Division of Intramural Research

NAEHS Council Update

September, 2016

DIR RECRUITMENTS

Deputy Scientific Director
The National Institute of Environmental Health Sciences (NIEHS) is seeking an accomplished scientist to serve as the Deputy Scientific Director of our Division of Intramural Research (DIR). This is an exciting leadership opportunity to provide scientific oversight and help set the research agenda for the DIR. This individual will lead a team that is directly focused on intramural scientific research. Responsibilities include strategic planning and management, faculty evaluation, recruitment of scientific peer reviewers and oversight of review panels for intramural scientists, training within the DIR, coordination of research activities funded by non-NIEHS entities, development and/or recommendation of research policies, priorities, and procedures, and communication with other federal entities including other NIH Institutes and external organizations. The successful candidate will work closely with the Scientific Director to manage all scientific aspects of the DIR. Dr. Thomas Kunkel, Genome Integrity & Structural Biology Laboratory, is chair of the Search Committee.

Investigator in Epidemiology
The National Institute of Environmental Health Sciences is recruiting for a full-time Tenure-Track Epidemiologist. The successful candidate will be expected to develop an outstanding, investigator-initiated independent epidemiology research program on human health outcomes. Applicants are welcome with expertise in any of the following areas: reproduction, pregnancy outcomes, pediatric outcomes, early origins of disease, life course epidemiology, adult health/chronic disease, or other areas of environmental epidemiology. Biologically-based epidemiological research (including genetics, epigenetics, metabolomics, microbiomics, and biomarkers) is especially encouraged. Successful candidates will be expected to have the ability to work independently and as part of multi-disciplinary and/or collaborative teams. Candidates should have a Doctoral degree and a record of accomplishment in epidemiology, including a strong publication record and research experience. Dr. Janet Hall, Clinical Research Branch, is chair of the search committee.

Biostatistician
The National Institute of Environmental Health Sciences is seeking an experienced biostatistician at the rank of Staff Scientist in the Biostatistics and Computational Biology Branch (BCBB) of the Division of Intramural Research (DIR). The incumbent will collaborate extensively with researchers in the DIR and the Division of the National Toxicology Program (DNTP). The successful candidate will also play a major role in analyses for the National Toxicology Program (NTP), he/she will provide statistical leadership and ensure the statistical integrity of its research program. In addition, the position involves management and oversight of statistical support service contracts. Development of new statistical methods is encouraged, but will not be a major component of the job. Drs. Kathy Laber, Comparative Medicine Branch and Paul Foster, Toxicology Branch, are co-chairs of the search committee.

NEW HIRES AND CHANGES IN DIR LEADERSHIP

Deputy Chief of the Comparative Medicine Branch
Dr. Rebecca Wiltshire, Senior Director, Laboratory Animal Services at Children's Hospital of Philadelphia, Philadelphia, PA has accepted a position as Deputy Chief of the Comparative Medicine Branch. She is expected to start in September 2016.

NEWLY TENURED DIR PRINCIPAL INVESTIGATORS

At the May 2, 2016 meeting of the NIH Central Tenure Committee held in Bethesda **Dr. Carmen J. Williams** of the Reproductive and Developmental Biology Laboratory was awarded tenure.

Carmen J. Williams, M.D., Ph.D.
Reproductive Medicine Group
Reproductive and Developmental Biology Laboratory
DIR, NIEHS

Millions of reproductive age couples are unable to control their fertility despite efforts to either prevent or achieve pregnancy. Furthermore, many women who do achieve pregnancy have problems carrying the pregnancy to term. Endocrine disrupting chemicals are implicated as contributing factors to these public health issues. The long-term goal of the Reproductive Medicine Group is to improve our understanding of factors and cellular mechanisms that regulate human reproductive health so that effective prevention and treatment strategies can be developed. We focus on understanding at a molecular level the basic reproductive biology of fertilization and preimplantation embryogenesis and how the female reproductive tract environment supports these processes. An important part of this work is to investigate how estrogenic endocrine disruptors affect the function of the female reproductive tract. Such basic studies are in line with the mission of the NIEHS to "discover how the environment affects people in order to promote healthier lives". Our work encompasses both fundamental research and exposure research in reproduction. The group focuses its efforts on two projects. The first project centers on signaling mechanisms regulating egg activation and preimplantation embryo development and in particular the role of calcium in this process. The second project concerns how endocrine disrupting chemicals alter female reproductive tract development in ways that affect its function in supporting fertilization and embryo development and lead to the development of uterine cancer. Collectively, our studies will have broad translational application via information gained regarding normal preimplantation embryogenesis that is relevant to stem cell biology and identification of preventable environmental exposures that have adverse reproductive health consequences.

At the August 1, 2016 meeting of the NIH Central Tenure Committee held in Bethesda **Dr. Guang Hu** of the Epigenetics and Stem Cell Biology Laboratory was awarded tenure.

Guang Hu, Ph.D.
Stem Cell Biology Group
Epigenetics and Stem Cell Biology Laboratory
DIR, NIEHS

Pluripotent stem cells, such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have two defining characteristics: self-renewal and pluripotency. Self-renewal describes their capability to go through cycles of cell division and maintain the undifferentiated state, while pluripotency describes their capability to differentiate into all cell types from the three germ layers. Due to these unique properties, pluripotent stem cells hold great promises for both basic and translational research. In addition, they can also provide new tools and insights for environmental health sciences. For example, pluripotent stem cells can be used to study the impact of environmental chemicals on human development, to derive cell types that are otherwise difficult to obtain for toxicology studies, and to derive patient-specific cells to study the interactions between genetic and environmental factors in diseases with complex causes.

The long-term goal of our research is to better understand the molecular mechanisms that regulate the pluripotent state. We have previously carried out a genome-wide RNAi screen in ESCs and identified a list of novel regulators of ESC self-renewal. We have since investigated the function of several of the identified factors, including the Ccr4-Not and INO80 complexes, in ESCs, iPSCs, and mouse embryonic development, and uncovered novel mechanisms such as mRNA deadenylation, mRNA export, mRNA alternative polyadenylation, and chromatin remodeling in the regulation of the pluripotent state. In the future, we will continue to investigate the function of these complexes, and will also use genetic and genomic tools to identify and characterize additional regulators of pluripotent stem cell fates. Our work will provide new insights to mammalian development and facilitate the use of pluripotent stem cells for translational research. Further, it will also promote the development of novel pluripotent stem cell-based culture models for toxicology and environmental health science studies.

At the August 1, 2016 meeting of the NIH Central Tenure Committee held in Bethesda **Dr. R. Scott Williams** of the Genome Integrity and Structural Biology Laboratory was awarded tenure.

R. Scott Williams, Ph.D.
Genome Stability Structural Biology Group
Genome Integrity and Structural Biology Laboratory
DIR, NIEHS

DNA strand breaks occur continuously as our cells duplicate their chromosomes, and as a consequence of oxidation or environmental exposure to chemical mutagens and DNA-damaging radiation. Inflammation, cellular respiration, routine DNA metabolism, and xenobiotics including pharmaceutical drugs all lead to the production of cytotoxic DNA strand breaks. DNA breaks are chemically heterogeneous, and typically lack DNA 5′-phosphate and/or 3′-hydroxyl moieties required for DNA repair synthesis and ligation. Failure to resolve damage at DNA ends is associated with genomic instability, mutagenesis, neurological disease, aging and carcinogenesis. The Williams laboratory employs a multidisciplinary approach that integrates complementary biochemical, mutational, structural and proteomic studies to identify and characterize, with atomic resolution, cellular DNA damage recognition, signaling and reversal processes acting as the cellular first line of defense to genotoxic insult. In our work, molecular structural studies form the bedrock for informed structure-guided mutagenesis that probes biochemical and cellular functions. Current ongoing work in the group is aimed at deciphering functions the DNA end processing factors Aprataxin (APTX), Tyrosyl-DNA phosphodiesterase 2 (TDP2) and the CtIP/Ctp1 tumor suppressor. Defects in processes we are studying destabilize the genome, alter organismal functions and susceptibility to genotoxic stressors, and are linked to human diseases that emerge and progress over a lifespan. For example, *TDP2* mutations are found in individuals with intellectual disability, seizures and ataxia, deficiencies in APTX RNA/DNA deadenylase cause Ataxia with Oculomotor Apraxia (AOA1) and *CtIP* mutations are linked to Seckel syndrome. We envision that a detailed molecular understanding of the mechanisms of genome repair we are studying will further open new doors for the development of novel targeted DNA repair inhibitors for treatment of cancers that have acquired resistance to DNA crosslinkers, alkylating agents, topoisomerase poisons, and other DNA-targeted chemotherapeutics.

SCIENTIFIC UPDATE BY A DIR PRINCIPAL INVESTIGATOR

Regulation of embryo development by the oviductal environment

Carmen J. Williams, M.D., Ph.D.
Reproductive Medicine Group
Reproductive and Developmental Biology Laboratory
DIR, NIEHS

Environmental exposures, particularly during developmentally sensitive time windows, can impact on later health outcomes in children and adults. Although this was a novel idea that encountered widespread skepticism when first proposed more than 25 years ago, it is now generally accepted due to robust documentation of these effects in both animal models and humans. Now known as DOHaD (Developmental Origins of Health and Disease), the field is moving toward defining the mechanisms by which early exposures modulate long-term physiology. A major window of developmental sensitivity is the very beginning of embryo development: during fertilization and preimplantation development within the oviduct as the embryo moves toward the uterus. This talk will provide data regarding how the oviduct responds to environmental cues in modulating survival and development of the early embryo.

TRAINING AND MENTORING

The Fellows Award for Research Excellence

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

The NIEHS had 18 winners of FARE awards:

FARE Awardee	Mentor	Group and Laboratory/Branch
Georgia M. Alexander, Ph.D.	Serena M. Dudek, Ph.D.	Synaptic and Developmental Plasticity Group, Neurobiology Laboratory
Jonathan T. Busada, Ph.D.	John A. Cidlowski, Ph.D.	Molecular Endocrinology Group, Signal Transduction Laboratory
Derek W. Cain, Ph.D.	John A. Cidlowski, Ph.D.	Molecular Endocrinology Group, Signal Transduction Laboratory
Yu-Wei Chen, Ph.D.	Patricia Jensen, Ph.D.	Developmental Neurobiology Group, Neurobiology Laboratory
Shannon L. Farris, Ph.D.	Serena M. Dudek, Ph.D.	Synaptic and Developmental Plasticity Group, Neurobiology Laboratory
Bo He, Ph.D.	John A. Cidlowski, Ph.D.	Molecular Endocrinology Group, Signal Transduction Laboratory
Ming Ji, Ph.D.	Xiaoling Li, Ph.D	Metabolism, Gene, and Environment Group, Signal Transduction Laboratory
Yuan Yuan Li, Ph.D.	Leping Li, Ph.D.	Biostatistics & Computational Biology Branch
Hoai Nghia Nguyen, Ph.D.	Stephen B. Shears, Ph.D.	Inositol Signaling Group, Signal Transduction Laboratory
Barbara C. Nicol, Ph.D.	Humphrey Yao, Ph.D.	Reproductive Developmental Biology Group, Reproductive and Developmental Biology Laboratory
Rajneesh Pathania, Ph.D.	Raja Jothi, Ph.D.	Systems Biology Group, Epigenetics and Stem Cell Biology Laboratory
Mathew A. Quinn, Ph.D.	John A. Cidlowski, Ph.D.	Molecular Endocrinology Group, Signal Transduction Laboratory
Erin M. Romes, Ph.D.	Robin E. Stanley, Ph.D.	Nucleolar Integrity Group, Signal Transduction Laboratory

Matthew J. Schellenberg, Ph.D.	R. Scott Williams, Ph.D.	Genome Stability and Structural Biology Group, Genome Integrity and Structural Biology Laboratory
Sheng Song, Ph.D.	Jau-Shyong Hong, Ph.D.	Neuropharmacology Group, Neurobiology Laboratory
Shuang Tang, M.D., Ph.D.	Xiaoling Li, Ph.D	Metabolism, Gene, and Environment Group, Signal Transduction Laboratory
Seddon Y. Thomas, Ph.D.	Donald N. Cook, Ph.D.	Immunogenetics Group, Immunity, Inflammation and Disease Laboratory
Ma Wan, M.D, Ph.D.	Douglas A. Bell, Ph.D.	Environmental Genomics Group, Genome Integrity and Structural Biology Laboratory

Summer Internship Program Best Poster Awards

NIEHS takes a leadership role in science research and education. Scientists at NIEHS are committed to sharing with students the intensity, excitement, sense of discipline, and tremendous satisfaction that careers in science can impart to those who pursue them. To this end, the DIR established the Summer Internship Program for which internships are given to outstanding high school and college undergraduate and graduate students interested in pursuing careers in the biomedical/biological sciences. Participants are selected by intramural scientific mentors and spend between 8 to 12 weeks (during May through September) working on individual research projects that bring them exposure to the latest biochemical, molecular, and analytical techniques. There is a poster session at the end of the summer where participants display the results of their research efforts and respond to questions as though they were participating in a national scientific society meeting. The poster session was held on Thursday, July 28, and awards were presented for Best Poster in three categories, High School Interns, Undergraduate Interns and Graduate Interns. At the Awards Ceremony the following awards were presented:

<u>High School Intern:</u>

Neil Shah, Wake Early College of Health and Sciences, Reproductive and Developmental Biology Laboratory, Mentor: Fei Zhao, Reproductive Developmental Biology Group, Poster Title: "Decoding Reproductive System Development: A Novel Cell Population Influencing the Formation of the Female Reproductive Tract"

<u>Undergraduate Intern:</u>

Asha Anand, North Carolina State University, Reproductive and Developmental Biology Laboratory, Mentor: Manas Ray, Mouse Knockout Core, Poster Title: "Utilizing CRISPR/Cas9 to Disrupt Galectin-3 and Protein Kinase C Delta to Study Their Role in LC3-associated Phagocytosis"

Lucas Van Gorder, North Carolina State University, Neurobiology Laboratory, Mentor: Negin Martin, Viral Vector Core, Poster Title: "Validating CRISPR/Cas9 Delivery and Use with Adeno-associated Virus"

Graduate Intern:
Rachel Nethery, University of North Carolina, Chapel Hill, Epidemiology Branch, Mentor: Richard Kwok, Poster Title: "The Residential Neighborhood Environment and its Impact on GuLF STUDY Participants"

NIGMS PRAT Fellowship

The National Institute of General Medical Sciences (NIGMS) Postdoctoral Research Associate (PRAT) Program is a competitive postdoctoral fellowship program to pursue research in one of the laboratories of the National Institutes of Health (NIH) or the Food and Drug Administration (FDA). PRAT is a 3 year program providing outstanding laboratory experiences, access to NIH's extensive resources, mentorship, career development activities and networking. The program places special emphasis on training fellows in all areas supported by NIGMS, including cell biology, biophysics, genetics, developmental biology, pharmacology, physiology, biological chemistry, computational biology, immunology, neuroscience, technology development and bioinformatics.

Lee F. Langer, Ph.D., a fellow in the Chromatin and Gene Expression Group, Epigenetics and Stem Cell Biology Laboratory, was awarded a 2016 Prat Fellowship from NIGMS. Dr. Langer will be mentored by Dr. Trevor Archer.

Society for the Study of Reproduction Trainee Awards

Bart T. Phillips, Ph.D., a postdoctoral fellow in the Macromolecular Structure Group, Epigenetics and Stem Cell Biology Laboratory was selected for a Larry Ewing Memorial Trainee Travel Fund (LEMTTF) grant to present his research at the 2016 Annual Meeting of the Society for the Study of Reproduction, July 16-19, 2016. He was also selected to give a platform presentation. His mentor is Traci Hall, Ph.D.

Fei Zhao, Ph.D., a visiting postdoctoral fellow, Reproductive Developmental Biology Group, Reproductive and Developmental Biology Laboratory, won the Trainee Research Award (platform presentation category) at 2016 Annual Meeting for the Society for the Study of Reproduction (SSR) on July 16-19, 2016. His abstract was selected from 475 abstracts as one of the 6 finalists for the platform presentation category. The 6 finalists presented their work to the entire SSR membership at the annual meeting (~900 attendees). The award committee selected him as the winner of the final competition. His mentor is Humphrey Yao, Ph.D.

DIR RESEARCH ACCOMPLISHMENTS FOR FY 2016

Long lasting changes to the DNA of newborns due to having a mother who smoked during pregnancy

NIEHS epidemiologist Stephanie London formed the international Pregnancy And Childhood Epigenetics (PACE) consortium to study the impact of exposure during pregnancy on methylation, an epigenetic mark, in offspring. Epigenetics refers to changes to the DNA that are not changes to the sequence. In this inaugural paper from PACE, data were combined from 16 birth and child cohorts resulting in identification of more than 6,000 CpG sites in newborns differentially methylation in relation to maternal smoking during pregnancy. Nearly half of the sites were not previously associated with smoking and methylation in either newborns or adults. Notable, most of these signals persist later into childhood. This study shows that many of the same epigenetic changes that are found in adult due to their own smoking can be seen in newborns of mothers who smoked**.**

Joubert BR, Felix JF, Yousefi P, Bakulski KM, Just AC, Breton C, Reese SE, Markunas CA, Richmond RC, Xu CJ, Küpers LK, Oh SS, Hoyo C, Gruzieva O, Söderhäll C, Salas LA, Baïz N, Zhang H, Lepeule J, Ruiz C, Ligthart S, Wang T, Taylor JA, Duijts L, Sharp GC, Jankipersadsing SA, Nilsen RM, Vaez A, Fallin MD, Hu D, Litonjua AA, Fuemmeler BF, Huen K, Kere J, Kull I, Munthe-Kaas MC, Gehring U, Bustamante M, Saurel-Coubizolles MJ, Quraishi BM, Ren J, Tost J, Gonzalez JR, Peters MJ, Håberg SE, Xu Z, van Meurs JB, Gaunt TR, Kerkhof M, Corpeleijn E, Feinberg AP, Eng C, Baccarelli AA, Benjamin Neelon SE, Bradman A, Merid SK, Bergström A, Herceg Z, Hernandez-Vargas H, Brunekreef B, Pinart M, Heude B, Ewart S, Yao J, Lemonnier N, Franco OH, Wu MC, Hofman A, McArdle W, Van der Vlies P, Falahi F, Gillman MW, Barcellos LF, Kumar A, Wickman M, Guerra S, Charles MA, Holloway J, Auffray C, Tiemeier HW, Smith GD, Postma D, Hivert MF, Eskenazi B, Vrijheid M, Arshad H, Antó JM, Dehghan A, Karmaus W, Annesi-Maesano I, Sunyer J, Ghantous A, Pershagen G, Holland N, Murphy SK, DeMeo DL, Burchard EG, Ladd-Acosta C, Snieder H, Nystad W, Koppelman GH, Relton CL, Jaddoe VW, Wilcox A, Melén E, London SJ. *Am. J. Hum. Genet.,* 98:680-696, 2016.

Endotoxin Exposure in the United States

Inhaled endotoxin induces airway inflammation and is an established risk factor for asthma. The 2005-2006 National Health and Nutrition Examination Survey included measures of endotoxin and allergens in homes as well as specific IgE to inhalant allergens. Our objective was to understand the relationships between endotoxin exposure, asthma outcomes, and sensitization status for 15 aeroallergens in a nationally representative sample. Participants were administered questionnaires in their homes. Reservoir dust was vacuum sampled to generate composite bedding and bedroom floor samples. We analyzed 7,450 National Health and Nutrition Examination Survey dust and quality assurance samples for their endotoxin content using extreme quality assurance measures. Data for 6,963 subjects were available, making this the largest study of endotoxin exposure to date. Log-transformed endotoxin concentrations were analyzed using logistic models and forward stepwise linear regression. Analyses were weighted to provide national prevalence estimates and unbiased variances. Endotoxin exposure was significantly associated with wheeze in the past 12 months, wheeze during exercise, doctor and/or emergency room visits for wheeze, and use of prescription medications for wheeze.

Models adjusted for age, sex, race and/or ethnicity, and poverty-to-income ratio and stratified by allergy status showed that these relationships were not dependent upon sensitization status but were worsened among those living in poverty. Significant predictors of higher endotoxin exposures were lower family income; Hispanic ethnicity; participant age; dog(s), cat(s), cockroaches, and/or smoker(s) in the home; and carpeted floors. In conclusion, in this U.S. nationwide representative sample, higher endotoxin exposure was significantly associated with measures of wheeze, with no observed protective effect regardless of sensitization status.

Thorne PS, Mendy A, Metwali N, Salo P, Co C, Jaramillo R, Rose KM, Zeldin DC. Endotoxin Exposure: Predictors and Prevalence of Associated Asthma Outcomes in the United States. *Am. J. Respir. Crit. Care Med.,* 192:1287-1297, 2015.

NIEHS Sister Study data yields epidemiological findings on douching, talc use, and risk of ovarian cancer

Previous studies had reported an association between genital talc use and ovarian cancer, and Johnson and Johnson recently lost a huge settlement over use of baby powder by women who developed ovarian cancer. Women who use genital talc also tend to use douching, which is associated with increased levels of phthalates. The Sister Study, the first to examine the association between douching and ovarian cancer, found a positive association between douching and ovarian cancer, but no association between talc use and ovarian cancer. Although based on a small number of ovarian cancer cases, the findings may be important to the health of women.

Gonzalez NL, O'Brien KM, D'Aloisio AA, Sandler DP, Weinberg CR. Douching, Talc Use, and Risk of Ovarian Cancer. *Epidemiology*, epub ahead of print, doi: 10.1097/EDE.0000000000000528.

A pregnant woman's exposure to nearby smokers may increase the risk of facial clefts in her offspring

Mothers who smoke are known to have an increased risk of facial clefts in their babies. New evidence strongly suggests that this risk may be present even when mothers are passively exposed to cigarette smoke from others. These data from a large, multi-study consortium are the first to show that passive smoke exposure may cause birth defects.

Kummet CM, Moreno LM, Wilcox AJ, Romitti PA, DeRoo LA, Munger RG, Lie RT, Wehby GL. Passive Smoke Exposure as a Risk Factor for Oral Clefts-A Large International Population-Based Study. *Am. J. Epidemiol.*, 183:834-841, 2016.

Pesticides linked to rheumatoid arthritis in women

Earlier studies have found that farming was associated with rheumatoid arthritis, a systemic autoimmune disease affecting the joints and other organs in the body. We studied wives of licensed pesticide applicators who enrolled in the Agricultural Health Study cohort who have been followed since 1993-1997 for the development of cancer and other health conditions. Some of these women applied pesticides themselves. We found that women who had or developed rheumatoid arthritis since enrolling were forty percent more likely to have used at least one of the pesticides studied. RA was specifically associated with use of the fungicide maneb/mancozeb (three-fold risk) and the herbicide glyphosate (40% increase in risk). Risk of

developing RA was also associated with applying chemical fertilizers and cleaning farm equipment with solvents. In contrast, exposure to farm animals in both childhood and as an adult appeared to reduce the chances of developing RA, consistent with prior literature suggesting that early life exposures to endotoxins can influence the adult immune system.

Parks CG, Hoppin JA, DeRoos AJ, Costenbader KR, Alavanja MC, Sandler DP. Rheumatoid arthritis in Agricultural Health Study Spouses: Associations with pesticides and other farm exposures. *Environ. Health Perspect*., epub ahead of print, doi: 10.1289/EHP129

ALS in US military veterans associated with exposures to pesticides, engine exhaust, and burning agents

We compared 621 US military veterans with ALS to 958 veterans without ALS. Veterans with longer deployments had greater risk of ALS, although the condition was not related to branch of service. Exposures to pesticides, engine exhaust, and burning agents were associated with increased ALS risk. These results may explain at least in part the apparent increase of ALS in veteran populations.

Beard JD, Engel LS, Richardson DB, Gammon MD, Baird C, Umbach DM, Allen KD, Stanwyck CL, Keller J, Sandler DP, Schmidt S, Kamel F. Military service, deployments, and exposures in relation to amyotrophic lateral sclerosis etiology. *Environ. Int.*, 91:10-15, 2016.

Downstream events in DNA base lesion repair pathway

Downstream events in DNA base lesion repair pathway, i.e., after the DNA synthesis step, include the sequential hand-off of the repair intermediate to the DNA ligase step. If the hand-off to ligase is defective, stalled repair intermediates can accumulate leading to cell death. NIEHS scientists found that pol β can insert the oxidized nucleotide 8-oxodGTP during repair, however, in contrast to normal insertion, the enzyme re-opens and releases the reaction products. In this case, the newly incorporated 8-oxoG base is no longer annealed to the template base cytosine. This appears to interrupt the repair process leading to a stalled repair intermediate. These observations help us to understand the biological implications of oxidative stressors that oxidize the nucleotide pool, and aspects of this research are described in the following papers.

Freudenthal BD, Beard WA, Perera L, Shock DD, Kim T, Schlick T, Wilson SH. Uncovering the polymerase-induced cytotoxicity of an oxidized nucleotide. *Nature*, 517:635-9, 2015.

Caglayan, M., Horton, J.K., Stefanick, D.F, and Wilson, S.H. Oxidized nucleotide insertion by pol β confounds ligation during base excision repair. *Nat. Commun*. (in press)

Sassa A, Caglayan, M, Beard WA, Wilson SH, Nohmi T, Honma M, Yasui M. Impact of ribonucleotide backbone on translesion synthesis and repair of 7,8-dihydro-8-oxoguanine. *J. Biol. Chem.* (in press).

Cilli P, Ventura I, Minoprio A, Meccia E, Martire A, Wilson SH, Bignami M, Mazzei F. Oxidized dNTPs and the OGG1 and MUTYH DNA glycosylases combine to induce CAG/CTG repeat instability. *Nucleic Acids Res.*, 44:5190-203, 2016.

Defects in degradation pathway results in autoimmunity

Components of the autophagy pathway, which functions in cell survival during starvation, are also important for the clearance of dead cells and preventing autoimmunity. Defects in these components result in a lupus-like disease with age. These studies allow us to study the non-canonical roles of autophagy machinery and its effects on immunity.

Martinez J, Cunha LD, Park S, Yang M, Lu Q, Orchard R, Li Q-Z, Yan M, Janke L, Guy C, Linkermann A, Virgin HW, and Green DR. Noncanonical autophagy inhibits the autoinflammatory, lupus-like response to dying cells. *Nature,* 533:115-119, 2016.

Genetic predisposition for beta cell fragility underlies type 1 and type 2 diabetes

Induction of the unfolded protein response (UPR) has been reported to play an important role in both the development of type 1 and type 2 diabetes. Reduced expression of the transcription factor GLIS3 enhances the susceptibility to both type 1 and type 2 diabetes that involves increased apoptosis and senescence of pancreatic beta cells.

Dooley J, Tian L, Schonefeldt S, Delghingaro-Augusto V, Garcia-Perez JE, Pasciuto E, Di Marino D, Carr EJ, Oskolkov N, Lyssenko V, Franckaert D, Lagou V, Overbergh L, Vandenbussche J, Allemeersch J, Chabot-Roy G, Dahlstrom JE, Laybutt DR, Petrovsky N, Socha L, Gevaert K, Jetten AM, Lambrechts D, Linterman MA, Goodnow CC, Nolan CJ, Lesage S, Schlenner SM, Liston A. Genetic predisposition for beta cell fragility underlies type 1 and type 2 diabetes. *Nat. Genet.,* 48:519-527, 2016.

Transforming the brain's representations of space

Scientists at NIEHS have discovered a novel mechanism by which certain memories may be formed in the brain. They discovered that existing spatial representations in hippocampal area CA2 are rapidly updated in response to an animal experiencing social or novel object stimuli. Their report provided the first evidence of a specific mechanism for encoding social and contextual information in this little known part of the brain.

Alexander GM, Farris S, Pirone JR, Zheng C, Colgin LL, Dudek SM. Social and novel contexts modify hippocampal CA2 representations of space. *Nat. Commun.,* 7:10300, 2016.

A link between the transcriptional activation functions of GATA3 with chromatin remodeling

In this study, the central question of how DNA binding proteins locate their cognate recognition sites within chromosomal DNA to elicit downstream events was explored in a model cellular reprogramming system. It was determined that the transcription factor GATA3 has the capacity to bind DNA in regions where local chromatin structure inhibits access of structural probes. Such binding was accompanied, in some cases, with local reorganization of chromatin to form a functional enhancer capable of increasing expression of nearby genes. Using mutant versions of GATA3, it was determined that DNA binding was not sufficient for local chromatin remodeling which required the transcriptional activation domain of GATA3. These results are broadly applicable to the process of cellular reprogramming, including iPS cell generation.

Takaku M, Grimm SA, Shimbo T, Perera L, Menafra R, Stunnenberg HG, Archer TK, Machida S, Kurumizaka H, Wade PA. GATA3-dependent cellular reprogramming requires activation-domain dependent recruitment of a chromatin remodeler. *Genome Biol.*, 17:36, 2016.

Estrogen Receptor Beta (ESR2) promotes growth and survival of the ectopic endometrial tissue

A novel mouse model was generated that allowed the expression of ESR2 in the uterus of mice. Notably, gain of ESR2 function stimulated the progression of endometriosis in this mouse model by aiding the ability of this tissue evade endogenous immune surveillance for cell survival. This study identified the mechanism by which ESR2 inhibited cell death and interacted with inflammatory machinery to cellular adhesion and proliferation properties. Furthermore, ESR2 enhanced the invasion activity of endometriotic tissues for establishment of ectopic lesions.

Han SJ, Jung SY, Wu SP, Hawkins SM, Park MJ, Kyo S, Qin J, Lydon JP, Tsai SY, Tsai MJ, DeMayo FJ, O'Malley BW. Estrogen Receptor β Modulates Apoptosis Complexes and the Inflammasome to Drive the Pathogenesis of Endometriosis. *Cell*, 163:960-974, 2015.

Nutrient sensing limits gut inflammation

GCN2 allows cells to sense stress-inducing signals, such as amino acid starvation, and coordinate an appropriate response. These studies demonstrate that GCN2 controls intestinal inflammation by suppressing inflammasome activation via activating autophagy. These results reveal a mechanism that couples amino acid sensing with control of intestinal inflammation via GCN2.

Ravindran R, Loebbermann J, Nakaya H, Khan N, Gama L, Machiah D, Sharma P, Lawson B, Wang Y-C , Hakimpour P, Kaufman P, Li S, Martinez J, and Pulendran B. The amino acid sensor GCN2 controls gut inflammation by inhibiting inflammasome activation. *Nature*, 531:523-527, 2016.

Connecting base excision repair (BER) pathways

NIEHS investigators and collaborators have been instrumental in defining the main mammalian base excision repair (BER) pathways using purified enzymes, cells and cell extracts. This project includes studies of the cellular role of BER in the context of overall cellular DNA repair. The project also evaluates the effect of genotoxic agents and DNA synthesis inhibitors on cell cycle control and on the mechanism of cell death. Many of these compounds have been implicated in DNA polymerase inhibition through a chain termination mechanism, producing toxic DNA strand breaks. Although much is known about BER in mammalian cells, researchers are only beginning to understand the multifaceted connections between this DNA repair pathway, environmental exposures and disease. Therefore, NIEHS researchers are continuing to explore the mechanism of BER, and have recently contributed important new insight to this field in the following papers.

Freudenthal BD, Beard WA, Cuneo MJ, Dyrkheeva NS, Wilson SH. Capturing snapshots of APE1 processing DNA damage. *Nat. Struct. Mol. Biol.,* 22:924-31, 2015.

Horton JK, Gassman NR, Dunigan BD, Stefanick DF, Wilson SH. DNA polymerase β-dependent cell survival independent of XRCC1 expression. *DNA Repair (Amst).,* 26:23-29, 2015.

Prasad R, Dyrkheeva N, Williams J, Wilson SH. Mammalian Base Excision Repair: Functional Partnership between PARP-1 and APE1 in AP-Site Repair. *PLoS One*, 10:e0124269, 2015.

Kirby TW, Gassman NR, Smith CE, Pedersen LC, Gabel SA, Sobhany M, Wilson SH, London RE. Nuclear Localization of the DNA Repair Scaffold XRCC1: Uncovering the Functional Role of a Bipartite NLS. *Sci. Rep.,* 5:13405, 2015.

Çağlayan M, Horton JK, Prasad R, Wilson SH. Complementation of aprataxin deficiency by base excision repair enzymes. *Nucleic Acids Res.,* 43:2271-2281, 2015.

Kirby TW, Gassman NR, Smith CE, Zhao M-L, Horton, JK, Wilson SH, London RE. DNA polymerase β contains a functional nuclear localization signal at Its N-terminus. *Nucleic Acids Res.* (in press).

Speed and accuracy of human DNA polymerase (pol) β

Accurate replication and repair of the genome is vital to genome integrity in all cells, and DNA polymerases provide many of the DNA synthesis and other functions that are essential in the replication and repair processes. To understand the speed and accuracy of DNA polymerases, NIEHS investigators applied time-lapse crystallography, various kinetic assays and computational techniques to study human DNA polymerase (pol) β, considered a model enzyme for understanding nucleotidyl transfer reactions by polymerases. This crystallography approach provides novel snapshots of structural intermediates of the enzyme and substrates and products as they passes through the catalytic cycle. There is an "open to closed" conformational transition in the pol β that hastens correct and deters incorrect nucleotide insertion into DNA, and this can be visualized, and the structures are being subjected to computational analyses to gain a better understanding of the conformational dynamics. Importantly, after correct nucleotide insertion, the enzyme remains in the closed conformation, and the active site has a new divalent metal ion-binding site, termed the product metal. Thus, slow product release could be coupled to the reverse reaction of DNA synthesis, pyrophosphorolysis, since a divalent metal ion is associated with the product pyrophosphate and the enzyme is closed and poised for nucleotidyl transfer. In other studies of the pol β, the mechanism of discrimination against mismatched primer extension was discovered and special features of the enzyme's use of two divalent metals in the active site. These features and discoveries are evaluated in the following paper.

Perera L, Freudenthal BD, Beard WA, Shock DD, Pedersen LG, Wilson SH. Requirement for transient metal ions revealed through computational analysis for DNA polymerase going in reverse. *Proc. Natl. Acad. Sci. U.S.A.,* 112:E5228-E5236, 2015

Batra VK, Beard WA, Pedersen LC, Wilson SH. Structural analysis of mispaired DNA termini transitioning to DNA polymerase pre-catalytic complexes supports an induced fit fidelity mechanism. *Structure*, (in press)

Perera L, Beard WA, Pedersen LG, Wilson SH. The bimetallic magnesium covalent bond in enzyme active sites. *Nat. Chem. Biol.*, (in press)

Harnessing a natural anti-inflammatory protein to attack inflammatory diseases
The natural occurring anti-inflammatory protein tristetraprolin (TTP) is expressed in almost all vertebrate species, including mice and humans, and is responsible for controlling the expression of harmful cytokines in the regulation of the innate immune response. The authors showed many years ago that deficiency of this protein in mice led to a serious inflammatory disease, with arthritis, dermatitis, and many other abnormalities. In the current work, they found that modifying the sequence of the gene encoding TTP, so that the protein was moderately overexpressed, prevented the development of mouse models of immune inflammatory disease that are used as models of human multiple sclerosis, psoriasis, and rheumatoid arthritis. This study identifies TTP as a potential target candidate for the development of new therapies for these and related conditions in man.

Patial S, Curtis AD 2nd, Lai WS, Stumpo DJ, Hill GD, Flake GP, Mannie MD, Blackshear PJ. Enhanced stability of tristetraprolin mRNA protects mice against immune-mediated inflammatory pathologies. *Proc. Natl. Acad. Sci. USA.*, 113:1865-70, 2016.

Macrophages engage distinct pathways during inflammation
Depending on the immunological stimulus, macrophages can adjust their metabolic outputs to suit their bioenergetics demands. These studies demonstrate that a pro-inflammatory stimuli shifts macrophage from a Myc (proliferative) program to a HIF1α (glycolytic) program.

Liu L, Lu Y, Martinez J, Wang T, Wang J, Yang M, Liu G, Green DR, and Wang R. Pro-inflammatory stimulation suppresses proliferation and shifts the regulation of macrophage metabolism from a Myc-dependent to a HIF1α-dependent manner. *Proc. Natl. Acad. Sci. USA*, 113:1564-1569, 2016.

Antihypertensive drugs not linked to breast cancer risk
A recent case-control study had shown that calcium channel blockers, a class of drugs commonly used to treat hypertension, were associated with a two-fold increase in risk of breast cancer in women who used them for 10 or more years; raising considerable concern about the use of these drugs in women. But those concerns have largely been put to rest by a recent prospective cohort study by the NIEHS, which has been following more than 50,000 women at increased breast cancer risk since 2003. Although 1372 women developed invasive breast cancer, women who used calcium channel blockers or other classes of antihypertensive drugs had the same breast cancer risk as women who did not use the drugs.

Wilson LE, D'Aloisio AA, Sandler DP, Taylor JA. Long-term use of calcium channel blocking drugs and breast cancer risk in a prospective cohort of US and Puerto Rican women. *Breast Cancer Res.*, 18:61, 2016.

A cancer mutagen floating against the current
An antiviral component of the human innate immune system - the APOBEC cytidine deaminases-was recently identified as a prominent source of mutations in cancers. It was found that these enzymes behave in the way opposite to all other known cancer mutagens. While the rate of most mutations in cancer genomes is known to be elevated in late-replicating regions that

are characterized by reduced chromatin accessibility and low gene density, a marked enrichment of APOBEC mutations in early-replicating regions was observed. This unusual mutagenesis profile should be accounted for in statistical analyses of cancer genome mutation catalogs aimed at understanding the mechanisms of carcinogenesis as well as at highlighting genes that are significantly mutated in cancer.

Kazanov MD, Roberts SA, Polak P, Stamatoyannopoulos J, Klimczak LJ, Gordenin DA, Sunyaev SR. APOBEC-Induced Cancer Mutations Are Uniquely Enriched in Early-Replicating, Gene-Dense, and Active Chromatin Regions. *Cell Rep*., 13:1103-1109, 2015.

NRF2 protein turns on genetic switch that may protect against neurodegenerative disease
A complex involving the protein NRF2 and a genetic switch controlling a brain molecule known as tau may reduce the development of neurodegenerative disorders. Some forms of tau tend to stick together, making the neurofibrillary tangles found in the brains of people with Alzheimer's disease, progressive supranuclear palsy, corticobasal degeneration, and sometimes Parkinson's disease. Other forms of tau are less likely to aggregate into tangles. This study integrated genome-wide maps of NRF2 binding with genome databases and experimental work to determine that NRF2 binds to an inherited version of the tau gene, called the T allele, and this binding appears to turn on production of one of the protective forms of tau.

Wang X, Campbell MR, Lacher SE, Cho HY, Wan M, Crowl CL, Chorley BN, Bond GL, Kleeberger SR, Slattery M, Bell DA. A Polymorphic Antioxidant Response Element Links NRF2/sMAF Binding to Enhanced MAPT Expression and Reduced Risk of Parkinsonian Disorders. *Cell Rep.,* epub ahead of print, doi: 10.1016/j.celrep.2016.03.068

Importance of an RNA binding protein in fetal nutrition.
ZFP36L3 is an RNA binding protein that is expressed only in the placenta and yolk sac of developing rodents, and acts, like other members of its protein family, to promote the destruction of its target messenger RNAs and thus regulate gene expression. Stumpo and colleagues found that mice deficient in ZFP36L3 had smaller than normal litters of pups, and that gene expression of many target mRNAs was altered in the developing placentas of these mice. One consequence of this genetic deletion was the markedly decreased expression the transferrin receptor in the placenta, resulting in decreased iron uptake by the fetuses. Since fetal iron deficiency has been linked to later cognitive defects in man, current work is exploring the effect of this developmental deficiency on brain development in the ZFP36L3-deficient adult animals.

Stumpo DJ, Trempus CS, Tucker CJ, Huang W, Li L, Kluckman K, Bortner DM, Blackshear PJ. Deficiency of the placenta- and yolk sac-specific tristetraprolin family member ZFP36L3 identifies likely mRNA targets and an unexpected link to placental iron metabolism. *Development*, 143:1424-1433, 2016.

New genetic tools enable studies of brain structure and function
NIEHS investigators designed genetically modified mice that will help neurobiologists address one of the most fundamental questions in brain research — what are the identities and functions of different cell types in the brain? The new mouse lines will let scientists identify specific

populations of brain cells and determine how they control behavior in an animal. The findings will advance our understanding of neurological disorders such as Alzheimer's disease, drug addiction, and depression.

Plummer NW, Evsyukova IY, Robertson SD, de Marchena J, Tucker CJ, Jensen P. Expanding the power of recombinase-based labeling to uncover cellular diversity. *Development*, 142:4385-4393, 2015.

Sciolino NR, Plummer NW, Chen YW, Alexander GM, Robertson SD, Dudek SM, McElligott ZA, Jensen P. Recombinase-Dependent Mouse Lines for Chemogenetic Activation of Genetically Defined Cell Types. *Cell Rep.*, 15:2563-2573, 2016.

Ubiquitous environmental contaminant found not to interfere with fertility

A previous study suggested that the ubiquitous environmental contaminant, perfluorooctane sulfonamide, decreased female fertility. NIEHS investigators addressed this question using a much larger study and found no evidence of an adverse effect.

Whitworth KW, Haug LS, Sabaredzovic A, Eggesbo M, Longnecker MP. Plasma concentrations of perfluorooctane sulfonamide (PFOSA) and time to pregnancy among primiparous women. *Epidemiology*, epub ahead of print, doi: 10.1097/EDE.0000000000000524

Estrogen regulation of oviduct function crucial for embryo survival

In female mammals, eggs made in the ovaries travel to the uterus via tubes called oviducts (or Fallopian tubes). Eggs are fertilized by sperm in the oviduct, and then they develop as embryos in the oviduct for several days. Here NIEHS investigators use mice to show that the hormone estrogen is needed to change the fluid within the oviduct so that it is not toxic to developing embryos. Disrupting estrogen signaling in the oviduct led to embryo death by increasing oviduct fluid levels of enzymes that destroy proteins. These findings in the mouse identify altered oviduct fluid components as a possible contributor to human infertility.

Winuthayanon W, Bernhardt ML, Padilla-Banks E, Myers PH, Edin ML, Lih FB, Hewitt SC, Korach KS, Williams CJ. Oviductal estrogen receptor α signaling prevents protease-mediated embryo death. *Elife*, 4:e10453, 2015.

Chemical mechanism of DNA synthesis by DNA polymerases

Understanding the chemical mechanism of DNA synthesis by the class of enzymes termed DNA polymerases has been a topic of long standing interest in biological research. By making use of a novel interdisciplinary approach involving X-ray crystallography, enzyme kinetics and computational studies, NIEHS scientists and collaborators recently obtained novel insight into the mechanism of DNA synthesis and how an oxidized pyrimidine base in DNA, 5-chlorocytosine, causes mutations. Use of the model DNA polymerase, human pol β, and altered DNA substrates revealed novel understanding of the mechanism of mutagenesis and how this could links chronic inflammation and cancer.

Fedeles BI, Freudenthal BD, Yau E, Singh V, Chang SC, Li D, Delaney JC, Wilson SH, Essigmann JM. Intrinsic mutagenic properties of 5-chlorocytosine: A mechanistic

connection between chronic inflammation and cancer. *Proc. Natl. Acad. Sci. U.S.A.,* 112:E4571-E4580, 2015.

A protein defect in the rare genetic disease ANE syndrome causes disruption of molecular interactions in ribosome assembly

Five boys in the same family were born with a disease called ANE syndrome, which causes baldness, mental retardation, short height, failure to develop normally during puberty, and problems with missing teeth. The disease is caused by a single amino acid change in the protein, RBM28. While little is known about the role of human RBM28, it is known that the equivalent protein in yeast – known as Nop4 – plays a critical role in assembling ribosomes, the essential, large machines that make proteins. McCann et al. asked how changing one amino acid in a protein of over 400 amino acids could cause disease and whether this involves interrupting the assembly of ribosomes. The experiments show that introducing the same mutation into yeast Nop4 impaired the ability of Nop4 to form the network of proteins needed for ribosomes to assemble and that the mutation also alters the shape of the human RBM28 protein. A challenge for the future is to determine how disrupting ribosome assembly, an essential cellular need, could lead to the specific symptoms of ANE syndrome.

McCann KL, Teramoto T, Zhang J, Tanaka Hall TM, Baserga SJ. The molecular basis for ANE syndrome revealed by the large ribosomal subunit processome interactome. *Elife*, 5: e16381, 2016.

Novel anti-inflammatory effects of statin drugs

'Statins' are medications taken by millions of people in the U.S. and worldwide in order to reduce bloodstream levels of 'bad cholesterol' (low density lipoprotein [LDL]-cholesterol) and to increase levels of 'good cholesterol' (high density lipoprotein [HDL]-cholesterol). In recent years, it has been increasingly recognized that these drugs also have important inflammation-reducing activities. NIEHS investigators collaborated with NHLBI investigators to isolate bloodstream HDL from volunteers who had taken rosuvastatin for 4 weeks, aiming to identify proteins that associate with HDL particles with the use of mass spectrometry. Interestingly, the investigators found that rosuvastatin increased the levels of alpha-1-antitrypsin (A1AT), an important anti-inflammatory protein, within HDL particles, thereby enhancing A1AT anti-inflammatory function through protecting it from inactivation. Taken together, this study has identified an important, novel mechanism, separate from cholesterol reduction, through which statins may be protective through reducing inflammation.

Gordon SM, McKenzie B, Kemeh G, Sampson M, Perl S, Young NS, Fessler MB, Remaley AT. Rosuvastatin Alters the Proteome of High Density Lipoproteins: Generation of alpha-1-antitrypsin Enriched Particles with Anti-inflammatory Properties. *Mol. Cell. Proteomics*, 14:3247-57, 2015.

Genetic dissection and molecular analysis of mechanisms of repair

Genome stability in response to DNA damage and under normal growth is dependent on extensive mechanisms of damage recognition and repair. Using yeast NIEHS investigators explored repair mechanisms in response to radiation induced double-strand breaks and the single-strand DNA damaging agent methyl methanesulfonate (MMS). With a Pulse Field Gel

Electrophoresis-shift approach that they developed, they determined resection at IR-DSBs in WT and mutants lacking exonuclease1 or Sgs1 helicase. A severe reduction in resection tract length had only a modest effect on repair of multiple, dirty DSBs in G2-arrested cells. This study provided the first opportunity to directly relate resection length at DSBs to the capability for global recombination repair between sister chromatids. In a related study they found that at MMS lesions, the Shu complex promotes Rad51-dependent HR as the primary repair/tolerance mechanism over error-prone translesion DNA polymerases. The Shu complex's promotion of Rad51 pre-synaptic filaments was shown to be critical for high-fidelity bypass of multiple replication-blocking lesion.

Westmoreland J, Resnick MA. Recombinational repair of radiation-induced double-strand breaks occurs in the absence of extensive resection assay. *Nucleic Acids Res*., 44: 695-704, 2016.

Godin SK, Zhang Z, Herken BW, Westmoreland JW, Lee AG, Mihalevic M, Yu Z, Sobol RW, Resnick MA, Bernstein KA. The Shu complex promotes error-free tolerance of alkylation-induced base-excision repair products. *Nucleic Acids Res*., epub ahead of print, doi: 10.1093/nar/gkw535

Characterization of the subunit-specific RH domain unfolding step in HIV-1 reverse transcriptase

Formation of the mature HIV-1 reverse transcriptase (RT) p66/p51 heterodimer requires subunit-specific processing of the p66/p66' homodimer precursor. Since the ribonuclease H (RH) domain contains an occult cleavage site located near its center, cleavage must occur either prior to folding or subsequent to unfolding. Previous NMR studies by NIEHS researchers have identified a slow, subunit-specific RH domain unfolding process proposed to result from a residue tug-of-war between the polymerase and RH domains on the functionally inactive, p66' subunit. During the past year these researchers were able to capture a partially unfolded RH domain intermediate based on observation of a domain swapped dimer. In this type of structure, a partially unfolded molecule becomes stabilized by forming a set of complementary interactions with a second unfolded molecule. The study revealed several intrinsically destabilizing characteristics of the isolated domain that facilitate excursions of Tyr427 from its binding pocket and separation of helices B and D. These studies provide independent support for the subunit selective RH domain unfolding pathway in which instability of the Tyr427 binding pocket facilitates its release followed by domain transfer, acting as a trigger for further RH domain destabilization and subsequent unfolding. It was also shown that addition of an RH active site-directed isoquinolone ligand retarded the subunit-selective RH domain unfolding behavior of the p66/p66 homodimer, demonstrating the feasibility of directly targeting RT maturation with therapeutics.

Zheng X, Pedersen LC, Gabel SA, Mueller GA, DeRose EF, London RE. Unfolding the HIV-1 reverse transcriptase RNase H domain--how to lose a molecular tug-of-war. *Nucleic Acids Res*., 44:1776-1788, 2016.

Tdp2 protects cells from Topoisomerase 2 DNA-protein crosslinks

The DNA repair protein Tyrosyl-DNA phosphodiesterase 2 (Tdp2), regulates genome stability and resistance to anticancer topoisomerase 2 (Top2) drugs by reversing drug-induced Top2 DNA-protein crosslink structures. In this study, NIEHS investigators employed molecular

imaging, biochemical and cell biology to elucidate the catalytic mechanism of Tdp2. Their work defines the mechanistic framework for understanding Tdp2 genome protective functions, and a platform for targeting Tdp2 activity in cancer therapy. Additionally it was established that human genetic variants (single nucleotide polymorphisms) undermine the activity of Tdp2 in human cells and confer sensitivity to the anti-cancer agent etoposide, suggesting that Tdp2 status may be used as a biomarker in humans to predict the individual sensitivity to chemotherapy.

Schellenberg MJ, Perera L, Strom CN, Waters CA, Monian B, Appel CD, Vilas CK, Williams JG, Ramsden DA, Williams RS. Reversal of DNA damage induced Topoisomerase 2 DNA-protein crosslinks by Tdp2. *Nucleic Acids Res*., 44:3829-3844, 2016.

The novel p53 target TNFAIP8 variant 2 counterbalances p53-dependent tumor suppression

The Tumor Necrosis Factor-alpha Induced Protein-8 gene (TNFAIP8) is a stress response gene recently identified as p53 transcriptional target by NIEHS researchers. A transcriptional variant of this gene TNAFIP8-v2, is overexpressed in several cancers suggesting TNFAIP8 could be considered as a chemotherapeutic target. Reducing the expression of TNFAIP8-V2 by genetic approaches resulted in the transactivation of p53 target genes and in a p53-dependent cell cycle arrest and DNA damage sensitization. Furthermore in response to the chemotherapeutic agent doxorubicin, p53 regulates TNFAIP8-V2 expression through binding to an intragenic enhancer. It is proposed that TNFAIP8 v2 promotes human cancer by broadly repressing p53 functions.

Lowe J, Nguyen T, Resnick MA, Grimm SA, Gabor KA, Peddada SD, Anderson CW, Menéndez D, Fessler MB. The novel p53 target TNFAIP8 variant 2 is increased in cancer and offsets p53-dependent tumor suppression. *Cell Death Differ.*, (in press).

Short term disruption of a teen's deep sleep does not lead to insulin resistance

Cross-sectional studies in children and adults and a small number of interventional studies in adults suggest that short slow wave sleep (SWS) duration and SWS fragmentation are risk factors for metabolic syndrome. The current studies, which represent the first SWS disruption studies with metabolic phenotyping to be conducted in children, demonstrate that a single night of SWS disruption does not diminish insulin sensitivity in pubertal children, challenging the existing dogma. These results imply that adolescents may have a unique metabolic resiliency against acute sleep disruption; follow-up studies will be important in settings of more prolonged SWS restriction or disruption.

Shaw ND, McHill AW, Schiavon M, Kangarloo T, Mankowski PW, Cobelli C, Klerman EB, Hall JE. Effect of Slow-Wave Sleep Disruption on Metabolic Parameters in Adolescents. *Sleep*, epub ahead of print, pii: sp-00126-16.

Air pollution and the risk of Parkinson's disease

Increasing evidence suggests that air pollution may increase the risk of age-related neurodegenerative diseases such as Parkinson's and Alzheimer's disease. The associations of ambient air pollutants such as particulate matter (PM) less than 10μm in diameter (PM10), less than 2.5μm in diameter (PM2.5), and nitrogen dioxide (NO2) in relation to PD risk were investigated by comparing data from 1566 Parkinson's patients and 3133 individuals without

Parkinson's disease. Overall, little evidence was observed for associations of these air pollutants with Parkinson's disease. However, the data suggest that higher exposure to PM10 and PM2.5 may be associated with higher risk of Parkinson's disease among women nonsmokers. This evidence is suggestive and needs to be confirmed by other studies.

Liu R, Young MT, Chen JC, Kaufman JD, Chen H. Ambient Air Pollution Exposures and Risk of Parkinson Disease. *Environ. Health Perspect.*, epub ahead of print, doi: 10.1289/EHP135

Low-level arsenic exposure before birth associated with early puberty and obesity in female mice

Female mice exposed in utero, or in the womb, to low levels of inorganic arsenic through drinking water displayed signs of early puberty and became obese as adults, according to the findings by NIEHS investigators. Inorganic arsenic compounds (such as those found in water) are highly toxic while organic arsenic compounds (such as those found in seafood) are less harmful to health. The finding is significant because the exposure level of 10 parts per billion used in the study is the current U.S. Environmental Protection Agency (EPA) and World Health Organization (WHO) standard, or maximum allowable amount, for arsenic in drinking water. The study serves as a good starting point for examining whether low-dose arsenic exposure could have similar health outcomes in humans.

Rodriguez K, Ungewitter E, Crespo Y, Liu C, Nicol B, and Yao HHC. In utero exposure to arsenate during the second half of gestation leads to early onset of vaginal opening and obesity in female CD-1 mice. *Environ. Health Perspect*., 124:336–343, 2016.

Bisphenol A studies in humans

Cashiers (n = 77) and non-cashiers (n = 25) were recruited for a study to examine the effects of handling thermal paper receipts that use bisphenol A (BPA) or alternate compounds as a developer on blood and urine levels of BPA, bisphenol S (BPS) and 4-hydroxyphenyl 4-isoprooxyphenylsulfone (BPSIP). Each receipt contained 1-2% by weight of the paper of BPA, BPS, or BPSIP. The post-shift geometric mean total urinary BPS concentration was significantly higher than the pre-shift mean in 33 cashiers who handled receipts containing BPS. The mean urine BPA concentrations in 31 cashiers who handled BPA receipts were as likely to decrease as to increase after a shift, but the mean post-shift concentrations were significantly higher than those in non-cashiers. BPSIP was detected more frequently in the urine of cashiers handling BPSIP receipts than in the urine of non-cashiers. Only a few cashiers had detectable levels of total BPA or BPS in serum, whereas BPSIP tended to be detected more frequently. In related studies on the pharmacokinetics of BPA it was shown that Conjugation reactions are rapid and nearly complete with unconjugated BPA comprising less than 1% of the total d6-BPA in blood at all times. Elimination of conjugates into urine largely occurs within 24h.

Thayer KA, Taylor KW, Garantziotis S, Schurman SH, Kissling GE, Hunt D, Herbert B, Church R, Jankowich R, Churchwell MI, Scheri RC, Birnbaum LS, Bucher JR. Bisphenol A, Bisphenol S, and 4-Hydroxyphenyl 4-Isoprooxyphenylsulfone (BPSIP) in Urine and Blood of Cashiers. *Environ. Health Perspect.*, 124:437-444, 2016.

Thayer KA, Doerge DR, Hunt D, Schurman SH, Twaddle NC, Churchwell MI, Garantziotis S, Kissling GE, Easterling MR, Bucher JR, Birnbaum LS. Pharmacokinetics of bisphenol A in humans following a single oral administration. *Environ. Int.,* 83:107-15, 2015.

Complex effects of an RNA instability factor on gene expression

Tristetraprolin (TTP) is an RNA binding protein that promotes the decay of messenger RNAs coding for harmful cytokines, such as tumor necrosis factor (TNF), in the innate immune response to infectious diseases and other environmental stimuli. Many studies have evaluated its effects on gene expression in cell culture models, but its complex effects in many cells and tissues have made study of its effects in intact animals difficult. Patial and colleagues developed a "triple knockout mouse" to evaluate the effect of TTP on gene expression in mouse tissues in the absence of the complicating effects of TNF, and found that TTP influenced the levels of many primary and secondary target messenger RNAs in mouse tissues.

Patial S, Stumpo DJ, Young WS 3rd, Ward JM, Flake GP, Blackshear PJ. Effects of Combined Tristetraprolin/Tumor Necrosis Factor Receptor Deficiency on the Splenic Transcriptome. *Mol. Cell. Biol.*, 36:1395-1411, 2016.

Genome integrity consequences of environmental chemicals that induce oxidative stress

Earlier studies by NIEHS investigators have important implications for research on the genome integrity consequences of environmental chemicals that induce oxidative stress. One recent example of this is the improved understanding of how a cultured cell model system responds to the environmental chemical Bisphenol A (BPA). After inducing oxidative stress with an oxidizing agent, BPA treatment of the cells suppressed DNA repair of oxidized DNA bases. It was also discovered that the exposure to the chemical mixture of potassium bromate and BPA results in a range of cellular effects in addition to the effect on DNA repair. These results pointing to the oxidative stress-inducing capacity of BPA and to the robust cellular adaptive response to BPA exposure are described in the following papers**.**

Gassman NR, Coskun E, Stefanick DF, Horton JK, Jaruga P, Dizdaroglu M, Wilson SH. Bisphenol a promotes cell survival following oxidative DNA damage in mouse fibroblasts. *PLoS One*, 10:e0118819, 2015.

Gassman NR, Coskun E, Jaruga PE, Dizdaroglu M, Wilson SH. Bisphenol A alters cellular microenvironment to promote survival after oxidative stress. *Environ. Health Perspect.*, (in press).

New study launched to investigate human toxicity of airborne PCBs

Significant questions remain about the potential toxicity of low-molecular weight PCBs (polychlorinated biphenyls) that were used to construct housing world-wide. Danish and NIH investigators have launched a large new study that capitalizes on the national health registry system in Demark as well as detailed records available about the presence of PCBs in public housing in that country. The specific health effects to be studied include cancer, fertility, and neurodevelopment.

Bräuner EV, Andersen ZJ, Frederiksen M, Specht IO, Hougaard KS, Ebbehøj N, Bailey J, Giwercman A, Steenland K, Longnecker MP, Bonde JP. Health Effects of PCBs in

Residences and Schools (HESPERUS): PCB - health Cohort Profile. *Sci. Rep.*, 6:24571, 2016.

How do oscillatory brain activities emerge in the absence of pacemakers?
The hippocampal theta rhythm emerges as rhythmic and synchronized activities among the hippocampus and hippocampus-associated brain regions during active exploration, providing a potential means for brain inter-regional communication. However, after decades of research, the origins of the theta rhythm remain elusive, at least partly due to the difficulty in recording from all three essential regions for theta generation, namely the hippocampus itself, the septum, and the entorhinal cortex. For this reason, NIEHS researchers established an in vitro theta model in a septo-entorhinal-hippocampal brain slice tri-culture system by pairing septal cholinergic inputs with hippocampal local activities. The study reveals a mechanism for theta rhythms to emerge as the functional results of dynamic interactions among the septum, hippocampus, and the entorhinal cortex, in the absence of clear pacemakers.

Gu Z, Yakel JL. Inducing theta oscillations in the entorhinal hippocampal network in vitro. *Brain Struct. Funct.*, epub ahead of print, doi:10.1007/s00429-016-1256-3

Contribution of Alveolar Type II Cell Cyclooxygenase-2 to Basal Airway Function, Lung Inflammation and Lung Fibrosis
Cyclooxygenase (COX)-2 has been shown to be involved in regulating basal airway function, bacterial lipopolysaccharide (LPS)-induced airway hyperresponsiveness (AHR) and lung inflammation, and bleomycin-induced lung fibrosis; however, the cellular source of COX-2 related to these effects is unknown. In this study, NIEHS scientists generated mice with alveolar type II (ATII) cell-specific knockdown of COX-2 (AT2CC-/-) to examine the role of ATII cell-derived prostaglandins in these processes. Specific knockdown of COX-2 was confirmed by real-time RT-PCR and western blot analyses. LC/MS/MS analysis showed that ATII cells produced prostaglandins. Basal airway responsiveness of AT2CC-/- mice was decreased compared to that of wild-type mice. LPS-induced hypothermic response, infiltration of inflammatory cells into the airway, and lung inflammation were enhanced in AT2CC-/- mice relative to wild-type controls; however, LPS-induced AHR and pro-inflammatory cytokine/chemokine expression were similar between the genotypes. After 21 days of bleomycin administration, AT2CC-/- mice behaved in a similar manner to wild-type mice. Thus, ATII cell-derived COX-2 plays an important role in regulating basal airway function and LPS-induced lung inflammation, but does not play a role in bleomycin-induced fibrosis. These findings provide insight into the cellular source of COX-2 related to these lung phenotypes.

Cheng J, Dackor RT, Bradbury JA, Li H, DeGraff LM, Hong LK, King D, Lih FB, Gruzdev A, Edin ML, Travlos GS, Flake GP, Tomer KB, Zeldin DC. Contribution of alveolar type II cell-derived cyclooxygenase-2 to basal airway function, lung inflammation, and lung fibrosis. *FASEB J*., 30:160-173, 2016.

Nuclear Localization of the DNA Repair Scaffold XRCC1: Uncovering the Functional Role of a Bipartite NLS
The human DNA repair system provides a major basis for protection against the toxicity of environmental agents. Efficient DNA repair is dependent on the intranuclear assembly of

damage-dependent repair complexes. Since proteins are generally not synthesized in the nucleus, DNA repair is dependent on nuclear localization of these repair proteins. During the past year NIEHS investigators characterized the nuclear localization signal (NLS) of XRCC1 structurally using X-ray crystallography and functionally using fluorescence imaging. Crystallography and binding studies revealed the bipartite nature of the XRCC1 NLS interaction with Importin α (Impα) in which the major and minor binding motifs are separated by >20 residues, and resolved previous inconsistent determinations. Binding studies of peptides containing the bipartite NLS, as well as its major and minor binding motifs, to both wild-type and mutated forms of Impα revealed pronounced cooperative binding behavior that is generated by the proximity effect of the tethered major and minor motifs of the NLS. This cooperativity stems from the increased local concentration of the second motif near its cognate binding site that is a consequence of the stepwise binding behavior of the bipartite NLS. Conversely, the stepwise dissociation of the NLS from Impα facilitates unloading by providing a partially complexed intermediate that is available for competitive binding by Nup50 or the Importin β binding domain. This behavior provides a basis for meeting the intrinsically conflicting high affinity and high flux requirements of an efficient nuclear transport system.

Kirby TW, Gassman NR, Smith CE, Pedersen LC, Gabel SA, Sobhany M, Wilson SH, London RE. Nuclear Localization of the DNA Repair Scaffold XRCC1: Uncovering the Functional Role of a Bipartite NLS. *Sci. Rep.*, 5:13405, 2015.

Steroidogenic Factor 1 (SF1) impacts uterine gland formation in a mouse model of endometriosis

SF1 regulates the expression of enzymes that produce estrogen and help the uterine explants grow independent of estrogen from the ovaries. In a novel mouse model the expression of SF1 in the mouse endometrium promoted the development of enlarged endometrial glands and interfered with steroid hormone regulation of the uterus helping the growth of ectopic endometriotic lesions in a mouse model of endometriosis. Uterine-specific SF1-regulated genes involved in gland development and epithelium-stroma interaction were identified. The present results indicate that SF1 directly contributes to the abnormal uterine gland morphogenesis, an inhibition of steroid hormone signaling and activation of an immune response, in addition to previously postulated estrogen production.

Vasquez YM, Wu SP, Anderson ML, Hawkins SM, Creighton CJ, Ray M, Tsai SY, Tsai MJ, Lydon JP, DeMayo FJ. Endometrial Expression of Steroidogenic Factor 1 Promotes Cystic Glandular Morphogenesis. *Mol. Endocrinol.,* 30:518-532, 2016.

The powerhouse of the cells has other essential functions than energy production

Mitochondria are organelles recognized primarily as the major energy producers in the cells. Using genetic models to manipulate mitochondrial function, our study identified two essential functions of mitochondria that do not involve energy production: the tricarboxylic acid cycle and the maintenance of the membrane potential. It was found that the former is necessary to maintain the epigenome, which can control how genes are expressed, and that the latter is required for cell proliferation. This work sets the stage to better understand how mitochondrial function affects the cell and organismal biology beyond its role in generating energy.

Martínez-Reyes I, Diebold LP, Kong H, Schieber M, Huang H, Hensley CT, Mehta MM, Wang T, Santos JH, Woychik R, Dufour E, Spelbrink JN, Weinberg SE, Zhao Y, DeBerardinis RJ, Chandel NS. TCA Cycle and Mitochondrial Membrane Potential Are Necessary for Diverse Biological Functions. *Mol. Cell*, 61:199-209, 2016.

Environmental and other factors at illness onset may be associated with chronic autoimmune muscle diseases in children

The objectives of this investigation were to determine predictors of disease course for a group of rare childhood systemic autoimmune diseases called juvenile idiopathic inflammatory myopathies, in which the immune system attacks skeletal muscle, skin and other organ systems. Approximately one-third of children with juvenile myositis appear to recover fully within two years of diagnosis with adequate immunosuppressive therapy, one-third develop a relapsing-remitting course of illness, and one-third have a chronic course of illness that is prolonged beyond two years, often lasting many years despite adequate therapies. Factors associated with the chronic or relapsing courses of illness included the p155/140 myositis autoantibody, the most frequently identified autoantibody present in the sera of patients with juvenile myositis. Additional factors associated with a chronic illness course included a documented infection within 6 months of illness onset, as well as severe illness at onset and a higher clinical severity score. Additional associations with a chronic course compared to a monocyclic course, that were not as strongly associated, included certain photosensitive skin rashes as well as the ultraviolet index based in the month prior to diagnosis on residential location. These findings indicate that myositis autoantibodies, in particular anti-p155/140 autoantibodies, as well as early clinical features and environmental exposures are associated with a chronic course in patients with juvenile myositis. This is the first study that found environmental factors at illness onset to be associated with the course of illness in this pediatric autoimmune muscle disease.

Habers GE, Huber AM, Mamyrova G, Targoff IN, O'Hanlon TP, Adams S, Pandey JP, Boonacker C, van Brussel M, Miller FW, van Royen-Kerkhof A, Rider LG; Childhood Myositis Heterogeneity Study Group. Brief Report: Association of Myositis Autoantibodies, Clinical Features, and Environmental Exposures at Illness Onset With Disease Course in Juvenile Myositis. *Arthritis Rheumatol.,* 68:761-768, 2016.

Analyses of a new major allergen from house dust mites called Der p 23.

A newly discovered dust mite allergen called Der p 23 was assessed in an American population for the first time, and biochemically characterized. Surprisingly the percentage of patients with antibodies to Der p 23 is extremely high, on a par with the two other major allergens from mites, Der p 1 and Der p 2. The total antibody response to Der p 23 accounts for a small percentage of the IgE response to mite allergens. Curiously, the prevalence and amount of specific IgE to Der p 23 is disproportionately high compared to the abundance of other Dermatophagoides allergens. This study helps understand the immunological and biochemical characteristics of different mite allergens that skew the immune response towards allergy.

Mueller GA, Randall TA, Glesner J, Pedersen LC, Perera L, Edwards LL, DeRose EF, Chapman MD, London RE, Pomés A. Serological, genomic and structural analyses of the major mite allergen Der p 23. *Clin. Exp. Allergy*, 46:365-376, 2016.

The best biomarker of oxidative stress

Oxidative stress is elevated in numerous environmental exposures and diseases. Millions of dollars have been spent to try to ameliorate this damaging process using anti-oxidant therapies. Currently, the best accepted biomarker of oxidative stress is the lipid oxidation product 8-iso-prostaglandin F2α (8-iso-PGF2α), which has been measured in over a thousand human and animal studies. The results in this manuscript demonstrate two sources of 8-iso-PGF2α formation in vivo. The sources of inflammation and oxidative stress can be distinguished by measuring the 8-iso-PGF2α/PGF2α ratio in plasma. The results presented in our study further challenge the long held notion that 8-iso-PGF2α is solely a product of chemical lipid peroxidation in vivo and is a selective marker for oxidative stress. Quantifying the contributions of both pathways provides vital mechanistic insights into diseases and environmental exposures. These mechanistic insights are imperative to correctly interpret experimental findings based on this biomarker of oxidative stress.

Van't Erve TJ, Lih FB, Jelsema C, Deterding LJ, Eling TE, Mason RP, Kadiiska MB. Reinterpreting the best biomarker of oxidative stress: The 8-iso-prostaglandin F2α/prostaglandin F2α ratio shows complex origins of lipid peroxidation biomarkers in animal models. *Free Radic. Biol. Med.*, 95:65-73, 2016.

Widespread use of an RNA binding domain throughout evolution

Mammals express a small family of three or four proteins, depending on the species, that bind to messenger RNA and promote its rapid decay, thus providing a brake on gene expression. These proteins are involved in the control of gene expression in the inflammatory response to environmental stimuli such as infectious agents, and also in the regulation of blood formation and fetal nutrition during pregnancy. Wells and colleagues found that the RNA binding domains of these proteins, which are responsible for the first step in their RNA decay promoting function, can be found in almost all eukaryotes, including plants, which last shared a common ancestor with man more than a billion years ago. Moreover, domains from plants, humans, insects and yeasts seem to be interchangeable in functional assays, arguing that this recognition motif has persisted essentially unchanged for more than a billion years.

Wells ML, Hicks SN, Perera L, Blackshear PJ. Functional equivalence of an evolutionarily conserved RNA binding module. *J. Biol. Chem.*, 290:24413-24423, 2015.

Functional rescue of tumor associated p53 mutants enhance immune and apoptosis pathways mediated by TLR3.

The innate immune Toll like receptors (TLRs) proteins, which provide front-line protection against pathogens, assure rapid inflammatory responses. In response to common anticancer agents, the tumor suppressor and transcription factor, p53 upregulate most members of TLR gene family and consequently enhance TLR-dependent production of pro-inflammatory cytokines in various human immune-related primary cells as well as cancer-derived cells. p53 is the most frequent gene mutated in human cancers. The impact of p53 mutants in TLR gene expression was evaluated. While many mutants retained the ability to drive TLR expression at WT levels, others exhibited null, limited, or change-of-spectrum transactivation of TLR genes. Using TLR3 signaling as a model, it was shown that some cancer-associated p53 mutants amplify cytokine, chemokine and apoptotic responses after stimulation by the TLR3 cognate ligand. Functional

rescue of loss-of-function p53 mutants by the p53 reactivating drug RITA restored TLR3 gene expression in a mutant p53 cell lines and also enhanced DNA damage induced-apoptosis via TLR3 signaling. We propose that chemotherapeutic manipulation of normal or mutantp53 responses along with immune challenges that include TLRs could enhance inflammatory/immune type responses to environmental factors.

Menéndez D, Lowe JM, Snipe J, Resnick MA. Ligand dependent restoration of human TLR3 signaling and death in p53 mutant cells. *Oncotarget*, (in press)

Interactions Between Mammalian WDR12 and Midasin

Researchers from NIEHS determined the crystal structure of the ubiquitin-like (UBL) domain of Ytml, from *Saccharomyces cerevisiae*, which is a homologue of the mammalian ribosomal protein WD repeat domain 12 (WDR12). Ytm1 is an essential ribosome assembly factor that associates with two other assembly factors, Nop7 and Erb1 to form the Nop7 complex, which is required for maturation of the large ribosomal subunit. Ribosomes carry out the fundamental role of making proteins within living systems, so this research has important implications for understanding how ribosomes are assembled. Crystallographic studies revealed that the UBL domain of Ytm1 is structurally homologous with the UBL domain of another ribosome assembly protein Rsa4, which is the yeast homolog of mammalian Notchless (Nle1). Subsequent binding studies revealed that the metal ion-dependent adhesion site (MIDAS) domain of Midasin binds to both WDR12 and Nle1 through their UBL domains. This interaction is dependent upon metal ion-coordination and a well-conserved extension region within the MIDAS domain. These studies demonstrate that the interactions between the UBL domains of WDR12 and Nle1 with Midasin are evolutionarily conserved in the eukaryotic ribosome assembly pathway. Moreover both WDR12 and Nle1 have been linked to human health implications and are possible novel targets for therapeutic intervention.

Romes EM, Sobhany M, Stanley RE. The crystal structure of the ubiquitin-like domain of ribosome assembly factor Ytm1 and characterization of its interaction with the AAA-ATPase midasin. *J. Biol. Chem.,* 291:882-893, 2016.

How cells achieve high accuracy of chromosomal DNA replication

The accuracy of DNA replication is a crucial factor for the mechanisms by which cells and organisms produce mutations. To gain understanding in this area NIEHS scientists are studying the accuracy (fidelity) of DNA replication in the bacterium *Escherichia coli*, which is a useful model system for these questions. The bacterial chromosome is replicated by the DNA polymerase III holoenzyme (HE), whose accuracy we have studies in detail. But in addition, other DNA polymerases play a role (*E. coli* has five such accessory DNA polymerases) and they also affect the overall error rate. The important role of the 5'-deoxynucleoside-triphosphates (dNTPs), which are the building blocks used by the polymerases for synthesizing DNA, has also been demonstrated.

Tse L, Kang TM, Yuan J, Mihora D, Becket E, Maslowska KH, Schaaper RM, Miller JH. Extreme dNTP pool changes and hypermutability in dcd ndk strains. *Mutat Res.*, 784-785:16-24, 2016.

Singh D, Schaaper RM, Hochkoeppler A. A continuous spectrophotometric enzyme-coupled assay for deoxynucleoside triphosphate triphosphohydrolases. *Anal. Biochem.*, 496:43-49, 2016.

Itsko M, Schaaper RM. Transcriptome Analysis of Escherichia coli during dGTP Starvation. *J. Bacteriol.*, 198:1631-1644, 2016.

Glis3 is essential for spermatogenesis

In this study, a novel and essential role for Glis3 in the regulation of the dynamics early postnatal spermatogenesis was identified. Glis3 is expressed in spermatogonial stem and progenitor cells and loss of GLIS3 functions affects self-renewal and prevents further differentiation and maturation of spermatocytes.

Kang HS, Chen LY, Lichti-Kaiser K, Liao G, Gerrish K, Bortner CD, Yao HH, Eddy EM, Jetten AM. Transcription Factor GLIS3: a New and Critical Regulator of Postnatal Stages of Mouse Spermatogenesis. *Stem Cells*, epub ahead of print, doi: 10.1002/stem.2449.

Muscle gene expression patterns in autoimmune muscle disease can predict responses to some biologic therapies.

Autoimmune muscle diseases (myositis) comprise a group of complex autoimmune disorders influenced by genetic and environmental factors. To identify if the gene expression patterns in muscle of these patients can predict clinical responses to a biologic agent called rituximab, which blocks B lymphocyte responses, the investigators examined muscle biopsies from before and after treatment. Myeloid type I interferon (IFN) signature genes were expressed at higher levels at baseline in the skeletal muscle of rituximab responders than in non-responders, whereas classic non-myeloid IFN signature genes were expressed at higher levels in non-responders at baseline. The decrease in the type I IFN signature following administration of rituximab was also associated with the decreases in muscle-infiltrating CD19+ B cells and CD68+ macrophages in responders. These data add further evidence for defining the type I IFN signature as both a predictor of therapeutic responses and a biomarker of myositis disease activity.

Nagaraju K, Ghimbovschi S, Rayavarapu S, Phadke A, Rider LG, Hoffman EP, Miller FW. Muscle myeloid type I interferon gene expression may predict therapeutic responses to rituximab in myositis patients. *Rheumatology (Oxford)*, epub ahead of print, doi: 10.1093/rheumatology/kew213.

Structure determined of a nuclease from Streptococcus pyogenes that enhances virulence

S. pyogenes is a leading cause of severe invasive disease in humans including toxic shock syndrome and necrotizing fasciitis ("flesh eating bacteria") that leads to over 160,000 deaths a year worldwide. In this study NIEHS researchers determined crystal structures of a nuclease (Sda1) from *S. pyogenes* that enhances the virulence of the bacteria by allowing it to escape from the host immune response. Understanding how Sda1 functions may help in the rational design of drugs that specifically target *S. pyogenes* and do not kill symbiotic bacteria in the gut.

Moon AF, Krahn JM, Lu X, Cuneo MJ, Pedersen LC. Structural characterization of the virulence factor Sda1 nuclease from Streptococcus pyogenes. *Nucleic Acids Res.*, 44:3946-3957, 2016.

The design and exploitation of metabolically-stable surrogates of highly reactive signaling entities.
NIEHS scientists developed and validated the development of stable analogues of an intracellular signal that mediates environmental stress responses; these reagents greatly aid further research in environmental health science.

Riley AM, Wang H, Shears SB, L Potter BV. Synthetic tools for studying the chemical biology of InsP8. *Chem. Commun. (Camb).*, 51:12605-12608, 2015.

RORγ antagonists: potential therapy of autoimmune disease
RORγ is critical for Th17 cell differentiation and IL-17 production, both of which play a critical role in autoimmune disease. In collaboration with GSK, NIEHS investigators have characterized several RORγ antagonists. These antagonists inhibit Th17 differentiation and activation of the IL17 promoter. Treatment of psoriatic-like skin lesions of imiquimod-treated mice as well as of skin biopsy explant cultures from psoriatic patients with RORγ antagonists reduced inflammation and the expression of psoriasis signature genes. Based on these data, it is expected that topical delivery of this RORγ-specific inverse agonist will impact local cytokine expression, leading to clinical improvement in psoriasis patients.

Smith SH, Peredo CE, Takeda Y, Bui T, Neil J, Rickard D, Millerman E, Therrien JP, Nicodeme E, Brusq JM, Birault V, Viviani F, Hofland H, Jetten AM, Cote-Sierra J. Development of a Topical Treatment for Psoriasis Targeting RORγ: From Bench to Skin. *PLoS One*, 11:e0147979, 2016.

House dust programs pulmonary dendritic cells to promote type 2 T-cell responses.
Common house dust contains many allergens and other products that, when inhaled, can lead to allergic asthma. A type of cell known as the dendritic cell is thought to be essential for allergic sensitization, but the molecular and cellular mechanisms that affect the actions of dendritic cells are poorly understood. Moran and colleagues have demonstrated that inhaled house dust extracts (HDEs) indirectly activate dendritic cells. Thus, bronchoalveolar lavage fluid from HDE-exposed mice activated lung dendritic cells to induced robust differentiation of T helper 2 cells, which have been linked to allergic asthma. By contrast, direct exposure of dendritic cells to HDE did not have this effect. This indirect reprogramming of dendritic cells was associated with the upregulation of several molecules, whose requirement will be investigated in future studies. Identifying these factors could lead to novel therapeutic targets for allergic asthma.

Moran TP, Nakano K, Whitehead GS, Thomas SY, Cook DN, Nakano H. Inhaled house dust programs pulmonary dendritic cells to promote type 2 T-cell responses by an indirect mechanism. *Am. J. Physiol. Lung Cell. Mol. Physiol.,* 309:L1208-L1218, 2015.

DNA research offers clues on cell mutations
NIEHS researchers studying how mutations arise in DNA discovered that misincorporation of RNA (ribonucleotides) into DNA occurs frequently and has important effects on the health of the cell. Failure to remove ribonucleotides results in mutations and rearrangement of genetic

information. These discoveries may have implications for human diseases that include autoimmune disorders and cancer.

Conover HN, Lujan SA, Chapman MJ, Cornelio DA, Sharif R, Williams JS, Clark AB, Camilo F, Kunkel TA, Argueso JL. Stimulation of Chromosomal Rearrangements by Ribonucleotides. *Genetics*, 201:951-961, 2015.

A novel analytical strategy to identify fusion transcripts between repetitive elements and protein coding-exons

Repetitive elements (REs) comprise 40-60% of the mammalian genome and were initially thought to be 'junk' DNA. More recently, they have been shown to influence the expression of genes in various ways, including by the formation fusion transcript (FTs) with adjacent protein coding genes. NIEGS investigators developed a bioinformatics method to predict FTs from Next Generation sequencing tools. Using data from a study in which mice were exposed to cocaine, the investigators found 438 genes that express FTs with different types of repeats. This new method will help to facilitate additional studies to better understand the functional role of FTs in the biology of organisms.

Wang T, Santos JH, Feng J, Cahill M, Fargo DC, Shen L, Nestler EJ, Woychik RP. A novel analytical strategy to identify fusion transcripts between repetitive elements and protein coding-exons using RNA-seq. *PLoS One*, 11:e0159028, 2016.

Structural insight into the protein involved in generating high affinity antibodies.

Activation-Induced Deoxycytidine Deaminase (also known as AID) plays an important role in antibody diversification that leads to high affinity antibodies by initiating two critical processes called somatic hypermutation and class-switch recombination. Disruption of the AID gene leads to HIGM-2 syndrome a disorder that results in defects in class-switch recombination. In this study NIEHS scientists determined the first crystal structure of human AID that helps understand how various mutations in AID leads to disease as well as to better understand how AID engages DNA to convert the cytosine base to uracil resulting in mutations.

Pham P, Afif SA, Shimoda M, Maeda K, Sakaguchi N, Pedersen LC, Goodman MF. Structural analysis of the activation-induced deoxycytidine deaminase required in immunoglobulin diversification. *DNA Repair (Amst).*, 43:48-56, 2016.

Spatiotemporal expression of GLIS3 during pancreatic development

The transcription factor Glis-similar 3 (Glis3) has been implicated in the development of neonatal, type 1 and type 2 diabetes. This study demonstrates that Glis3 protein exhibits a temporal and cell type-specific pattern of expression during embryonic and neonatal pancreas development that is consistent with a regulatory role for Glis3 in promoting endocrine progenitor generation, regulating insulin and Ppy expression in beta and PP cells, respectively, and duct morphogenesis. The findings of the study will help us to better understand the functions of Glis3 in the pancreas as well as in diabetes.

Kang HS, Takeda Y, Jeon K, Jetten AM. The Spatiotemporal Pattern of Glis3 Expression Indicates a Regulatory Function in Bipotent and Endocrine Progenitors during Early Pancreatic Development and in Beta, PP and Ductal Cells. *PLoS One*, 11:e0157138, 2016.

A 'futile' protein: an enzyme that degrades what it makes.
NIEHS investigators characterized a highly unusual dual domain signaling protein, which hosts a phosphatase domain that degrades the product formed by a separate kinase domain. The signaling activities are regulated by an oxygen-sensitive iron-sulfur cluster, so the protein's functionality is likely sensitive to oxygen stress bought on by environmental insults.

Wang H, Nair VS, Holland AA, Capolicchio S, Jessen HJ, Johnson MK, Shears SB. Asp1 from Schizosaccharomyces pombe binds a $[2Fe\text{-}2S]^{2+}$ cluster which inhibits inositol pyrophosphate 1-phosphatase activity. *Biochemistry,* 54:6462-6474, 2015.

Improving Potency Estimating in High-Throughput Screening Experiments Using an Information Theoretic Approach
Quantitative high-throughput screening (qHTS) data typically includes concentration-response profiles for thousands of chemicals generated in a single experiment. The concentration for half-maximal activity in the Hill equation model (i.e., AC50) is the most common potency metric in these data sets. However, the AC50 parameter is subject to large uncertainties. In this study, a new potency measure is introduced, based on weighted Shannon entropy. The new potency measure does not depend on the assumption of a pre-specified concentration-response relationship and estimates potency with greater precision and less bias compared to the conventional AC50 value.

Shockley KR. Estimating Potency in High-Throughput Screening Experiments by Maximizing the Rate of Change in Weighted Shannon Entropy. *Sci. Rep.*, 6:27897, 2016.

Loss of Glis2/NPHP7 causes kidney epithelial cell senescence
Loss of Glis2 causes progressive kidney atrophy, interstitial inflammatory infiltration, and fibrosis, and is associated with induction of cell senescence. The cystic phenotype in Kif3a deficient mice is rescued by the loss of Glis2.

Lu D, Rauhauser A, Li B, Ren C, McEnery K, Zhu J, Chaki M, Vadnagara K, Elhadi S, Jetten AM, Igarashi P, Attanasio M. Loss of Glis2/NPHP7 causes kidney epithelial cell senescence and suppresses cyst growth in the Kif3a mouse model of cystic kidney disease. *Kidney Int.*, 89:1307-1323, 2016.

Biomarkers for oxidative stress responses induced by Zinc in human cells
Determining mechanism-based biomarkers that distinguish adaptive and adverse cellular processes is critical to understanding the health effects of environmental exposures. NIEHS researchers characterized oxidative stress responses of the tracheobronchial airway to a model oxidant, zinc (Zn), and explored the genomic mechanisms mediating the switch from recoverable adaptive cellular response to adverse events. Normal, adaptive, and cytotoxic Zn exposure conditions were determined with traditional apical endpoints, and differences in global gene expression around the tipping point of the responses were used to delineate underlying molecular

mechanisms. The researchers then identified candidate genes for use as early biomarkers in differentiating adaptive and adverse cellular responses. Bioinformatic analyses of differentially expressed genes indicate early enrichment of stress signaling pathways, including those mediated by the transcription factors p53 and NRF2.

Currier JM, Cheng WY, Menéndez D, Conolly R, Chorley BN. Developing a Gene Biomarker at the Tipping Point of Adaptive and Adverse Responses in Human Bronchial Epithelial Cells. *PLoS One*, 11:e0155875, 2016.

An ion channel required for male fertility.
NIEHS researchers discovered a gene required for male fertility. This gene codes for an ion channel, a molecule that allows calcium ions to enter cells. When this gene is mutated or absent, sperm fail to develop in the testis. Modification of calcium channels in the testis by drugs may provide a novel strategy to enhance or regulate male fertility.

Davis FM, Goulding EH, D'Agostin DM, Janardhan KS, Cummings CA, Bird GS, Eddy EM, Putney JW. Male infertility in mice lacking the store-operated Ca^{2+} channel Orai1. *Cell Calcium*, 59:189-197, 2016.

A mechanism for regulating transfer of a human signaling protein to the cell nucleus.
Specificity in cell signaling can be brought about by regulating access of a signaling entity to the specific cell compartment in which it is active. Here, NIEHS scientists identify a pentapetide 'localization' sequence that directs the PPIP5K2 signaling enzyme to the nucleus. They further demonstrate that the localization sequence is not functional by default; it's activity is switched on and off by a protein phosphorylation event.

Yong ST, Nguyen HN, Choi JH, Bortner CD, Williams J, Pulloor NK, Krishnan MN, Shears SB. Identification of a functional nuclear translocation sequence in hPPIP5K2. *BMC Cell. Biol.,* 16:17, 2015.

New statistical method for uncovering genes affecting multiple disease-related traits
Scientific evidence suggests that intricate interactions of genetic risk factors with environmental exposures play a major role in the development of diseases. In studies of relative contribution of an individual's genetic composition to disease susceptibility and progression, multiple traits can be measured and analyzed jointly. These traits may share common etiology and comprise binary, categorical, and quantitative measurements. NIEHS researchers propose a statistical method for simultaneous testing of multiple correlated phenotypes, including quantitative binary, categorical or a combination thereof, with the flexibility of adjusting for other covariates. The approach is unique in providing a nuanced depiction of genetic effects and interactions via association curves fit over genetic regions.

Vsevolozhskaya OA, Zaykin DV, Barondess DA, Tong X, Jadhav S, Lu Q. Uncovering Local Trends in Genetic Effects of Multiple Phenotypes via Functional Linear Models. *Genet. Epidemiol.*, 40:210-221, 2016.

Improved methods developed to support human studies of perfluoroalkly substance toxicity

Studies have reported a number of potentially toxic effects from exposure to perfluoroalkyl substances (PFAS), even at the low levels encountered by the general public. To support more detailed investigations of health effects among children, a pharmacokinetic model of PFAS concentrations in child blood was developed, which is based on maternal blood measurements and history of child breastfeeding.

Verner MA, Ngueta G, Jensen ET, Fromme H, Völkel W, Nygaard UC, Granum B, Longnecker MP. A Simple Pharmacokinetic Model of Prenatal and Postnatal Exposure to Perfluoroalkyl Substances (PFASs). *Environ. Sci. Technol.,* 50:978-986, 2016.

Gene Expression Changes in the Nasal Cavity after Exposure to the Carcinogenic Compound N,N-dimethyl-p-toluidine (DMPT).

N,N-dimethyl-p-toluidine (DMPT) is an accelerant for methyl methacrylate monomers in medical devices and a nasal cavity carcinogen in a two-year cancer study of F344/N rats. Early changes in the nasal cavity were explored here after short-term DMPT exposure. NIEHS scientists found gene expression transcription patterns characteristic of an antioxidative damage response, cell proliferation and decreasing apoptosis in nasal tissue. These molecular changes suggest that oxidative damage in the nasal cavity is a mechanism of DMPT damage and/or carcinogenic effects.

Dunnick JK, Merrick BA, Brix A, Morgan DL, Gerrish K, Wang Y, Flake G, Foley J, Shockley KR. Molecular Changes in the Nasal Cavity after N, N-dimethyl-p-toluidine Exposure. *Toxicol. Pathol.*, epub ahead of print, doi: 10.1177/0192623316637708.

Two novel methods and a software package to facilitate epigenome-wide association studies

The Illumina Methylation BeadChip is widely used in epigenome-wide association studies to search for human disease related DNA CpG sites, however, this array-based measurement is subject to strong background noise and systematic bias caused by array design and experimental procedures. NIEHS investigators developed two novel methods to reduce background noise and correct for probe-type bias. The new methods greatly improved signal to noise ratios. They incorporated the methods into a software package ENmix, which is freely available from Bioconductor website.

Xu Z, Niu L, Li L, Taylor JA. ENmix: a novel background correction method for Illumina HumanMethylation450 BeadChip. *Nucleic Acids Res.,* 44:e20, 2016.

Niu L, Xu Z, Taylor JA. RCP: a novel probe design bias correction method for Illumina Methylation BeadChip. *Bioinformatics*, epub ahead of print, doi: 10.1093/bioinformatics/btw285