Division of Intramural Research

NAEHS Council Update

September 2009

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 (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.

Investigators in Bioinformatics
The NIEHS is seeking an investigator in Bioinformatics/Computational Biology. Candidates will be considered for Senior Investigator or Tenure-Track Investigator, depending upon qualifications. The incumbent will develop and direct a strong research group to carry out independent and collaborative research in the general area of bioinformatics and computational biology, particularly as related to biological networks, analysis of high-dimensional data, proteomics, comparative and functional genomics, gene expression, and epigenetics. This work will provide a bioinformatics infrastructure and innovative data mining approaches to advance intramural research aimed at understanding biological responses to environmental stressors, in the context of cell biology, animal experimentation, clinical research and epidemiology. Dr. Thomas Kunkel, Laboratory of Molecular Genetics, is chair of the search committee. A candidate has been identified for the position.

Tenure-Track Reproductive Epidemiologist
The Epidemiology Branch, National Institute of Environmental Health Sciences, NIH, invites applications for a tenure-track epidemiologist to develop an independent investigator-initiated research program. Applicants must have an M.D. and/or Ph.D. in epidemiology or related field, at least two years of post-degree research experience, and a record of accomplishment, including relevant peer-reviewed publications. Expertise is welcome in areas of reproduction, infertility, pregnancy, child development, and early origins of later outcomes. Research on environmental and/or genetic contributors to outcomes is encouraged. Applicants will be evaluated on their demonstrated ability to conduct biologically-based, interdisciplinary, population-level research in reproductive or developmental epidemiology. Dr. E. Mitch Eddy, Laboratory of Reproductive and Developmental Toxicology, is chair of the search committee.

Tenure-Track X-Ray Crystallographer
The Laboratory of Structural Biology in the Division of Intramural Research of the National Institute of Environmental Health Sciences is seeking a Tenure-Track Principal Investigator in X-ray crystallography. Applicants should have a doctoral degree, a clear record of accomplishment in X-ray crystallography, and plans to develop a strong and original research program to investigate the structure and function of proteins involved in determining biological responses to environmental stress. While applicants proposing research in all areas related to the structure of biological macromolecules will be considered, we are particularly interested in candidates proposing research plans that coincide with areas of strength in the NIEHS Intramural Program, including but not limited to signal transduction, nuclear hormone receptor signaling, epigenetics, DNA replication and repair, and pulmonary biology. Dr. Michael Resnick, Laboratory of Molecular Genetics, is chair of the search committee. A candidate has been identified for the position.

Tenure-Track Embryonic Stem Cell Biologist
The Laboratory of Molecular Carcinogenesis is recruiting a Tenure-Track Investigator - Embryonic Stem Cell Biologist with intellectual and research strengths in, but not necessarily limited to, regulation of gene expression, development, chromatin and epigenetics. The successful applicant will be expected to establish a high-quality independent research program in stem cell biology, relevant to cancer, within a group with diverse research interests and backgrounds but focused upon the molecular and environmental mechanisms of carcinogenesis. Applicants should have a Ph.D, M.D. or equivalent doctoral degree with 3 years of postdoctoral research experience, and a strong publication record. Research experience with cancer models is desirable but not mandatory. Dr. Traci Hall, Laboratory of Structural Biology, is chair of the search committee. Dr. Guan Hu from the Department of Medicine, Division of Genetics, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, has accepted the position and is scheduled to start October, 2009.

Tenure-Track Developmental Neurobiologist
The Laboratory of Neurobiology is recruiting a Tenure-Track Investigator to lead a high-quality independent research program on any fundamental aspect of developmental neurobiology with the potential for identifying and preventing the deleterious effects of environmental exposures on human cognitive development. Applicants should have a Ph.D., M.D., or equivalent doctoral degree with at least 3 years of postdoctoral research experience in developmental neurobiology and a strong publication record. Applicants using fluorescence imaging and genetic model organisms are particularly encouraged to apply, but the emphasis will be on identifying an outstanding scientist with an innovative research program. Dr. Jan Drake, Laboratory of Molecular Genetics, is chair of the search committee. Dr. Patricia Jensen from the Department of Genetics at the Harvard Medical School in Boston, Massachusetts, has accepted the position and is scheduled to start August 31, 2009.

Tenure-Track Developmental Biologist

A position is available for a Developmental Biologist to establish an independent basic research program and form a research group in the Laboratory of Reproductive and Developmental Toxicology, Division of Intramural Research. Applications are invited from scientists with demonstrated ability for creative and productive research in cellular and molecular mechanisms of mammalian development. Of particular interest are investigators using rodent models to study cell interactions, epigenetics or other basic biomedical problems relating to the impact of the environment on development. The successful candidate will interact with investigators studying diverse problems in reproductive biology, developmental toxicology, hormone mechanisms, signal transduction, cell cycle regulation, cell growth and differentiation, apoptosis, gene regulation, mutagenesis and DNA repair, and cancer biology. Minimum qualifications are an M.D., Ph.D., D.V.M. or equivalent doctoral degree in the biomedical sciences, at least three years of postdoctoral experience, and publications in high quality journals. Dr. Darryl Zeldin, Acting Clinical Director and Laboratory of Respiratory Biology, is chair of the search committee.

Chief, Toxicology Branch/Senior Scientist

The NIEHS is seeking a dynamic, highly motivated scientist to serve as a Senior Scientist to oversee the operations of the Toxicology Branch (TB) of the National Toxicology Program (NTP). The Chief, TB, supervises senior scientists, staff scientists, and support staff who design, interpret, review, and report toxicology and carcinogenicity studies for substances studied by the NTP. As a senior official representing NTP, the Chief, TB, is the primary expert and interface on toxicology with many agencies and offices and provides guidance on relevant research, policy, and practices. He/she identifies, assesses, and integrates advancements in molecular and cellular techniques with traditional rodent toxicology testing methods. To accomplish this, he/she builds strong relationships with intramural and extramural investigators to design and carry out studies that further our understanding of toxicological mechanisms. The Chief, TB, works with computational and bioinformatics scientists at NTP, NIEHS, and other institutes and agencies to organize data collection and analysis, works with NTP chemists to evaluate compounds, and works with other NTP staff to oversee the development of technical reports on study findings. He/she also evaluates and develops new methodologies and technologies for toxicology assessments, writes technical reports and prepares manuscripts in collaboration with investigators within and outside NTP. The training of NTP postdoctoral fellows to prepare outstanding future leaders in toxicological research is also a critical part of this position. Finally, the Chief, TB, develops collaborations and relationships with other agencies and institutes, including EPA, FDA, advocacy groups and industry coalitions. Dr. Christopher Portier, Office of Risk Assessment and Laboratory of Molecular Toxicology, is chair of the search committee. Candidates are currently being interviewed.

GRAND OPENING OF THE NIEHS CLINICAL RESEARCH UNIT

On Monday, July 27, 2009, opening ceremonies were held for the new NIEHS Clinical Research Unit in Rodbell Auditorium of the Rall Building at NIEHS main campus in Research Triangle Park, NC. Dr. Joe Graedon of The People's Pharmacy served as master of ceremonies. Opening remarks were made by Dr. Linda Birnbaum, director of the NIEHS and National Toxicology Program. Greeting from the main NIH campus in Bethesda were presented by Dr. Michael Gottesman the NIH Deputy Director for Intramural Research. A local partner's perspective was presented by Dr. Robert Califf, Vice Chancellor for Clinical Research, Duke University. Comments were also made by a number of political officials including Kay Hagen, U.S. Senator from NC; David Price, U.S. Representative from NC, 4th District; Bob Etheridge, U.S. Representative from NC, 2nd District; Brad Miller, U.S. Representative from NC, 13th District; Walter Dalton, Lieutenant Governor of NC; Lanier Cansler, NC Secretary of Health and Human Services; Michael Page, Durham County Commissioner; and William Bell, Durham Mayor.

Dr. Darryl Zeldin, Acting Director, NIEHS Clinical Research Program, presented awards to individuals responsible for making the opening of the Clinical Research Unit possible. The awardees were: William Blair, Chief, Facilities Management Branch, NC; Debra Del Corral, Space Management Specialist, Office of Management; Vickie Englebright, Administrative Coordination Specialist, Office of the Scientific Director; Stavros Garantziotis, Staff Clinician, Medical Director, Clinical Research Unit; Crystal Green, Mechanical Engineer, Facilities Operation Branch; Clyde Hasty, Safety and Occupational Health Specialist, Health and Safety Branch; Gregory Holland, IC Liaison, Office of Research Facilities; Christopher Hunt, Safety Officer, Health and Safety; Scott Merkle, Chief, Health and Safety Branch; Valeria Shropshire, Industrial Hygienist, Health and Safety Branch; Michael Spencer, Clinical Program Coordinator, Clinical Research Program; and F. Mitchell Williams, Chief, Operations and Security Branch.

Presentation of awards was followed by a ribbon-cutting ceremony at the Clinical Research Unit which included a reception and tours of the unit.

A Clinical Research Symposium was held in the afternoon as part of the ceremonies. Dr. Stavros Garantziotis, Staff Clinician, Medical Director, Clinical Research Unit, served as Symposium Moderator. Presentations were by Dr. Robert Califf, Vice Chancellor for Clinical Research, Duke University, Durham, NC, who spoke on "Addressing Key Issues on Evaluating Mechanisms of Disease in Humans"; Dr. Philip Landrigan, Professor and Chair of Community and Preventive Medicine, Mt. Sinai Medical Center, New York, NY, who spoke on "The National Children's Study — The Need and the Promise"; and Dr. Franck Mauvais-Jarvis, Associate Professor of Medicine, Division of Endocrinology, Metabolism and Molecular Medicine, Northwestern University, Chicago, IL, who spoke on "Estrogen Receptors and Pancreatic Islet Survival in Diabetes: An Example of Bidirectional Translational Research."

DIR RESEARCH UPDATE

An Epigenetic Pathway Specifies Phenotype in Breast Cancer Cells

Paul Wade, Ph.D., Eukaryotic Transcription Regulation Group
Laboratory of Molecular Carcinogenesis

Estrogen receptor alpha (ER-α) is a key regulatory molecule in mammary epithelial cell development and a critical prognostic indicator in breast cancer. Our laboratory has defined a molecular pathway downstream of ER that regulates important aspects of breast cancer cell physiology, shape and behavior. The receptor (ER-α) directs the synthesis of a regulatory component of a chromatin remodeling enzyme, the Mi-2/NuRD complex, which in turn participates in regulation of a variety of genes. This pathway ties action of estrogen receptor to growth properties and phenotypic characteristics of breast cancer cells. Further, elucidation of this epigenetic pathway has provided insights into how the local environment of breast cancer cells can influence their epigenome.

DIR SCIENTIFIC ACCOMPLISHMENTS 2009

Completion of Enrollment in the Sister Study.
More than 50,000 women at increased risk for breast cancer due to having a sister with the disease have enrolled in the NIEHS Sister Study. This cohort will be followed over time for the development of breast cancer and other outcomes; women who develop breast cancer will be studied over time to learn more about environmental and genetic contributors to disease. Biologic and environmental samples and questionnaire data collected at baseline along with tumor tissue from those who develop breast cancer will facilitate research on a wide range of topics.

Genome-Wide Association Study (GWAS) of Childhood Asthma in a Mexican Population Identifies a Novel Susceptibility Locus.
NIEHS investigators completed the first GWAS at the institute. The investigators did genome wide association genotyping of childhood asthma in Mexico City. The study identified a novel locus associated with asthma – near a gene called *TLE4* which is plausibly involved in asthma. The analysis also took ancestry into account – this is possible when disease prevalence and allele frequencies differ between ethnic groups. This ancestry analysis provided supportive evidence for the finding from the GWAS.

Hancock DB, Romieu I, Shi M, Sienra-Monge J-J, Wu H, Chiu GY, Li H, del Rio-Navarro BE, Willis-Owens SAG, Weiss ST, Raby BA, Gao H, Eng C, Chapela R, Burchard EG, Tang H, Sullivan PF, London SJ. Genome-Wide Association Study Implicates Chromosome 9q21.31 as a Susceptibility Locus for Asthma in Mexican Children. *PloS Genet.* (in press).

Uterine Fibroids have Highly Variable Growth Rates
Fibroids are the leading cause of hysterectomy in the United States, but little is known about tumor growth. A study in premenopausal women that tracked growth of 262 fibroids showed a median growth rate of 9% per six months, but great variation with 7% shrinking and 34% growing at greater than 20% per 6 months. Fibroids from the same woman grew at very different rates, despite being in the same hormonal milieu. While fibroids in black and white women under 35 years of age grew similarly, growth rates were low for tumors in the older white women, an age-difference not seen in blacks. Tumor growth rate did not vary with parity, body mass index, location of fibroid in the uterus, or fibroid size.

Peddada SD, Laughlin SK, Miner K, Guyon JP, Haneke K, Vahdat HL, Semelka RC, Kowalik A, Armao D, Davis B, Baird DD. Growth of uterine leiomyomata among premenopausal black and white women. *Proc. Natl. Acad. Sci. USA* 105:19887-19892, 2008.

Immune Response Genes are Risk and Severity Factors for Autoimmune Muscle Diseases in Adults and Children
To enhance understanding of the causes of the autoimmune disease myositis, investigators at NIEHS asked whether certain immune response genes, including those

encoding the pro-inflammatory proteins tumor necrosis factor alpha and interleukin-1, as well as immunoglobulins, increase risk for the development of a juvenile form of myositis, dermatomyositis, and also increase the severity of illness. The researchers showed that polymorphisms in the genes for tumor necrosis factor alpha and interleukin-1beta, which are associated with increased production of these pro-inflammatory cytokines, as well as polymorphisms in genes encoding the heavy chains of immunoglobulin G (called GM), are important risk factors for the development of juvenile dermatomyositis. The tumor necrosis factor alpha promoter polymorphism was also associated with the development of calcification and ulceration, a severe rash in this population. Certain GM polymorphisms were also found to be risk or protective factors for some forms of myositis in adults. These findings of unique genetic susceptibility factors have implications for diagnosis, pathogenesis and treatment. These results also support the hypothesis that the autoimmune muscle diseases comprise multiple, distinct entities, which develop as the result of different gene-environment interactions.

Mamyrova G., O'Hanlon TP, Sillers L, Malley K, James-Newton L, Parks CG, Cooper GS, Pandey JP, Miller FW, Rider LG, for the Childhood Myositis Heterogeneity Collaborative Study Group. Cytokine Gene Polymorphisms as Risk and Severity Factors for Juvenile Dermatomyositis. *Arthrit. Rheumat.* 58:3941–3950, 2008.

O'Hanlon TP, Rider LG, Schiffenbauer A, Targoff IN, Malley JK, Pandey JP, Miller FW. Immunoglobulin Gene Polymorphisms are Susceptibility Factors in Clinical and Autoantibody Subgroups of the Idiopathic Inflammatory Myopathies. *Arthrit. Rheumat.* 58:3239–3246, 2008.

Association of Obesity with Allergy in the U.S.

Obesity is a growing problem in the U.S. and in most of the developed countries of the world. At the same time, allergic disease prevalence has increased. There has been some disagreement about whether or not obesity is related to allergy symptoms or to higher serum IgE levels (indicators of allergic sensitization). In addition, it has been suggested that systemic inflammation, measured by C-reactive protein (CRP) level which is often elevated in overweight individuals, may be involved in this relationship. NIEHS investigators examined the association of obesity with allergen-specific serum IgE levels and allergy symptoms in U.S. children and adolescents, and also assessed the role of CRP in this complex relationship, using nationally representative data from the National Health and Nutrition Examination Survey (NHANES) 2005-2006. They found that allergic sensitization to foods was more common among obese children and that total IgE levels were higher among obese and overweight children than among normal weight children. CRP levels were positively associated with total IgE and that relationship was influenced by body mass index (BMI), suggesting that there could be an inflammatory component to the association between BMI and IgE levels. These findings suggest that obesity may be a contributor to the increased prevalence of allergic disease, particularly food allergy, in children. Thus, efforts to reduce or prevent childhood obesity may have the added benefit of reducing allergic disease, especially to foods.

Visness CM, Daniels JL, Kaufman JS, London SJ, Yeatts KB, Siega-Riz AM, Calatroni A, Zeldin DC. Association of Obesity with IgE and Allergy Symptoms in Children and Adolescents: Results from NHANES 2005-2006. *J. Allergy Clin. Immunol.* 123: 1163-1169, 2009.

Gergen PJ, Arbes SJ, Calatroni A, Mitchell HE, Zeldin DC. Total IgE and Asthma Prevalence in the U.S. Population: Results from the National Health and Nutrition Examination Survey (NHANES) 2005-2006. *J. Allergy Clin. Immunol.* (in press).

Potentially Deleterious *CYP2C9* Polymorphism Identified in an African American Patient with Major Hemorrhage on Warfarin Therapy.

Polymorphisms in the human drug metabolizing enzyme CYP2C9 can metabolism of the anticoagulant drug warfarin, with potential life-threatening bleeding episodes in some patients.. In a large collaborative study with the University of Alabama, NIEHS researchers evaluated the effects of CYP2C9 polymorphisms on dose and adverse effects of warfarin in patients on long-term therapy. Polymorphisms affected both pharmacokinetics and life-threatening adverse effects. A new polymorphism was discovered in an African American patient who had a life-threatening bleeding episode.

Goldstein JA, Blaisdell JA, Limdi NA. A potentially deleterious new *CYP2C9* polymorphism identified in an African American patient with major hemorrhage on warfarin therapy. *Blood Cells Mol. Dis.*, 42:155-158, 2009.

Folic Acid Supplementation during Pregnancy May Increase Asthma Risk.

NIEHS is collaborating with a large birth cohort of over 100,000 women in Norway (the Norwegian Mother and Child Cohort) to study risk factors for early life outcomes. These investigators examined whether maternal folate supplementation might be related to risk of asthma in the offspring. They were interested in this hypothesis because of results from mouse models, done largely at NIEHS, showing that supplementation of pregnant mice with folate led to increased allergic asthma in the offspring due to epigenetic mechanisms (changes in DNA that do not alter the sequence). Children of mothers who took folate supplements had slightly higher risk of asthma related phenotypes at 18 months of age. Norway is an excellent place to examine this association because food is not fortified with folate as it is in the US and other countries. Also recommendations for use of folate in pregnancy are more recent in Norway than in the US.

Håberg SE, London SJ, Stigum H, Nafstad P, Nystad W. Folic Acid Supplements in Pregnancy and Early Childhood Respiratory Health. *Arch. Disease Childhood*. 94:180-184, 2009.

Sun Exposure May Trigger Certain Autoimmune Diseases in Women

NIEHS researchers determined if there were relationships between the level of UV exposure at the onset of the disease and the type of myositis and autoantibodies that people developed. They found that among women who develop myositis, the odds for developing the form called dermatomyositis instead of the form called polymyositis are increased about 4-fold for each additional unit of ambient UV radiation exposure. They

also found an association between UV exposure and dermatomyositis in women and not in men, and it could be that inherent differences in how women and men respond to UV radiation may play a role in the development of certain autoimmune diseases. Patients with autoimmune diseases make a variety of autoantibodies that are unique to different conditions. One autoantibody specifically associated with dermatomyositis is called the anti-Mi-2 autoantibody and UV radiation increases levels of the Mi-2 protein to which this autoantibody binds. In addition to finding an association between the level of UV radiation and the proportion of women who developed dermatomyositis compared to polymyositis, the researchers also found an association between UV levels and the proportion of women with the anti-Mi-2 autoantibody.

Love LA, Weinberg CR, McConnaughey DR, Oddis CV, Medsger TA, Reveille JD, Arnett FC, Targoff IN, Miller FW. Ultraviolet Radiation Intensity Predicts the Relative Distribution of Dermatomyositis and Anti-Mi-2 Autoantibodies in Women. *Arthrit. Rheumat.* (in press).

Pesticides Associated with Asthma in farmers.

Among over 19,000 farmers in the Agricultural Health Study, NIEHS researchers evaluated whether use of specific pesticides was related to adult onset asthma. They categorized asthma cases as allergic (N=127) and non-allergic (N=314) based on their history of eczema or hay fever. A history of a high pesticide exposure event, such as a spill, was associated with a doubling of adult onset asthma risk. Twelve pesticides, including three organophosphate insecticides and paraquat, were associated with allergic asthma and four with non-allergic asthma. Animal studies support the findings for organophosphate insecticides and paraquat. Two commonly used pesticides, diazinon and malathion, were associated with increased asthma among farmers.

Hoppin JA, Umbach DM, London SJ, Henneberger PK, Kullman GJ, Coble J, Alavanja MCR, Beane Freeman LE, Sandler DP. Pesticide use and adult-onset asthma among male farmers in the Agricultural Health Study. *Eur. Resp. J.* (in press).

Chemical Exposures in the Workplace Increase Risk of Amyotrophic Lateral Sclerosis (ALS).

ALS (Lou Gehrig's disease) is a devastating disease of the neuromuscular system that often leads to respiratory failure and death within two to three years after diagnosis. At present there is no cure for the disease and known treatments slow progression only slightly. In addition, although ALS likely is caused by a complex interplay of genetic susceptibility with environmental exposures, little is known about this process. NIEHS researchers found higher risk of ALS among construction workers excluding supervisors and among precision metal workers. Workplace exposure to chlorinated hydrocarbons, glycols, glycol ethers, and hexane were each associated with a 50% increase in ALS risk. These results suggest that certain occupations and workplace exposures may contribute to the development of ALS.

Fang F, Quinlan P, Ye W, Barber M, Umbach DM, Sandler DP, Kamel F. Occupational exposures and the risk of amyotrophic lateral sclerosis. *Environ. Hlth. Perspect.* (in press).

Scrunching during DNA Repair Synthesis.
Repair of damaged DNA often requires DNA polymerases to fill in short single-stranded gaps, in reactions that should be efficient to avoid release of potentially toxic repair intermediates. NIEHS scientists recently solved several crystal structures revealing that, when bound to a two-nucleotide gap, pol λ "scrunches" the template strand, allowing the distance between the 5' and 3' ends of the gap to remain constant. In doing so, a binding pocket comprised of three amino acids holds the yet uncopied template base in an extrahelical position. Alanine replacement of these three amino acids reduces processive synthesis and the efficiency of DNA repair. These results show that, akin to scrunching of nucleotides by RNA polymerase during initiation of transcription, nucleotide scrunching contributes to the efficiency of gap filling DNA synthesis associated with DNA repair.

Garcia-Diaz M, Bebenek K, Larrea A, Havener JM, Perera L, Krahn J, Pedersen LC, Ramsden DA, Kunkel TA. Template strand scrunching during DNA gap repair synthesis by human DNA polymerase λ, *Nature Struct. Molec. Biol.* (in press).

Regulation of a Ubiquitous Chloride Channel by Phosphoinositide IP4 Establishes a Signaling Paradigm Controlling Diverse Physiological Processes.
Certain intracellular signaling molecules exert effects upon a broad range of biological processes, dependent upon their cellular contexts. NIEHS scientists raised the biological relevance of inositol 3,4,5,6-tetrakisphosphate (IP4) from that of a signaling molecule with a limited repertoire of roles (regulating plasma membrane chloride efflux in some epithelial cells), to a near-ubiquitous second messenger of potentially high physiological significance. They do this by demonstrating that the long-sought molecular target for IP4 is a widely expressed chloride transporter [ClC-3], that has established roles in a diverse range of physiological processes.

Mitchell J, Wang X, Gentsch M, Nelson DJ, Shears B. An Expanded Biological Repertoire for Ins(3,4,5,6)P_4 through its Modulation of ClC-3 Function. *Curr. Biol.* 18:1600-1605, 2008.

First Glimpse of a DNA Synthesis Enzyme Making a Mutation
DNA polymerase ß, the enzyme responsible for copying DNA during chromosome replication, makes occasional mistakes and inserts the wrong base into newly formed and copied DNA. This type of mistake can be harmful in adults, contributing to diseases like cancer, since the error can be retained as a mutation. Working with a purified human DNA polymerase, a NIEHS group determined the structure of the enzyme as it was poised to make an error. The new structure offered a big surprise: The polymerase makes a mistake because it inserts a new nucleotide without reference to the DNA template

strand being copied. This new information has implications for new approaches toward preventing mutations associated with environmental stress and aging.

Beard WA, Shock DD, Batra VK, Pedersen LC, Wilson SH. DNA polymerase ß substrate specificity: Side chain modulation of the 'A-rule'. *J. Biol. Chem.* (in press).

Lin P, BatraVK, Pedersen LC, Beard WA, Wilson SH, Pedersen LG. Incorrect nucleotide insertion at the active site of a G:A mismatch catalyzed by DNA polymerase ß. *Proc. Natl. Acad. Sci. U.S.A.* 105:5670-5674, 2008.

Batra VK, Beard WA, Shock DD, Pedersen LC, Wilson SH. Structures of DNA polymerase ß with active-site mismatches suggest a transient abasic site intermediate during misincorporation. *Mol. Cell* 30:315-324, 2008.

DNA Mismatch Repair.

Post-replication mismatch repair (MMR) is critical for genome stability. Defects in this pathway can result in in cancer, drug resistance, infertility, altered immunity and altered cellular responses to environmental stresses. In a recent study of MMR, NIEHS investigators in collaboration with investigators at the University of North Carolina at Chapel Hill identified ATP-dependent conformational changes that occur in MutLα, the key protein that harbors an essential endonuclease activity.

Sacho EJ, Karyrov FA, Modrich P, Kunkel TA, Erie DA. Direct visualization of asymmetric adenine nucleotide induced conformational changes in MutLα. *Molec. Cell* 29:112-121, 2008.

SIRT1, A Nuclear Anti-Aging Protein, Promotes Fatty Acid Oxidation in Liver

SIRT1 is a member of the high-profile sirtuin family of proteins that plays a vital role in the regulation of metabolism, stress responses, genome stability, and ultimately aging. A NIEHS group of scientists is investigating the role of this longevity protein in age-associated metabolic diseases, such as obesity, type-2 diabetes, and atherosclerosis. Utilizing a mouse model that specifically deleted SIRT1 gene in the liver, they demonstrate that SIRT1 regulates lipid metabolism through activation of a transcription factor that mediates fatty acid oxidation. As a result, mice lacking SIRT1 in the liver accumulate lipids in their livers when challenged with a high-fat high cholesterol diet. The elevated lipid levels in turn leads to the development of chronic inflammation and stress. Their findings define a new role for SIRT1 as a key regulator of hepatic lipid metabolism, and suggest that therapeutic strategies designed to activate SIRT1 activity may be beneficial for the treatment of a number of obesity-associated metabolic diseases.

Purushotham A, Schug TT, Xu Q, Surapureddi S, Guo X, Li X. Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation. *Cell Metab.* 9:327-338, 2009.

SNPs in Human Ion Channel Genes Increase Susceptibility to Disease by Creating New Phosphorylation Sites in the Channel Proteins

NIEHS scientists have determined that a common SNP in a cardiac potassium channel

gene, hERG1, creates a phosphorylation site within the channel protein that inhibits channel activity. These channels are necessary for rhythmic excitability of cardiac muscle and endocrine cells, and they are regulated by phosphorylation However, in human channels with the K897T polymorphism, in which a single nucleotide change alters the translation of codon 897 from a lysine to a threonine, hormonal signaling through kinases has the opposite effect on phosphorylation of the channel and on its activity. Human cardiac myocytes with less channel activity have longer action potentials, as indicated by longer QT intervals in the electrocardiogram, and an increased risk of developing an arrhythmia, which can be fatal. The finding suggests that human genetic differences can alter susceptibility to disease by changing phosphorylation sites in proteins and altering their regulation, a phenomenon the authors have named "phosphorylopathy." The authors identify 15 other SNPs in nine channel genes that are predicted to create or destroy putative phosphorylation sites. They are currently investigating these genes in collaboration with clinical investigators at NIEHS.

Gentile S, Martin N, Scappini E. Williams J, Erxleben C, Armstrong DL. The human ERG1 channel polymorphism, K897T, creates a phosphorylation site that inhibits channel activity. *Proc. Natl. Acad. Sci. U.S.A.* 105:14704-14708, 2008.

Loss of the Transcriptional Regulator Glis3 Leads to Development of Polycystic Kidney Disease

Study of mice deficient in the transcriptional regulator Glis3, a Krüppel-like zinc finger protein, demonstrated that Glis3-deficient mice develop polycystic kidney disease which ultimately results in renal failure. In addition, the study showed that Glis3 interacts with the co-activator Wwtr1 and is part of a primary cilium signaling pathway. Glis3 is critical for maintaining normal renal functions.

Kang H., Beak JY, Kim Y-S, Herbert R, Jetten AM. Glis3 is associated with primary cilia and TAZ and is implicated in polycystic kidney disease. *Mol. Cell. Biol*. 29:2556-2569, 2009.

ZFP36L2 is a Novel Modulator of Hematopoiesis.

Evaluation of ZFP36L2 knockout mice revealed that definitive hematopoiesis was severely disrupted in the embryo and adult. This derived from the decreased number and/or function of hematopoietic stem cells. Since ZFP36L2 is an RNA binding and destabilizing protein, a suggested mechanism for this effect is the abnormal accumulation of one or more transcripts, whose products are toxic to hematopoietic stem cells. These studies link the regulation of mRNA turnover to definitive hematopoeisis.

Stumpo DJ, Broxmeyer HE, Ward T, Cooper S, Hasngoc G, Chung YJ, Shelley WC, Richfield EK, Ray MK, Yoder MC, Aplan PD, Blackshear PJ. Targeted disruption of Zfp3612, encoding a CCCH tandem zinc finger RND-binding protein, results in defective hematopoiesis *Blood* (in press).

Studies on the Inter-Relationship between TRPC and Orai Channels.

Previous studies by NIEHS researchers had shown both a functional interaction between TRPCs and Orai and a role for TRPCs in store operated calcium entry. (SOCE). Studies performed during the last year revealed, somewhat unexpectedly, a role for Orai in receptor operated calcium entry (ROCE), previously thought to be a process mediated by just TRPC channels. This finding supports a model in which Orai and TRPCs form heteromeric channels able to function as both ROCE and SOCE channels. This model stands in opposition to models by others that propose SOCE channels to be formed by only Orai molecules and ROCE channels being formed only by TRPC channels.

Liao Y, Plummer NW, George MD, Abramowitz J, Zhu MX, Birnbaumer L. A role for Orai in TRPC-mediated Ca2+ entry suggests that a TRPC:Orai complex may mediate store and receptor operated Ca^{2+} entry. *Proc. Natl. Acad. Sci. U.S.A.* 106:3202-3206, 2009.

Double Strand DNA Breaks Can Induce Lymphocyte Differentiation Responses.
Inappropriate responses to DNA damage can result in severe consequences including cell death, or genomic instability and disease development, such as cancer. NIEHS scientists have collaborated with investigators at Washington University in St. Louis to study the genetic program activated in developing lymphocytes in response to inducible physiologic DNA double strand breaks (DSBs) generated in the setting of lymphocyte antigen receptor gene assembly, using a mouse pre-B cell model system. It was found that many of the genes regulated by RAG-mediated DSBs encode proteins involved in lymphocyte development and function and have no known function in the canonical DNA DSB response. These findings have established a new paradigm whereby physiologic DNA DSBs regulate processes important for normal cellular functions. Thus, physiological DNA double-strand breaks provide cues that can regulate cell-type-specific processes not directly involved in maintaining the integrity of the genome, and genotoxic DNA breaks could disrupt normal cellular functions.

Bredemeyer AL, Helmink BA, Innes CL, Calderon B, McGinnis LM, Mahowald GK, Gapud EJ, Walker LM, Collins JB, Weaver BK, Mandik-Nayak L, Schreiber RD, Allen PM, May MJ, Paules RS, Bassing CH, Sleckman BP. DNA double-strand breaks activate a multi-functional genetic program in developing lymphocytes. *Nature* 456**:**819-823, 2008.

Studies on the Physiologic and Pathophysiologic Roles of Signal Transducing G Proteins
In collaboration with investigators at the Mayo Clinic (Scottsdale, AZ) NIEHS scientists discovered that allergic inflammation in the lung promotes recruitment of circulating tumor cells to the lung that is dependent on expression of the Gi2 G protein in the endothelium through which these cells need to extravasate identifying lung inflammation as a risk factor for metastatic lung cancer.

In a collaboration with scientists the University of California at Irvine, NIEHS investigators found epithelial dysregulation in colitis-associated colon cancer in Gi2 deficient mice.

Previous studies in collaboration with scientist from the University of Düsseldorf (Germany), had uncovered an unexpected obligatory role for the Gi3 G protein in the antiautophagic action of insulin in liver. It was surmised that the step in which Gi3 was participating was intracellular and likely involved the activation of the mTOR/S6 kinase pathway. New studies in collaboration with investigators from Brown University (Providence RI) showed that activation of the Akt/mTOR signaling pathway by EGF receptor is dependent on Gi1 and Gi3, thus complementing the earlier findings. The role of Gi3 in an intracellular process is a novel finding.

Taranova AG, Maldonado D, Vachon CM, Jacobsen EA, Abdala-Valencia H, McGarry, MP, Ochkur SI, Protheroe CA, Doyle A, Grant CS, Cook-Mills J, Birnbaumer L, Lee, NA, Lee JJ. Allergic pulmonary inflammation promotes the recruitment of circulating tumor cells to the lung. *Cancer Res*. 68:8582-8589, 2008.

Edwards RA, Wang K, Davis JS, Birnabumer L. A role for epithelial dysregulation in early onset colitis-assocaited colon cancer in Gi2-/- mice. *Inflam. Bowel Dis.* 14:898-907, 2008.

Cao C, Huang X, Han Y, Wan Y, Birnbaumer L, Feng G-S, Marshall J, Jiang M, Chu, W-M. Requirement of Gαi1 and Gαi3 for activation of the Akt/mTORC1 pathway by epidermal growth factor. *Sci. Signal.* 2: ra17, 2009.

Studies on The Physiologic and Pathophysiologic Roles of the TRPC calcium-Permeable Non-selective Cation Channels.

In collaboration with investigators from the Technical University of Munich (Germany) NIEHS investigators discovered an obligatory role for TRPC3 in synaptic transmission and motor control dependent on cerebellar Purkinje cells. In contrast and in collaboration scientists from the University of Texas Medical School at Dallas, NIEHS investigators discovered that TRPC3 is a risk factor for acute pancreatitis caused by chronic subpramaximal stimulation by agonists which promotes abnormal store depletion activated influx of calcium and attendant abnormal intracellular trypsin activation. These studies also established that TRPC3 is a store operated channel in vivo, and extend similar conclusions for TRPC1 in a store operated channel in vivo reached earlier.

Hartmann J, Dragicevic, E, Adelsberger H, Henning H, Sumser M, Blum R., Dietrich A, Abramowitz J, Freichel V, Birnbaumer L, Konnerth, A. TRPC3 channels required for synaptic transmission and motor coordination. *Neuron* 59:392-398, 2008.

Kim, MS, Hong JH, Shin DM, Abramowitz, J, Birnbaumer L, Muallem S. Deletion of TRPC3 in mice reduces Store-Operated Ca2+ influx and the severity of acute pancreatitis. *Gastroenterology* (in press).

Calcium Handling Regulates the Brain's Ability to Change.

The brain can regulate the strength of its connections, but recent evidence has suggested that it does so only at some stages of development and in only certain brain regions. NIEHS investigators describe how such plasticity of connections is negatively regulated in one brain region. By quickly pumping calcium out of the cell, neurons in the

hippocampal CA2 region prevent increases in synaptic strength. These findings also provide critical insight into why this same area is resistant to damage from stroke, trauma and epilepsy.

Simons SB, Escobedo Y, Yasuda R, Dudek SM. Regional differences in hippocampal calcium handling provides a cellular mechanism for limiting plasticity *Proc. Natl. Acad. Sci. U.S.A*. 2009. doi:10.1073/pnas.0904775106

Male Infertility Caused by a Single Amino Acid Change in One Protein

The male descendants of a mouse treated with a drug at the Jackson Laboratory known to cause gene mutations were infertile. NIEHS investigators found that sperm from these mice were malformed and unable to swim effectively, similar to what is seen in some infertile men. Genetic methods were used to map the mutation to a region on one chromosome and by sequencing genes a mutation was found in a single nucleotide that caused substitution of one amino acid for another in the CAPZA3 protein. This protein is present only in male germ cells, but a very similar protein is present in other cell types that regulate actin filament organization. Additional studies determined that CAPZA3 is required to regulate actin organization during sperm assembly and that the amino acid substitution disrupts this function.

Geyer CB, Inselman AI, Sunman JA, Bornstein S, Handel MA, Eddy EM. 2009. A missense mutation in the *Capza3* gene and disruption of F-actin organization in spermatids of *repro32* infertile male mice. *Devel. Biol.* 330:142-152, 2009.

The RNA Binding Protein TTP is a Regulator of an Important Cell Cycle Protein Kinase.

Polo-like kinase 3 (Plk3) is an important regulator of the cell cycle, and has been implicated in apoptosis. NIEHS researchers found that the transcript encoding this protein in mouse fibroblasts was stabilized in the absence of the RNA binding protein tristetraprolin, or TTP. TTP bound to several canonical binding sites in the Plk3 transcript, and could promote its decay. This work identifies TTP-mediated mRNA decay as an important control locus for the cellular levels of Plk3, and thus the cell cycle.

Horner TJ, Lai WS, Stumpo DJ, Blackshear PJ. Stimulation of Polo-Like Kinase 3 mRNA Decay by Tristetraprolin. *Mol. Cell. Biol.*, 29:1999-2010, 2009.

Yeast Model used to Explore Genomic Hypermutation Resulting From Environmental Damage

Following the generation of long stretches of single-strand DNA in the vicinity of chromosomal double-strand breaks or uncapped telomeres of the budding yeast *Saccharomyces cerevisiae* the DNA can be restored to double-strand. Importantly, restoration occurs even if there are multiple lesions, which are not repairable in single strand DNA. The error-prone DNA polymerase zeta provides synthesis past the lesions resulting in the efficient generation of mutations. This work establishes a simple

molecular mechanism for simultaneous generation of multiple mutations spanning many kilobases without severe mutation load in the rest of the double-strand genome, where repair is efficient. These findings indicate that environmental toxicants and drugs that can lead to and/or generate lesions in single strand DNA are of special health concern since single-strand regions in the genome may be at high risk for mutation and subsequent disease. This model was subsequently used to provide the first direct evidence that cells possess a highly efficient system for recognition and initiation of resection at γ-radiation induced dirty ends and that the resection is largely dependent on the Rad50/Mre11/Xrs2 complex.

Yang Y, Sterling J, Storici F, Resnick MA, Gordenin DA. Hypermutability of damaged single-strand DNA formed at double-strand breaks and uncapped telomeres in yeast *Saccharomyces cerevisiae*. *PLoS Genetics* 4(11):e1000264, 2008.

Westmoreland J, Ma W, Yan Y, Van Hulle K, Malkova A, Resnick, MA. *RAD50* is required for efficient initiation of resection and recombinational repair at random, γ-induced double-strand break ends. *PLoS Genetics* (in press).

Detection of Mutations in Mitochondrial DNA in Newborns Exposed in utero to AZT

AZT based therapies during pregnancy have proven very effective in the prevention of HIV infection from mother to child. However, the long term effect of AZT exposure to newborns is unknown. NIEHS scientists in collaboration with researchers at Lovelace Respiratory Research Institute searched for mtDNA mutations in healthy newborns after exposure in utero to AZT/3TC. Using human umbilical cord tissue they identified a three-fold increase in mitochondrial DNA sequence variants in those newborns exposed to antiviral therapy after prepartum AZT based HIV-1 prophylaxis as compared to controls. This research emphasizes a greater need to understand the consequence of anti-HIV therapy to newborns exposed in utero to these therapies. In related studies, researchers at the NIEHS scientists have unraveled why one genetic variations cause some people to be more sensitive than others to the side effects of anti-HIV drugs. They found that the R964C mutation in DNA polymerase γ causes the enzyme to have a higher selection for D4T and helps to explain the D4T-induced mitochondrial toxicity displayed by patients harboring this R964C mutation.

Torres SM, Walker DM, McCash CL, Carter MM, Ming J, Cordova EM, Pons RM, Cook Jr DL, Seilkop SK, Copeland WC, Walker VM. Mutational analysis of the mitochondrial tRNA genes and flanking regions in umbilical cord tissue from uninfected infants receiving AZT-based therapies for prophylaxis of HIV-1. *Env. Mol. Mut.* 50:10-26, 2009.

Bailey CM, Kasiviswanathan R, Copeland WC, Anderson KS. R964C mutation of DNA polymerase γ imparts increased stavudine toxicity by decreasing nucleoside analog discrimination and impairing polymerase activity. *Antimicrob. Agents Chemother.* 53:2610-2612, 2009.

Hypertension in Mice Lacking CYP2J5

The cytochrome P450 (CYP) enzymes participate in a wide range of biochemical functions including metabolism of arachidonic acid and steroid hormones. Mouse CYP2J5 is abundant in the kidney where its products, the cis-epoxyeicosatrienoic acids (EETs), modulate sodium transport and vascular tone. To define the physiological role of CYP2J5 in the kidney, knockout mice were generated using a conventional gene targeting approach. Cyp2j5 (-/-) mice develop normally and exhibit no overt renal pathology. While renal EET biosynthesis was apparently unaffected by the absence of CYP2J5, deficiency of this CYP in female mice was associated with increased blood pressure, enhanced proximal tubular transport rates, and exaggerated afferent arteriolar responses to angiotensin II and endothelin I. Interestingly, plasma 17β-estradiol levels were reduced in female Cyp2j5 (-/-) mice and estrogen replacement restored blood pressure and vascular responsiveness to normal levels. There was no evidence of enhanced estrogen metabolism, or altered expression or activities of steroidogenic enzymes in female Cyp2j5 (-/-) mice, but their plasma levels of luteinizing hormone and follicle stimulating hormone were inappropriately low. Together, the findings illustrate a sex-specific role for CYP2J5 in regulation of blood pressure, proximal tubular transport and afferent arteriolar responsiveness via an estrogen-dependent mechanism.

Athirakul K, Bradbury JA, Graves JP, DeGraff LM, Ma J, Zhao Y, Couse J, Quigley R, Harder DR, Zhao X, Imig JD, Pedersen TL, Newman JW, Hammock BD, Conley AJ, Korach KS, Coffman TM, Zeldin DC. Increased Blood Pressure in Mice Lacking Cytochrome P450 2J5. *FASEB J.* 22:4096-4108, 2008.

The protein kinase C substrate MARCKS is critical for normal radial glia organization in the developing brain.

In a collaboration between scientists at UNC and at NIEHS, it was found that the absence of the prominent protein kinase C (PKC) substrate protein MARCKS led to several abnormalities in the formation of the normal radial glial scaffold during forebrain development. These in turn led to abnormal radial neuron migration and neuronal ectopias resembling the cobblestone lissencephalies. Surprisingly, PKC phosphorylation was not crucial for this function of MARCKS, but the amino terminal myristoylation was, implicating membrane association of this protein in the formation of the normal radial glial scaffold, the foundation for the lamination of the developing brain.

Weimer JM, Yokota Y, Stanco A, Stumpo DJ, Blackshear PJ Anton ES. MARCKS modulates radial progenitor placement, proliferation, and organization in the developing cerebral cortex. *Development* (in press).

Identification of a Gene Involved in Regulation of Germ Cell Development

Homeobox genes encode transcription factors whose expression organizes programs of development. A number of homeobox genes expressed in reproductive tissues have been identified recently, including a cluster on the X chromosome in mice. NIEHS researchers identified *Rhox13*, a novel member of this cluster that is expressed specifically in the testis and ovary in the fetus and in the testis but not in the ovary after birth. The pattern

of expression of this gene and the characteristics and distribution of the RHOX13 protein strongly suggest it is a key regulator of the program of germ cell development.

Geyer CB, Eddy EM. Identification and characterization of Rhox13, a novel X-linked mouse homeobox gene. *Gene* 423:194-200, 2008.

Structure and Function of an Elusive Bacterial DNA Replication Protein

DNA Polymerase III holoenzyme, the protein complex responsible for duplicating the chromosome of the bacterium *E. coli*, is the best characterized chromosomal replicase to date. However, the function of the *theta* (θ) subunit, a tightly-bound component of the Pol III core polymerase, is still unclear. NIEHS investigators have demonstrated that (i) within the pol III core θ plays an important role in stabilizing the Pol III ε proofreading subunit, (ii) the function of θ can be substituted for by a bacteriophage P1 protein, named Hot, and (iii) the Hot protein, while not essential, provides a selective growth advantage to the phage and host alike, presumably by supporting the bacterial replication machinery responsible for replicating both phage and host.

Cisneros GA, Perera L, Schaaper RM, Pedersen LC, London RE, Pedersen LG, Darden TA. Reaction mechanism of the ε subunit of *E. coli* DNA polymerase III: Insights into active site metal coordination and catalytically significant residues. *J. Am. Chem. Soc.* 131:1550-1556, 2009.

Mutation at Very High Temperatures

Most mutations are harmful, so organisms try hard to avoid new mutations. Thermophilic microbes that live at temperatures that are lethal to the unadapted, but high temperatures can induce mutagenic damage in genes. Instead of having especially high mutation rates, however, thermophiles growing at 75˚C have mutation rates lower than their moderate-temperature cousins. The reason is probably that the most common mutations are much more harmful at high temperatures, so that a thermophile is forced to invest particularly heavily in avoiding new mutations.

Drake JW. Avoiding dangerous missense: Thermophiles display especially low mutation rates. *PLoS Genetics* 5:e1000520, 2009.

A New Method to Link Disease Processes and Environmental Health

Pathogenesis of complex diseases involves the integration of genetic and environmental factors over time, making it particularly difficult to tease apart relationships between phenotype, genotype, and environmental factors using traditional experimental approaches. A method is proposed that finds enriched pathways relevant to a studied condition using the measured molecular data and also the structural information of the pathway viewed as a network of nodes and edges. Tests are performed using simulated data and genomic data sets and the method is compared to two existing approaches. The analysis provided demonstrates the method proposed is very competitive with the current approaches and also provides biologically relevant results. Using this novel method on gene-centered databases, the NIEHS investigators then developed a network of complex diseases and environmental factors through the identification of key molecular pathways

associated with both genetic and environmental contributions. Identification of key regulatory pathways that integrate genetic and environmental modulators define disease associated targets that will allow for efficient screening of large numbers of environmental factors, screening that could set priorities for further research and guide public health decisions.

Thomas R, Gohlke JM, Stopper GF, Parham FM, Portier CJ. Choosing the right path: enhancement of biologically relevant sets of genes or proteins using pathway structure. *Genome Biol.* 10:R44, 2009.

Gohlke JM, Thomas R, Zhang Y, Rosenstein MC, Davis AP, Murphy C, Becker KG, Mattingly CJ, Portier CJ. Genetic and environmental pathways to complex diseases. *BMC Syst. Biol.* 3:46, 2009.

Gene Dosage and the Development of Lupus Nephritis.
NIEHS scientists demonstrated that lowering the dose of a key gene important for the generation of switched antibodies and high affinity memory B cells by half significantly reduces the severity and delays the onset of lupus nephritis in lupus-prone mice. The reduction was dues to a decrease in the levels of pathogenic antibodies. This opens up the door to the possibility of therapeutically targeting this protein in the treatment of Lupus.

Jiang C, Zhao M, Diaz M. Activation-induced deaminase heterozygous MRL/lpr mice are delayed in the production of high-affinity pathogenic antibodies and in the development of lupus nephritis. *Immunology* 126:102-113, 2008.

Quantitative Proteomic Analysis Reveals Novel Early Signaling Events Triggered in the Macrophage by Exposure to Bacteria.
Lipopolysaccharide (LPS), a glycolipid from the outer cell wall of Gram-negative bacteria, is well-known to trigger a pro-inflammatory signaling cascade in immune cells such as macrophages, but important details of the cell's early signaling response remain undescribed. Mass spectrometry-based proteomics is a powerful technique that can be used to identify changes in proteins during cell signaling. Using quantitative proteomic analysis, NIEHS scientists identified dynamic relocalization within the plasma membrane of multiple cell signaling proteins following LPS exposure, and then unified these changes into putative cell signaling networks using a bioinformatics approach. Notably, a previously undescribed role in the LPS signaling pathway for the 26S proteasome, a multiprotein proteolytic complex, was identified and then biochemically confirmed.

Dhungana S, Merrick BA, Tomer K, Fessler MB. Quantitative Proteomic Analysis of Macrophage Rafts Reveals Compartmentalized Activation of the Proteasome and of Proteasome-mediated ERK Activation in Response to Lipopolysaccharide. *Mol. Cell. Proteom.* 8:201-213, 2009.

Nrf2-Directed Antioxidant Defense System is Essential in Fighting Against Airway Viral Disease
Respiratory syncytial virus (RSV) is a seasonal ubiquitous airway pathogen that infects high risk groups including infants and young children, as well as immune compromised

adults and the elderly worldwide; most (>95%) children are known to be infected by the virus by age 2. RSV infection is associated with severe lower respiratory illness characterized by bronchiolitis and respiratory failure, and is the leading cause of infant hospitalization, but its pathogenesis is not fully understood. The transcription factor Nrf2 directs antioxidant enzyme and defense protein induction to orchestrate the body's antioxidant-oxidant balance against oxidative insults and inflammation. Our study used mice genetically deficient in a transcription factor *Nrf2* to determine that lack of Nrf2 and its downstream effectors exacerbates RSV airway disease phenotypes. Furthermore, oral administration of the broccoli extract sulforaphane, which potentiates Nrf2 activity, protected against RSV infectivity and viral airway inflammation. Results demonstrate that Nrf2-mediated host defense mechanism plays a pivotal role in airway oxidative viral disease, and indicate that targeting oxidative stress may limit pulmonary RSV infectivity, and suggest a potential novel therapeutic means for RSV disease.

Cho HY, Imani F, Miller-DeGraff L, Walters D, Melendi GA, Yamamoto M, Polack FP, Kleeberger SR. Antiviral activity of Nrf2 in a murine model of respiratory syncytial virus disease. *Am. J. Respir. Crit. Care Med.* 179:138-150, 2009.

Allergic Sensitization Through the Airway Primes Th17-Dependent Neutrophilia and Airway hyperresponsiveness.

It is well-known that the allergic inflammation seen in lungs of asthmatics is associated with a specific type of immune cell called the T helper (Th2) cell. However, NIEHS scientists in collaboration with investigators at Children's Hospital of Pittsburgh found that allergic sensitization (immunization) to inhaled allergens also leads to activation of a more recently-described immune cell type, called the Th17 cell. Upon subsequent inhalation of this same type of allergen, Th17 cells release IL-17 into the airway, which in turns leads to neutrophil recruitment and airway hyperresponsiveness, two hallmarks of severe asthma. These findings suggest that therapeutic strategies targeting both Th17 and Th2 cells might be particularly effective for treating individuals with asthma.

Wilson RH, Whitehead GS, Nakano H, Free ME, Kolls JK, Cook DN. Allergic sensitization through the airway primes Th17-dependent neutrophilia and airway hyperresponsiveness. *Am. J. Respir. Crit. Care Med.* (in press).

Discovering Functional Genetic Variation in the mu-Opioid Receptor, The Primary Biological Target for Opioid Analgesics.

A collaborative study with NIEHS investigators identified new genetic variants that appear to affect individual responsiveness to opioid drugs. Up to a third of individuals treated with these drugs develop substantial side effects, and there is over a 10-fold variation in the responses. A combination of approaches from molecular genetics, bioinformatics and statistical genetics allowed identifying novel functional variants within the mu-opioid receptor that may lead to improved prediction of side effects and facilitate development of more effective drugs.

Shabalina SA, Zaykin DV, Gris P, Ogurtsov AY, Gauthier J, Shibata K, Tchivileva IE, Belfer I, Mishra B, Kiselycznyk C, Wallace MR, Staud R, Spiridonov NA, Max MB, Goldman D, Fillingim RB, Maixner W, Diatchenko L. Expansion of the human mu-opioid receptor gene architecture: novel functional variants. *Hum. Mol. Genet.* 18:1037-1051, 2009.

RAP80 Interacts with the Tumor Suppressor p53 and Modulates its Activity.
The nuclear protein RAP80 and p53 play an important role in DNA damage response signaling. This study identifies a link between RAP80 and p53. RAP80 interacts with p53 and modulates its stability while p53 regulates the expression of the RAP80 gene. The findings imply an important role for this pathway in genome stability and oncogenesis.

Yan J, Menendez D, Yang X-P, Resnick MA, Jetten AM. A regulatory loop composed of RAP80-HDM2-p53 provides RAP80 enhanced p53 degradation by HDM2 in response to DNA damage. *J. Biol. Chem.* 284:19280-19289, 2009.

Dietary Fatty Acids Found to Stimulate Cancer Cells through Novel Pathway
NIEHS scientists recently uncovered the pathway by which a common dietary omega-6 fatty acid, arachidonic acid, stimulates cancer cells to adhere to surfaces found in the human body. This adhesion, a critical step in the metastatic spread of tumors, was found to depend on the interaction of several common cellular proteins that had not previously been known to associate. The results open the door for researchers to develop therapeutic approaches that block the interaction of these proteins, thereby reducing the spread of malignant cancers.

Garcia MC, Ray DM., Rubino M, Lackford B, Olden K, Roberts JD. Arachidonic Acid Stimulates Cell Adhesion through a Novel p38MAPK-RhoA Signaling Pathway that Involves Heat Shock Protein 27. *J. Biol. Chem.* 284:20936-20945, 2009.

A New Cause of Ozone Wheezing and Potential Treatments
NIEHS researchers in collaboration with extramural scientists at Duke Medical School and National Jewish Hospital have discovered a cause of airway irritation and wheezing after exposure to ozone, a common urban air pollutant. Using an animal model, the researchers were also able to identify several ways to stop the airways from narrowing. These findings help identify potential new targets for drugs which may eventually help physicians better treat emergency room patients suffering from wheezing, coughing and shortness of breath. The researchers found several proteins which can mediate the hyaluronan effect and can be used as treatment targets. They were also able to block the airway responsiveness by binding the native hyaluronan away, as well as by administering a slightly modified form of hyaluronan.

Garantziotis S, Li Z, Potts EN, Kimata K, Zhuo L, Morgan DL, Savani RC, Noble PW, Foster WM, Schwartz DA, Hollingsworth JW. Hyaluronan mediates

ozone-induced airway hyperresponsiveness in mice. *J. Biol. Chem.* 284:11309-11317, 2009.

NuRD Complex Defines a Novel Pathway for Heterochromatin Assembly.
NIEHS investigators defined a novel role for the NuRD (nucleosome remodeling deacetylase) complex in assembly of pericentromeric heterochromatin in a subset of mammalian cells. Unexpectedly, they observed major differences in how different cell types assemble pericentromeric heterochromatin, an essential nuclear structure. In a subset of cells, including rapidly proliferating lymphoid cell, NuRD complex is associated with these sequences during chromosome replication in striking nuclear structures.

Chadwick LH., Chadwick B, Jaye DL, Wade PA. The Mi-2/NuRD complex specifies a molecular pathway for chromatin assembly and maturation in rapidly proliferating lymphoid cells. *Chromosoma* 118:445-457, 2009.

Critical Roles of ROR Nuclear Receptor in Th17 Differentiation and Immunity.
In collaboration with extramural investigators, NIEHS scientists discovered that RORα and RORγt are both essential for optimal Th17 differentiation. Interleukin-1 (IL-1) signaling is required for the early programming of the Th17 cell lineage and for Th17 cell-mediated autoimmunity. IL-1 regulates the expression of the transcription factors RORγt during Th17 cell differentiation. The study indicates a critical role of IL-1 in Th17 cell differentiation. This pathway may serve as a unique target for Th17 cell-mediated immunopathology. In addition, we showed that CCR6 expression in Th17 cells is regulated by TGF-beta and requires the nuclear receptors, RORα and RORγt. Lack of CCR6 in Th17 cells reduces the severity of experimental autoimmune encephalomyelitis and Th17 and Treg recruitment into inflammatory tissues. The data indicate an important role of CCR6 in Treg and Th17 cell migration.

Yamazaki T, Yang XO, Chung Y, Fukunaga A, Nurieva R, Pappu B, Martin-Orozco N, Kang HS, Ma L, Panopoulos AD, Craig S, Watowich SS, Jetten AM, Tian Q, Dong C. CCR6 regulates the migration of inflammatory and regulatory T cells. *J. Immunol.* 181:8391-8401, 2008.

Chung Y, Chang SH, Martinez GJ, Yang XO, Nurieva R, Kang HS, Ma L, Watowich S, Jetten AM, Tian Q, Dong C. Critical regulation of early Th17 cell differentiation by IL-1 signaling. *Immunity* 30:567-587, 2009.

Peroxymonocarbonate Plays a Role in Superoxide Dismutase Peroxidase Activity.
The enzyme superoxide dismutase (SOD) exerts a protective effect by scavenging and detoxifying reactive superoxide radicals. However, it also has been found to function as a peroxidase, creating radical species such as the carbonate radical. In this study, NIEHS researchers demonstrated using both electron spin resonance (ESR) and nuclear magnetic resonance (NMR) that peroxymonocarbonate, which is formed by the reaction of peroxide ion with carbon dioxide, plays a key role in this activity, and may be important for evaluating proposed roles of this enzyme in the pathology of ALS (amyotropic lateral sclerosis).

Bonini MG, Gabel SA, Ranguelova K, Stadler K, DeRose EF, London RE, Mason RP. Direct magnetic resonance evidence for peroxymonocarbonate involvement in the Cu, Zn-superoxide dismutase peroxidase catalytic cycle. *J. Biol. Chem.* 284:14618-14627, 2009.

TAK1 Binding Protein 1 (TAB1): Mediates Osmotic Stress-Induced TAK1 Activation but is Dispensable for TAK1-Mediated Cytokine Signaling of TAB1.
TAB1 binds to TAK1 kinase, an indispensable intermediate in several cytokine signaling pathways including tumor necrosis factor (TNF), iterleukin-1 (IL1), and transforming growth factor-β (TGF-β) signaling pathways. TAK1 also participates in stress-activated intracellular signaling pathways, such as osmotic stress signaling pathways. The role of TAB1 in TAK1 signaling was determined by analyzing TAB1-deficient mouse embryonic fibroblasts (MEFs). Tumor necrosis factor and iterleukin-1-induced activation of TAK1 was entirely normal in *Tab1*-deficient MEFs, and could activate both mitogen-activated protein kinases and NFκB. In contrast, osmotic stress induced activation of TAK1 was largely impaired in *Tab1*-deficinet MEFs. These results demonstrate that TAB1 mediates TAK1 activation only in a subset of TAK1 pathways that are mediated through spontaneous oligomerization of TAB1-TAK1.

Inagaki M, Omori E, Kim J-Y, Komatsu Y, Scott G, Ray MK, Yamada G, Matsumoto K, Mishina Y, Ninomiya-Tsuji J. TAK1 binding protein 1, TAB1, Mediates Osmotic Stress-induced TAK1 Activation but is Dispensable for TAK1-mediated Cytokine Signaling. *J. Biol. Chem.* 238:33080-33086, 2008**.**

Inagaki M, Komatsu Y, Scott G, Yamada G**,** Ray M, Ninomiya-Tsuji J, Mishina Y. Generation of a conditional null allele for *Tab1* in mouse. *Genesis* 46: 431-439; 2008.

Translesion DNA Synthesis.
When replication forks stall upon encountering unrepaired lesions that result from environmental insults (e.g., sunlight, chemical damage), specialized DNA polymerases are called into action to perform translesion synthesis (TLS) that avoids cytotoxicity, but often at the expense of mutagenesis that can have adverse health consequences. NIEHS scientists have recently analyzed the properties of several of these TLS polymerases – Pol θ, Pol η, a mutant derivative of the model replicative enzyme RB69 Pol, and Pol ζ.

Arana ME, Seki M, Rogozin IB, Wood RD, Kunkel TA. Low fidelity DNA synthesis by human DNA polymerase theta. *Nucl. Acids Res*. 36:3847-3856, 2008.

McCulloch SD, Kokoska RJ, Garg P, Burgers PM, Kunkel TA. The efficiency and fidelity of 8-oxo-guanine bypass by DNA polymerases δ and η. *Nucl. Acids Res.* 37:3774-3787, 2009.

Zhong X, Pedersen LC, Kunkel TA. Characterization of a replicative DNA polymerase mutant with reduced fidelity and increased translesion synthesis capacity. *Nucl. Acids Res*. 36:3892-3904, 2008.

Stone JE, Kissling GE, Lujan SA, Rogozin IB, Stith CM, Burgers PMJ, Kunkel TA. Low fidelity DNA synthesis by the L979F mutator derivative of *Saccharomyces cerevisiae* DNA polymerases ζ. *Nucl. Acids Res.* 37: 2830-2840, 2009.

Novel Mechanisms of DNA Replication Fidelity

The accuracy of DNA replication is a crucial factor for the process by which organisms undergo mutation. To gain understanding in this area NIEHS investigators are studying the fidelity of DNA replication by the DNA polymerase III holoenzyme complex (HE) that is responsible for duplicating the chromosome of the bacterium *Escherichia coli*. They have shown that the tau subunit of HE plays an important fidelity role and that two accessory DNA polymerases (Pol II and Pol IV) also contribute to the chromosomal error rate, in an error-free and error-prone manner, respectively, while DNA Pol I fulfills a specific role in the error-free filling of the Okazaki fragment gaps.

Gawel D, Pham PT, Fijalkowska IJ, Jonczyk, Schaaper RM. Role of accessory DNA polymerases in DNA replication in *Escherichia coli*: analysis of the *dnaX36* mutator mutant. *J. Bacteriol.* 190:1730-1742, 2008.

Gawel D, Hamilton MD, Schaaper RM A novel mutator of *Escherichia coli* carrying a defect in the *dgt* gene encoding a dGTP triphosphohydrolase. *J. Bacteriol*. 190:6931-6939, 2008.

Makiela K, Jaszczur M, Banach-Orlowska M, Jonczyk P, Schaaper RM, Fijalkowska IJ. Role of *Escherichia coli* DNA polymerase I in chromosomal DNA replication fidelity. *Mol. Microbiol.* (in press).

Global Analysis of Transcription Using New Technologies Uncovers Novel Regulatory Mechanism.

NIEHS investigators describe the use of genome-wide localization strategies such as chromatin immunoprecipitation followed by microarray (ChIP-chip) or deep sequencing (ChIP-seq) to study details of transcription mechanism. The use of these techniques to determine the distribution of RNA polymerase II genome-wide has lead to the discovery that the RNA polymerase is bound to the majority of genes in the genome, but frequently fails to be released from the promoter region into the gene. They find that this phenomenon, called promoter-proximal stalling of RNA polymerase, represents a major rate limiting step in gene expression, and discuss the implications of this finding for our current models of transcription regulation.

Gilchrist DA, Fargo D, Adelman K. Using ChIP-chip and ChIP-seq to study the regulation of gene expression: genome-wide localization studies reveal widespread regulation of transcription elongation. *Methods* 48:398-408, 2009.

NMR Analysis of HIV Reverse Transcriptase Suggests Mode of Interaction with Non-Nucleoside Inhibitors

HIV reverse transcriptase (RT) is a key enzyme in the life cycle of human immunodeficiency virus that causes AIDS, and an important drug target. Two classes of

drugs nucleoside inhibitors and non-nucleoside inhibitors (NNI) are used clinically. The mechanism of interaction of non-nucleoside inhibitors with RT has been a mystery, since the NNI binding pocket is not observed in the absence of the inhibitors. NIEHS investigators labeled the enzyme with [methyl-^{13}C]methionine in order to study residues located near the NNI binding pocket. The observed methionine resonances indicate substantial mobility of one of these residues, M230, supporting the conclusion that dynamic behavior of the enzyme near the NNI binding site plays an important role in the NNI interaction.

Zheng X, Mueller GA, DeRose EF, London RE. Solution characterization of [methyl-^{13}C]methionine HIV-1 reverse transcriptase by NMR spectroscopy. *Antiviral Res.* (in press).

Structure Guided Design of Specific Heparan Sulfates for Future Therapeutics
In collaboration with investigators the University of North Carolina School of Pharmacy, NIEHS researchers determined the crystal structure of a heparan sulfate sulfotransferase. Based on this structure they were able to change the specificity of the enzyme for its substrates allowing for the production of heparan sulfate with unique sulfation patterns. This information will assist in the goal of using an enzymatic based approach for the design of heparan sulfate/heparin drugs with specific therapeutic applications ranging from blood coagulation to cancer therapies.

Bethea HN, Xu D, Liu J, Pedersen LC.. Redirecting the substrate specificity of heparan sulfate 2-O-sulfotransferase by structurally guided mutagenesis. *Proc. Natl. Acad. Sci. U.S.A.* 105:18724-18729, 2008.

New Activity in a Human DNA Polymerase Elucidated.
NIEHS scientists have identified a new activity, called the 5'-dRP-lyase activity, found in the human DNA polymerase theta enzyme. The human DNA polymerase theta was discovered in 1999 by NIEHS researchers but the exact function in the cell has remained elusive. Now, with the discovery of a dRP lyase activity in DNA polymerase theta, researches indicate that this polymerase functions in DNA repair pathways to remove base damage in DNA, known as base excision repair. The dRP activity was mapped to part of the enzyme that functions to copy DNA.

Prasad R, Longley MJ, Sharief FS, Hou EW, Copeland WC, Wilson SH. Human DNA polymerase θ possesses 5′-dRP lyase activity and functions in single-nucleotide base excision repair *in vitro. Nucleic Acids Res.* 37:1868-1877, 2009.

Cell-Surface form of Nucleolin binds P-selectin and Promotes Tumor Cell Adhesion
NIEHS scientists determined that the cell-surface form of nucleolin binds P-selectin — a vascular adhesion molecule — to the surface of Colo-320 human colon carcinoma cells. This binding leads to the activation of signal transduction pathways that promote tumor cell adhesion. The protein nucleolin predominantly exists inside the cell and shuttles between the nucleus and the cytoplasm. However the cell-surface form of nucleolin acts

as a P-selectin receptor molecule. This work sheds light on how the immune system can modulate the ability of cancer cells to establish new colonies at distant sites in the body. The researchers also elucidated the downstream steps in this pathway. P-selectin binding to the cell initiated tyrosine phosphorylation of nucleolin and the phosphoinositide-3 kinase and p38 mitogen-activated protein kinase signaling pathways, which in turn activated the transmembrane glycoprotein α5β1 integrin. These actions resulted in the increase of cell attachment and cell spreading on fibronectin substrates. The finding that cell-surface nucleolin is a P-selectin receptor provides a potential target for novel therapeutic drugs that can slow or inhibit the progress of metastatic disease.

Reyes-Reyes EM, Akiyama SK. Cell-surface nucleolin is a signal transducing P-selectin binding protein for human colon carcinoma cells. *Exp. Cell Res*. 314:2212-2223, 2008.

Biochemical Causes of Mitochondrial Disease

Depletion and mutation of mitochondrial DNA are observed commonly in patients that exhibit an array of mitochondrial disorders. Researchers at the NIEHS have focused on 6 mutations in the gene for the mitochondrial DNA polymerase, POLG, linked to the mitochondrial disease Alper's syndrome. This rare inherited neurological disease typically affects young children who do not often live into their teens due to the cerebral degeneration associated with the disease. Recent work has linked polymerase gamma mutations to Alper's syndrome. They found that four of these mutations in POLG to harbor less than 1% wild type activity and two displayed defects in DNA binding which they propose form a DNA binding cleft in the active site.

Kasiviswanathan R, Longley MJ, Chan SSL, Copeland WC. Disease mutations in the human mitochondrial DNA polymerase thumb subdomain impart severe defects in mtDNA replication. *J. Biol. Chem.* 284:19501-19510, 2009.

Protein phosphatase 5 protects neurons against amyloid β toxicity

Amyloid β (Aβ) is thought to promote neuronal cell loss in Alzheimer's disease (AD), in part through the generation of reactive oxygen species (ROS) and subsequent activation of mitogen activated protein kinase (MAPK) pathways. Protein phosphatase 5 (PP5) is a ubiquitously expressed phosphatase which has been implicated in several cell stress response pathways and shown to inactivate MAPK pathways through key dephosphorylation events. Therefore, NIEHS investigators in collaboration with investigators at Purdue University examined whether PP5 protects dissociated embryonic rat cortical neurons in vitro from cell death evoked by Aβ. Neurons in which PP5 expression was decreased were more susceptible to Aβ toxicity. In contrast, overexpression of functional PP5, prevented MAPK phosphorylation and neurotoxicity induced by Aβ. PP5 also prevented cell death caused by direct treatment with H_2O_2, but did not prevent Aβ-induced production of ROS. The neuroprotective effect of PP5 requires its phosphatase activity and lies downstream of Aβ-induced generation of ROS. Thus, PP5 plays a pivotal neuroprotective role against cell death induced by Aβ and

oxidative stress. Consequently, PP5 might be an effective therapeutic target in AD and other neurodegenerative disorders in which oxidative stress is implicated.

Sanchez-Ortiz E, Hahm BK, Armstrong DL, Rossie S. Protein phosphatase 5 protects neurons against amyloid β toxicity. *J. Neurochem.* (in press).

Fly Mutator Gene is Homologous to Human Mediator of DNA Repair.
Telomeres are structures at the ends of eukaryotic chromosomes required for chromosome stability. A gene in the fruit fly *Drosophila* that is responsible for protecting chromosomes from a rare form of aberration, loss of a telomere, has been found to be a homolog of a human gene that encodes a component of structures that form at double strand DNA breaks. The proteins made by these two genes act as a scaffold, connecting a chromosomal protein that marks the site of the break to a protein complex necessary for repair. These results suggest a model for formation of new telomeres in a specific cell type of *Drosophila* and provide a basis to understand the paucity of telomere formation elsewhere.

Dronamraju R, Mason JM. Recognition of double strand breaks by a mutator protein (MU2) in *Drosophila melanogaster*. *PLoS Genet.* 5:e1000473, 2009.

RNA Recognition Properties of PUF Proteins can be adapted by Simple Substitutions.
Researchers at the NIEHS determined the three-dimensional solution structure of the double-stranded RNA-binding domain of the enzyme Drosha, which is involved in the first step of processing regulator microRNA. The structure identifies unique features to this domain and suggests that its function is to bind substrate RNA.

Mueller GA, Miller MT, DeRose EF, Ghosh M, London RE, Hall TMT. Solution structure of the Drosha double-stranded RNA-binding domain. *Silence* (in press).

Transforming Growth Factor-beta is the underlying Cause of Virus-Induced Asthmatic Exacerbations
Asthma is characterized by chronic inflammation of the airways resulting in airways fibrosis and narrowing. Symptoms of asthma include difficulty breathing, wheezing, coughing and chest tightness. Furthermore, asthma is associated with approximately 5000 deaths/year. The annual cost of asthma in the USA is upwards of 18 billion dollars. Thus far, there is no cure for asthma and it is predominantly managed by inhaled corticosteroids. Infections with common respiratory viruses such as common cold virus, and respiratory syncytial virus can cause severe exacerbations of asthma leading to hospitalization and death. A key difference between normal subjects and asthmatics is the level of transforming growth factor-beta (TGF-β) which is reported to be higher in the asthmatic lung. In our research, we have shown that replication of RSV and human common cold virus is significantly enhanced by the presence of TGF-β. This suggests

that the presence of TGF-β in the asthmatic lungs may be the underlying cause of virus-induced exacerbations.

Gibbs JD, Ornoff DM, Igo HA, Zeng JY, Imani F. Cell cycle Arrest through RSV-Induced TGF-β1 Enhances Virus Replication in Lung Epithelial Cells. *J. Virol.* (in press).

ADHD Medications Do Not Cause Genetic Damage in Children

In contrast to recent findings, methylphenidate and amphetamine, two of the most common stimulant medications used to treat attention deficit hyperactivity disorder (ADHD), do not appear to cause cytogenetic (chromosomal) damage in children who take them as prescribed. The researchers looked at three measures of cytogenetic damage in white blood cells of each child participating in the study and found no evidence of any changes after three months of continuous treatment. The study was designed to determine the reproducibility of findings from a previously published paper that reported methylphenidate-induced chromosomal changes in children with ADHD. That paper raised concern for the medical community and for parents, given that some of the changes have been associated with an increased risk of cancer. The current study, which did not replicate the findings from the previous study, extends the literature by using a larger sample size than previous studies, investigating more than one commonly prescribed medication, and providing high confidence data and well-characterized results that can be generalized to other ADHD populations.

Witt KL, Shelby MD, Itchon-Ramos N, Faircloth M, Kissling GE, Chrisman AK, Ravi H, Murli H, Mattison DR, Kollins SH. Methylphenidate and amphetamine do not induce cytogenetic damage in lymphocytes of children with ADHD. *J. Am. Acad. Child. Adolesc. Psychiat.* 47:1375-1383, 2008.

Genistein Exposure Immediately After Birth Disrupts Female Fertility by Altering Reproductive Tract Function.

Previous work demonstrated that neonatal female mice exposed for five days to genistein, an isoflavone abundant in soy protein, are completely infertile as adults. NIEHS researchers have now determined that the defect caused by genestein lies within the uterus of the female. First they showed that preimplantation embryos from these genestein-treated mice developed normally if they were removed from the mother's reproductive tract and transferred into uteri of untreated surrogate females. Thus the defect lies within the dam, and not the fetus. Then they examined the fate of the pre-implantation embryos if left within their natural mother. The reason for infertility of the genistein-treated mothers was two-fold: about half of the embryos died in the oviduct and never were transported to the uterus. The remainder was lost due to a failure of the uterus to support implantation. These findings suggest that in the mouse model, neonatal exposure to genistein has permanent detrimental effects on the function of the female reproductive tract.

Jefferson WN, Padilla-Banks E, Goulding EH, Lao SC, Newbold RR, Williams CJ. Neonatal exposure to genistein disrupts ability of mouse female reproductive tract to support preimplantation embryo development and implantation. *Biol. Reprod.* 80:425-431, 2009.

An Enzyme Required for Energy Production in Sperm

The PGK enzyme is responsible for a key step in glycolysis, the anaerobic energy-producing process. It is the product of the *Pgk1* gene in most tissues, but of the *Pgk2* gene in male germ cells. A knock-out of the *Pgk2* gene in mice resulted in severe impairment of male fertility. Mating behavior, reproductive organ structure and function, sperm counts, and sperm ultrastructure were unaffected, indicating that PGK2 is not required for completion of spermatogenesis. Although sperm motility and sperm energy production were markedly reduced, some energy production occurred and a low efficiency alternative pathway was identified that bypasses the PGK2 step in sperm.

Danshina PV, Geyer CB, Dai Q, Goulding EH, Willis WD, McCarrey JR, Eddy EM, O'Brien DA. Phosphoglycerate kinase 2 [PGK2] is essential for sperm function and male fertility. *Biol. Reprod.* (in press).

Cadmium Produces Free Radicals in Rats

The environmental and industrial pollutant metal cadmium (Cd) induces the *in vivo* generation of free radicals in murine liver cells, according to researchers from NIEHS. This work is the first to demonstrate that Cd-induced radical formation is dependent on the activation of Kupffer cells, liver macrophages and iron-catalyzed reactions. The research team used electron spin resonance (ESR) spectroscopy to examine which adducts were produced in rats following the administration of cadmium chloride ($CdCl_2$) and the spin trapping agent α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (POBN). Depletion of hepatic glutathione by diethyl maleate significantly increased free radical production, whereas inactivation of Kupffer cells by gadolinium chloride and chelation of iron by desferal inhibited it. Treatment with the xanthine oxidase inhibitor allopurinol, the catalase inhibitor aminobenzotriazole or the cytochrome P-450 inhibitor 3-amino-1, 2, 4-triazol had no effect.

Liu J, Qian SY, Guo Q, Jiang J, Waalkes MP, Mason RP, Kadiiska MB . Cadmium generates reactive oxygen- and carbon-centered radical species in rats: Insights from in vivo spin-trapping studies. *Free Radic. Biol. Med.* 45:475-481, 2008.

Celecoxib Affects Raft-related removal of Amyloid-beta by Microglia: Implications for Treatment of Alzheimer's Disease.

Certain non-steroidal anti-inflammatory drugs, e.g. the COX-2-specific NSAID, celecoxib, raise $A\beta_{42}$ levels. Using a newly developed procedure NIEHS investigators demonstrated that prolonged celecoxib exposure could disrupt rafts in a manner similar to that seen in an elevated $A\beta_{42}$ environment. This resulted in aberrant receptor recruitment to rafts and impaired receptor-mediated phagocytosis by microglial cells. Thus, they

propose that maintaining raft integrity is crucial to determining microglial phagocytic outcomes and disease progression.

Persaud-Sawin DA, Banach L, Harry GJ. Celecoxib affects raft-related removal of amyloid-beta by microglia: implications for treatment of Alzheimer's disease. *Glia.* 57:320-335, 2009.

Persaud-Sawin DA, Lightcap S, Harry GJ. Development of a new method for isolating rafts from brain tissue. *J. Lipid Res.* 50:759-767, 2009.

Background-Level Exposure to the Endocrine-Disrupting Compounds, Polychlorinated Biphenyls, Found to be Unrelated to Occurrence of Male Birth Defects

Recent evidence suggests that polychlorinated biphenyls may have adverse effects on the male reproductive system but data on their relationship with risk of male birth defects were needed. NIEHS researchers showed that at current levels of exposure to these compounds risk of the defects was unaffected.

McGlynn KA, Guo X, Graubard BI, Brock JW, Klebanoff MA, Longnecker MP. Maternal pregnancy levels of polychlorinated biphenyls and risk of hypospadias and cryptorchidism in male offspring. *Environ. Health Perspect.* (in press).

***In vitro* Binding Assay Recapitulates Mutant p53 Protein Phenotype.**

To evaluate how DNA sequence variation affects the binding of the p53 tumor suppressor protein to gene regulatory elements, NIEHS investigators developed a multiplex fluorescent microsphere method. The p53 DNA binding data generated is useful for creating computational models of p53 binding, evaluating the effect of polymorphism in gene regulatory elements and in evaluating the phenotype of mutant p53 proteins in tumors.

Noureddine MA, Menendez D, Campbell MR, Bandele OJ, Horvath MM, Wang X, Pittman GS, Chorley BN, Resnick MA, Bell DA. Probing the functional impact of sequence variation on p53-DNA interactions using a novel microsphere assay for protein-DNA binding with human cell extracts. *PLoS Genet.* 5:e1000462, 2009.

Multiple Risk Factors May Increase Risk for Liver Cancer

Multiple risk factors are associated with development of liver cancer in humans. The prevalence of heritable mutations resulting from random spontaneous or environmental carcinogen induced mutations in tumor suppressor genes that suppress cancer are unknown except in the human population except in families with high incidence of cancers that develop at an early age (e.g., Li-Fraumeni syndrome). NIEHS investigators showed that expression of the hepatitis B antigen along with exposure to the fungal toxin, Aflatoxin B1 in mice with a heritable mutation in the p53 tumor suppressor gene increased the incidence of liver cancer in male mice. These results demonstrate that the p53 deficient mouse is a good model for human cancer and the investigation of oncogenic

virus and chemical carcinogen interactions that are associated with liver cancer.

Cullen JM, Brown DL, Kissling GE, Foley JF, Rizzo J, Marion PL, Parron VI, French JE. Aflatoxin B1 and/or hepatitis B virus induced tumor spectrum in a genetically engineered hepatitis B virus expression and Trp53 haploinsufficient mouse model system for hepatocarcinogenesis. *Toxicol. Pathol.* 37:333-342, 2009.

Crystal Structure Determination of the Signal Transduction Regulator RACK1
NIEHS investigators have determined the crystal structure of RACK1 from *Arabidopisis thaliana.* In mammals RACK1 (receptor for activated C-kinases 1) is a scaffold protein that interacts with a myriad of signaling proteins including kinases, phosphatases, ion channels, and G proteins. As no mammalian structure of RACK1 has been determined yet, this structure provides a foundation for dissecting RACK1 mediated cellular signaling mechanisms in both plants and animals.

Ullah,H, Scappini EL, Moon AF, Williams LV, Armstrong DL, Pedersen LC. Structure of a signal transduction regulator, RACK1, from Arabidopsis thaliana. *Protein Sci.* 17:1771-1780, 2008.

Inducible Nitric Oxide Synthase Is Involved With Streptozotocin-induced Diabetes
Recent studies from researchers at NIEHS and the Universidad de la Republica in Uruguay indicate that inducible nitric oxide synthase (iNOS) is a significant source of the free radical intermediates that are formed in streptozotocin (STZ)-induced diabetic rats. The finding sheds new light on the mechanisms involved in diabetes. Earlier published reports, using STZ-induced diabetic rats as a model, suggested that oxidative stress and free radicals are contributing to diabetes and its complications through various mechanisms. To determine the source of the free radicals, the research team employed electron paramagnetic resonance (EPR) spectroscopy, *in vivo* spin-trapping, isotope labeling experiments and immunological techniques. The results indicated that iNOS was the main source of radical generation, and isotope labeling determined that the lipid-derived radicals detected by EPR spectra were induced by hydroxyl radicals. L-arginine pretreatment and 1400W, a specific iNOS inhibitor, reduced EPR signals to baseline levels, which indicated that peroxynitrite was the source of the hydroxyl radicals. Immunohistochemistry of the liver and kidney of the diabetic rats determined the correlation and co-localization between iNOS, nitrotyrosine and 4-hydroxynonenal as a lipid peroxidation end product in the tissues.

Stadler K, Bonini MG, Dallas S, Jiang J, Radi R, Mason RP, Kadiiska MB. Involvement of inducible nitric oxide synthase in hydroxyl radical-mediated lipid peroxidation in streptozotocin-induced diabetes. *Free Radic. Biol. Med.* 45:866-874, 2008.

Biomarkers of Exposure are Critical to Estimation of Internal Dose
Biomarkers of environmental or occupational exposure and their potential health effect are critical to understanding the exposure response and development of toxicity and/or

disease. Exposure may occur through multiples sources of contact, air, water/food, skin, etc. and lead to variable rates of metabolism and internalized mass or quantity of a toxic chemical. The work described here demonstrates the benefit of predicting the interaction between an absorbed chemical and the interaction of a reactive intermediate metabolite with a biological molecule that provides a stable biomarker of that exposure. The predicted S-arylcysteine-keratin adduct was synthesized chemically and used to produce highly specific antibodies that were used to develop a rapid and non-invasive procedure for removing non-viable skin cells by tape stripping, solubilizing the keratin, and quantifying the amount of keratin-S-aryl adducts that result from multiple repeated exposures to a polyaromatic hydrocarbon, naphthalene. Estimating the amount of chemical entering through the skin versus that inhaled or ingested facilitates and helps to further explain the total body burden and predicting the internal dose.

Kang-Sickle J-CC, Fox DD, Nam T-G, Jayaraj K, Ball LM, French JE**,** Klapper DG, Gold A, Nylander-French, LA. S-Arylcysteine adducts in keratin as biomarkers of dermal exposure to aromatic hydrocarbons. *Chem. Res. Toxicol.* 21:852-858, 2008.

Mouse Allergen Exposure and Asthma

The majority of the studies investigating the role of residential mouse allergen exposures in asthma have focused on inner-city populations. NIEHS researchers examined whether elevated mouse allergen levels were associated with occupants' asthma status in a nationally representative sample of US households. Data for this study were collected as part of the National Survey of Lead and Allergens in Housing. This cross-sectional study surveyed 831 housing units inhabited by 2456 individuals in 75 different locations throughout the US. Information on demographics, household characteristics, and occupants' health status was obtained by questionnaire and environmental observations. Concentrations of mouse urinary protein in vacuumed dust collected from various indoor sites were assessed with a polyclonal immunoassay. Of the surveyed homes, 82% had detectable levels of mouse allergen and, in 35% of the homes, mouse allergen concentrations exceeded 1.6 μg/g, a level that has been associated with increased mouse allergen sensitization rates. Current asthma, defined as having doctor-diagnosed asthma and asthma symptoms in the past 12 months, was positively associated with increased mouse allergen levels. The observed association was modified by atopic status; in allergic individuals, elevated mouse allergen levels increased the odds of having asthma symptoms, but the association was not seen in those who did not report allergies. Thus, in allergic asthma, residential mouse allergen exposure is an important risk factor for asthma morbidity.

Salo PM, Jaramillo R, Cohn RD, London SJ, Zeldin DC. Exposure to Mouse Allergen in US Homes is Associated with Asthma Symptoms. *Environ. Hlth. Perspect.* 117:387-391, 2009.

Determinants of Endotoxin in U.S. Housing

The relationship of domestic endotoxin exposure to allergy and asthma has been widely investigated. However, few studies have evaluated predictors of household endotoxin and

none have done so for multiple locations within homes and on a national scale. NIEHS investigators assayed 2552 house dust samples in a representative nationwide sample to understand the determinants of household endotoxin in samples from the bedroom floors, family room floors, beds, kitchen floors, and family room sofa. House dust was vacuum-sampled from five locations within homes and assayed for endotoxin. Demographic and housing information was assessed through questionnaire and on-site evaluation of 2,456 residents of 831 homes selected to represent the demographics of the U.S. Meteorological data were obtained for each U.S. primary sampling location and tested as predictors of indoor endotoxin to determine if wetter or warmer microclimates would produce higher endotoxin levels as seen with some allergens. Geometric mean endotoxin concentration ranged from 20.6 to 84.4 EU/mg for the 5 sampling locations and endotoxin load ranged from 4,530 to 20,100 EU/m^2. The major determinants of endotoxin concentration were low income, number of residents, children in the home, dog ownership, west census region, cigarette smoking, and evidence of cockroaches and presence of food debris. Thus, increased endotoxin in household reservoir dust is principally associated with poverty, people, pets, smoking, roaches and geography.

Thorne PS, Cohn R, Arbes SJ, Zeldin DC. Predictors of Endotoxin Levels in U.S. Housing. *Environ. Hlth. Perspect.* 117:763-771, 2009.

Pesticide Exposure among the General Population in the Netherlands Found to be Unusually High

NIEHS scientists, in collaboration with Dutch investigators, have discovered that the levels of metabolites of organophosphate pesticides in urine were unusually high among pregnant women in Rotterdam. The source of contamination was likely food imported from countries with less stringent regulation of pesticide use. Low-level exposure to these compounds before birth has been related to increased risk of abnormalities resembling autism and attention-deficit hyperactivity disorder in U.S. populations, but the findings were equivocal. Additional studies in the Netherlands by U.S. investigators will be extremely useful in the future to examine potential adverse effects on neurodevelopment of such exposures.

Ye X, Pierik FH, Hauser R, Duty S, Angerer J, Park MM, Burdorf A, Hofman A, Jaddoe VWV, Mackenbach JP, Steegers EAP, Tiemeier H, Longnecker MP. Urinary metabolite concentrations of organophosphorous pesticides, bisphenol A, and phthalates among pregnant women in Rotterdam, the Netherlands: The Generation R Study. *Environ. Res.* 108:260-7, 2008.

Bisphenol A exposure among the General Population in Norway found to be Unusually High

NIEHS scientists, in collaboration with Norwegian investigators, have discovered that the levels of metabolites of bisphenol A in urine were unusually high among pregnant women in Norway. The source of contamination was likely food from cans lined with epoxy resins leaching bisphenol A. Low-level exposure to these compounds before birth has been related to increased risk of neurodevelopmental abnormalities in rodents. Human data on health effects of early-life exposure are needed. Additional studies in the

Norway by U.S. investigators will be extremely useful in the future to examine potential adverse effects on neurodevelopment of such exposures.

Ye X, Pierik FH, Angerer J, Meltzer HM, Jaddoe VWV, Tiemeier H, Hoppin JA, Longnecker MP. Levels of metabolites of organophosphate pesticides, phthalates, and bisphenol A in pooled urine specimens from pregnant women participating in the Norwegian Mother and Child Cohort Study (MoBa). Int. J. Hygiene Env. Health 212:481-491, 2009.

Chemicals Produce Unique Toxicity "Fingerprints"
NIEHS investigators demonstrated that different chemicals produce unique gene expression patterns as measured in either liver tissue or blood samples. The patterns could be further associated with certain toxicity profiles of these test agents. These results may lead to a more precise prediction of the potential toxicity of new chemicals.

Lobenhofer EK, Auman JT, Blackshear P, Boorman G, Bushel PR, Cunningham ML, Fannin R, Foley J, Fostel J, Gerrish K, Grissom S, Heinloth A, Irwin R, Malarkey D, Merrick BA, Sieber SO, Tucker CJ, Ward S, Wilson R, Hurban P, Tennant RW, Paules, RS. Identification of variability in the phenotypic response to toxicant exposure using gene expression profiles from either the target organ or whole blood. *Genome Biol.* 9:R100, 2008.

NTP's High Throughput Screening Program Identifies Compounds that Mimic the Effect of Hypoxia on Cellular Pathways.
NTP's high throughput screening program has developed a broad set of cell-based assays in collaboration with the NIH Chemical Genomics Center and the EPA. The goal is to develop predictive patterns of responses among these many assays that will be predictive of which toxicity pathways are affected by environmental agents. If successful the methodology will allow for rapid preliminary testing of thousands of compounds, for classification and pritoritization for additional study, where warranted. The study published here is an important representative example of how quantitative high throughput screening methods, combined with a well-reasoned strategy using secondary assays to more completely characterize the toxic response, was used to identify several chemicals among 1353 tested that trigger key events in cellular pathways that respond to hypoxia.

Xia M, Huang R, Sun Y, Semenza GL, Aldred SF, Witt KL, Inglese J, Tice RR, Austin CP. Identification of Chemical Compounds that Induce 1 HIF-1α Activity. *Toxicol. Sci.* 2009; doi:10.1093/toxsci/kfp123.

Mutations in K-ras and p53 Cancer Genes and Alterations in K-ras and Erk MAP Kinase Pathways Contribute to Cumene-Induced Lung Cancer.
Exposure to cumene induced alveolar/bronchiolar adenomas and carcinomas in B6C3F1 mice. Cumene-induced lung tumors have an increased incidence of mutations in K-ras (87% vs. 12%) and p53 (52% vs. 0%) compared to spontaneous lung tumors. The study results suggest that DNA damage and genomic instability contribute to cumene-induced

lung cancer and may be of relevance to humans since similar mutations are present in lung cancer of humans. In related studies the Erk MAP kinase signaling pathway is significantly altered in cumene-induced lung cancer with K-*ras* mutations compared to tumors without K-*ras* mutations. Cumene-induced carcinomas with K-*ras* mutations also have greater malignant potential than those without mutations as reported in human lung cancer. Gene expression analysis suggested the formation of alveolar/bronchiolar carcinomas in cumene-exposed mice typically involves mutation of K-*ras*, which results in increased Erk MAP kinase signaling.

Hong HH, Ton TV, Kim Y, Wakamatsu N, Clayton NP, Chan PC, Sills RC, Lahousse SA. Genetic alterations in K-ras and p53 cancer genes in lung neoplasms from B6C3F1 mice exposed to cumene. *Toxicol. Pathol.* 36:720-726, 2008.

Wakamatsu N, Collins JB, Parker JS, Tessema M, Clayton NP, Ton TV, Hong HH, Belinsky S, Devereux TR, Sills RC, Lahousse SA. Gene expression studies demonstrate that the K-ras/Erk MAP kinase signal transduction pathway and other novel pathways contribute to the pathogenesis of cumene-induced lung tumors. *Toxicol. Pathol.* 36:743-752, 2008.

Novel Detoxification Mechanisms Dependent on the Molybdenum Cofactor.
The base analog N6-hydroxylaminopurine (HAP), which is an analog of the normal DNA and RNA constituent adenine, is an extremely potent mutagen in all organisms from bacteria to man. NIEHS researchers discovered a novel detoxification system for this compound in the bacterium *E. coli*, which requires the Molybdenum cofactor. They have defined the genes as well as the proteins responsible for this activity. These proteins constitute a novel family of Molybdoproteins. These proteins may play a wider role in the general detoxification of N-hydroxylated compounds.

Kozmin SG, Schaaper RM. YcbX and YiiM, two novel determinants for resistance of E. coli to N-hydroxylated base analogs. *Mol. Microbiol.* 68:51-65, 2008.

Mfsd2a Encodes a Novel Major Facilitator Superfamily Domain-Containing Protein with a Function in Thermogenesis
NIEHS scientists identified a novel major facilitator superfamily domain-containing protein referred to as Mfsd2a. This gene is induced in brown adipose tissues during fasting and thermogenesis suggesting a role for this protein in these physiological processes.

Angers M, Uldry M, Kong D, Gimble JM, Jetten AM. Mfsd2a encodes a novel major facilitator superfamily domain-containing protein highly induced in brown adipose tissue during fasting and adaptive thermogenesis. *Biochem. J.* 416**:**347-355, 2008.

Estrogen receptor alpha (ERα) phospho-serine-118 is highly expressed in human uterine fibroids.

It is thought that the growth of uterine leiomyomas may be mediated by the interaction of estrogen receptor alpha (ERα) and growth factor pathways. NIEHS investigators have shown that non-genomic activation of ERα through ERα-phospho-Ser118 is important in leiomyoma growth and activation of ERα-Ser118 is possibly mediated by phospho-p44/42 MAPK. These data provide a novel mechanism and molecular target for intervention strategies for clinical cases of fibroids.

Hermon TL, Moore AB, Yu L, Kissling GE, Castora FJ, Dixon D. Estrogen receptor alpha (ERalpha) phospho-serine-118 is highly expressed in human uterine leiomyomas compared to matched myometrium. *Virchows Arch.* 453:557–569, 2008.

Similar Chemicals Can Produce Different Toxic Outcomes

Assessment of the similarities and differences in the toxic effects of chemicals provides a valuable resource for regulating chemical exposures. While some studies have reported on gene expression "fingerprints" as a way to classify chemicals, these studies indicate that grouping of chemicals based on such profiling may not be predictive for chemical classes that reportedly produce similar biological effects.

Ross PK, Woods CG, Bradford BU, Kosyk O, Gatti DM, Cunningham ML, Rusyn I. Time-course comparison of xenobiotic activators of CAR and PPARalpha in mouse liver. *Toxicol. Appl. Pharmacol.* 235:199-207, 2009.

Gene Network Dynamics Could Permit Members in a Clonal Cell Population to Initiate an Effective Response to Changing Conditions

While several studies have provided important insights into general principles of biological networks, the link between network organization and the genome-scale dynamics of the underlying entities (genes, mRNA and proteins) and its role in systems behavior remain unclear. In a collaborative study NIEHS investigators showed that transcription factor (TF) dynamics and regulatory network organization are tightly linked. By classifying TFs in the yeast regulatory network into three hierarchical layers (top, core, and bottom) and integrating diverse genome-scale datasets, we find that the TFs have static and dynamic properties that are similar within a layer and different across layers. At the protein level, the top-layer TFs are relatively abundant, long-lived, and noisy compared to the core- and bottom-layer TFs. While variability in expression of top-layer TFs might confer a selective advantage, as this permits at least some members in a clonal cell population to initiate a response to changing conditions, tight regulation of the core- and bottom-layer TFs may minimize noise propagation and ensure fidelity in regulation. The investigators propose that the interplay between network organization and TF dynamics could permit differential utilization of the same underlying network by distinct members of a clonal cell population.

Jothi R, Balaji S, Wuster A, Grochow JA, Gsponer J, Przytycka TM, Aravind L, Babu MM. Genomic analysis reveals a tight link between transcription factor dynamics and regulatory network architecture. *Mol. Sys. Biol.* (in press).

Structural Changes in Anthrax Protective Antigen During Translocation into the Cell.
NIEHS investigators have used oxidation and mass spectrometry to identify amino acids in the *Bacillus anthracis* protective antigen (PA) that undergo changes in reactivity as the protective antigen is internalized during infection. They identified specific conformational changes and showed that internalization exposes PA to an acidic environment which then induces a change in the shape of the PA. These data were then used to refine structural models of the new conformation.

Smedley JG III, Sharp JS, Kuhn JF, Tomer KB. Probing the pH-dependent prepore to pore transition of *Bacillus anthracis* protective antigen with differential oxidative protein footprinting. *Biochemistry* 47:10694-10704, 2008.

Multiple Ways for Signaling Pathways to be Altered in Lymphoma.
Heritable and acquired suppression or increased expression of molecules required for maintaining normal cell function may lead to cancer. Notch1 mutations have been reported in human lymphoblastic leukemia. NIEHS researchers reported a variable incidence of chemical specific induced mutations associated with mouse lymphoma as a model for the human disease. These results suggest that there are many ways to disrupt the Notch signaling pathway associated with development of this blood cancer.

Karlson A, Rasmussen A, French JE, Söderkvist P. Notch1 is a frequent mutational target in chemically induced lymphoma in mouse. *Intl. J. Cancer* 123:2720-2724, 2008.

Understanding Susceptibility to Preterm Birth
The rate of preterm birth in the US is an alarming 12.5% and rising, and preterm birth is a major cause of death and long-term disability, including blindness, in affected infants. While it is clear that some women are unusually susceptible to this pregnancy complication, and that environmental factors are involved, the genetic contributors to susceptibility have not been identified. Recent studies have evaluated patterns of recurrence and risk within extended families, and the growing body of evidence suggests that susceptibility is strictly maternally transmitted. NIEHS scientists reviewed the available epidemiologic evidence leading to this conclusion and demonstrated that the usual approaches to designing studies of the genetics of preterm birth will not elucidate the likely causal mechanisms. They propose powerful alternative family-based designs aimed at identifying the genetic variants involved.

Weinberg CR, Shi M. The genetics of preterm birth: Using what we know to design better association studies. *Am. J. Epidemiol.* (in press).

Sugars on Alzheimer's Proteins Located Near the Site Involved in Plaque Formation.

The beta-Amyloid (Aβ) protein, derived from Alzheimer's Precursor Protein (APP), is a major constituent of the plaque formation that is characteristic of Alzheimer's Disease. NIEHS researchers have identified three specific O-glycosylation sites on APP and characterized the composition of the glycans at each site. One of these sites is close to the secretase cleavage site. Lack of glycosylation of this site may be involved in the incorrect cleavage of APP that leads to plaque formation.

Perdivara I.R. Petrovich B, Alliquant LJ, Deterding, Tomer KB, Przybylski M. Elucidation of O-glycosylation structures of the ß-amyloid precursor protein by liquid chromatography-mass spectrometry using electron transfer dissociation and collision induced dissociation. *J. Proteome Res.* 8:631-642, 2009.

Prediction of Metal Ion Position in Proteins

In collaboration with scientists at Brooklyn College of the City University of New York, NIEHS scientists successfully predicted metal ion positions in the solution structure of osteocalcin. The structure provides insight into the role of this protein in lead toxicity.

Dowd TL, Li L, Gundberg CM. The ^{1}H NMR structure of bovine Pb^{2+}-osteocalcin and implications for lead toxicity. *Biochim. Biophys. Acta*, 1784:1534-1545, 2008.

New Software for Discovery of DNA Regulatory Sequences

A NIEHS investigator developed a computational tool for discovering functional elements in large DNA sequences from genome-wide studies. The software tool is freely available for public use at http://www.niehs.nih.gov/research/resources/software/gadem/.

Li L. GADEM: A genetic algorithm guided formation of spaced dyads coupled with an EM algorithm for motif discovery. *J. Comput. Biol.*16: 317-329, 2009.

Toxicity Studies of Flame Retardant Mixtures

Polybrominated diphenyl ethers (PBDE) are a class of chemicals frequently used as flame retardants and are found in our environment including in water, fish, mammals, and human milk and tissues. The NTP has identified liver as a target organ from exposure to this class of flame retardants. Studies are in progress to determine the carcinogenic potential of the PBDE flame retardants.

Dunnick JK, Nyska A. Characterization of liver toxicity in F344/N rats and B6C3F1 mice after exposure to a flame retardant containing lower molecular weight polybrominated diphenyl ethers. *Exp. Toxicol. Pathol.* 61:1-12, 2009.

Rodent Model Detects Heart Damage Earlier Than Traditional Methods

Sudden cardiac death is seen in humans without warning. Even without clinical signs of cardiac toxicity, this rodent model detects heritable or induced heart damage that may lead to more severe cardiac toxicity and heart failure.

Nyska A, Cunningham M, Snell M, Malarkey D, Sutton D, Dunnick J. The pivotal role of electron microscopic evaluation of the cardiotoxicity of bis(2-chloroethoxy)methane in rats and mice. *Toxicol. Pathol.* (in press).

Tool for Analyzing Gene Expression Data

NIEHS investigators developed a computational tool for analyzing large-scale toxicogenomic gene expression data. The method detects correlation between gene expression and toxicological biomarkers such as liver toxicity.

Lin R, Dai S, Irwin RD, Heinloth AN, Boorman GA, Li L. A Method for Gene Set Enrichment Analysis of Toxicogenomics Data. *BMC Bioinformatics*, 9:481, 2008.

Opioid Agonists and Antagonists

NIEHS investigators, in collaboration with several international scientific laboratoriesdeveloped new types of synthetic opioid analogues based on their exhibited selectivity for one of two closely related opioid membrane receptors, δ- or μ-opioid receptors. These analogues may be useful in the treatment of addictions to morphine, heroin, and alcohol as well as in the management of addictive eating disorders leading to obesity.

Li Q, Marczak ED, Okada Y, Wilson W, Lazarus LH, Swartzwelder HS. The novel μ-opioid receptor antagonist [*N*-allyl-Dmt^1]-endomorphin-2 attenuates alcohol-induced GABAergic neurotransmission in rat hippocampus. *Alcohol Alcoholism* 44:13-19, 2009.

Ballet S, Feytens, D, De Wachter R, De Vlaeminick M, Marczak ED, Salvadori S, de Graf C, Rognan D, Negri L, Lattanzi R, Lazarus LH, Tourwé D, Balboni G. Conformationally constrained opioid ligands: the Dmt-Aba and Dmt-Aia vs. Dmt-Tic pharmacophore. *Bioorg. Med. Chem*. 19:433-437, 2009.

Chao DM, Balboni G, Lazarus LH, Salvadori, Xia Y. Na^+ mechanism of δ-opioid receptor induced protection from anoxic K^+ leakage in the cortex. *Cell. Mol. Life Sci*. 2009, doi 10.1007/s00018-009-8759-5).

Balboni G, Trapella C, Sasaki Y, Ambo A, Marczak ED, Lazarus LH, Salvadori S. Influence of the side chain adjacent to benzimidazole on the bioactivity profile of Dmt-Tic pharmacophore opioids. *J. Med. Chem*. (in press).

Marczak ED, Jinsmaa Y, Myers P, Blankenship-Paris T, Wilson R, Balboni G, Salvadori S, Lazarus LH. Orally administered H-Dmt-Tic-Lys-NH-CH_2-Ph (MZ-2), a potent μ-/δ-opioid receptor antagonist, regulates obese-related factors in mice *Eur. J. Pharmacol.* 616:115-121, 2009.

TRAINING AND MENTORING

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 Wednesday, July 29, 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

Michelle Corea is a student at Durham Academy in Durham, NC, who worked in the Laboratory of Reproductive and Developmental Toxicology. Poster: Corea, M., Jefferson, W., Jakub, K., and Williams, C. "Metastasis Associated Protein 3 (MTA3) Localizes to Autophagosomes in Mouse Preimplantation Embryos." Her mentor was Carmen J. Williams, M.D., Ph.D., head of the Reproductive Medicine Group.

Best Poster by Undergraduate Interns

John Peart is a student at North Carolina State University in Raleigh, NC, who worked in the Laboratory of Pharmacology. Poster: Peart, J., Cannon, R., and Miller, D.S. "Downregulation of P-glycoprotein Transport Activity at the Blood-Brain Barrier by Sphingosine-1-phosphate." His mentor was David S. Miller, Ph.D., head of the Intracellular Regulation Group.

Best Poster by Graduate Interns

Amber Haynes is a graduate student at Tulane University, New Orleans, LA, who worked in the Laboratory of Respiratory Biology. Poster: Haynes, A, Sever, M.L., and Zeldin, D.C. "Dust Mite Allergen Reduction Study: Preliminary Analysis of In-Home Test kit Data." Her mentor was Darryl F. Zeldin, M.D., head of the Environmental Cardiopulmonary Disease Group.

The Fellows Award for Research Excellence

The Fellows Award for Research Excellence (FARE) was started in 1995 to recognize scientific excellence among NIH intramural trainees. 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 20 winners of FARE awards:

Recipient	***Laboratory/Branch***	***Mentor***	***Abstract Title***
Jim Aloor	Laboratory of Respiratory Biology	Michael Fessler	HIV-1 envelope protein gp41 triggers pro-inflammatory responses in the macrophage through Toll like receptors-2 and -4 and their adaptors.
Omari Bandele	Laboratory of Molecular Genetics	Douglas Bell	Single nucleotide polymorphisms alter sequence-specific p53-DNA binding to gene regulatory elements.
Abee Boyles	Epidemiology Branch	Allen Wilcox	Maternal alcohol consumption and infant clefts: the role of alcohol metabolism gene variants.
Ryan Dackor	Laboratory of Respiratory Biology	Darryl Zeldin	Prostaglandin E2 Protects Murine Lungs from Bleomycin-Induced Pulmonary Fibrosis and Lung Dysfunction
David Draper	Laboratory of Respiratory Biology	Michael Fessler	ABCG1 is a negative regulator of pulmonary host defense.

Dana Hancock	Epidemiology Branch	Stephanie London	Chromosome 9q21.31 as a novel susceptibility locus for childhood asthma: evidence from a genome-wide association study in Mexicans.
Andrew Kraft	Laboratory of Molecular Toxicology	Jean Harry	The neurites of striatal neurons expressing mutant huntingtin protein are sites of microglial association and proliferation, which facilitates the progression of degenerative phenotypes.
Erica Lannan	Laboratory of Signal Transduction	John Cidlowski	Identification and classification of inflammatory genes co-regulated by dexamethsone and TNF-alpha.
Andres Larrea	Laboratory of Molecular Genetics	Thomas Kunkel	Scrunching During DNA Replication
Hideki Nakano	Laboratory of Respiratory Biology	Donald Cook	The impact of surface ALDH1a2 on pulmonary dendritic cells for generation of regulatory T cells leading to immunotolerance to inhaled antigens.
Aparna Purushotham	Laboratory of Signal Transduction	Xiaoling Li	Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation.
Li Qian	Laboratory of Pharmacology	Jau-Shyong Hong	Potent neuroprotective effects of long-acting β2-adrenergic receptor agonists: a potential new therapeutic indication through a novel mechanism.
Ritu Rana	Laboratory of Pharmacology	Joyce Goldstein	Mediator 25 functions as an essential coactivator for HNF4α and associates with RNA Pol II to regulate human *CYP2C9* gene expression.
Rongqin Ren	Laboratory of Signal Transduction	John Cidlowski	Mechanisms of glucocorticoid dependent cardiac hypertrophy.
Ramendra Saha	Laboratory of Neurobiology	Serena Dudek	Rapid induction of neuronal *arc* is mediated by a promoter proximal RNA polymerase-II stalling mechanism.

Thaddeus Schug	Laboratory of Signal Transduction	Xiaoling Li	Macrophage-specific SIRT1 regulates NF-κB-dependent transcription and inflammation by deacetylation.
Jeremy Smyth	Laboratory of Signal Transduction	James Putney	Phosphorylation of the endoplasmic reticulum calcium sensor STIM1 underlies suppression of store-operated calcium entry during mitosis.
Jeffrey Stumpf	Laboratory of Molecular Genetics	William Copeland	Yeast homologues of eighteen disease mutations in DNA polymerase gamma cause mtDNA depletion and mutagenesis.
Jeffrey Sunman	Laboratory of Molecular Carcinogenesis	Steven Akiyama	The hemopexin domain of matrix metalloproteinase-9 (MMP-9) induces a pro-migratory phenotype in human melanoma cells independent of MMP-9 catalytic activity.
Xueqian Wang	Laboratory of Pharmacology	David Miller	The aryl hydrocarbon receptor regulates P-glycoprotein at the blood-brain barrier.