Division of Intramural Research

NAEHS Council Update

September 2014

DIR RECRUITMENTS

Director, Clinical Research Program
The NIEHS is searching for a senior investigator to direct its Clinical Research Program. The Director, Clinical Research Program, is responsible for the development, administration, coordination and oversight of investigator-initiated clinical research; provides general advice to the Director and Scientific Director, NIEHS, on matters relating to human and clinical studies; supervises the Office of Research Compliance; and develops policies and programs for the execution of clinical research at NIEHS. The Clinical Director is responsible for creating and maintaining a research environment in which clinical findings influence the direction of laboratory studies and laboratory findings are applied back to the clinical and clinical research communities. The incumbent will facilitate intramural clinical research by identifying opportunities for translating basic science into clinical studies. The Clinical Director will ensure that Institute research reflects the highest standards of scientific excellence and ethical conduct for the protection of human subjects. The incumbent will review matters pertaining to the provision of patient care in research protocols and oversee research allocation, scientific review, and recruitment of staff. The Clinical Director will provide advice and training on the conduct of clinical studies, facilitate clinical research collaborations between intramural and extramural investigators, and develop long-range clinical research goals and objectives relevant to the mission of NIEHS. It is expected that the successful candidate will oversee a personal clinical research program that will involve some combination of outpatient oriented studies within the Clinical Research Unit, epidemiological studies, basic laboratory studies, or inpatient studies at the Clinical Research Center in Bethesda. Emphasis will be placed upon investigators with a primary research interest in clinical research; however, the selected candidate may have a modest independent basic laboratory research program, particularly if the basic research intersects with the candidate's clinical studies. Dr. Carter Van Waes, Clinical Director, National Institute on Deafness and Other Communication Disorders is chair of the search committee. A Candidate has been identified.

Investigators in the areas of Molecular and Cellular Signaling, Neuroscience, and Developmental or Reproductive Biology
The National Institute of Environmental Health Sciences is recruiting for multiple full-time appointments at either the tenure-track or tenure-eligible level in the areas of Molecular and Cellular Signaling, Neuroscience, and Developmental or Reproductive Biology. The successful candidates are expected to lead innovative, independent research programs on any fundamental aspect of research in the above three areas of interest which form a basis for understanding the effects of the environment on human health. Applicants should have a Ph.D., M.D. or equivalent doctoral degree with at least 3 years of postdoctoral research experience in their field and an outstanding publication record. The emphasis will be on identifying exceptional scientists with innovative and productive research programs. Evaluation of applications has begun. Dr. Trevor Archer, Chief, Laboratory of Molecular Carcinogenesis, is chair of the search committee. Candidates have been identified.

Investigators in the areas of Biostatistics, Bioinformatics, and Computational Biology
The National Institute of Environmental Health Sciences is recruiting for multiple full-time appointments at either the tenure-track or tenure-eligible level in the areas of biostatistics,

bioinformatics, and computational biology. The successful candidate will develop and direct a high-quality, independent research program on analytic methods applicable to understanding the effects of the environment on human health. Applicants should have a Ph.D. or equivalent doctoral degree and a proven record in the development of analytic methods with biomedical application. Experience working with epidemiologic or high dimensional genetic, epigenetic, and genomic datasets is highly desirable, but not required. Evaluation of applications has begun. Dr. Jack A. Taylor, Epidemiology Branch and Laboratory of Molecular Carcinogenesis, is chair of the search committee. A tenure-track and tenure-eligible candidate have been identified.

NEW HIRES AND CHANGES IN DIR LEADERSHIP

Clinical Director
Dr. Janet Hall has tentatively accepted our offer to be Clinical Director at NIEHS. Dr. Hall is currently a Professor of Medicine at Harvard University and serves as Associate Chief of the Reproductive Endocrine Unit at Massachusetts General Hospital. Dr. Hall received her M.D. in 1981 from McMaster University, completed her residency in Internal Medicine at McMaster University and an Endocrinology fellowship at Massachusetts General Hospital. She is an international recognized physician-scientist who studies human reproductive physiology and pathophysiology with a view to translating this information to benefit women with reproductive disorders. She is the Past-President of the Endocrine Society which has over 17,000 members worldwide. She also has served on numerous NIH Special Emphasis Panels and numerous Editorial Boards. Dr. Hall is expected to begin work at NIEHS on September 21, 2014.

Earl Stadtman Tenure-Track Investigators
Dr. Robin Stanley from the Laboratory of Molecular Biology at National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD, has been offered a tenure-track position in DIR at NIEHS. Dr. Stanley studies the regulation of autophagy and ribosome biogenesis by the target of rapamycin (TOR) signal transduction pathway. Dr. Stanley has accepted our offer and will have a primary appointment in the Signal Transduction Laboratory and a secondary appointment in the Genomic Integrity and Structural Biology Laboratory. Dr. Stanley started at NIEHS July 28, 2014.

Biostatistics, Bioinformatics, and Computational Biology
Dr. Shanshan Zhao from the Fred Hutchinson Cancer Research Center, Seattle, WA, has been offered a tenure-track position in DIR at NIEHS. Dr. Zhao has statistical interests in mediation analysis, error, and high dimensional data. Dr. Zhao has accepted our provisional tenure-track offer. She will have a primary appointment in the Biostatics and Computational Biology Branch and a secondary appointment in the Epidemiology Branch. Her tentative start date is January 1, 2015.

Dr. Ruth Pfeiffer, from the Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda, MD, is a candidate for a tenure-eligible position in the Biostatistics and Computational Biology Branch. She is currently in discussions with the Scientific Director. Her statistical research interests are in development of methods for family data accounting for ascertainment, mixture models and application, methods for analysis of high dimensional data, absolute risk modeling and power considerations for association studies.

Molecular and Cellular Signaling, Neuroscience, and Developmental or Reproductive Biology
Dr. Dante Bortone from the Center for Neural Circuits and Behavior, Howard Hughes Medical Institute, University of California-San Diego, La Jolla, CA, has been offered a tenure-track position in DIR at NIEHS. Dr. Bortone studies the developmental origin of neurons in the visual cortex. Dr. Bortone has accepted our provisional tenure-track offer. He will have a primary appointment in the Neurobiology Laboratory and a secondary appointment in the Reproductive and Developmental Biology Laboratory. His tentative start date is in the Spring 2015.

Dr. Guohong Cui from the Laboratory of Integrative Neuroscience, National Institute on Alcohol Abuse and Alcoholism, NIH, Rockville, MD, has been offered a tenure-track position in DIR at NIEHS. Dr. Cui studies the development and function of brain circuits involved in reward processing and locomotion. Dr. Cui has accepted our provisional tenure-track offer. He will have a primary position in the Neurobiology Laboratory and a secondary appointment in the Reproductive and Developmental Biology Laboratory. His tentative start date is October 1, 2014.

Dr. Jennifer Martinez from the Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN, has been offered a tenure-track position in DIR at NIEHS. Dr. Martinez studies how cells of the innate immune system process extracellular material, and how these events affect subsequent immune responses. Dr. Martinez has accepted our provisional tenure-track offer. She will have a primary appointment in the Immunity, Inflammation and Disease Laboratory and a secondary appointment in the Signal Transduction Laboratory. Her tentative start date is March 1, 2015.

Dr. Anant Parekh from the Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK, is a candidate for a tenure-eligible position in the Signal Transduction Laboratory. He is currently in discussions with the Scientific Director. Dr. Parekh's research addresses fundamental questions in intracellular calcium signaling, plasma membrane CRAC channels and systems physiology with the aim of providing new molecular insight into the patho-physiology of nasal polyposis and allergies including atopic dermatitis and asthma, immune disorders.

Dr. Francesco DeMayo from the Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, is a candidate for a tenure-eligible position in the Reproductive and Developmental Biology Laboratory. He is currently in discussions with the Scientific Director. Dr. DeMayo investigates the molecular regulation of cellular differentiation and physiology in the lung and uterus in order to shed light on molecular pathways to aid in the diagnosis and treatment of human disease with the goal of helping the design treatments for pulmonary diseases and infertility.

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 17 winners of FARE awards:

FARE Awardee	Mentor	FARE Abstract Title
Margaret A. Adgent, Ph.D.	Walter Rogan	Urinary triclosan and enterolactone: A cross sectional study of environmental influence on gut microbiome function
Georgia M. Alexander, Ph.D.	Serena M. Dudek	Neuronal Activity in Hippocampal Area CA2 During Spatial Processing
Qing Cheng, Ph.D.	Jerrel L. Yakel	Activation of α7 nicotinic acetylcholine receptors increased intracellular cAMP levels in cultured hippocampal neurons
Senthilkumar Cinghu, Ph.D.	Raja Jothi	Nucleolin regulates the homeostatic balance between self-renewal and differentiation in embryonic stem cells
Quaker E. Harmon, M.D., Ph.D.	Allen J. Wilcox	Risk of fetal death with preeclampsia
Mallikarjuna R. Metukuri, Ph.D.	Xiaoling Li	Deletion of intestinal SIRT1 activates Paneth cells, enhances intestinal inflammation, and alters gut microbiota
Thuy-Ai T. Nguyen, Ph.D.	Michael A. Resnick	The p53 protein interactome is also a p53-regulated cistrome
Barbara Nicol, Ph.D.	Humphrey Yao	Uncovering New Paradigm in Testis Differentiation Using Mouse Genetic Models
Andrew J. Oldfield, Ph.D.	Raja Jothi	NF-Y specifies cell identity by promoting chromatin accessibility for master transcription factors at active enhancers
Matt A. Quinn, Ph.D.	John A. Cidlowski	Imbalance of endogenous glucocorticoids and estrogen leads to the development of autoimmune hepatitis like symptoms in mice
Sivapriya Ramamoorthy, Ph.D.	John A. Cidlowski	Glucocorticoid Receptor Isoform Knock-in Mice have Unique Responses to Glucocorticoids

Deirdre K. Robinson, B.S.	Suzanne E. Fenton	Assessing early developmental and pubertal effects in CD-1 mice following in utero exposure to bisphenol (BP) analogs
Natacha Steinckwich-Besancon, Ph.D.	James W. Putney	Role of the calcium sensor protein, STIM1, in neutrophil chemotaxis and infiltration into psoriatic inflamed skin
Percy Tumbale, Ph.D.	Scott Williams	Molecular Mechanism of the Aprataxin-linked Neurodegenerative Disorder - Ataxia with Oculomotor Apraxia Type 1 (AOA1)
Erica K. Ungewitter, Ph.D.	Humphrey Yao	Gli-similar 3 is a master regulator of retrotransposon silencing in male fetal germ cells
Qingshan Wang, M.D.	Jau-Shyong Hong	Ultra-low dose of diphenyleneiodonium attenuates progressive dopaminergic neurodegeneration and motor deficits in multiple rodent Parkinson's disease models
Pengyi Yang, Ph.D.	Raja Jothi	Master transcription factors establish cell type-specific transcription attenuators for rheostat control of gene expression

The NIH Pathway to Independence Award (K99/R00)

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

Kin Chan, Ph.D., received a K99/R00 grant for his proposal entitled, "Signatures of environmental carcinogen exposure within single-strand DNA." Dr. Chan will train in the Laboratory of Molecular Genetics under the mentorship of Drs. Michael Resnick, Ph.D., and Dmitry Gordenin.

Tracy M. Clement, Ph.D., received a K99/R00 grant for her proposal entitled, "Mechanisms of a Novel Actin Related Protein in Male Gametes Ensuring Fertility." Dr. Clement will train in the Laboratory of Reproductive and Developmental Toxicology under the mentorship of Dr. E. Mitch Eddy.

Bret Freudenthal, Ph.D., received a K99/R00 grant for his proposal entitled, "DNA Repair Strategies that Impact Genomic Stability During Oxidative Stress." Dr. Freudenthal will train in the Laboratory of Structural Biology under the mentorship of Dr. Samuel H. Wilson.

Anne Marie Z. Jukic, Ph.D., received a K99/R00 grant for her proposal entitled, "Vitamin D and reproduction: An investigation of human fertility and early pregnancy." Dr. Jukic will train in the Epidemiology Branch under the mentorship of Dr. Allen Wilcox.

Summer Internship Program Best Poster Awards

NIEHS takes a leadership role in science research and education. Scientists at NIEHS are committed to sharing with students the intensity, excitement, sense of discipline, and tremendous satisfaction that careers in science can impart to those who pursue them. To this end, the DIR established the Summer Internship Program for which internships are given to outstanding high school and college undergraduate students interested in pursuing careers in the biomedical/biological sciences. Participants are selected by intramural scientific mentors and spend between 8 to 12 weeks (during May through September) working on individual research projects that bring them exposure to the latest biochemical, molecular, and analytical techniques. There is a poster session at the end of the summer where participants display the results of their research efforts and respond to questions as though they were participating in a national scientific society meeting. The poster session was held on Thursday, July 24, 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>
Tanika Bantukul, Enloe High School, Laboratory of Reproductive and Developmental Toxicology; Mentors: Erica Ungewitter and Humphrey Yao; Poster Title: "Phenotypic Consequences of Mid-Gestational Exposure to Low and High Levels of Di(2-ethylhexyl) Phthalate in Male Mice"

<u>Undergraduate Intern:</u>
Emma Gierman, Elon University, Laboratory of Signal Transduction; Mentor: Perry Blackshear; Poster Title: "The Effects of Microtubule-Targeting Compounds on Tristetraprolin Production in a Murine Macrophage Cell Line"

<u>Graduate Intern:</u>
Kaitlyn Gam, Tulane University School of Public Health and Tropical Medicine, Epidemiology Branch; Mentor: Dale Sandler; Poster Title: "Oil Exposure and Pulmonary Health among Oil Spill Clean-up Workers and Non-Workers in the GuLF STUDY Cohort"

Endocrine Society FLARE Internship

The FLARE program, which launched in August 2012 by the Endocrine Society, provides training and professional development opportunities for senior graduate students, postdocs, and clinical research fellows from underrepresented groups doing hormone health research. The FLARE awards are supported by the National Institute of Diabetes and Digestive and Kidney Diseases, part of the National Institutes of Health.

Diana Cruz-Topete, Ph.D., was one of seven young scientists to receive a 2014 Future Leaders Advancing Research in Endocrinology (FLARE) internship at a joint meeting of the International Society of Endocrinology and the Endocrine Society June 21-24 in

Chicago. Dr. Cruz-Topete is a research fellow in the Molecular Endocrinology Group, Laboratory of Signal Transduction; her mentor is John Cidlowski, Ph.D.

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.

Amanda Conway, Ph.D., an IRTA fellow in the Systems Biology Group, Laboratory of Molecular Carcinogenesis, is the recipient of a 2014 NIGMS PRAT Fellowship for her project "The role of nuclear export receptor CRM1 in gene regulation"; her mentor is Raja Jothi, Ph.D.

NIEHS Postdoctoral Alumni

DIR has recently analyzed where postdoctoral trainees have gone upon completing their training, what they are doing and the level of the positions they took. Below is a summary of the analysis of 244 postdoctoral trainees that left NIEHS from July 1, 2010 through July 15, 2014.

Where did they go?

Academic Institution	101
Government Agency	36
For-profit Company	70
Non-profit Organization	9
Private Medical Practice	0
Independent/Self-Employed	0
Unknown or Undecided	27
Deceased	1
TOTAL	244

What is the level of their position?

Tenure Track faculty	50
Non-tenure Track Faculty	18
Professional Staff	105
Support Staff	10
Management	9
Trainee	24
Unknown or Undecided	27
Deceased	1
TOTAL	244

What are they doing?

Additional Postdoctoral Training	20
Internship	0
Additional Advanced Degree [1 DDS, 1 PA]	2
Primarily Teaching	12
Primarily Basic Research	65
Primarily Clinical Research	10
Primarily Clinical Practice	3
Primarily Applied Research	47
Primarily Patient Care	0
Regulatory Affairs	5
Science Administration/Project Management	7
Intellectual Property/Licensing And Patenting	2
Consulting	5
Public Policy	0
Science Writing or Communications	14
Grants Management	4
Business Development or Operations	1
Computation/Informatics	9
Sales/Marketing	3
Technical/Customer Support	6
Unknown or Undecided	27
Other (Finance Administration)	1
Deceased	1
TOTAL	244

DIR RESEARCH ACCOMPLISHMENTS FOR FY 2014

Aprataxin and threats of RNA contamination in DNA

Recent evidence indicates that transient RNA contamination in DNA exceeds all known DNA damage combined, by 1-2 orders of magnitude. However, the consequences of RNA contamination of DNA, and the identity of protein factors operating in this RNA-DNA realm to protect genomic integrity remain largely unknown. NIEHS scientists investigated effects of RNA on the critical genome maintenance step of DNA ligation, and discovered that RNA triggers ligation failure and production of RNA-DNA lesions. Aprataxin (Aptx), a protein mutated in inherited neurological disease, is required to resolve RNA-DNA derived lesions. Molecular structural analysis of human Aptx/RNA-DNA complexes define the molecular mechanism for RNA-DNA damage detection and reversal, and provide a molecular framework for understanding human APTX mutations causing the heritable neurological disease Ataxia Oculomotor Apraxia 1 (AOA1).

Tumbale P, Williams JS, Schellenberg MJ, Kunkel TA, Williams RS. Aprataxin resolves adenylated RNA-DNA junctions to maintain genome integrity. *Nature,* 506: 111-115, 2014.

Hypermutation by antiviral enzymes was detected in cancer genomes

Cancer is the disease associated with multiple forms of genome instability. Recently, in part due to efforts of NIEHS scientists, antiviral enzymes APOBEC were implicated in accidental hypermutation of chromosomal DNA. Following up this discovery the NIEHS team was invited to participate in The Cancer Genome Atlas (TCGA) analysis workgroups in the attempts to integrate the data coming from multiple TCGA platforms. They have found that APOBEC mutagenesis is the most prominent source of mutations in bladder cancers but is barely detected in another urologic cancer – chromophobe renal cell carcinomas. However, even in the latter case, APOBEC enzymes caused clusters of localized hypermutation. Identification of hypermutation causes and understanding its mechanisms can contribute into identification of cancer drivers and help in developing cancer early detection and prevention.

Cancer Genome Atlas Research Network (Gordenin DA, Roberts SA, Klimczak LJ, Fargo D). Comprehensive molecular characterization of urothelial bladder carcinoma. *Nature*, 507: 315-322, 2014.

Cancer Genome Atlas Research Network (Gordenin DA, Roberts SA, Klimczak LJ, Fargo D). The somatic genomic landscape of chromophobe renal cell carcinoma. *Cancer Cell*, in press.

New genes for lung function identified by a large global effort

A global consortium of researchers that was headed by NIEHS scientists has discovered six new loci — specific regions of the genetic code — that associate with individual variations in an important clinical metric of lung health. The findings point to previously unexplored pathways and mechanisms underlying lung function and could lead to the identification of novel therapeutic targets for lung diseases. The scientists identified six gene regions that appear to influence an individual's lung function, as measured by a widely used measurement called forced

vital capacity (FVC). FVC is used to diagnose and monitor lung diseases, and it may be severely reduced in some restrictive lung diseases such as pulmonary fibrosis, or scarring of the lung.

Loth DW, Artigas MS, Gharib SA, Wain LV, Franceschini N, Koch B, Pottinger TD, Smith AV, Duan Q, Oldmeadow C, Lee MK, Strachan DP, James AL, Huffman JE, Vitart V, Ramasamy A, Wareham NJ, Kaprio J, Wang XQ, Trochet H, Kähönen M, Flexeder C, Albrecht E, Lopez LM, de Jong K, Thyagarajan B, Alves AC, Enroth S, Omenaas E, Joshi PK, Fall T, Viñuela A, Launer LJ, Loehr LR, Fornage M, Li G, Wilk JB, Tang W, Manichaikul A, Lahousse L, Harris TB, North KE, Rudnicka AR, Hui J, Gu X, Lumley T, Wright AF, Hastie ND, Campbell S, Kumar R, Pin I, Scott RA, Pietiläinen KH, Surakka I, Liu Y, Holliday EG, Schulz H, Heinrich J, Davies G, Vonk JM, Wojczynski M, Pouta A, Johansson A0, Wild SH, Ingelsson E, Rivadeneira F, Völzke H, Hysi PG, Eiriksdottir G, Morrison AC, Rotter JI, Gao W, Postma DS, White WB, Rich SS, Hofman A, Aspelund T, Couper D, Smith LJ, Psaty BM, Lohman K, Burchard EG, Uitterlinden AG, Garcia M, Joubert BR, McArdle WL, Musk AB, Hansel N, Heckbert SR, Zgaga L, van Meurs JB, Navarro P, Rudan I, Oh YM, Redline S, Jarvis DL, Zhao JH, Rantanen T, O'Connor GT, Ripatti S, Scott RJ, Karrasch S, Grallert H, Gaddis NC, Starr JM, Wijmenga C, Minster RL, Lederer DJ, Pekkanen J, Gyllensten U, Campbell H, Morris AP, Gläser S, Hammond CJ, Burkart KM, Beilby J, Kritchevsky SB, Gudnason V, Hancock DB, Williams OD, Polasek O, Zemunik T, Kolcic I, Petrini MF, Wjst M, Kim WJ, Porteous DJ, Scotland G, Smith BH, Viljanen A, Heliövaara M, Attia JR, Sayers I, Hampel R, Gieger C, Deary IJ, Boezen HM, Newman A, Jarvelin MR, Wilson JF, Lind L, Stricker BH, Teumer A, Spector TD, Melén E, Peters MJ, Lange LA, Barr RG, Bracke KR, Verhamme FM, Sung J, Hiemstra PS, Cassano PA, Sood A, Hayward C, Dupuis J, Hall IP, Brusselle GG, Tobin MD, London SJ. Genome-wide association analysis identifies six new loci associated with forced vital capacity. *Nat. Genet.*, 46: 669-677, 2014.

Embryonic Stem Cell gene expression maintained by a chromatin remodeler

Embryonic stem cells can differentiate into all cell types found in the adult body upon stimulation, and they can also continue to proliferate as stem cells under the right culture condition. Their unique properties are maintained by a specific gene expression program. In this paper, NIEHS investigators showed that a chromatin remodeling complex, INO80, and plays a critical role in the establishment of the stem cell state by controlling the activation of embryonic stem cell-specific gene expression. They further showed that this INO80-mediated gene regulation is important in both normal embryonic development and the reprogramming of somatic cells into the induced pluripotent stem cells.

Wang L, Du Y, Ward JM, Shimbo T, Lackford B, Zheng X, Miao YL, Zhou B, Han L, Fargo DC, Jothi R, Williams CJ, Wade PA, Hu G. INO80 facilitates pluripotency gene activation in embryonic stem cell self-renewal, reprogramming, and blastocyst development. *Cell Stem Cell*, 14: 575-591, 2014

mRNA export plays important roles in Embryonic Stem Cells
The THO complex regulates mRNA export from the nucleus to the cytosol for translation. NIEHS scientists demonstrated that THO preferentially regulates the export of embryonic stem cell gene mRNAs and is required for maintaining the stem cell state, early embryonic development, and somatic cell reprogramming. Their findings revealed a novel layer of post-transcriptional gene regulation in stem cells and development.

Wang L, Miao YL, Zheng X, Lackford B, Zhou B, Han L, Yao C, Ward JM, Burkholder A, Lipchina I, Fargo DC, Hochedlinger K, Shi Y, Williams CJ, Hu G. The THO complex regulates pluripotency gene mRNA export and controls embryonic stem cell self-renewal and somatic cell reprogramming. *Cell Stem Cell*, 13: 676-690, 2013.

Obesity confers increased risk for colorectal cancer
Using a mouse model, NIEHS investigators identified molecular alterations to chromosomes at sites that regulate gene expression in obese versus lean animals. The changes occur at genomic regions involved in colorectal cancer progression. These data predict that obesity increases the probability that mutation in a key gatekeeper gene in the colon will lead to a clinically detectable tumor.

Li R, Grimm SA, Mav D, Shah R, Kosak J, Chrysovergis K, Wang X, Eling TE, Wade PA. Obesity, rather than diet, drives epigenomics alterations in colonic epithelium resembling cancer progression. *Cell Metabolism*, 19: 702-711, 2014.

Women who work with organic solvents had a greater risk for developing breast cancer
Organic solvents are ubiquitous in occupational settings where they may contribute to risks for carcinogenesis. However, there is limited information on organic solvents as human breast carcinogens. NIEHS researchers examined the relationship between occupational exposure to solvents and breast cancer in a prospective study of 47,661 women with an occupational history in the Sister Study cohort. They observed that women who started working with solvents before their first full-term birth had a greater risk for breast cancer.

Ekenga CC, Parks CG, D'Aloisio AA, DeRoo LA, Sandler DP. Breast cancer risk after occupational solvent exposure: the influence of timing and setting. *Cancer Res*., 74: 3076-3083, 2014.

How to accurately replicate huge nuclear genomes.
The nuclear genomes of eukaryotic cells are huge, yet they need to be replicated with incredibly high accuracy to maintain species identity and prevent cell death and disease. This year NIEHS investigators performed a study that provides the first ever comprehensive genome-wide view of nuclear genome replication fidelity. The results have a number of important implications for evolution and disease etiology**.**

Lujan SA, Clausen AR, Clark AB, MacAlpine HK, MacAlpine DM, Malc EP, Mieczkowski PA, Burkholder AB, Fargo DC, Gordenin DA, Kunkel TA.

Heterogeneous polymerase fidelity and mismatch repair bias genome variation and composition. *Genome Res.,* in press

Speed and accuracy of DNA polymerases

Accurate replication and repair of the genome is vital to genomic stability in all cells, and DNA polymerases provide many of the DNA synthesis and other functions that are essential in the replication and repair processes. To understand the speed and accuracy of DNA polymerases, the group applied time-lapse crystallography to study human DNA polymerase beta. This provided snapshots of structural intermediates of the enzyme as it passed through the catalytic cycle. Conformational adjustments in the polymerase and substrates that hasten correct and deter incorrect nucleotide insertion into DNA were visualized for the first time and the structures were subjected to computational analysis. Importantly, the structures revealed that the product release step is much slower than other steps. The slow product release step appears to be coupled to downstream events in DNA repair such as the hand-off to DNA ligase. When the hand-off to ligase is defective, ligase can produce a stalled repair intermediate. It was found that DNA polymerase β can tailor the block, restoring the integrity of the base excision repair pathway. Hand-off of products and substrates also was examined for the repair of the oxidized CpG dinucleotide, and important features of the metabolism of this critical dinucleotide were revealed. These results have important implications for understanding the consequences of environmental stressors on genomic stability.

Cağlayan M, Batra VK, Sassa A, Prasad R, Wilson SH. Role of polymerase β in complementing aprataxin deficiency during abasic-site base excision repair. *Nat. Struct. Mol. Biol.,* 21: 497-499, 2014.

Li Y, Freudenthal BD, Beard WA, Wilson SH, Schlick T. Optimal and variant metal-ion routes in DNA polymerase β's conformational pathways. *J. Am. Chem. Soc.*, 136: 3630-3639, 2014.

Freudenthal BD, Beard WA, Wilson SH. Watching a DNA polymerase in action. *Cell Cycle*, 13: 691-692, 2014.

Sassa A, Cağlayan M, Dyrkheeva NS, Beard WA, Wilson SH. Base Excision Repair of Tandem Modifications in a Methylated CpG Dinucleotide. *J. Biol. Chem*., doi: 10.1074/jbc.M114.557769. Epub ahead of print.

Patiently paused polymerase: waiting near the promoter for input

Gene expression is controlled by the integrated activity of multiple signaling networks that converge on numerous distinct transcription factors. However, it remained unclear how these factors work together to orchestrate gene expression programs, given the transient nature of transcription factor binding and the low probability that multiple factors would be associated with a particular gene at one time. To elucidate this phenomenon, NIEHS scientists studied the kinetic interplay between transcription factors and the transcription machinery, and found that RNA polymerase II (Pol II) recruited to a gene promote is extremely stable once it has begun transcription elongation, and can remain paused during early elongation for tens of minutes while awaiting a subsequent signal to enter productive mRNA synthesis. This extended lifetime of promoter-proximally paused Pol II provides an opportunity to span and integrate information from multiple, temporally separate signaling and/or transcription factor binding events.

Henriques T, Gilchrist DA, Nechaev S, Bern M, Muse GW, Burkholder A, Fargo DC, Adelman K. Stable pausing by RNA polymerase II provides an opportunity to target and integrate regulatory signals. *Mol. Cell*, 52: 517-528, 2013.

NF-Y specifies cell identity by orchestrating cell type-specific enhanceosome assembly

Proteins that are expressed in specific tissues (e.g., heart, liver, brain, etc.) are usually associated with functions unique to those tissues. Conversely, those that are found across all tissue types are widely thought to play general 'house-keeping' functions. NIEHS researchers report a novel and tissue-specific role for one such ubiquitously expressed protein called NF-Y. The authors demonstrate dual roles for NF-Y in house-keeping and tissue type specification depending on which regions of the DNA it interacts with. When NF-Y binds DNA closer to the gene promoters, it helps maintain the general wellness of the cell by ensuring proper proliferation (house-keeping role). In contrast, when NF-Y interacts with regions (enhancers) away from genes, its helps specify tissue identity. The authors go on to show that NF-Y plays a pioneer role by acting like a first responder to external stimuli in order to initiate the recruitment of other proteins necessary to correctly define specific tissue types. This is made possible by NF-Y's ability to facilitate access for other proteins to interact with the DNA.

Oldfield, AJ, Yang P, Cinghu S, Freudenberg JM, Zheng X, Yellaboina S, Hu G, Jothi R. Histone-fold protein NF-Y promotes chromatin accessibility for cell type-specific master transcription factors, *Molecular Cell,* in press.

SIRT1 regulates cellular retinoic acid signaling and modulates embryonic stem cell differentiation

Retinoid homeostasis is critical for normal embryonic development. Both the deficiency and excess of these compounds are associated with congenital malformations. In this study NIEHS investigators demonstrate that SIRT1, the most conserved mammalian NAD^+-dependent protein deacetylase, contributes to homeostatic retinoic acid (RA) signaling and modulates mouse embryonic stem cell (mESC) differentiation in part through deacetylation of cellular retinoic acid binding protein, CRABPII. They found that SIRT1 interacts with and deacetylates this protein factor, regulating its nuclear localization. Consequently, SIRT1 deficiency induces hyper-acetylation and nuclear accumulation of CRABPII, enhancing RA signaling and accelerating mESC differentiation in response to RA. Consistently, SIRT1 deficiency is associated with elevated RA signaling and development defects in mice. These findings reveal a novel molecular mechanism that regulates RA signaling, and highlight the importance of SIRT1 in regulation of ESC pluripotency and embryogenesis.

Tang S, Huang G, Fan W, Chen Y, Ward JM, Xu X, Xu Q, Kang A, McBurney MW, Fargo DC, Hu G, Baumgart-Vogt E, Zhao Y, Li X. SIRT1-mediated deacetylation of CRABPII regulates cellular retinoic acid signaling and modulates embryonic stem cell differentiation. *Molecular Cell*, in press.

DNA Polymerase μ Lacks Movement During Catalysis

Using X-ray crystallography to determine the structures of polymerase μ at different points of catalysis, it was revealed that unlike other DNA polymerase, polymerase μ shows very little

movement with the exception of a loop (loop1) that moves upon DNA binding. This structure reveals how certain residues in this loop contribute to polymerase μ ability to function on double strand breaks in DNA. This may be important in understanding mechanisms of double strand break repair resulting from DNA damage caused by environmental insult or during immunological maturation.

Moon AF, Pryor JM, Ramsden DA, Kunkel TA, Bebenek K, Pedersen LC. Sustained active site rigidity during synthesis by human DNA polymerase μ. ***Nat. Struct. Mol. Biol***., 21: 253-260, 2014.

Brain region required for social aggression

Most animals, including humans, can act aggressively when confronted with social threats. New research from the NIH has identified a region of the brain that may be responsible for interpreting these social cues and responding with aggressive behavior. Researchers from NIEHS and the National Institute of Mental Health (NIMH) demonstrated that a specific area of the hippocampus, a part of the brain involved in learning and memory, allows animals to assess and respond to social threats. The study reported that activation of a receptor for the social neuropeptide vasopressin, very much like activation of oxytocin receptors, enhanced effectiveness of neuronal connections in a subregion of the hippocampus (area CA2). The results may help scientists better understand the network of neurons involved in social recognition memory and aggression.

Pagani JH, Zhao M, Cui Z, Williams Avram SK, Caruana DA, Dudek SM, Young WS. Role of the vasopressin 1b receptor in rodent aggressive behavior and synaptic plasticity in hippocampal area CA2. ***Mol. Psychiatry.*** doi: 10.1038/mp.2014.47. Epub ahead of print.

A fresh look at the genetics of breast cancer

NIEHS scientists found evidence suggesting that the risk of breast cancer is influenced by nonstandard genetic mechanisms. They analyzed data from the NIEHS Sister Study, a cohort of 50,884 breast-cancer-free sisters of women who have had breast cancer. Genome-wide association studies have largely overlooked certain genetic mechanisms: the maternal genome can act prenatally, the effect of a gene variant can depend on its parent of origin, and mitochondrial variants can influence risk. Because these mechanisms produce asymmetry in family history of breast cancer cases, the scientists analyzed data to compare rates in maternal versus paternal grandmothers. Significantly more maternal grandmothers than paternal grandmothers had developed breast cancer. The scientists developed algebraic formulae to quantify the contributions of the nonstandard mechanisms to that asymmetry and showed that the small difference observed between maternal and paternal lineages could arise from a single nonstandard mechanism with a large effect. Ongoing analyses using families in the Two Sister Study will be able to pinpoint those nonstandard mechanisms more directly.

Weinberg CR, Shi M, Deroo LA, Taylor JA, Sandler DP, Umbach DM. Asymmetry in family history implicates nonstandard genetic mechanisms: application to the genetics of breast cancer. ***PLoS Genet.,*** 10: e1004174, 2014.

Intestinal SIRT1 deficiency protects bile acid induced liver damage in mice

SIRT1 is the most conserved mammalian NAD^+-dependent protein deacetylase that plays a vital role in the regulation of a number of metabolic processes in various metabolic tissues. Dysfunction of SIRT1 in mammals has been associated with a number of metabolic diseases such as type-2 diabetes and fatty liver disease. However, the role of SIRT1 in nutrient absorption and sensing in small intestine, a key metabolic organ that provides the first interface between nutrients and animal metabolism, is still completely unknown. NIEHS investigators recently explored the function of SIRT1 in intestinal metabolism utilizing a novel intestine-specific SIRT1 KO mouse model. Using a combination of molecular, cellular, and genetic approaches, we demonstrate that intestinal SIRT1 is an important regulator of ileal bile acid absorption that feedback modulates systemic bile acid and cholesterol homeostasis. They found that deletion of SIRT1 in mouse increases acetylation levels of an important co-factor of an transcriptional factor that is critically involved in intestinal bile acid homeostasis, reducing intestinal bile acid absorption and protecting animals from high-bile acid diet induced liver damage. Intriguingly, they further found that deletion of SIRT1 in liver or intestine have distinct impacts on systemic bile acid metabolism, which demonstrates that the same molecular mechanism can yield distinct pathophysiologies in different tissues. This study points out that tissue specificity should be considered when applying SIRT1 small molecule modulators-based therapeutic strategies to bile acid and cholesterol diseases.

Kazgan N, Metukuri MR, Purushotham A, Lu J, Rao A, Lee S, Pratt-Hyatt M, Lickteig A, Csanaky IL, Zhao Y, Dawson PA, Li X. Intestine-specific deletion of SIRT1 in mice impairs DCoH2-HNF-1α-FXR signaling and alters systemic bile acid homeostasis. *Gastroenterology,* 146: 1006-1016, 2014.

Prevalence of Allergic Sensitization in the U.S.

Allergic sensitization is an important risk factor for the development of atopic disease. The National Health and Nutrition Examination Survey (NHANES) 2005-2006 provides the most comprehensive information on IgE-mediated sensitization in the general US population. NIEHS scientists investigated clustering, sociodemographic and regional patterns of allergic sensitization and examined risk factors associated with IgE-mediated sensitization. Data for this cross-sectional analysis were obtained from NHANES 2005-2006. Participants aged ≥1 year were tested for sIgEs to inhalant and food allergens. Serum samples were analyzed using the Pharmacia ImmunoCAP System. Information on demographics and participant characteristics was collected by questionnaire. Of the study population aged 6 and older, 44.6% had detectable sIgEs, while 36.2% of children aged 1-5 years were sensitized to ≥1 allergen. Allergen-specific IgEs clustered into 7 groups that mostly reflected biological cross-reactivity. Although sensitization to individual allergens and allergen types showed regional variation, the overall prevalence of sensitization did not differ across census regions, except in early childhood. In multivariate modeling, young age, male gender, non-Hispanic black race/ethnicity, geographic location (census region), and reported pet avoidance measures were most consistently associated with IgE-mediated sensitization. It was concluded that the overall prevalence of allergic sensitization does not vary across US census regions, except in early life, although allergen-specific sensitization differs by sociodemographic and regional factors. Biological cross-reactivity may be a major, but not a sole, contributor to the clustering of allergen-specific IgEs.

Salo PM, Arbes SJ Jr, Jaramillo R, Calatroni A, Weir CH, Sever ML, Hoppin JA, Rose KM, Liu AH, Gergen PJ, Mitchell HE, Zeldin DC. Prevalence of allergic sensitization in the United States: Results from the National Health and Nutrition Examination Survey (NHANES) 2005-2006. *J. Allergy Clin. Immunol.,* 134: 350-359, 2014.

People with the Epsilon 4 Variant of the Apolipoprotein E (APOE) Gene Have More Severe Inflammatory Responses

Investigators at NIEHS report, that a fairly common variant, or 'allele', of the gene that codes for the lipid-binding protein APOE, namely, APOE4, is associated with more severe inflammation. The research team found that whole blood samples from subjects with APOE4 produced much higher levels of inflammatory proteins than did blood from subjects with the more common E3 version of the gene (i.e., APOE3) upon stimulation with bacterial molecules. Similarly, research volunteers with APOE4, as well as mice engineered to contain human APOE4, developed larger swings in body temperature and higher blood inflammation when exposed directly to low levels of bacterial molecules. Finally, the team also found that APOE4 was associated with more severe organ injury among patients admitted to the hospital with severe sepsis. These new findings may someday allow for gene-targeted management of patients with inflammatory and infectious diseases.

Gale SC, Gao L, Mikacenic C, Coyle SM, Rafaels N, Murray Dudenkov T, Madenspacher JH, Draper DW, Ge W, Aloor JJ, Azzam KM, Lai L, Blackshear PJ, Calvano SE, Barnes KC, Lowry SF, Corbett S, Wurfel MM, Fessler MB. APOe4 is associated with enhanced in vivo innate immune responses in human subjects. *J. Allergy Clin. Immunol.,* 134: 127-134.e9, 2014.

Smoking alters the DNA of both women and their children

Smoking is known to cause a variety of adverse health effects, both in adults who smoke and in babies born to mothers who smoke. In a pair of studies, NIEHS researchers show that smoking is associated with a variety of epigenetic changes to DNA in both adults who smoke and in babies born to smoking mothers. These changes, which may be associated with either increased or decreased gene activity, may be linked to the adverse health effects found in smoking adults and in their children.

Harlid SS, Xu Z, Panduri V, Sandler DP, Taylor JA. CpG Sites Associated with Cigarette Smoking: Analysis of Epigenome-Wide Data from the Sister Study. *Environ. Health Perspect*., 122: 673-678, 2014.

Markunas CA, Xu Z, Harlid SS, Wade PA, Lie RT, Taylor JA, Wilcox AJ. Identification of DNA methylation changes in newborns related to maternal smoking during pregnancy. *Environ. Health Perspect*., doi: 10.1289/ehp.1307892. Epub ahead of print.

The tumor suppressor p53 can greatly enhance human inflammatory responses.

There is a well-established relationship between inflammation and the appearance of cancers. NIEHS scientists have established that in human macrophages, which are involved in many immune responses, activation of the tumor suppressor p53 can greatly enhance inflammatory responses in a manner that appears unique to this type of cell. This effect requires cooperation

with the NF-κB inflammatory system, whereas these two systems typically have opposing effects. Importantly, macrophages associated with tumors have activated p53 that can lead to altered inflammatory responses. These discoveries reveal that p53 may have significant functions in carcinogenesis outside of its well-described function as a tumor suppressor, which could have important implications in tumor progression as well as tumor responses to chemotherapy

Lowe JM, Menendez D, Bushel PR, Shatz M, Kirk EL, Troester MA, Garantziotis S, Fessler MB, Resnick MA. p53 and NF-κB coregulate proinflammatory gene responses in human macrophages. *Cancer Res.,* 74: 2182-2192, 2014.

Poly(ADP-ribose) polymerase-1 and cancer chemotherapy

The effect of inhibitors of the catalytic activity of the DNA repair enzyme poly(ADP-ribose) polymerase-1 (PARP-1) on cell viability is an important topic in cancer chemotherapy research. This is because the inhibitors kill cancers with DNA repair deficiencies, whereas normal cell are resistant. Nevertheless, there is wide variability in the efficiency of cell killing by PARP inhibitors, and factors modifying the cell killing phenotype are largely unknown. NIEHS investigators and collaborators recently discovered that the stability of a protein complex of base excision repair factors is important in the PARP inhibitor-based cell killing phenotype. It was also found that base excision repair deficiency invokes PARP inhibitor cell killing in a fashion reminiscent of that observed with double-strand break repair deficiency mediated by loss of BRCA1.

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

Horton JK, Stefanick DF, Prasad R, Gassman NR, Kedar PS, Wilson SH. Base excision repair defects invoke hypersensitivity to PARP inhibition. *Mol. Cancer Res*., doi: 10.1158/1541-7786.MCR-13-0502. Epub ahead of print.

Prasad R, Horton JK, Chastain PD 2nd, Gassman NR, Freudenthal BD, Hou EW, Wilson SH. Suicidal cross-linking of PARP-1 to AP site intermediates in cells undergoing base excision repair. *Nucleic Acids Res*., 42: 6337-6351, 2014.

Horton JK, Wilson SH. Strategic Combination of DNA-Damaging Agent and PARP Inhibitor Results in Enhanced Cytotoxicity. *Front. Oncol*., 30: 257, 2013.

How cells achieve high accuracy of chromosomal DNA replication

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

the 5'-deoxynucleoside-triphosphates (dNTPs), the building blocks used by the polymerases for synthesizing DNA, and how cells must control the dNTP levels to keep replication accurate.

Ahluwalia D, Schaaper RM. Hypermutability and error catastrophe due to defects in ribonucleotide reductase. *Proc. Natl. Acad. Sci. USA*, 110: 18596-18601, 2013.

Gawel D, Fijalkowska I J, Jonczyk P, Schaaper RM. Effect of dNTP pool alterations on leading and lagging-strand replication fidelity in E. coli. *Mut. Res.,* 759: 22-28, 2014.

Itsko M, Schaaper RM. dGTP starvation in Escherichia coli provides new insights into the thymineless-death phenomenon, *PLoS Genetics,* 10: e1004310, 2014.

An integrative framework uncovers key determinants of ES cell identity and homeostasis

Just as every individual has a unique personality, every cell type in an adult body is characterized by a unique set of proteins that are necessary to maintain its identity. Identification of protein-coding genes associated with specific biological phenotypes is a fundamental step toward understanding the molecular basis underlying development and pathogenesis. NIEHS scientists developed a computational framework that can integrate previously published gene expression data, profiled across various cell types, to identify key genes defining a cell identity of interest. Using this approach, they identify key determinants of embryonic stem (ES) identity including Nucleolin, an abundantly expressed protein in cancer and stem cells. They show that Nucleolin regulates the homeostatic balance between self-renewal and differentiation in ESCs by controlling the Nanog-p53 bistable switch. These findings connect the dots on a previously unknown regulatory circuitry involving genes associated with traits in both ESCs and cancer and might have profound implications for understanding cell fate decisions in cancer stem cells.

Cinghu S, Yellaboina S, Freudenberg JM, Ghosh S, Zheng X, Oldfield AJ, Lackford B, Zaykin DV, Hu G, Jothi R. An integrative framework for identification of key cell identity genes uncovers determinants of ES cell identity and homeostasis. *Proc. Natl. Acad. Sci. USA*, 111: E1581-E1590, 2014.

Stress hormone signaling found to prevent heart disease

Chronic stress is increasingly recognized for its contribution to the development and progression of heart disease, a leading cause of death in the Western world. Glucocorticoids are hormones that mediate the stress response by binding the glucocorticoid receptor (GR) and regulating gene expression. To determine the role of stress hormone signaling in heart muscle cells, NIEHS researchers generated knockout mice lacking GR specifically in cardiomyocytes. They found that these mice die prematurely from heart failure. These results suggest that the normal pulsatile secretion of glucocorticoids on a daily basis is critical for maintaining cardiovascular function and that targeting glucocorticoid signaling in cardiomyocytes may provide a new approach for treating heart disease.

Oakley RH, Ren R, Cruz-Topete D, Bird GS, Myers PH, Boyle MC, Schneider MD, Willis MS, Cidlowski JA. Essential role of stress hormone signaling in cardiomyocytes for the prevention of heart disease. *Proc. Natl. Acad. Sci. USA.,* 110: 17035-17040, 2013.

Phosphorylation of stem-loop binding protein drives histone mRNA regulation by reducing conformational entropy

As DNA is replicated during cell division, it must be packaged by histones. To match the level of available histones to DNA replication, histone mRNA expression is controlled by a 3′-end stem-loop structure unique to replication-dependent histone mRNAs. In *Drosophila*, this regulation is mediated by histone mRNA stem-loop–binding protein (dSLBP), which is a disordered protein when not bound to RNA. NIEHS investigators show that phosphorylation of dSLBP dramatically increases binding affinity for stem-loop RNA, but the phosphorylated C-terminal tail of dSLBP does not contact RNA. Instead, phosphorylation promotes compaction of dSLBP that reduces the entropic energy barrier to binding histone mRNA.

Zhang J, Tan D, DeRose EF, Perera L, Dominski Z, Marzluff WF, Tong L, Hall TMT. Molecular mechanisms for the regulation of histone mRNA stem-loop-binding protein by phosphorylation. *Proc. Natl. Acad. Sci. USA.*, 111: E2937-E2946, 2014.

Regulation of Embryonic Stem Cells by Alternative Polyadenylation

The majority of mammalian genes has multiple polyadenylation sites and produce alternatively polyadenylated mRNAs. APA not only expands the proteome diversity by altering the mRNA coding region, but also provides an important means for post-transcriptional gene control by altering the 3' UTR. In this paper, Lackford et al showed that the Fip1 gene regulates the expression of embryonic stem cell genes by promoting efficient recognition of upstream polyadenylation sites and production of mRNAs with shorter 3' untranslated region, thus revealing a previously un-characterized mechanism of post-transcriptional gene regulation in stem cell maintenance.

Lackford B, Yao C, Charles GM, Weng L, Zheng X, Choi EA, Xie X, Wan J, Xing Y, Freudenberg JM, Yang P, Jothi R, Hu G, Shi Y. Fip1 regulates mRNA alternative polyadenylation to promote stem cell self-renewal. *EMBO J.*, 33: 878-889, 2014.

Nrf2 increases ABC transporter expression at blood-brain barriers and reduces drug entry into the brain

Previous studies indicated that Nrf2, a redox-sensor and ligand-activated transcription factor that plays a critical role in cellular defenses against oxidative stress, was neuroprotective in stroke and traumatic brain injury. However, activation of Nrf2 at the blood-brain and blood-spinal cord barriers increases expression and transport activity of three xenobiotic efflux pumps. As a consequence, uptake of therapeutic drugs into the CNS is reduced. Induction of transporter expression and activity involves a sequence of events involving Nrf2 signaling through p53, p38 MAPK and $NF_{\kappa}B$. These findings suggest caution when activating Nrf2 for neuroprotection, since increased drug efflux transporter expression would impair subsequent CNS pharmacotherapy.

Wang X, Campos CR, Peart JC, Smith LK, Boni JL, Cannon RE, Miller DS. Nrf2 upregulates ATP binding cassette transporter expression and activity at the blood-brain and blood-spinal cord barriers. *J. Neurosci.*, 34: 8585-8593, 2014.

Age-related changes in DNA may explain increase cancer frequency among elderly
The frequency of many cancers increases exponentially as people age, but the underlying mechanisms for this effect are not well known. A recent study by NIEHS researchers shows that as people age they acquire epigenetic modifications to their genes, and that these changes are associated with gene silencing. The researchers show that these same modifications are found in a diverse array of human cancers, and they suggest that age-related epigenetic silencing of genes makes it easier for the cells of older people to become cancerous.

Xu Z, Taylor JA. Genome-wide age-related DNA methylation changes in blood and other tissues relate to histone modification, expression, and cancer. *Carcinogenesis*, 35: 356-364, 2014.

First genome-wide study of autoimmune muscle disease shows genetic overlap with other autoimmune diseases.
Autoimmune muscle disease is known to have both genetic and environmental risk factors. NIEHS scientists performed the first genome-wide association study (GWAS) of 1178 adult and juvenile patients who also have autoimmune muscle and skin disease, called dermatomyositis (DM), and controls (n = 4,724). Patients with DM had a strong signal in the human leukocyte antigen (HLA) region and an analysis of 141 non-HLA genes previously associated with autoimmune diseases showed that 3 genes were associated with DM. These genes were all related to immune responses but none was previously reported to be associated with DM. The findings confirm HLA as the major genetic region associated with DM and indicate that DM shares non-HLA genetic features with other autoimmune diseases, suggesting the presence of additional novel risk loci. This first identification of autoimmune disease genetic predispositions shared with DM may lead to enhanced understanding of pathogenesis and novel diagnostic and therapeutic approaches.

Miller FW, Cooper RG, Vencovský J, Rider LG, Danko K, Wedderburn LR, Lundberg IE, Pachman LM, Reed AM, Ytterberg SR, Padyukov L, Selva-O'Callaghan A, Radstake TR, Isenberg DA, Chinoy H, Ollier WE, O'Hanlon TP, Peng B, Lee A, Lamb JA, Chen W, Amos CI, Gregersen PK; Myositis Genetics Consortium. Genome-wide association study of dermatomyositis reveals genetic overlap with other autoimmune disorders. *Arthritis Rheum*., 65: 3239-3247, 2013.

EETs and Organ Regeneration
Epoxyeicosatrienoic acids (EETs) are produced in the endothelium and regulate inflammation, angiogenesis, and vascular tone; however, the role of these epoxyeicosanoids in normal organ and tissue regeneration remains unknown. NIEHS investigators hypothesized that endothelial cells stimulate organ and tissue regeneration via production of bioactive EETs. To determine whether endothelial-derived EETs affect physiologic tissue growth in vivo, they used genetic and pharmacological tools to manipulate endogenous EET levels. They showed that endothelial-derived EETs play a critical role in accelerating tissue growth in vivo, including liver regeneration, kidney compensatory growth, lung compensatory growth, wound healing, corneal neovascularization, and retinal vascularization. Administration of synthetic EETs recapitulated these results, whereas lowering EET levels, either genetically or pharmacologically, delayed tissue regeneration, demonstrating that pharmacological modulation of EETs can affect normal

organ and tissue growth. They also showed that soluble epoxide hydrolase inhibitors, which elevate endogenous EET levels, promote liver and lung regeneration. Thus, the observations indicated a central role for EETs in organ and tissue regeneration and their contribution to tissue homeostasis**.**

Panigrahy D, Kalish BT, Huang S, Bielenberg DR, Le HD, Yang J, Edin ML, Lee CR, Benny O, Mudge DK, Butterfield CE, Mammoto A, Mammoto T, Inceoglu B, Jenkins RL, Simpson MA, Akino T, Lih FB, Tomer KB, Ingber DE, Hammock BD, Falck JR, Manthati VL, Kaipainen A, D'Amore PA, Puder M, Zeldin DC, Kieran MW. Epoxyeicosanoids promote organ and tissue regeneration. *Proc. Natl. Acad. Sci. USA.*, 110: 13528-13533, 2013.

Smad7 role pancreatic islet cell development

Studies by NIEHS investigators highlight a crucial role for the smad complex and in particular Smad7 in pancreatic islet cell development and proliferation after Beta cell loss and during embryonic development of the pancreatic endocrine compartment.

El-Gohary Y, Tulachan S, Guo P, Welsh C, Wiersch J, Prasadan K, Paredes J, Shiota C, Xiao X, Wada Y, Diaz M, Gittes G. Smad signaling pathways regulate pancreatic endocrine development. *Dev. Biol.*, 378: 83-93, 2013.

El-Gohary Y, Tulachan S, Wiersch J, Guo P, Welsh C, Prasadan K, Paredes J, Shiota C, Xiao X, Wada Y, Diaz M, Gittes G. A smad signaling network regulates islet cell proliferation. *Diabetes*, 63: 224-236, 2014.

Circadian regulation of hepatic gluconeogenesis

The circadian clock plays a critical role in the regulation of glucose and lipid metabolism and energy homeostasis. The retinoic acid-related orphan receptor γ (RORγ) directly regulates the expression of glucose metabolic genes in the liver downstream of the hepatic circadian clock, thereby enhancing gluconeogenesis, and decreasing insulin sensitivity and glucose tolerance. Inhibition of RORγ activity by antagonists might be beneficial for the management of glucose metabolic diseases including type 2 diabetes.

Takeda Y, Kang HS, Freudenberg J, DeGraff LM, Jothi R, Jetten AM. Retinoic acid-related orphan receptor γ (RORγ): a novel participant in the diurnal regulation of hepatic gluconeogenesis and insulin sensitivity. *PLoS Genet.,* 10: e1004331, 2014.

Trif-dependent induction of Th17 immunity by lung dendritic cells

Allergic asthma is a widespread disease characterized by airway hyperresponsiveness (AHR). Although inhaled glucocorticoids are an effective therapy for many asthmatics, others respond poorly to this treatment, and improved interventional strategies are needed for these individuals. An improved understanding of the cellular and molecular basis of glucocorticoid-resistant asthma might lead to improved strategies for its treatment. NIEHS investigators have found that the adaptor protein, TRIF (TIR-domain-containing adapter-inducing interferon-β), is critical for neutrophilic inflammation, production of the cytokine, interleukin (IL)-17, and consequent AHR.

These findings suggest small molecules that can antagonize the function of TRIF might have great potential in treating individuals with glucocorticoid-resistant forms of asthma.

Hsia BJ, Whitehead GS, Thomas SY, Nakano K, Gowdy KM, Aloor JJ, Nakano H, Cook DN. Trif-dependent induction of Th17 immunity by lung dendritic cells. *Mucosal Immunol.,* doi: 10.1038/mi.2014.56. Epub ahead of print.

Nicotine strengthens neuronal communication in the hippocampus

NIEHS scientists investigated the cellular mechanism of nicotine's positive action on cognition via electrophysiology and fluorescence imaging. They found that activation of presynaptic α7 nicotinic acetylcholine receptors (nAChR) enhanced glutamatergic synaptic transmission between dentate granule cells to CA3 pyramidal neurons. The α7 nAChR-mediated enhancement was dependent on intracellular calcium rise and protein kinase A. The study sheds light into the molecular mechanisms of the positive cognitive actions of α7 nAChR agonists and development of therapeutic treatments for cognitive impairments.

Cheng Q, Yakel, JL. Presynaptic alpha7 nicotinic acetylcholine receptors enhance hippocampal mossy fiber glutamatergic transmission via PKA activation. *J. Neurosci*., 34: 124-133, 2014.

Risk factor for lung disease in premature infants found

NIEHS scientists and their colleagues have identified a gene named *Chrm2* that may make premature infants susceptible to developing a chronic lung disease called bronchopulmonary dysplasia (BPD). Since previous studies unveiled a genetic component to BPD, the scientists exposed neonatal mice to either supplemental oxygen or room air. The mice that breathed supplemental oxygen sustained more lung inflammation than the control group, but the severity of the damage varied depending on the genetic background of an individual mouse. The researchers then used a statistical technique called genome wide association mapping and identified chromosomal regions containing genetic sequence differences that could account for the differential responses. *Chrm2* was among the candidate genes identified, and they found that mice with a functional mutation in the *Chrm2* gene, which codes for receptors that contribute to neurogenic inflammation of the airways, experienced less lung injury and inflammation. Because this damage is similar to the respiratory injury seen in human BPD, the researchers may be able to use *Chrm2* both as a therapeutic target to prevent neonatal lung injury and as a way to identify individuals at risk of developing BPD.

Nichols JL, Gladwell W, Verhein KC, Cho HY, Wess J, Suzuki O, Wiltshire T, Kleeberger SR. 2 Genome-wide associated mapping of acute lung injury in neonatal inbred mice. *FASEB J.,* 28: 2538-2550, 2014.

Cytochrome P450s and Vascular Function

CYP4F enzymes metabolize arachidonic acid to 20-hydroxyeicosatetraenoic acid (20-HETE). To investigate the role of CYP4F2 in vascular disease, NIEHS scientists generated mice with endothelial expression of human CYP4F2 (Tie2-CYP4F2-Tr). LC/MS/MS analysis revealed increases in 20-HETE levels in tissues and endothelial cells of Tie2-CYP4F2-Tr mice relative to wild-type controls. Tie2-CYP4F2-Tr endothelial cells demonstrated increases in growth and tube

formation that were 20-HETE-dependent and associated with upregulation of pro-oxidant NADPH oxidase and pro-angiogenic VEGF. Increases in VEGF and NADPH oxidase levels were abrogated by inhibitors of NADPH oxidase and MAPK, respectively, suggesting the possibility of crosstalk between pathways. IL-6 levels in Tie2-CYP4F2-Tr mice were upregulated via NADPH oxidase- and 20-HETE-dependent mechanisms. Although Tie2-CYP4F2-Tr aortas displayed increased vasoconstriction, vasorelaxation and blood pressure were unchanged. The findings indicate that human CYP4F2 significantly increases 20-HETE production, CYP4F2-derived 20-HETE mediates endothelial proliferation and angiogenesis via VEGF- and NADPH oxidase-dependent manners, and the Tie2-CYP4F2-Tr mouse is a novel model for examining the pathophysiological effects of CYP4F2-derived 20-HETE in the vasculature.

Cheng J, Edin ML, Hoopes SL, Li H, Bradbury JA, Graves JP, DeGraff LM, Lih FB, Garcia V, Shaik JS, Tomer KB, Flake GP, Falck JR, Lee CR, Poloyac SM, Schwartzman ML, Zeldin DC. Vascular characterization of mice with endothelial expression of cytochrome P450 4F2. *FASEB J.*, 28: 2915-2931, 2014.

Chemical mechanisms of 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 the chemical reaction. A strategic mutation was engineered into the model DNA polymerase, human DNA polymerase β, and the altered enzyme was examined. The results revealed novel understanding of the mechanism of the chemical reaction of nucleotide insertion into DNA.

Wu S, Beard WA, Pedersen LG, Wilson SH. Structural comparison of DNA polymerase architecture suggests a nucleotide gateway to the polymerase active site. *Chem. Rev.,* 114: 2759-2774, 2014.

Oertell K, Chamberlain BT, Wu Y, Ferri E, Kashemirov BA, Beard WA, Wilson SH, McKenna CE, Goodman MF. Transition state in DNA polymerase β catalysis: rate-limiting chemistry altered by base-pair configuration. *Biochemistry*, 53: 1842-1848, 2014.

Bienstock RJ, Beard WA, Wilson SH. Phylogenetic relationship of DNA polymerase β and the X-family DNA polymerases. *DNA Repair* (In Press).

Beard WA, Wilson SH. Structure and mechanism of DNA polymerase β. *Biochemistry,* 53: 2768-2780, 2014.

Selective unfolding of one Ribonuclease H domain of HIV reverse transcriptase is linked to homodimer formation

HIV-1 reverse transcriptase (RT), a critical enzyme of the HIV life cycle and an important drug target, undergoes complex and largely uncharacterized conformational rearrangements that underlie its asymmetric folding, dimerization and subunit-selective ribonuclease H domain (RH) proteolysis. NIEHS scientists used a combination of NMR spectroscopy and X-ray crystallography to characterize the p51 and p66 monomers and the conformational maturation of

the p66/p66' homodimer. The p66 monomer exists as a loosely structured molecule in which the fingers/ palm/connection, thumb and RH substructures are connected by flexible (disordered) linking segments. The initially observed homodimer is asymmetric and includes two fully folded RH domains, while exhibiting other conformational features similar to that of the RT heterodimer. The RH' domain of the p66' subunit undergoes selective unfolding, consistent with destabilization due to residue transfer to the polymerase' domain on the p66' subunit. A simultaneous increase in the intensities of resonances near the random coil position is also observed. These results demonstrate that RH' unfolding is coupled to homodimer formation. Understanding the structural maturation of this enzyme reveals new targets for potential therapeutic intervention.

Zheng X, Pedersen LC, Gabel SA, Mueller GA, Cuneo MJ, DeRose EF, Krahn JM, London RE. Selective unfolding of one Ribonuclease H domain of HIV reverse transcriptase is linked to homodimer formation. *Nucleic Acids Res.,* 42: 5361-5377, 2014.

Non-genomic signaling by nuclear receptor for thyroid hormone is essential for brain development

Man-made chemicals, or "xenobiotics," in our air, water and food pose risks to human health, in part by disrupting endocrine signaling. However, no one has identified a molecular process that is disrupted by concentrations of xenobiotics found in humans, which could directly explain the recent increase in developmental disorders of human intellectual disabilities (ID), such as autism spectrum disorders or attention-deficit hyperactivity disorder. Thyroid hormone, more than any other hormone, is required for normal brain development, and T3-deficiency resulting from iodine-deficient diets remains the leading global cause of ID. However, in affluent countries where salt is iodinated, the incidence of early learning disorders has also increased dramatically over the past few decades. Inherited mutations in the hormone-binding domain of human TRß are also known to increase the incidence of ID. NIEHS scientists have discovered that TRß, a zinc-finger receptor for thyroid hormone, regulates synaptic maturation and plasticity in the mouse hippocampus through the phosphatidylinositol 3-kinase (PI3K), and they are testing whether this molecular process is disrupted by xenobiotics.

Martin NP, Fernandez de Velasco EM, Mizuno F, Scappini EL, Gloss B, Erxleben C, Williams JG, Stapleton HM, Gentile S, Armstrong DL. A rapid cytoplasmic mechanism for PI3 kinase regulation by the nuclear thyroid hormone receptor, TRß, and genetic evidence for its role in the maturation of mouse hippocampal synapses in vivo. *Endocrinology*, doi: 10.1210/en.2013-2058, Epup ahead of print.

New signaling pathway for vitamin D metabolites

NIEHS investigators identified a novel signaling pathway, in which vitamin D metabolites, such as $20(OH)D_3$ and $20,23(OH)_2D_3$, act as antagonists of RORα and RORγ and inhibit interleukin 17 expression. These vitamin D metabolites open new possibilities to regulate immune responses and inflammation, and therefore may be useful in the management of autoimmune diseases.

Slominski AT, Kim TK, Takeda Y, Janjetovic Z, Broz˙yna AA, Skobowiat C, Wang J, Postlethwaite A, Li W, Tuckey RC, Jetten AM. RORα and ROR γ are expressed in human skin and serve as receptors for endogenously produced noncalcemic 20-hydroxy- and 20, 23-dihydroxyvitamin D. *FASEB J.,* 28: 2775-2789, 2014.

Unlocking the Code of Heparan Sulfate/Heparin Synthesis

Heparan sulfates play important roles in inflammation, signaling, coagulation and development. X-ray crystallography was used to determine the structure of an important enzyme in heparan sulfate/heparin biosynthesis, heparan sulfate 2-O-sulfotransferase, with a heparan sulfate substrate bound. This structure explains why during biosynthesis 2-O-sulfation occurs after 2-N sulfation and before 6-O-sulfation. Currently this enzyme is being used for the production of experimental therapeutics. The knowledge gained from this structure may allow for the tailoring of the enzyme's specificity to expand and improve upon the chemoenzymatic synthesis of future therapeutics for the treatment of such ailments such as cancer, inflammation and deep vein thrombosis.

Liu C, Sheng J, Krahn JM, Perera L, Xu Y, Hsieh PH, Dou W, Liu J, Pedersen LC. Molecular mechanism of substrate specificity for heparan sulfate 2-O-sulfotransferase. *J. Biol. Chem.*, 289: 13407-13418, 2014.

Phthalate Exposure and Allergy

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

Hoppin JA, Jaramillo R, London SJ, Bertelsen RJ, Salo PM, Sandler DP, Zeldin DC. Phthalate exposure and allergy in the U.S. population: results from NHANES 2005-2006. *Environ. Health Perspect.,* 121: 1129-1134, 2013.

Flame Retardants Can Mimic Estradiol Binding to Human Proteins.

Flame retardants such as Tetrabromobisphenol A (TBBPA) have been previously shown to inhibit human estrogen sulfotransferase (SULT1E1), an important enzyme in estradiol metabolism. X-ray crystallography was used to determine the structure of TBBPA bound to SULT1E1. This structure revealed that this flame retardant bound to the estradiol binding site in

manner similar to that of estradiol suggesting how TBBPA binding to SULT1E1 can inhibit its function.

Gosavi RA, Knudsen GA, Birnbaum LS, Pedersen LC. Mimicking of estradiol binding by flame retardants and their metabolites: a crystallographic analysis. *Environ. Health Perspect.,* 121: 1194-1199, 2013.

Production of GDNF by peritubular myoid cells supports mouse spermatogonial stem cell maintenance

Peritubular myoid (PM) cells surround the seminiferous tubule and together with Sertoli cells form the cellular boundary of the spermatogonial stem cell (SSC) niche. However, it remains unclear what role PM cells have in determining the microenvironment in the niche required for maintenance of the ability of SSCs to undergo self-renewal and differentiation into spermatogonia. NIEHS scientists tested the hypothesis that T-regulated GDNF expression by PM cells contributes to the maintenance of SSCs using an adult mouse PM cell primary culture system and germ cell transplantation. They found that T induced GDNF expression at the mRNA and protein levels in PM cells. When spermatogonia isolated from neonatal mice co-cultured with PM cells with or without T and transplanted to the testes of germ cell-depleted mice, the number and length of transplant-derived colonies was increased considerably by in vitro T treatment. These results support the novel hypothesis that T-dependent regulation of GDNF expression in PM cells has a significant influence on the microenvironment of the niche and SSC maintenance.

Chen LY, Brown PB, Willis WB, Eddy EM. Peritubular myoid cells have an essential role in male mouse spermatogonial stem cell maintenance. *Endocrinology*, in press

XRCC1 interaction with the REV1 C-terminal domain suggests a role in post replication repair

Repair of DNA damage is generally a complex process that utilizes multiple enzymes. This process is facilitated by scaffold proteins that bring together sets of DNA repair enzymes in order to create an efficient process and to limit the release of incompletely repaired intermediates that may be highly labile and pose toxic consequences for the cell. NIEHS investigators demonstrated that a region in the first linker domain of X-ray cross complementing group 1 protein (XRCC1) – a scaffold protein that plays a central role in the repair of DNA single strand breaks, interacts with a protein that organizes trans-lesion synthesis. These proteins help the cell to deal with DNA lesions under conditions for which rapid repair become more critical than removal of the lesion and replacement with the correct, complementary DNA. The interaction between these proteins suggests that XRCC1 can play a significant role in this process, most probably due to its association with DNA polymerase beta, which has been shown to be capable of translesion synthesis.

Gabel SA, DeRose EF, London RE. XRCC1 interaction with the REV1 C-terminal domain suggests a role in post replication repair. *DNA Repair (Amst)*, 12: 1105-1113, 2013.

Identifying respiratory syncytial virus risk using gene markers
NIEHS researchers led a collaborative team that developed a cell model to investigate genes involved in respiratory syncytial virus (RSV) infection, a respiratory virus common in infants and children. The model identified candidate genes, and in particular one viral gene, which may be used as a biomarker to validate RSV infections. Using RSV, the researchers infected human lymphoblastoid cell lines (LCLs) from several groups, including Northern European, African American, and Japanese. They found that RSV infectivity differed among individuals and between ethnic groups. In addition, they utilized LCL microarray gene expression data from HapMap, an international project sponsored by the National Human Genome Research Institute to develop a map of human haplotypes, genes that are inherited together, to determine patterns of human DNA variation. Out of the 62 genes that correlated with RSV infection, they found that slight sequence changes, or polymorphisms, in the gene for the influenza myxovirus (MX1) was associated with increased expression of RSV in LCLs, and an increased risk of severe RSV disease in an Argentinian infant cohort. The data suggest MX1 is a susceptibility marker for RSV infection, and their translational approach may be used to predict which other genes confer RSV risk.

Ciencewicki JM, Wang X, Marzec J, Serra ME, Bell DA, Polack FP, Kleeberger SR. A genetic model of differential susceptibility to human respiratory syncytial virus (RSV) infection. *FASEB J*., 28: 1947-1956, 2014.

Polyunsaturated fatty acids in diet reduce the risk of Parkinson's disease associated with pesticide exposure.
Pesticide exposure is associated with increased risk of Parkinson's disease. In a study of farmers using pesticides, NIEHS investigators found that those whose diet included greater amounts of polyunsaturated fatty acids had reduced risk of Parkinson's disease. Moreover, risk associated with pesticide exposure was lower in these farmers than in those who consumed smaller amounts of polyunsaturated fatty acids. These results suggest that a diet high in in polyunsaturated fatty acids might reduce risk of Parkinson's disease.

Kamel F, Goldman SM, Umbach DM, Chen H, Richardson G, Barber MR, Meng C, Marras C, Korell M, Kasten M, Hoppin JA, Comyns K, Chade A, Blair A, Bhudhikanok GS, Webster Ross G, William Langston J, Sandler DP, Tanner CM. Dietary fat intake, pesticide use, and Parkinson's disease. *Parkinsonism Relat. Disord.,* 20: 82-87, 2014.

Research on the physiology of pregnancy provides crucial input for evaluating environmental risks.
Recent epidemiologic data indicate that in the general population of pregnant women, there is an association between blood levels of environmental contaminants in the perfluoroalkyl substances (PFAS) class and lower birthweight of the offspring. If this is true, it would indicate that current exposure levels in the U.S. general population are toxic, which would have major implications for regulatory agencies and preventive strategies. However, whether the relation of birthweight-PFAS association is causal or due to confounding depends on the relationship between birthweight and kidney function in pregnancy. The new report provides critical new information

about the kidney function-birthweight relationship, and makes it clear that the association of PFAS with birthweight is at least partly an artifact due to confounding.

Morken NH, Travlos GS, Wilson RE, Eggesbø M, Longnecker MP. Maternal glomerular filtration rate in pregnancy and fetal size. *PLoS One*, 9: e101897, 2014.

A new druggable target on a small molecule cell-signaling kinase

PPIP5Ks are enzymes that synthesize inositol pyrophosphates, a specialized a specialized class of intracellular signals. A novel ligand-binding site on the surface of PPIP5K2 was identified adjacent to the catalytic pocket. This new site captures substrate from the bulk phase, prior to transfer into the catalytic pocket; this is unprecedented for a small molecule kinase. In addition to demonstrating a "catch-and-pass" reaction mechanism, a substrate analogue that inhibits the substrate capture site on PPIP5K2 was synthesized. This work suggests that the substrate-binding site offers new opportunities for targeted drug design.

Wang H, Godage HY, Riley AM, Weaver JD, Shears SB, Potter BV. Synthetic inositol phosphate analogs reveal that PPIP5K2 has a surface-mounted substrate capture site that is a target for drug discovery. *Chem. Biol.,* 21: 689-699, 2014.

Equivalent but dissimilar: Mutational and structural analysis of the tandem zinc finger domains of tristetraprolin

Tristetraprolin (TTP) is a protein that binds to AU-rich elements (a region of mRNA that contains the sequence AUUUAUUU) of the mRNA to promote their degradation. In mice, it has been found that deficiency in TTP leads to develop a complex syndrome of inflammatory diseases. TTP contains two Zn binding domains (known as tandem Zn finger domains). Mutations of some residues lead to decreased RNA binding and thereby, introduce excessive accumulation of mRNA that can be toxic to the cell. In this study, NIEHS investigators have extensively looked at the correlations of the mutations of TTP with its ability to bind to RNA. It was concluded that while the majority of conserved residues are required for productive binding, not all residues at sequence-equivalent positions in the two zinc fingers of the TZF domain of TTP are functionally equivalent.

Lai WS, Perera L, Hicks SN, Blackshear PJ. Mutational and structural analysis of the tandem zinc finger domain of tristetraprolin. *J. Biol. Chem.*, 289: 565-580, 2014.

Pharmacologic exposure to hormones in early pregnancy related to obesity in offspring

Data from animal experiments and a previous epidemiologic study indicate that exposure to pharmacologic estrogens early in life can affect subsequent risk of obesity. The present report was based on analysis of data from pharmacy records in Norway and a cohort of pregnant women whose children had growth data. Women who inadvertently took hormonal contraceptives had children with varying risks of obesity, depending on the specific formulation of contraceptives used. The data provide evidence supporting the environmental obesogen hypothesis.

Jensen ET, Daniels JL, Stürmer T, Robinson WR, Williams CJ, Moster D, Juliusson PB, Vejrup K, Magnus P, Longnecker MP. Maternal hormonal contraceptive use and

offspring overweight or obesity. *Int. J. Obes. (Lond).*, doi: 10.1038/ijo.2014.114. Epub ahead of print.

Exposure to diethylstilbestrol (DES) during pregnancy increases obesity risk in offspring
Data from animal experiments indicate that exposure to pharmacologic estrogens early in life can affect subsequent risk of obesity. This report describes an analysis of data from a population studied in the 1960s, when DES was still being used. Women who were given DES in early pregnancy had offspring who were more likely to be obese. The data provide evidence supporting the environmental obesogen hypothesis.

Jensen ET, Longnecker MP. Pharmacologic sex hormones in pregnancy in relation to offspring obesity. *Obesity (Silver Spring)*, doi: 10.1002/oby.20778. Epub ahead of print.

New insight into how neonatal exposure to DES affects uterus function in mice
DES exposure, just after birth, causes changes in specific epigenetic marks that regulate how genes are expressed in the uterus. Epigenetic changes mean that the underlying DNA sequence remains normal, but gene expression has been modified. In this case, the epigenetic changes were observed in a gene called Six1. Persistence of the epigenetic changes probably explains why the Six1 protein is abnormally expressed in the uterus of older adult mice, when it normally is not there. These findings suggest that epigenetic changes, induced by early life exposures to estrogenic compounds, may explain the long-term effects of this exposure on fertility and the later development of cancer.

Jefferson WN, Chevalier DM, Phelps JY, Cantor AM, Padilla-Banks E, Newbold RR, Archer TK, Kinyamu HK, Williams CJ: Persistently altered epigenetic marks in the mouse uterus following neonatal estrogen exposure. *Mol. Endocrinol.*, 27: 1666-1677, 2013.

Dry roasting peanuts causes certain known allergens to bind a human innate immune receptor.
NIEHS scientists examined the molecular changes that occur in peanuts when they are dry roasted. Adducts that formed during the cooking process were identified and it was demonstrated that these adducts bind specifically to a human cell receptor known as RAGE. Surprisingly, not all of the known peanut allergens were found to be significantly modified. This is the first direct demonstration of this receptor RAGE binding to peanut allergens, although it has been suggested that RAGE plays a role in biasing the immune response towards allergy.

Mueller GA, Maleki SJ, Johnson K, Hurlburt BK, Cheng H, Ruan S, Nesbit JB, Pomés A, Edwards LL, Schorzman A, Deterding LJ, Park H, Tomer KB, London RE, Williams JG. Identification of Maillard reaction products on peanut allergens that influence binding to the receptor for advanced glycation end products. *Allergy*, 68: 1546-1554, 2013.

Strategy for reduction of exposure to DDT identified
DDT is still used in selected countries for control of malaria and other infectious diseases. People who live in homes that are sprayed with DDT are highly exposed, with unknown consequences to health. In this report NIEHS researchers identify an inexpensive, practical, and specific strategy for reducing exposure due to indoor spraying, based on a study conducted of women in South Africa where blood levels of DDT were measured.

Whitworth KW, Bornman RM, Archer JI, Kudumu MO, Travlos GS, Wilson RE, Longnecker MP. Predictors of plasma DDT and DDE concentrations among women exposed to indoor residual spraying for malaria control in the South African Study of Women and Babies (SOWB). *Environ. Health Perspect*., 122: 545-552, 2014.

Generation of a novel transgenic mouse line for studies of meiosis in male mice
Transgenic mice were generated using a heat shock protein 2 (*Hspa2*) gene promoter to express green fluorescent protein (GFP) at the beginning of meiotic prophase I in spermatocytes. The expression and distribution of the GFP and HSPA2 proteins co-localized in spermatocytes and spermatids and fluorescence-activated cell-sorting (FACS) can be used to isolate purified populations of these cells. Unexpectedly, GFP expression was variegated in one transgenic line, being present in some cohorts of meiotic and post-meiotic germ cells and not in others. Although bisulfite sequencing revealed differences in the DNA methylation patterns in the promoter regions of the transgene of the variegated expressing GFP line, a uniformly expressing GFP reporter line, and the Hspa2 gene, these differences did not correlate with variegated expression. The Hspa2-GFP reporter mice provide a novel tool for studies of meiosis by allowing detection of GFP in situ and in isolated spermatogenic cells.

Brown PR, Odet F, Bortner CD, Eddy EM. Reporter Mice Express Green Fluorescent Protein at Initiation of Meiosis in Spermatocytes. *Genesis*, in press

Discovery of novel detoxification and mutation prevention mechanisms
The base analog N6-hydroxylaminopurine (HAP), which is an analog of the normal DNA and RNA constituent Adenine, is an extremely potent mutagen in all organisms from bacteria to man. NIEHS investigators have discovered a novel detoxification system for this compound in the bacterium E. coli, and have shown that this system requires the Molybdenum Cofactor. They have defined the genes as well as the proteins responsible for this activity. These proteins constitute a novel family of Molybdoproteins. These proteins may play a broad role in the detoxification of N-hydroxylated compounds.

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

Kozmin SG, Stepchenkova E I, Schaaper RM. TusA(YhhP) and IscS are required for molybdenum-cofactor-dependent base-analog detoxification. *Microbiol. Open*, 2: 743-755, 2013.

Kozmin SG, Stepchenkova E I, Chow S, Schaaper RM. A critical role for the putative NCS2 nucleobase permease YjcD in the sensitivity of Escherichia coli to cytotoxic and mutagenic purine analogs. *MBio.,* 4: e00661-13, 2013.

Inositol pyrophosphate defends cells against viral infection.
Inositol pyrophosphates are specialized intracellular signals with exceptionally intense electronegative charge. This study demonstrates that in cells infected with virus, one particular inositol pyrophosphate ("IP7"), stimulates a pro-inflammatory response – interferon transcription. Cells can be made more resistant to invasion by the flu virus by over-expression of the enzyme that synthesizes IP7. This work suggests a new pharmacological target that can aid in the defense against pandemics that threaten public health.

Pulloor NK, Nair S, Kostic AD, Bist P, Weaver JD, Riley AM, Tyagi R, Uchil PD, York JD, Snyder SH, García-Sastre A, Potter BV, Lin R, Shears SB, Xavier RJ, Krishnan MN. Human genome-wide RNAi screen identifies an essential role for inositol pyrophosphates in Type-I interferon response. *PLoS Pathog*., 10: e1003981, 2014.

Uncovering a novel enzymatic dialog between the microbiome and human gastrointestinal physiology
It was determined that a prominent gut bacterium, *Bacteroides thetaiotaomicron*, expresses a homolog (BtMINPP) of a mammalian signaling phosphatase (MINPP). BtMinpp is packaged inside outer membrane vesicles (OMVs) protecting the enzyme from degradation by gastrointestinal proteases. Moreover, an example of cross-kingdom cell-to-cell signaling was identified, showing that the BtMinpp-OMVs interact with intestinal epithelial cells to promote intracellular Ca^{2+} signaling.

Stentz R, Osborne S, Horn N, Li AW, Hautefort I, Bongaerts R, Rouyer M, Bailey P, Shears SB, Hemmings AM, Brearley CA, Carding SR. A bacterial homolog of a eukaryotic inositol phosphate signaling enzyme mediates cross-kingdom dialog in the mammalian gut. *Cell Rep.*, 6: 646-656, 2014

Study finds no risk of excess respiratory disease after exposure to DDT
DDT is still used in selected countries for control of malaria and other infectious diseases. The effects of DDT on human health are still poorly characterized. Previous studies have reported that exposure to DDT increases risk of respiratory disease in children. NIEHS investigators studied a population with unusually high exposure to DDT and found no adverse effects on childhood respiratory disease.

Cupul-Uicab LA, Terrazas-Medina EA, Hernández-Ávila M, Longnecker MP. Prenatal exposure to p,p'-DDE and p,p'-DDT in relation to lower respiratory tract infections in boys from a highly exposed area of Mexico. Environ. Res., 132: 19-23, 2014

Exposure to environmental contaminants found not to affect preeclampsia risk
Recent studies have reported that environmental contaminants in the perfluoroalkyl substances (PFAS) class increased risk of preeclampsia. Therefore NIEHS scientists examined the association of other persistent organic pollutants to see if they were similarly related to risk. In the population studied, which had unusually high exposure to persistent organic pollutants, no association with risk of preeclampsia was found.

Savitz DA, Klebanoff MA, Wellenius GA, Jensen ET, Longnecker MP. Persistent organochlorines and hypertensive disorders of pregnancy. *Environ. Res.,* 132: 1-5, 2014.

Sorting out true and false findings in large scale statistical experiments.
In experiments with many statistical tests there is a need to balance the frequency with which wrong claims are made with the frequency with which real effects remain undiscovered. NIEHS investigators proposed an approach where scientists can design statistical experiments in a way that allows controlling the proportion of false findings among the most statistically significant results. To control the proportion of false findings precisely requires external information such as the rate with which real effects occur in reality. That information is rarely available but we give guidelines about the effects of imprecision regarding that information.

Kuo CL, Zaykin D. The ranking probability approach and its usage in design and analysis of large-scale studies. *PLoS One*, 8: e83079, 2013.

Your old couch may affect the efficacy of drugs you take
BDE-47 is one of the chemical components of a frame retardant used for plastic products including foam stuffing in couches and mattresses. BDE-47 was found to induce a gene for drug metabolizing enzyme Cyp2b10 in livers from wild type mice but not from mice lacking the functional CAR gene. In cell culture assay systems developed with human derived cells, BDE-47 activated human CAR and induced major drug metabolizing enzyme genes CYP2B6 and CYP3A4 that exist in the human liver. Recent publications show that some individuals have a high content of BDE-47 in their body fat. Thus they may have altered chemical/drug metabolism in the liver compared to individuals who has lower exposure.

Sueyoshi T, Li L, Wang H, Moore R, Kodavanti PR, Lehmler HJ, Negishi M, Birnbaum LS. Flame retardant BDE-47 effectively activates nuclear receptor CAR in human primary hepatocytes. *Toxicol. Sci.,* 137: 292-302, 2014.

A simple method for detecting exposure to estrogen-like "endocrine disruptors" in infants
The "endocrine disruption" hypothesis states that hormonally active pollutant chemicals are interfering with endocrine function in the general population, including infants. Estrogen-mimicking pollutants are perhaps the best studied of the endocrine disrupting chemicals. Measuring exposure to such compounds, however, does not indicate whether the compound is potent enough and the dose is high enough to produce a response. NIEHS investigators adapted and validated a simple cytological method used in adult women, the Pap smear, for use in infants, and showed that newborns of either sex, who have recent exposure to high maternal estrogen while in utero, and 12 week olds, who have negligible estrogen exposure, have clearly different cytological finding. This simple non-invasive method can be used to assess whether a child's exposure to a putative endocrine disrupter is sufficient to provoke a response. There is no other tool now available that is as simple, cheap, safe, and readily interpretable.

Adgent MA, Flake GP, Umbach DM, Stallings VA, Bernbaum JC, Rogan WJ. Urogenital epithelial cells as simple markers of estrogen response in infants: methods and applications. *PLoS One,* 8: e77061, 2013.

Exposure to bisphenol A characterized in a human population

The ubiquitous environmental contaminant bisphenol A (BPA) is under intense study because of suspected health effects due to exposure. The optimal design of health effect studies requires detailed information on the pattern of exposure. In this report NIEHS scientists describe how we characterized BPA exposure in a population, which will support detailed future studies of health effects.

Jusko TA, Shaw PA, Snijder CA, Pierik FH, Koch HM, Hauser R, Jaddoe VW, Burdorf A, Hofman A, Tiemeier H, Longnecker MP. Reproducibility of urinary bisphenol A concentrations measured during pregnancy in the Generation R Study. *J. Expo. Sci. Environ. Epidemiol.,* doi: 10.1038/jes.2014.23. Epub ahead of print.

New statistical method developed for use in studies of women's fertility

In this report NIEHS investigators describe how they improved a powerful statistical procedure so that it can be applied to studies of fertility in women, to increase the statistical power of such studies. The benefit of the procedure was demonstrated in an analysis of real data.

Ding J, Zhou H, Liu Y, Cai J, Longnecker MP. Estimating effect of environmental contaminants on women's subfecundity for the MoBa study data with an outcome-dependent sampling scheme. *Biostatistics.* 2014 Epub ahead of print

New Method to Rank Chemicals by Bioactivity Level

Quantitative high-throughput screening (qHTS) experiments generate concentration-response profiles for thousands of chemicals at the same time. However, it is unclear how to prioritize chemicals for follow-up studies based on these results. The strategy developed here can be used to rank all tested chemicals in the absence of a prespecified model structure.

Shockley KR. Using weighted entropy to rank chemicals in quantitative high-throughput screening experiments. *J. Biomol. Screening,* 19: 344-353, 2014.