laboratory of Signal Transduction received the Presidential Poster Award at the 93rd Annual Meeting of the Endocrine Society held in Boston, Massachusetts, for his poster entitled "Glucocorticoids Regulate Gene Expression by a Novel Derepression Mechanism Involving Dismissal of the Hes1 Repressor." His mentor is John Cidlowski, Ph.D., Chief, Laboratory of Signal Transduction.

Summers of Discovery 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 Summers of Discovery 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 scientific mentors from the Intramural program 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 28, 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

Greeshma Somashekar attends the North Carolina School of Science and Mathematics, Durham, NC, and worked in the Laboratory of Respiratory Biology. Poster: Somashekar, G., Stober, V. and Garantziotis, S. "Intra-α-trypsin inhibitor heavy chain 4: Domain-based functional effects on immune cell activation and migration." Greeshma's mentor was Stravros Garantziotis, head of the Matrix Biology Group.

Best Poster by Undergraduate Interns (Tie)

Nicholas Fitzgerald attends North Carolina State University, Raleigh, NC, and worked in the Laboratory of Structural Biology. Poster: Fitzgerald, N., Kuhn, J., Schorzman, A., Gable, S., and Tomer, K. "Proteomics study of environmental toxins and stressors: Response of macrophages and cardiomyocytes to anthrax." Nicholas' mentor was Ken Tomer, head of the Mass Spectrometry Group.

Rahul Jaswaney attends Washington University, St. Louis, MO, and worked in the Laboratory of Toxicology and Pharmacology. Poster: Jaswaney, R., Trivedi, D., and Langenbach, R. "Elucidating the role of β-arrestin 2 in the development of angiotensin II-

induced abdominal aortic aneurysms." Rahul's mentor was Robert Langenbach, head of the Metabolism and Molecular Mechanisms Group.

Best Poster by Graduate Interns

Jack Dutton attends North Carolina State University, Raleigh, NC, and worked in the Comparative Medicine Branch. Poster: Dutton, J., Myers, P., Goulding, D., Rios, M., Clark, J., and Blankenship-Paris, T. "Development of a sustained release buprenorphine gel for postoperative analgesia in mice." Jack's mentor was Terry Blankenship-Paris head of Veterinary Medicine.