Division of Intramural Research

NAEHS Council Update

September 2015

DIR RECRUITMENTS

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

Deputy Chief of the Comparative Medicine Branch
The National Institute of Environmental Health Sciences (NIEHS) is searching for Veterinary Medical Officer to serve as Deputy Chief of the Comparative Medicine Branch (CMB), Facility Veterinarian, and Deputy Animal Program Director. CMB provides a broad range of services and collaborative support for NIEHS intramural research programs. The incumbent will be responsible for assisting the Chief CMB with the management of an AAALAC accredited animal care and use program and for support of NIEHS animal research programs that study the effects of environmental agents in order to develop methods of disease prevention and treatment. Candidates should have a Doctor of Veterinary Medicine (DVM) or equivalent degree, i.e., Veterinary Medical Doctor (VMD), obtained at a school or college of veterinary medicine accredited by the American Veterinary Medical Association Council on Education; have a permanent, full, and unrestricted license to practice veterinary medicine in a State, District of Columbia, the Commonwealth of Puerto Rico, or a territory of the United States; and be board certified by the American College of Laboratory Animal Medicine (ACLAM) or equivalent. A search committee is being formed.

Scientific Information Officer
The National Institute of Environmental Health Sciences is searching for an exceptional candidate to serve as the Scientific Information Officer (SIO). The SIO will head a dynamic office focused on the interface between cutting edge scientific computing and scientific exploration, discovery and translation. It is critical that our institute is able to fully harness advances in scientific computing and science information technology to meet our research mission. We are seeking an outstanding leader who will create an environment where scientific computing catalyzes our research program. The successful candidate will: 1). Advise the NIEHS Scientific Director, Division of the National Toxicology Program Director, NIEHS Leadership and other experts throughout the NIEHS on a variety of complex, unique and/or sensitive situations and issues in scientific computing; 2). Provide a vision for scientific computing and the extraction and use of knowledge from the data generated by and relevant to NIEHS research; 3). Lead the NIEHS in application of new methods and technologies emerging from the field of data science and "big data" as well as advancing the use of cloud and distributed computing

approaches; 4). Coordinate ongoing scientific computing activities with other Institutes/Centers throughout NIH, other federal agencies and other institutions as it relates to fostering the adoption and training of new, effective technologies and procedures for scientific computing and manipulating and interpreting large and complex data generated by researchers in the environmental health sciences community; 5). Set up relevant training and educational programs to ensure scientists at NIEHS are knowledgeable about the institute resources in these areas; 6). Work in concert with the Chief Information Officer (CIO) at NIEHS to ensure that NIEHS IT assets are planned for and deployed to meet needs; and 7). Work with other NIEHS groups that consume and provide scientific IT capabilities including the Integrated Bioinformatics Core Facility and Biostatistics/Computational Biology Branch to ensure effective use of NIEHS resources. Applicants should have a degree in a biological science, agriculture, natural resource management, chemistry, or related disciplines appropriate to this position. Dr. Traci Hall, Epigenetics and Stem Cell Biology Laboratory, is chair of the search committee. Potential candidates have been interviewed.

NEW HIRES AND CHANGES IN DIR LEADERSHIP

Reproductive and Developmental Biology
Dr. Francesco DeMayo from the Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, has accepted a position as a tenured Senior Scientist in the Reproductive and Developmental Biology Laboratory and will serve as Deputy Laboratory Chief. 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 design treatments for pulmonary diseases and infertility. He started at NIEHS on August 9, 2015.

Lasker Clinical Research Scholar
Dr. Natalie Shaw from the Department of Pediatrics, Harvard Medical School, Boston MA, has tentatively accepted a position as a NIH Lasker Clinical Research Scholar in DIR at NIEHS. She will have a primary appointment in the Clinical Research Branch and a secondary appointment in the Reproductive and Developmental Biology Laboratory. Dr. Shaw investigates the environmental and hormonal factors that control puberty. Using clinical research tools she explores how the sleep centers of the brain may influence the hypothalamic gonadotropin-releasing hormone (GnRH) neuronal network that drives the reproductive axis. She is scheduled to start at NIEHS September 8, 2015.

DIR RESEARCH UPDATE

SIRT1 in Metabolism, Tissue Homeostasis, and Human Diseases

Xiaoling Li, Ph.D.

Metabolism, Genes, and Environment Group
Signal Transduction Laboratory, DIR, NIEHS

The overarching goal of the Metabolism, Genes, and Environment Group is to understand how organisms monitor environmental changes and coordinate cellular signaling pathways to regulate processes associated with metabolism and animal physiology. Specifically, we study the functions of SIRT1, the most conserved class III histone deacetylase that plays vital roles in metabolism and stress responses. Our efforts at NIEHS have focused on the role of SIRT1 in cell signaling, metabolism, development, diseases, as well as environmental regulation of SIRT1 activity using culture cells and mouse models. In particular, our recent research revealed critical function of SIRT1 in embryonic stem cell biology, animal development, and cancer cell metabolism. Our studies advance our understanding of the role of SIRT1 in mediating gene-environment interaction during the process of development and diseases, which may provide the molecular basis for novel therapeutic targets against a number of human diseases.

BSC REVIEW OF THE EPIGENETICS AND STEM CELL BIOLOGY LABORATORY AND DR. R. S. WILLIAMS

The NIEHS DIR Board of Scientific Counselors reviewed the Epigenetics and Stem Cell Biology Laboratory and Dr. R.S. Williams, July 26-28, 2015.

Members of the Board of Scientific Counselors that Attended:

- Kenneth B. Adler, Ph.D., [BSC Chair], Professor, Dept. of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC
- Monica Justice, Ph.D. Program Head and Senior Scientist, Genetics & Genome Biology Program, SickKids Research Institute, The Peter Gilgan Centre for Research and Learning, Toronto, Ontario, Canada
- José E. Manautou, Ph.D., Associate Professor, Department of Pharmaceutical Sciences, University of Connecticut School of Pharmacy, Storrs, CT
- Ann M. Reed, M.D., Professor and Chair, Department of Pediatrics, Physician-in-Chief, Duke Children's Hospital, Duke University Medical Center, Durham, NC
- Donald P. McDonnell, Ph.D., Glaxo-Wellcome Professor and Chairman of Pharmacology and Cancer Biology, Duke University School of Medicine, Durham, NC
- Karen M. Vasquez, Ph.D., Professor, Division of Pharmacology and Toxicology, Dell Pediatric Research Institute, The University of Texas at Austin, Austin, TX
- Roland A. Owens, Ph.D., Ex-Officio BSC Member, Assistant Director, Office of Intramural Research, NIH, Bethesda, MD

Ad Hoc Reviewers that Attended:

- Hashim M. Al-Hashimi, Ph.D., Professor, Dept. of Biochemistry, Duke University School of Medicine, Durham, NC
- Christopher I. Amos, Ph.D., Professor, Dept. of Community and Family Medicine, Geisel School of Medicine at Dartmouth, Hanover, NH
- Michelle C. Barton, Ph.D., Professor, Department of Epigenetics and Molecular Carcinogenesis, The University of Texas MD Anderson Cancer Center, Houston, TX
- Mark D. Biggin, Ph.D., Principal Investigator, Genomics Division, Lawrence Berkeley National Laboratory, Berkeley, CA
- Stephen Buratowski, Ph.D., Professor, Department of Biological Chemistry & Molecular Pharmacology, Harvard Medical School, Boston MA
- Stephen Dalton, Ph.D., Professor, Department of Biochemistry and Molecular Biology, Paul D. Coverdell Center for Biomedical and Health Sciences, The University of Georgia, Athens, GA
- Mohanish Deshmukh, Ph.D., Professor, Neuroscience Center, Department of Cell Biology & Physiology, University of North Carolina, Chapel Hill, NC
- Fred Dyda, Ph.D., Principal Investigator, Laboratory of Molecular Biology, National Institute of Diabetes and Digestive Diseases and Stroke, NIH, Bethesda, MD
- Laura Elnitski, Ph.D., Senior Investigator, Translational and Functional Genomics Branch, Head, Genomic Functional Analysis Section, National Human Genome Research Institute, NIH, Rockville, MD

- Michael J. Garabedian, Ph.D., Professor, Depts. of Microbiology and Urology, NYU School of Medicine, New York, NY
- Anthony N. Imbalzano, Ph.D., Professor, Dept. of Cell and Developmental Biology, University of Massachusetts Medical School, North Worcester, MA
- Carol A. Lange, Ph.D., Professor, Departments of Medicine and Pharmacology, University of Minnesota, Minneapolis MN
- Susan P. Lees-Miller, Ph.D., Professor, Depts. of Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta, Canada
- Carlos T. Moraes, Ph.D., Lichtenstein Professor of Neurology, University of Miami Miller School of Medicine, Miami, FL
- John V. Moran, Ph.D., Gilbert S. Omenn Collegiate Professor of Human Genetics, Investigator, Howard Hughes Medical Institute, University of Michigan Medical School, Ann Arbor, MI
- Kathrin Plath, Ph.D., Professor, Department of Biological Chemistry, Univ. California, Los Angeles, Los Angeles, CA
- Ed Seto, Ph.D., Professor, Dept. of Molecular Medicine, University of South Florida, College of Medicine, Senior Member, Department of Molecular Oncology, Moffitt Cancer Center, Tampa, FL
- Keshav K. Singh, Ph.D., Professor, Dept. of Genetics, University of Alabama Birmingham School of Medicine, Birmingham, AL
- Daniel O. Stram, Ph.D., Professor, Division of Biostatistics and Genetic Epidemiology, Department of Preventive Medicine, University of Southern California Keck School of Medicine, Los Angeles, CA
- Patrick Sung, D. Phil., Ph.D., Professor, Depts. of Molecular Biophysics and Biochemistry and Therapeutic Radiology, Yale School of Medicine, New Haven, CT
- Weidong Wang, Ph.D., Senior Investigator, Laboratory of Genetics, National Institute of Aging, NIH, Baltimore, MD
- James R. Williamson, Ph.D., Professor, Dept. of Molecular Biology & Dept. Chemistry, The Scripps Research Institute, La Jolla, CA
- Jerry L. Workman, Ph.D., Investigator, Stowers Institute for Medical Research, Kansas City, MO.

Agenda:

Sunday, July 26 - Doubletree Hotel
Closed Evening Session

7:00 – 8:00 p.m.	Welcome and Discussion of Past Board Reviews, Drs. Darryl Zeldin, Trevor Archer and Bill Copeland,
8:00 – end	BSC Discussion Review, Dr. Ken Adler and panel

Monday, July 27 - NIEHS Rodbell Conference Rooms 101 ABC
Morning Session

8:30 – 8:45 a.m.	Welcome, Dr. Zeldin
8:45 – 9:05	Overview, Epigenetics & Stem Cell Biology Laboratory, Trevor Archer, Ph.D.
9:05 – 9:55	Chromatin and Gene Expression Group, Trevor Archer, Ph.D.

9:55 - 10:10	COFFEE BREAK
10:10 – 11:00	Stem Cell Biology Group, Guang Hu, Ph.D.
11:00 – 11:50	Macromolecular Structure Group, Traci M. Hall, Ph.D.
11:50 – 12:35 p.m.	Closed 1:1 Sessions with Investigators, Drs. Archer, Hu & Hall
12:35 – 1:30	Closed Working Lunch,

Afternoon Session

1:30 – 3:00	Poster Session—PI 1-5 Fellows and Staff Scientists, Rodbell Lobby
3:00 – 3:30	Closed Sessions with Fellows and Staff Scientists, 101ABC
3:30 – 3:45	COFFEE BREAK
3:45 – 4:35	Systems Biology Group, Raja Jothi, Ph.D.
4:35 – 5:25	Eukaryotic Transcriptional Regulation Group, Paul A. Wade, Ph.D.
5:25 – 5:55	Closed 1:1 Sessions with Investigators, Drs. Jothi & Wade
6:00	Return to Doubletree Hotel

Closed Evening Session

6:15 – end	BSC Discussion and completion of individual review assignments by each member, All BSC reviewers at hotel

Tuesday July 28 - NIEHS Rodbell Conference Rooms 101 ABC

Morning Session

8:30 – 9:20 a.m.	Mammalian Genome Group, Richard P. Woychik, Ph.D.
9:20 – 10:10	Transcriptional Responses to the Environment Group, Karen Adelman, Ph.D.
10:10 – 10:40	Closed 1:1 Sessions with Investigators, Drs. Woychik & Adelman
10:40 – 10:55	COFFEE BREAK
10:55 – 11:45	Genome Stability Structural Biology Group, R. Scott Williams, Ph.D.
11:45 – 12:00 noon	Closed 1:1 Session with Investigator, Dr. Scott Williams
12:00 – 1:00 p.m.	Closed Working Lunch, 101ABC

Afternoon Session

1:00 – 2:30	Poster Session—PI 6-8 Fellows and Staff Scientists, Rodbell Lobby
2:30 – 3:00	Closed Sessions with Fellows and Staff Scientists, 101ABC
3:00 – 3:15	COFFEE BREAK
3:15 – 4:00	Closed BSC Discussion and completion of individual review assignments by each member, All BSC reviewers
4:00 – 5:30	Closed Session and Debriefing to NIEHS/DIR Leadership
5:30	Adjurn.

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 16 winners of FARE awards:

FARE Awardee	Mentor	Group and Laboratory/Branch
Amanda E. Conway, Ph.D.	Raja Jothi, Ph.D.	Systems Biology Group, Epigenetics and Stem Cell Biology Laboratory
Shannon L. Farris, Ph.D.	Serena M. Dudek, Ph.D.	Synaptic and Developmental Plasticity Group, Neurobiology Laboratory
Kristin A. Gabor, Ph.D.	Michael B. Fessler, M.D.	Clinical Investigation of Host Defense Group, Immunity, Inflammation and Disease Laboratory
Bo He, Ph.D.	John A. Cidlowski, Ph.D.	Molecular Endocrinology Group, Signal Transduction Laboratory
Ashutosh Kumar, Ph.D.	Ronald P. Mason. Ph.D.	Free Radical Metabolism Group, Immunity, Inflammation and Disease Laboratory
Rui Liu, Ph.D.	Honglei Chen, M.D., Ph.D.	Aging and Neuroepidemiology Group, Epidemiology Branch
Julie M. Lowe, Ph.D.	Michael B. Fessler, M.D.	Clinical Investigation of Host Defense Group, Immunity, Inflammation and Disease Laboratory
Vijay R. More, Ph.D.	David S. Miller, Ph.D.	Intracellular Regulation Group, Signal Transduction Laboratory
Barbara C. Nicol, Ph.D.	Humphrey Yao, Ph.D.	Reproductive Developmental Biology Group, Reproductive and Developmental Biology Laboratory
Clinton D. Orebaugh, Ph.D.	Thomas A. Kunkel, Ph.D.	DNA Replication Fidelity Group, Genome Integrity and Structural Biology Laboratory
Sonika Patial, Ph.D.	Perry J. Blackshear, M.D., D. Phil.	Post-Transcriptional Gene Expression Group, Signal Transduction Laboratory
Matthew J. Schellenberg, Ph.D.	R. Scott Williams, Ph.D.	Genome Stability Structural Biology Group, Genome Integrity and Structural Biology Laboratory

Alisa A. Suen, Ph.D.	Carmen J. Williams, M.D., Ph.D.	Reproductive Medicine Group, Reproductive and Developmental Biology Laboratory
Seddon Y. Thomas, Ph.D.	Donald N. Cook, Ph.D.	Immunogenetics Group, Immunity, Inflammation and Disease Laboratory
Pengyi Yang, Ph.D.	Raja Jothi, Ph.D.	Systems Biology Group, Epigenetics and Stem Cell Biology Laboratory
Xiaofeng Zheng, Ph.D.	Guang Hu, Ph.D.	Stem Cell Biology Group, Epigenetics and Stem Cell Biology Laboratory

Summer Internship Program Best Poster Awards

NIEHS takes a leadership role in science research and education. Scientists at NIEHS are committed to sharing with students the intensity, excitement, sense of discipline, and tremendous satisfaction that careers in science can impart to those who pursue them. To this end, the DIR established the Summer Internship Program for which internships are given to outstanding high school and college undergraduate and graduate students interested in pursuing careers in the biomedical/biological sciences. Participants are selected by intramural scientific mentors and spend between 8 to 12 weeks (during May through September) working on individual research projects that bring them exposure to the latest biochemical, molecular, and analytical techniques. There is a poster session at the end of the summer where participants display the results of their research efforts and respond to questions as though they were participating in a national scientific society meeting. The poster session was held on Thursday, July 30, 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:

High School Intern:
Shivpriya Sridhar, Enloe High School, Cellular and Molecular Pathology Branch, DNTP, Mentor: Arun Pandiri, Poster Title: "Immunohistochemical characterization of Islets of Langerhans in F344/N rats exposed to cobalt by inhalation exposure"

Undergraduate Intern:
Bailey Lien, University of Pittsburgh, Neurobiology Laboratory, DIR, Mentor: Patricia Jensen, Poster Title: "A new mouse line for chemogenetic activation of locus coeruleus noradrenergic neurons"

Graduate Intern:
Craig McGowan, University of North Carolina, Chapel Hill, Epidemiology Branch, DIR, Mentor: Dale P. Sandler, Poster Title: "Respiratory Symptoms and Eye Irritation Related to Corexit 9500A and 9527A Exposure in the GuLF STUDY"

DIR RESEARCH ACCOMPLISHMENTS FOR FY 2015

EETs and Stem Cell Engraftment

Haematopoietic stem and progenitor cell (HSPC) transplant is a widely used treatment for life-threatening conditions including leukemia; however, the molecular mechanisms regulating HSPC engraftment of the recipient niche remain incompletely understood. Investigators developed a competitive HSPC transplant method in adult zebrafish, using in vivo imaging as a non-invasive readout. They used this system to conduct a chemical screen and identified epoxyeicosatrienoic acids (EET) as a family of lipids that enhance HSPC engraftment. EETs' pro-haematopoietic effects were conserved in the developing zebrafish embryo, where 11,12-EET promoted HSPC specification by activating a unique AP-1/runx1 transcription program autonomous to the haemogenic endothelium. This effect required the activation of the PI3K pathway, specifically PI3Kγ. In adult HSPCs, 11,12-EET induced transcriptional programs, including AP-1 activation, which modulate multiple cellular processes, such as migration, to promote engraftment. Finally, the investigators demonstrated that the EET effects on enhancing HSPC homing and engraftment are conserved in mammals. The study established a novel method to explore the molecular mechanisms of HSPC engraftment, and discovered a previously unrecognized, evolutionarily conserved pathway regulating multiple haematopoietic generation and regeneration processes. EETs may have clinical application in marrow or cord blood transplantation.

Li P, Lahvic JL, Binder V, Pugach EK, Riley EB, Tamplin OJ, Panigrahy D, Bowman TV, Heffner GC, McKinney-Freeman S, Schlaeger TM, Daley GQ, Zeldin DC, Zon LI. Epoxyeicosatrienoic Acids Enhance Embryonic Hematopoiesis and Adult Marrow Engraftment. *Nature.* 523:468-471, 2015.

Polymerase-induced cytotoxicity of an oxidized nucleotide

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

Freudenthal BD, Beard WA, Perera L, Shock DD, Kim T, Schlick T, Wilson SH. Uncovering the polymerase-induced cytotoxicity of an oxidized nucleotide. *Nature*, 517: 635-639, 2015

Finding a culprit for endogenous mutagenesis in human cancer

DNA editing enzymes are important but potentially dangerous proteins within the cell. When not tightly regulated, they can alter the DNA in the genome inappropriately, causing high levels of mutation and DNA damage that can lead to cancer. APOBEC3B is one well-characterized DNA editing protein that has been implicated in several cancers. A related enzyme, APOBEC3A, has

also been suspected as a potential mutagen, but its contribution to mutagenesis in cancers has been unclear. This study showed that APOBEC3A is not only capable of causing mutations in cancer genomes, like APOBEC3B, but that it actually does so at a much higher rate and in a distinct pattern. The authors suggested that APOBEC3A hypermutation plays important role in cancer, potentially making this enzyme an important target for future cancer therapies and diagnostics.

Chan K, Roberts SA, Klimczak LJ, Sterling JF, Saini N, Malc EP, Kim J, Kwiatkowski DJ, Fargo DC, Mieczkowski PA, Getz G, Gordenin DA. An APOBEC3A hypermutation signature is distinguishable from the signature of background mutagenesis by APOBEC3B in human cancers. *Nat. Genet.*, epub ahead of print, doi: 10.1038/ng.3378.

Ribonucleotides in DNA.
The interface between the DNA and RNA worlds is complicated by the fact that ribonucleotides are incorporated into DNA by replicative DNA polymerases. As one more step towards understanding the complex biology at this interface, NIEHS investigators have described the distribution of ribonucleotides incorporated into the leading and lagging strands during replication by each of the three major eukaryotic DNA polymerases.

Clausen AR, Lujan SA, Burkholder AB, Orebaugh CD, Williams JS, Clausen MF, Malc EP, Mieczkowski PA, Fargo DC, Smith DJ, Kunkel TA. Tracking replication enzymology in vivo by genome-wide mapping of ribonucleotide incorporation. *Nat. Struct. Mol. Biol*., 22:185-191, 2015.

Ctp1$^{CtIP/Sae2}$ bridges DNA strand breaks
Ctp1 (aka CtIP or Sae2) collaborates with the Mre11–Rad50–Nbs1 nuclease to initiate repair of DNA double strand breaks (DSBs), but the roles for Ctp1 in this process remain enigmatic. NIEHS researchers have imaged the first molecular structures of Ctp1 and discovered the protein links severed DNA strands to one another, acting as a molecular splint. This work defines a molecular mechanism for self-assembly of the tetrameric DNA bridging Ctp1 protein, implicates Ctp1 in the recognition and coordination of DNA strand break intermediates, and provides a new molecular insights into our understanding human CtIP mutations linked to Seckel and Jawad syndromes.

Andres SN, Appel CD, Westmoreland JW, Williams JS, Nguyen Y, Robertson PD, Resnick MA, Williams RS. 2015. Tetrameric Ctp1 coordinates DNA binding and DNA bridging in DNA double-strand-break repair. *Nat. Struct. Mol. Biol.*, 22:158-166, 2015.

Study solves ovarian cell mystery, shedding new light on reproductive disorders
NIEHS scientists have solved a long-standing mystery about the origin of ovarian theca cells, while also discovering how ovarian cells share information during proper development of an ovarian follicle, which holds the maturing egg. Researchers believe this new information on basic ovarian biology will help them better understand the cause of ovarian disorders, such as

premature ovarian failure and polycystic ovarian syndrome (PCOS), conditions that both result in hormone imbalances and infertility in women.

Liu C, Peng J, Matzuk MM, Yao HH-C. Lineage specification of ovarian theca cells requires multicellular interactions via oocyte and granulosa cells. *Nat. Commun.*, 6:6934, 2015.

Heart rate variability may predict Parkinson's risk

Clinical observations show that Parkinson's patients often have cardiac autonomic dysfunction such as decreased heart rate variability and orthostatic hypotension. Using data from the 20-year Atherosclerosis Risk in Communities study, NIEHS scientists provided the first epidemiological evidence that decreased heart rate variability might actually precede the clinical diagnosis of Parkinson's disease by years. This adds to the growing evidence that Parkinson's patients may develop a range of prodromal symptoms before the disease is clinically diagnosed, which may help to identify individuals at risk for the disease and to understand disease etiology.

Alonso A, Huang X, Mosley TH, Heiss G, Chen H. Heart rate variability and the risk of Parkinson disease: The Atherosclerosis Risk in Communities study. *Ann. Neurol*., 77:877-883, 2015.

Discovery of a molecular switch for lactation

Scientists at the NIEHS discovered that a specific type of calcium channel, known as store-operated calcium channels, provides the signal for ejection of milk from mammary glands during nursing. Pups born to female mice lacking the gene for this channel could not survive due to a complete failure of lactation and nursing.

Davis FM, Janoshazi A, Janardhan KS, Steinckwich N, D'Agostin DM, Petranka JG, Desai PN, Roberts-Thomson SJ, Bird GS, Tucker DK, Fenton SE, Feske S, Monteith GR, Putney JW Jr. Essential role of Orai1 store-operated calcium channels in lactation. *Proc. Natl. Acad. Sci. USA*., 112: 5827-5832, 2015.

DNA polymerase going in reverse

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, NIEHS investigators applied time-lapse crystallography and various kinetic techniques to study human DNA polymerase (pol) β. This crystallography approach provides novel snapshots of structural intermediates of the enzyme as it passes through the catalytic cycle. Open to closed conformational transition in the polymerase that hasten correct and deters incorrect nucleotide insertion into DNA are visualized, and the structures are being subjected to computational analyses to gain a better understanding of the conformational dynamics. Importantly, after correct nucleotide insertion, the enzyme remains in the closed conformation, and the active site has a new divalent metal ion-binding site. Thus, slow product release could be coupled to the reverse reaction of DNA synthesis, pyrophosphorolysis.

Perera L, Freudenthal BD, Beard WA, Pedersen LG, Wilson SH. DNA polymerase going in reverse: Roles of a transient metal ion as revealed by QM/MM analysis. *Proc. Natl. Acad. Sci. USA*, in press

Control of DNA packaging protein synthesis by phosphorylation of a regulatory protein
By examining phosphorylation of the histone mRNA stem-loop-binding protein (SLBP), NIEHS scientists and their collaborators have detailed a new mechanism by which histone protein expression is regulated. This research is important, because levels of histones, proteins that package DNA within the nucleus, have to be controlled carefully throughout the cell cycle. While DNA is replicating, histone levels must increase to package the new DNA, and following DNA replication, the presence of extra histones can be toxic to the cell. Using biochemical and structural methods, the scientists found that phosphorylation of SLBP dramatically increases its ability to bind histone mRNA, and hence control histone production. The negatively-charged phosphorylation sites do not contact the RNA, but instead increase SLBP's RNA binding by compacting the protein.

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

Human repair polymerase mu incorporates nucleotides differently than other polymerases
Most template DNA polymerases replicate a template strand by incorporating nucleotides onto the 3' end of the newly synthesized strand using the first available unpaired base on the 3' end of the templating strand. New research suggests that Polymerase Mu, within the context of gapped DNA, recognizes the unpaired nucleotide on the 5' end of the gap. This represents unprecedented behavior of a polymerase and we have captured this activity in a series of crystal structures demonstrating the mechanism by which this occurs.

Moon AF, Gosavi RA, Kunkel TA, Pedersen LC, Bebenek K. Creative template-dependent synthesis by human polymerase mu. *Proc. Natl. Acad. Sci. USA*., 112: E4530-E4536, 2015.

Lack of cross-reactivity between GST allergens could lead to new diagnostic tools
NIEHS scientists and their collaborators determined that glutathione S-transferase (GST) allergens from cockroach, roundworm, and 2 dust mite species did not contain cross-reactive sites. The discovery was the result of IgE reactivity assays and crystal structure data of allergen GSTs generated by the NIEHS members of the team. The finding is novel because it demonstrates that the allergic response to GST allergens is species specific, contradicting previous studies that suggested these GST allergens were cross-reactive. Allergists can use the information to offer a more accurate diagnosis of the sensitizing organism and suggest the appropriate treatment.

Mueller GA, Pedersen LC, Glesner J, Edwards LL, Zakzuk J, London RE, Arruda LK, Chapman MD, Caraballo L, Pomés A. Analysis of glutathione S-transferase allergen cross-reactivity in a North American population: Relevance for molecular diagnosis. *J. Allergy Clin. Immunol*., epub ahead of print, doi: 10.1016/j.jaci.2015.03.015.

Cell receptor for high density lipoprotein (HDL) found to have key role in survival from pneumonia

Scavenger receptor B-I (SR-BI) is a protein best known in its role as a receptor for cell uptake of high density lipoprotein (HDL, so called 'good cholesterol'), and has been studied extensively in atherosclerosis and cholesterol clearance. Investigators at the NIEHS recently identified a novel function for SR-BI – namely, that it is critical for survival from bacterial pneumonia. SR-BI-deficient mice were found to have markedly increased mortality during pneumonia, associated with higher bacterial burden in the lung and blood, higher serum inflammatory proteins, and increased organ injury. Several important roles for SR-BI in pneumonia were identified, including clearance of bacterial molecules from the lung, support of stress steroid hormone production by the adrenal glands, and support of bacterial killing function by white blood cells. Given that SR-BI-targeting drugs have recently been developed and that SR-BI variants exist in humans, these findings may offer new insights into the management of patients with pneumonia and sepsis.

Gowdy KM, Madenspacher JH, Azzam KM, Gabor KA, Janardhan KS, Aloor JJ, Fessler MB. Key role for scavenger receptor B-I in the integrative physiology of host defense during bacterial pneumonia. *Mucosal Immunol*., 8:559-571, 2015.

Identification of cells in the lung that control distinct forms of asthma

Allergic asthma is a chronic, inflammatory disease of the lung. It has recently become clear that there are different types of asthma, characterized by eosinophilic or neutrophilic inflammation. In this study, genetically modified mice were used to show that expression of the Toll-like receptor (TLR)4 in hematopoietic cells is critical for neutrophilic forms of asthma, whereas expression of this receptor in epithelial cells that line the airway is important for eosinophilic forms of asthma. These findings might facilitate the design of therapies targeting specific forms of asthma.

McAlees JW, Whitehead GS, Harley IT, Cappelletti M, Rewerts CL, Holdcroft AM, Divanovic S, Wills-Karp M, Finkelman FD, Karp CL, Cook DN. Distinct Tlr4-expressing cell compartments control neutrophilic and eosinophilic airway inflammation. *Mucosal Immunol*., 8:863-873, 2015.

Genistein Exposure Alters Glucocorticoid Signaling

NIEHS researchers discovered a unique mechanism by which genistein, an estrogen-like compound naturally occurring in soy products, regulates glucocorticoid receptor-mediated gene expression. Utilizing a whole genome approach, they found that genistein regulates a unique transcriptional response in human uterine endometrial cells compared to estradiol. Furthermore, co-administration of genistein with the synthetic glucocorticoid dexamethasone resulted in a unique pattern of gene expression compared to estradiol and dexamethasone. The findings from this study provide an in vitro model for understanding the consequences of genistein exposure and provide novel molecular targets for future studies.

Whirledge S, Senbanjo LT, Cidlowski JA. Genistein disrupts glucocorticoid receptor signaling in human uterine endometrial Ishikawa cells. *Environ. Health Perspect.*, 123: 80-87, 2015.

First genome-wide study of autoimmune muscle disease subgroups shows immune response genes located in an ancient haplotype as the major genetic risks

Autoimmune muscle diseases (myositis) comprise a group of complex disorders influenced by genetic and environmental factors. To identify genetic risk factors in patients of European ancestry, NIEHS investigators with the Myositis Genetics Consortium conducted a genome-wide association study (GWAS) of the major myositis subgroups, including 1710 children and adults with the myositis subgroups called polymyositis and dermatomyositis, and those with a disease-specific autoantibody called anti-Jo-1, and compared them with 4724 controls. Single-nucleotide polymorphisms showing strong associations ($P < 5 \times 10^{-8}$) in GWAS were identified in the major histocompatibility complex region for all myositis patients together, as well as for each of the four subgroups studied separately. Further analyses found that alleles comprising the human leukocyte antigen 8.1 ancestral haplotype (AH8.1) defined essentially all the genetic risk in all the groups studied, and multiple alleles of AH8.1 were required for the full risk effects. These findings establish that alleles of the AH8.1 comprise the primary genetic risk factors associated with the major myositis subgroups in geographically diverse Caucasian populations and suggest new approaches to diagnosis and therapy.

Miller FW, Chen W, O'Hanlon TP, Cooper RG, Vencovsky 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 WER, Scheet P, Peng B, Lee A, Byun J, Lamb JA, Gregersen PK, Amos CI, with the Myositis Genetics Consortium. Genome-wide Association Study Identifies HLA 8.1 Ancestral Haplotype Alleles as Major Genetic Risk Factors for Myositis Phenotypes. *Genes and Immunity*, epub ahead of print, doi: 10.1038/gene.2015.28.

Asymmetric conformational maturation of HIV-1 reverse transcriptase

HIV reverse transcriptase (RT) is a key enzyme in the life cycle of the virus that causes acquired immunodeficiency syndrome (AIDS) and an important drug target. The mature form of the enzyme consists of a catalytic p66 subunit, and a structural p51 subunit, and is a target of drugs used to treat AIDS. Both subunits are derived from a single peptide chain by a series of complex and largely uncharacterized conformational rearrangements that underlie its asymmetric folding, dimerization and subunit-selective ribonuclease H domain (RH) proteolysis. This process utilizes a metamorphic polymerase domain that is able to adopt two alternate structures that fulfill catalytic and structural roles, thereby minimizing its coding requirements. NIEHS scientists have investigated this conformational maturation using NMR studies of methyl-labeled RT for the slower processes in combination with molecular dynamics simulations for rapid processes. Starting from an inactive conformation, the p66 precursor undergoes a unimolecular isomerization to a structure similar to its active form, exposing a large hydrophobic surface that facilitates initial homodimer formation. The resulting p66/p66' homodimer exists as a conformational heterodimer, after which a series of conformational adjustments on different time scales can be observed. Formation of the inter-subunit RH:thumb' interface occurs at an early

stage, while maturation of the connection' and unfolding of the RH' domains are linked and occur on a much slower time scale.

Zheng X, Perera L, Mueller GA, DeRose EF, London RE. Asymmetric conformational maturation of HIV-1 reverse transcriptase. *eLife*, 4:e06359, 2015.

Nonmotor symptoms could effectively differentiate patients of Parkinson's disease from healthy controls.

NIEHS scientists conducted one of the largest analyses to date to evaluate whether nonmotor symptoms alone could differentiate drug-naïve Parkinson's patients from controls, and whether there are sex differences in the presentation of nonmotor symptoms. The scientists found that the presence of several nonmotor symptoms, decreased sense of smell in particular, could effectively differentiate patients with early Parkinson's from healthy controls. Further, the investigators also found potential sex differences in the presentation of nonmotor symptoms among Parkinson's patients. Research on Parkinson's nonmotor symptoms may eventually help to identify individuals at risk for the disease and to understand disease etiology.

Liu R, Umbach DM, Peddada SD, Xu Z, Troster AI, Huang X, Chen H. Potential sex differences in nonmotor symptoms in early drug-naive Parkinson disease. *Neurology*, 84:2107-2115, 2015.

Identification of protein that helps prepare for healthy egg-sperm union

The protein RGS2 can hold off an egg's development into an embryo in order to allow time for sperm to arrive and merge with the egg in a healthy fertilization process. The egg stores calcium during maturation, preparing it for fertilization when the sperm causes calcium to release within the egg, turning it into a developing embryo. Premature calcium release in the egg, before the sperm arrives, prevents fertilization. NIEHS investigators discovered that RGS2 suppresses premature calcium release during the maturation process, preserving fertilizability of the ovulated egg. This is the first demonstration of the protein's significant role in fertilization, and is important because RGS2 is being targeted as a clinical treatment for cardiac disease, hypertension, and anxiety.

Bernhardt ML, Lowther KM, Padilla-Banks E, McDonough CE, Lee KN, Evsikov AV, Uliasz TF, Chidiac P, Williams CJ, Mehlmann LM. Regulator of G-protein signaling 2 (RGS2) suppresses premature calcium release in mouse eggs. *Development*, 142: 2633-2640, 2015.

MMS exposure is associated with mtDNA mutagenesis.

Researchers from the NIEHS have identified a novel genetic and environmental interaction which alters mitochondrial DNA (mtDNA) replication efficiency. Maintenance of mtDNA is essential for cellular survival in eukaryotic cells, and the inability to properly replicate results in mitochondrial disease states and toxicity. Scientists investigated mtDNA replication efficiency, by examining yeast strains previously characterized with mutations in the mtDNA polymerase gene, in the presence of the alkylating base damaging agent, methyl methanesulfonate (MMS). The study demonstrated that MMS exposure increased mtDNA mutagenesis in strains with disease-associated mutations that disrupt polymerase activity. Approximately half of the

mutations arising from MMS exposure were cytosine to guanine transversions. Further observations suggested that MMS-induced mutagenesis did not arise by disrupting exonuclease activity. The authors have demonstrated that MMS exposure induced mtDNA mutagenesis in single-stranded mtDNA. Furthermore, this study supports a polymerase switching mechanism in mtDNA replication, which exposes single-stranded DNA to mutagenesis that was previously not described. These findings raise the question whether a similar mechanism occurs in mammalian mtDNA, and offers new insights for patients suffering from mitochondrial diseases and their susceptibility to DNA damaging agents.

Stumpf JD, Copeland WC. 2014. MMS exposure promotes increased mtDNA mutagenesis in the presence of replication-defective disease-associated DNA polymerase gamma variants. *PLoS Genet.,* 10:e1004748, 2014.

How cells achieve high accuracy of chromosomal DNA replication

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

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

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

Swerdlow SJ, Schaaper RM. Mutagenesis in the *lacI* gene target of *E. coli*: improved analysis for $lacI^d$ and *lacO* mutants. *Mutat. Res*., 770: 79-84, 2014.

Singh D, Gawel D, Itsko M, Hochkoeppler A, Krahn JM, London RE, Schaaper RM. Structure of dGTP triphosphohydrolase: A hexameric enzyme with DNA effector molecules. *J. Biol. Chem*., 290:10418-10429, 2015.

Maslowska KH, Makiela-Dzbenska K, Fijalkowska IJ, Schaaper RM. Suppression of the *E. coli* SOS response by dNTP pool changes. *Nucl. Acids Res.,* 43:4109-4120, 2015.

Dominant negative consequence of genetic mutation in mitochondrial disease

Mitochondrial disease is caused by the decline in energy production in the cell and can result in multi-organ failure. Many of the mitochondrial diseases are due to defects in the mitochondrial genome as a result of defects in the nuclear gene products (enzymes) that maintain this genome. Researchers at the NIEHS have studied the consequence of gene mutations in the accessory subunit (POLG2) of the mitochondrial DNA polymerase gamma, the enzyme responsible for replication of the mitochondrial genome. Using biochemistry and cell biological techniques, NIEHS researchers have determined that certain genetic variants in the POLG2 gene result in a dominant negative phenotype corresponding to an autosomal dominant disease.

Young MJ, Humble MM, DeBalsi KL, Sun KY, Copeland WC.POLG2 disease variants: analyses reveal a dominant negative heterodimer, altered mitochondrial localization and impaired respiratory capacity. *Hum. Mol. Genet*., epub ahead of print, doi: 10.1093/hmg/ddv240

Epigenetic changes may explain link between weight and breast cancer risk

Increased body mass index (BMI) is associated with increased risk of postmenopausal breast cancer, but the mechanism linking these has been unclear. This study shows that BMI influences the epigenetic profile of normal breast tissue, and that these changes preferentially involve genes involved in inflammatory response and energy metabolism. These findings support the hypothesis that increased adiposity is associated with a chronic inflammatory response, which in turn may lead to increased breast cancer risk by epigenetically altering the gene expression profile of normal breast tissue**.**

Hair BY1, Xu Z, Kirk EL, Harlid S, Sandhu R, Robinson WR, Wu MC, Olshan AF, Conway K, Taylor JA, Troester MA. Body mass index associated with genome-wide methylation in breast tissue. *Breast Cancer Res. Treat*., 151: 453-463, 2015.

Epigenetics, retroviral elements and evolution interact to regulate the response to DNA damage.

The tumor suppressor p53 is well known for its role in carcinogenesis. When activated by DNA damage, p53 binds to its DNA response elements and regulates transcription of genes involved in DNA repair and cell death. NIEHS scientists mapped p53 binding and chromatin status after DNA damage and found, surprisingly, that p53 strongly bound to thousands of DNA elements located in repressed chromatin that have recently evolved from human retroviral transposons. Characterizing the chromatin-mediated p53 stress response and the deregulation of transposons may prove to be clinically relevant for understanding outcomes in cytotoxic therapy for cancer.

Su D, Wang X, Campbell MR, Song L, Safi A, Crawford GE, Bell DA. Interactions of chromatin context, binding site sequence content, and sequence evolution in stress-induced p53 occupancy and transactivation. *PLoS Genet*., 11:e10004885, 2015.

The tumor suppressor p53 can greatly enhance human immune responses.

There is a well-established relationship between inflammation and the appearance of cancers as well as many diseases. NIEHS researchers have established inflammatory responses in various kinds of human tumor cells and primary lymphocytes can be dramatically enhanced by activated p53. This effect requires cooperation with the NF-κB inflammatory system, whereas these two systems typically have opposing effects, and it can be mediated by several molecular mechanisms including p38 MAP and PI3 kinases. 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. Since the expression of many synergistically induced genes is elevated in breast cancer patients responsive to chemotherapy, the researchers suggest that p53's capacity to enhance immune response could be exploited to increase antitumor immunity and to improve cancer treatment.

Shatz M, Shats I, Menendez D, Resnick MA. p53 amplifies inflammatory response in human primary and cancer cells through interaction with multiple signal transduction pathways. *Oncotarget*, 6: 16963-16980 2015.

Arsenic regulates gene expression in human cells at the level of messenger RNA stability.
Inorganic arsenic species are potent environmental toxins and causes of numerous health problems. Most studies have assumed that arsenic-induced changes in messenger RNA levels result from effects on gene transcription. NIEHS investogators evaluated the prevalence of changes in messenger RNA stability in response to sodium arsenite in human fibroblast cells. They found that arsenite modification of messenger RNA stability is relatively uncommon, but in some instances can result in significant changes in gene expression.

Qiu LQ, Abey S, Harris S, Shah R, Gerrish KE, Blackshear PJ. Global analysis of posttranscriptional gene expression in response to sodium arsenite. *Environ. Health Perspect*., 123: 324-330, 2015.

Characterization of the redox transition of the XRCC1 N-terminal domain
Repair of DNA lesions typically requires the involvement of multiple enzymes that are organized by interaction with a scaffold protein. XRCC1, a scaffold protein involved in repair of DNA single strand breaks, contains an N-terminal domain (X1NTD) that interacts specifically with DNA polymerase β. It was recently discovered that X1NTD contains a disulfide switch that allows it to adopt either of two metamorphic structures. NIEHS scientists demonstrated that formation of an N-terminal proline carbimate adduct resulting from the nonenzymatic reaction of Pro2 with CO_2, is essential for stabilizing the oxidized structure, X1NTDox. The kinetic response of X1NTDred to H_2O_2, monitored by NMR, was determined to be very slow, consistent with involvement of the buried, kinetically trapped Cys12 residue, but was significantly accelerated by addition of protein disulfide isomerase or by Cu^{2+}. NMR analysis of a sample containing the pol β polymerase domain, and both the reduced and oxidized forms of X1NTD, indicates that the oxidized form binds to the enzyme.

Gabel SA, Smith CE, Cuneo MJ, Mueller GA, Kirby TW, DeRose EF, Krahn JM, London RE. Characterization of the redox transition of the XRCC1 N-terminal domain. *Structure*, 22:1754-1763, 2014.

Simulated epidemiologic study provides insight into why association reported between environmental contaminant and birthweight
In a recent meta-analysis of epidemiologic data on perfluorooctanoic acid (PFOA) and birthweight, it was reported that higher concentrations of PFOA in pregnant women or cord blood were associated with lower birthweight. Using a pharmacokinetic model and a Monte-Carlo process, NIEHS investigators simulated an epidemiologic study and sampled observations, so that it was like the meta-analysis. The analysis of the simulated data showed the same type of associations as in the observed meta-analysis, indicating that at least some of the association was due to confounding by glomerular filtration rate rather than an adverse effect of the exposure.

Verner MA, Loccisano AE, Morken NH, Yoon M, Wu H, McDougall R, Maisonet M, Marcus M, Kishi R, Miyashita C, Chen MH, Hsieh WS, Andersen ME, Clewell HJ

3rd, Longnecker MP. Associations of Perfluoroalkyl Substances (PFASs) with Lower Birth Weight: An Evaluation of Potential Confounding by Glomerular Filtration Rate Using a Physiologically Based Pharmacokinetic Model (PBPK). *Environ. Health Perspect*., epub ahead of print, doi: 10.1289/ehp.1408837

Inhibition of protein phosphatases in the brain alters inhibitory signaling.
One of the major mechanisms regulating neuronal excitability in the brain is the inhibitory effect of neuropeptides that signal through G-protein coupled receptors. NIEHS investigators have discovered that the efficacy of this inhibitory signaling in the hippocampus, which involves potassium channel stimulation by the neuropeptide somatostatin, is regulated downstream of cyclic AMP by protein phosphatases that are the major targets of microbial toxins in algal blooms. Reductions in somatostatin releasing neurons and in protein phosphatase activity are known to be associated with aging and neurodegenerative diseases. The discovery links these two findings mechanistically, and raises concern about the contribution of exposures to water polluted by algal blooms in premature aging and dementia.

Lucas SJ, Armstrong DL. Protein phosphatase modulation of somatostatin receptor signaling in the mouse hippocampus. *Neuropharmacology*, 99: 232-241, 2015.

Reinterpreting the best biomarker of oxidative stress for the 21st century
An increase in the oxidation of biomolecules, or "oxidative stress" is believed to play an important role in the detrimental effects of diseases and environmental exposures on human health. Through rigorous validation studies, NIEHS investigators have identified the F2-isoprostanes as the best non-invasive biomarker of oxidative stress. However, there are confounding factors such as inflammation which compromise the interpretation of this marker. To properly interpret F2-isoprostane as a biomarker of oxidative stress, these investigators have successfully developed the first method which accounts for confounding factors, and allows for unperturbed determination of oxidative stress in human samples. With little effort to implement, this new method will correctly confirm the occurrence of oxidative stress in humans and advance continued research studies into the 21st century.

van 't Erve TJ, Lih FB, Kadiiska MB, Deterding LJ, Eling TE, Mason RP. Reinterpreting the best Biomarker of Oxidative Stress: The 8-iso-PGF2α / PGF2α Ratio Distinguishes Chemical from Enzymatic Lipid Peroxidation. *Free Radic. Biol. Med*., 83:245-251, 2015.

The anti-inflammatory protein tristetraprolin controls a complex network of cellular responses to inflammation.
Tristetraprolin is an anti-inflammatory protein that acts by binding to the messenger RNAs coding for cytokines involved in response to inflammation. It regulates cytokine release from the initial response cells, macrophages. The authors discovered that cytokines released in turn from the activated macrophages can affect the release of other proteins from secondary cells in tissues, proteins which recruit inflammatory cells to the site to help clear up the inflammation. Thus, a single protein can coordinately regulate the complex response to acute inflammation in several cell types.

Qiu LQ, Lai WS, Bradbury A, Zeldin DC, Blackshear PJ. Tristetraprolin (TTP) coordinately regulates primary and secondary cellular responses to proinflammatory stimuli. *J. Leukoc. Biol*., 97: 723-736, 2015.

Protective glove use reduces farmers' risk of Parkinson's disease related to pesticide use.
Pesticide use may increase risk of Parkinson's disease (PD). In a study of 69 PD cases and 237 matched controls, NIEHS scientists found that pesticide-related risk was smaller among farmers who used protective gloves while mixing or applying pesticides compared to farmers who did not use gloves. Similar results were found for use of good work hygiene practices, such as washing hands immediately after using pesticides. These results suggest an important approach to modifying PD risk.

Furlong M, Tanner CM, Goldman SM, Bhudhikanok GS, Blair A, Chade A, Comyns K, Hoppin JA, Kasten M, Korell M, Langston JW, Marras C, Meng C, Richards M, Ross GW, Umbach DM, Sandler DP, Kamel F. Protective glove use and hygiene habits modify the associations of specific pesticides with Parkinson's disease. *Environ. Int.*, 75:144-150, 2015.

Anti-Mullerian hormone concentrations found to be lower in women from rural South Africa exposed to pyrethroid insecticides for malarial control
The concentration of Anti-Mullerian hormone in women's blood reflects their ovarian reserve. This analysis included 420 women from Limpopo, South Africa studied in 2010-2011. These results are suggestive of decreased ovarian reserve associated with exposure to pyrethroid pesticides, which is consistent with laboratory animal data.

Whitworth KW, Baird DD, Steiner AZ, Bornman RM, Travlos GS, Wilson RE, Longnecker MP. Anti-Müllerian hormone and lifestyle, reproductive, and environmental factors among women in rural South Africa. *Epidemiology*, 26:429-435. 2015.

Simulated study provides insight into why association reported between environmental contaminant and age at menarche
In a recent epidemiologic study based on a cross-sectional study design, it was reported that higher concentrations of perfluoroalkyl substances (environmental contaminants) in adolescent females were associated with later age at menarche. Using a physiologically-based pharmacokinetic model and a Monte-Carlo process, NIEHS investigators simulated an epidemiologic cohort and sampled the observations cross-sectionally, so that it was like the observed study. The analysis of the simulated data showed the same type of associations as in the observed study, indicating that at least some of the association was due to growth dilution rather than an adverse effect of the exposure.

Wu H, Yoon M, Verner MA, Xue J, Luo M, Andersen ME, Longnecker MP, Clewell HJ 3rd. Can the observed association between serum perfluoroalkyl substances and delayed menarche be explained on the basis of puberty-related changes in physiology and pharmacokinetics? *Environ. Int.*, 82:61-68, 2015.

Developmental protein LIN28A modulates splicing and expression programs of genes implicated in biological processes that drive breast cancer.
Scientists at NIEHS established a role for LIN28A in the alternative splicing and steady state mRNA expression of genes that control cellular energy metabolism, immune response and receptor kinase signaling, processes implicated in various aspects of breast cancer biology.

Yang J, Bennett BD, Luo S, Inoue K, Grimm SA, Schroth GP, Bushel PR, Kinyamu HK, Archer TK. LIN28A modulates splicing and gene expression programs in breast cancer cells. *Mol. Cell. Biol.*, 35: 3225-3243, 2015.

Substrate-induced DNA polymerase activation.
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 investigators and collaborators recently obtained novel insight into the mechanism of the chemical reaction. Strategic mutations were engineered into the model DNA polymerase, pol β, and the altered enzyme was examined. The results revealed novel understanding of the mechanism of the chemical reaction of nucleotide insertion into DNA.

Beard WA, Shock DD, Batra VK, Prasad R, Wilson SH. Substrate-induced DNA polymerase β activation. *J. Biol. Chem.*, 289: 31411-31422, 2014.

MED25 found to regulate the human gene CYP2C9 by epigenetic changes.
The Mediator complex is necessary for transcriptional regulation of eukaryotic genes by modifying chromatin structure and initiating transcription. NIEHS researchers found MED25 was responsible for the epigenetic regulation (acetylation or methylation) of lysine27 (K27) of histone 3 (H3) on human cytochrome P450 2C9 (CYP2C9). MED25 is recruited to the CYP2C9 promoter by association by liver-enriched receptor hepatic nuclear factor 4α (HNF4α). H3 in the HNF4α binding region was enriched for acetyation of H3K27ac after MED25 overexpression and activation, but H3K27 was trimethylated when MED25 expression was silenced and CYP2C9 repressed. Chromatin conformation changes were dependent on MED25 expression. Thus, MED25 produces epigenetic changes resulting in transcriptional activation/deactivation of this important human gene.

Englert NA, Luo G, Goldstein JA, Surapureddi, S. Epigenetic modification of Histone 3 Lysine27: The Role of Med25 in the disassociation of Polycomb repressive complex2 from the promoter of the CYP2C9 gene. *J. Biol. Chem.*, 290: 244-2418, 2015.

Regulation of gene expression in the human pathogen *Candida albicans*
This study examined the regulation of gene expression at the level of messenger RNA stability in *Candida albicans*, an important fungal human pathogen. A single protein, Zfs1, regulated the expression of many genes by promoting the rapid destruction of their messenger RNAs. The Zfs1 protein is highly conserved in fungi, but its targets in *C. albicans* are different from those found in other fungi. Thus, although the mechanism by which this protein acts is conserved in evolution, the specific RNA targets to which this protein binds are species-specific.

Wells ML, Washington OL, Hicks SN, Nobile CJ, Hartooni N, Wilson GM, Zucconi BE, Huang W, Li L, Fargo DC, Blackshear PJ. Post-transcriptional regulation of transcript abundance by a conserved member of the tristetraprolin family in Candida albicans. *Mol. Microbiol*., 95: 1036-1053, 2015.

How to accurately replicate the nuclear genome.

The nuclear genomes of eukaryotic cells are replicated with incredibly high accuracy to maintain species identity and prevent cell death and disease. This year NIEHS scientists performed a study that provides a view of the three major steps that determine nuclear genome replication fidelity. The results have important implications for evolution and disease etiology.

St Charles JA, Liberti SE, Williams JS, Lujan SA, Kunkel TA. Quantifying the contributions of base selectivity, proofreading and mismatch repair to nuclear DNA replication in *Saccharomyces cerevisiae*. *DNA Repair*, 31:41-45, 2015.

KIKO mouse mode helps identify an unexpected hormone response motif activity of the female estrogen receptor

KIKO mice contain mutations that prevent direct estrogen response element DNA- binding. KIKO mice are infertile, due in part to the inability of estradiol (E2) to induce uterine epithelial proliferation. Analysis of these mutant mice demonstrates that ERα DNA-binding is crucial for biological and transcriptional processes in reproductive tissues and that ERα tethering may not contribute to estrogen responsiveness in vivo.

Hewitt SC, Li L, Grimm SA, Winuthayanon W, Hamilton KJ, Pockette B, Rubel CA, Pedersen LC, Fargo D, Lanz RB, DeMayo FJ, Schütz G, Korach KS. Novel DNA motif binding activity observed in vivo with an estrogen receptor α mutant mouse. *Mol. Endocrinol.*, 28: 899-911, 2014.

Liver metabolic disorder caused by drug intake factors.

More than 50% of therapeutic drugs activate receptor PXR in liver. Now it was found that this receptor activates SLC15A5 in human primary hepatocytes. SLC15A5 is known to mediate citrate uptake in cells and plays important roles in fatty acid and cholesterol synthesis. PXR activation augments lipid accumulation in hepatocytes and SLC15A5 gene knock down decreased the lipid content in a human liver derived cell line. Thus SLC15A5 gene induction via PXR activation by therapeutic drugs may affect liver fatty acid and cholesterol metabolism.

Li L, Li H, Garzel B, Yang H, Sueyoshi T, Li Q, Shu Y, Zhang J, Hu B, Heyward S, Moeller T, Xie W, Negishi M, Wang H: SLC13A5 is a novel transcriptional target of the pregnane X receptor and sensitizes drug-Induced steatosis in human liver. *Mol. Pharmacol*., 87: 674-681, 2015.

The sugar hyaluronan plays an important role in the development of lung injury after inhaled chlorine gas exposure

Chlorine gas exposure can and has happened either in industrial accidents (e.g. train derailments) or terrorism and chemical warfare. Chlorine gas inhalation leads to substantial lung injury and it is crucial to understand how to treat this. Researchers uncovered that the mechanism of chlorine-

gas-induced lung injury involves release of a shortened form of a sugar called hyaluronan, and they found out that they can treat chlorine-gas-induced lung injury by giving inhaled treatments with a healthy, long variant of hyaluronan. They also found out that pre-existing infections can lead to more short hyaluronan release in the lung after chlorine gas, and thus people with infections may respond even more to inhaled chlorine gas, but this can also be treated in the same way as above.

Lazrak A, Creighton J, Yu Z, Komarova S, Doran SF, Aggarwal S, Emala CW Sr, Stober VP, Trempus CS, Garantziotis S, Matalon S. Hyaluronan mediates airway hyperresponsiveness in oxidative lung injury. *Am. J. Physiol. Lung. Cell. Mol. Physiol*., 308:L891-L903, 2015.

Song W, Yu Z, Doran SF, Ambalavanan N, Steele C, Garantziotis S, Matalon S. Respiratory Syncytial Virus Infection Increases Chlorine Induced Airway Hyper-responsiveness. *Am. J. Physiol. Lung. Cell. Mol. Physiol*., 309: L205-L210, 2015.

Measurements of perfluorinated alkyl substances among women of reproductive age found to be highly reliable

NIEHS scientists investigated the association between PFAS (perfluoroalkyl substances) plasma concentrations of 100 women from Norway in two consecutive pregnancies to explore changes in plasma concentration related to reproductive factors. They found the reliability of PFAS measurements in maternal plasma to be moderate to high, and in these data, several factors, especially breastfeeding, were related to plasma concentrations.

Papadopoulou E, Haug LS, Sabaredzovic A, Eggesbø M, Longnecker MP. Reliability of perfluoroalkyl substances in plasma of 100 women in two consecutive pregnancies. *Environ. Res.*, 140: 421-429, 2015.

In-Home Test Kit for Dust Mite Allergens

Dust mite allergens can induce allergic sensitization and exacerbate asthma symptoms. Although dust mite reduction and control strategies exist, few asthmatics employ them. NIEHS investigators examined whether an in-home test kit, which quantifies dust mite allergen levels, resulted in behavioral changes in implementation and maintenance of mite reduction strategies and helped reduce allergen levels in homes of dust mite-sensitive children. The investigators enrolled 60 households of children aged 5-15 with parent-reported dust mite allergy into a randomized controlled trial. Intervention homes (N=30) received educational material about reducing dust mites and test kits at 1,2,5, and 8 months. Control homes (N=30) received only educational material. At baseline, 6 and 12 months, study staff visited all homes, collected dust samples from 3 locations and obtained information about parents' mite reduction behaviors by questionnaire. Allergen concentrations (Der f 2/Der p2) in dust were assessed by immunoassays. After adjusting for visit and location, allergen concentrations in intervention and control homes were compared using mixed effects model analysis. In the intervention homes, allergen concentrations in the child's bedroom and living room floors were significantly reduced over time compared to control homes. Although not all location-specific differences in allergen concentrations were statistically significant, combining data across locations, there was a differential reduction in allergen concentrations in the intervention group versus the control group.

Winn AK, Salo PM, Klein C, Sever ML, Zombeck A, Harris SF, Johndrow D, Crocket PW, Cohn RD, Zeldin DC. Efficacy of an In-home Test Kit in Reducing Dust Mite Allergen Levels: Results of a Randomized Controlled Pilot Study. *J. Asthma.,* in press, 2015.

Itch regulates Glis3 to control pancreatic ß cell and insulin production

The transcription factor Gli-similar 3 (Glis3) plays a critical role in the generation of pancreatic ß cells and the regulation insulin gene transcription and has been implicated in the development of several pathologies, including type 1 and 2 diabetes and polycystic kidney disease. However, little is known about the mechanisms of action of this protein. NIEHS researchers identified the E3 ubiquitin ligase, Itch, as a critical regulator of Glis3 activity and as such might play a role in modulating diabetes and other diseases in which Glis3 is implicated.

ZeRuth GT, Williams JG, Cole YC, Jetten AM. HECT E3 Ubiquitin Ligase Itch Functions as a Novel Negative Regulator of Gli-Similar 3 (Glis3) Transcriptional Activity. *PLoS One*, 10: e0131303, 2015.

Bisphenol A promotes cell survival following oxidative DNA damage

Bisphenol A (BPA) is a biologically active industrial chemical used in production of consumer products. Recent studies link BPA with the generation of reactive oxygen species, and base excision repair (BER) is responsible for removing oxidatively induced DNA lesions. Yet, the relationship between BPA and BER has yet to be examined. To determine the effect of BPA exposure on base excision repair of oxidatively induced DNA damage, cells compromised in double-strand break repair were treated with BPA alone or co-exposed with either potassium bromate ($KBrO_3$) or laser irradiation as oxidative damaging agents. BPA partially reversed the $KBrO_3$-induced cytotoxicity observed in these cells, and this was coincident with an increase in guanine base lesions in genomic DNA. The improvement in cell survival and the increase in oxidatively induced DNA base lesions were reminiscent of previous results with alkyl adenine DNA glycosylase-deficient cells, suggesting that BPA may prevent initiation of repair of oxidized base lesions. These results are consistent with the hypothesis that BPA can induce a suppression of oxidized base lesion DNA repair by the base excision repair pathway.

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

Accessing probability that a genetic association is genuine directly from significance tests.

There is a growing concern that lack of replicability of scientific findings is due, to a large extent, to misuse of significance testing. It is common strategy in genomic research to perform multiple statistical tests followed by selection of most significant results. Such selection results in a bias, i.e., in overstatement of the effect magnitude. However, when external information about possible effect magnitudes, such as the shape of the effect size distribution is available, this bias can be corrected. NIEHS investigators propose a straightforward method that is capable to convert significance values to valid probabilities that the corresponding findings are genuine**.**

Kuo CL, Vsevolozhskaya OA, Zaykin D. Assessing the Probability that a Finding Is Genuine for Large-Scale Genetic Association Studies. *PLoS One*, 10: e0124107, 2015.

Isoflavones enhance ROR α and γ to influence obesity, diabetes and autoimmune disorders
Isoflavones are naturally occurring plant chemicals, and their plant-based dietary intake may play a beneficial role in the treatment/prevention of obesity, cancer, osteoporosis, and cardiovascular disease. NIEHS scientists demonstrate that isoflavones enhance the activity of retinoic acid receptor-related orphan receptors α and γ which are involved in the regulation of obesity, diabetes and several autoimmune disorders.

Kojima H, Takeda Y, Muromoto R, Takahashi M, Hirao T, Takeuchi S, Jetten AM, Matsuda T. Isoflavones enhance interleukin-17 gene expression via retinoic acid receptor-related orphan receptors α and γ. *Toxicology*, 29: 32-39, 2015.

An Improved Method for Finding Effects of Genetic Variants on the X Chromosome
Investigators at the NIEHS have developed a more powerful approach to evaluating risk related to genetic variants on the X chromosome. The insight they exploited is that alleles related to risk will be over-represented in mothers of male cases and under-represented in mothers of female cases. They developed a unified statistical approach to capture both that asymmetry in the parents of cases, and the increased transmission of susceptibility alleles to affected offspring.

Wise AS, Shi M, Weinberg CR. Learning about the X from our parents. *Front. Genet.*, 6:15, 2015.

Towards predicting metastatic progression of skin melanoma
Despite the overall resemblance between primary and metastatic melanomas at genomic level, NIEHS investigators were able to assess a putative metastatic progression status for each tumor. They showed that loss of expression of characteristic epithelial cell lineage genes was highly correlated with the predicted metastatic progression scores for the primary tumors and the scores were significantly associated with clinical prognostic factors (e.g., staging of lymph nodes).

Li Y, Umbach DM, Krahn JM, Flake G, Li L. Towards predicting metastatic progression of melanoma based on gene expression data. *Pigment Cell Melanoma Res.*, 28: 453-463, 2015.

Social isolation during adolescence promotes alcohol intake
The study was designed using an establish mouse model of stress and alcohol consumption. Both female and male adolescent mice that were housed without companions showed a significant increase in voluntary alcohol intake. The effect of isolation was mitigated by providing the mice with environmental enrichment. The results of this study indicate that housing conditions during critical developmental periods can significantly modulate voluntary alcohol intake later in life.

Lopez M, Laber K. Impact of social isolation and enriched environment during adolescence on voluntary ethanol intake and anxiety in C57BL/6J mice. *Physiol. Behav.*, 148: 151-156, 2015.

Improving data analysis in quantitative high-throughput screening

Recent technological advancements have led to the generation of a large amount of quantitative high-throughput screening (qHTS) data, often consisting of concentration-response profiles for thousands of chemicals generated in a single experiment. However, nonlinear modeling of qHTS data presents importance challenges that are not problematic in linear modeling. Therefore, uncertainty in nonlinear model parameters should be carefully taken into account or robust data analysis approaches should be developed for chemical genomics and in vitro toxicity testing.

Shockley KR. Quantitative high-throughput screening data analysis: challenges and recent advances. *Drug Discovery Today*, 20: 296-300, 2015.