Division of Intramural Research

NAEHS Council Update

October 3, 2017

DIR RECRUITMENTS

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

Chief of the Administrative Research and Services Branch
The National Institute of Environmental Health Sciences (NIEHS) is seeking an accomplished individual to serve as the Chief of the Administrative Research and Services Branch (ARSB). This individual will serve as principal advisor to senior management on all phases of the administrative management of the Division of Intramural Research (DIR), the Division of the National Toxicology Program (DNTP), and Clinical Research Branch for NIEHS; and oversee the implementation of a variety of management services essential to the direction and operation of the Institute. The successful candidate will: Provide guidance and oversight for procurement, contracts, property management and operational management functions; Oversee and monitor the operating budget process to ensure the timely, appropriate, and efficient expenditure of funds against annual allotment; anticipate changes in funding levels; prepare proposals and justify current and increased expenditures; Serve as a principal advisor on all human resource management activities and ensures compliance with all applicable regulatory requirements; Oversee all administrative management matters associated with programs and operations; with responsibility for the analysis of organizational priorities and the development and implementation of administrative policies and procedures; Participate in and oversee the planning sessions related to the following space, telecommunications, travel, and/or timekeeping and leave; and Supervise the activities for administrative, technical and support staff. Dr. Jerry Yakel, Lab Chief of the Neurobiology Laboratory, is chair of the Search Committee.

Chief of the Biostatistics and Computational Biology Branch
The National Institute of Environmental Health Sciences (NIEHS) is seeking an accomplished individual to serve as the Chief of the Biostatistics and Computational Biology Branch (BCBB). The ideal candidate will be tenure-eligible based on an outstanding academic record of achievement, leadership capabilities, and broad interests in biostatistics and computational biology. In addition to directing their own independent research program, they will have responsibility for leading BCBB in new directions as biostatistics and environmental science data continually evolve. The successful candidate should have a keen interest in collaborating both with members of BCBB and with other investigators within NIEHS. Principal investigators in

the NIH intramural program have no formal teaching duties, are funded internally, and work with a great deal of protected time. They engage directly in research and methods development with postdoctoral fellows, students, and support staff, and collaborate with colleagues in solving important scientific problems. Dr. Jack Taylor, Epidemiology Branch, is chair of the search committee.

Biostatisticians

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

NEW HIRES

Dr. Xiaochang Zhang from Boston Children's Hospital and Harvard Medical School has accepted a position as an Earl Stadtman Tenure Track Investigator at NIEHS. He will have a primary appointment in the Neurobiology Laboratory and a secondary appointment in the Genome Integrity and Structural Biology Laboratory. Dr. Zhang investigates the roles of cell-type-specific alternative splicing in brain development and neuronal disorders. He is scheduled to start at NIEHS in February 2018**.**

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 platform presentation. 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	Group and Laboratory/Branch
Franziska Bollmann, Dr. rer. nat.	Perry Blackshear, M.D., D.Phil.	Post-Transcriptional Gene Expression Group, Signal Transduction Laboratory
Rachel M. Carroll, Ph.D.	Shanshan Zhao, Ph.D.	Biostatistics & Computational Biology Branch
Kelly E. Carstens, B.S.	Serena M. Dudek, Ph.D.	Synaptic and Developmental Plasticity Group, Neurobiology Laboratory
Qing Chen, Ph.D.	Guang Hu, Ph.D.	Stem Cell Biology Group, Epigenetics and Stem Cell Biology Laboratory
Amanda E. Conway, Ph.D.	Raja Jothi, Ph.D.	Systems Biology Group, Epigenetics and Stem Cell Biology Laboratory
Brian J. Deskin, Ph.D.	Raja Jothi, Ph.D.	Systems Biology Group, Epigenetics and Stem Cell Biology Laboratory
Kerry Dorr, Ph.D.	Anton Jetten, Ph.D.	Cell Biology Group, Immunity, Inflammation and Disease Laboratory
Chunfang Gu, Ph.D.	Stephen B. Shears, Ph.D.	Inositol Signaling Group, Signal Transduction Laboratory
Juhee Haam, Ph.D.	Jerry Yakel, Ph.D.	Ion Channel Physiology Group, Neurobiology Laboratory
Wan-Chi Lin, Ph.D.	Michael Fessler, M.D.	Clinical Investigation of Host Defense, Immunity, Inflammation and Disease Laboratory
Yu-Hua Lo, Ph.D.	Robin E. Stanley, Ph.D.	Nucleolar Integrity Group, Signal Transduction Laboratory
Oswaldo A. Lozoya, Ph.D.	Rick Woychik, Ph.D.	Mammalian Genome Group, Genome Integrity and Structural Biology Laboratory
Kathryn S. McClelland, Ph.D.	Humphrey Yao, Ph.D.	Reproductive Developmental Biology Group, Reproductive and Developmental Biology Laboratory

Bart T. Phillips, Ph.D.	Traci Hall, Ph.D.	Macromolecular Structure Group, Epigenetics and Stem Cell Biology Laboratory
Monica C. Pillon, Ph.D.	Robin E. Stanley, Ph.D.	Nucleolar Integrity Group, Signal Transduction Laboratory
Emmi Rotgers, M.D., Ph.D.	Humphrey Yao, Ph.D.	Reproductive Developmental Biology Group, Reproductive and Developmental Biology Laboratory
David W. Scoville, Ph.D.	Anton Jetten, Ph.D.	Cell Biology Group, Immunity, Inflammation and Disease Laboratory

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.

Kristen Upson, Ph.D., received a K99/R00 award from the National Institute of Nursing Research (NINR) entitled "Influence of diet, iron stores, and toxic metals on uptakes and effects on uterine fibroid risk in African American women." Dr. Upson will train in the Epidemiology Branch under the mentorship of Donna Baird, Ph.D.

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. This year the poster session was held on Thursday, July 27, 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:
Sofia Pauca, Mount Tabor High School, Neurobiology Laboratory, Mentor: Patricia Jensen, Ph.D., Developmental Neurobiology Group, Poster Title: "Deciphering the Role of Norepinephrine in Neocortical Development"

Undergraduate Intern:
Megan Stefkovich, University of Wisconsin-Madison, Reproductive and Developmental Biology Laboratory, Mentor: Kenneth Korach, Ph.D., Receptor Biology Group, Poster Title: "Non-genomic Estrogen Receptor α Involvement in Metabolic Regulation"

Graduate Intern:
Adrienna Bingham, The College of William and Mary, Biostatistics and Computational Biology Branch, Mentor: Leping Li, Ph.D., Poster Title: "Using Tumor Sample Gene Expression Data to Infer Tumor Purity Levels"

DIR RESEARCH ACCOMPLISHMENTS FOR FY 2016

International team discovers that mutations in the gene SMCHD1 cause human congenital arhinia (absent nose)

Scientists at NIEHS and Massachusetts General Hospital led an international team which assembled a cohort of 45 patients with congenital arhinia, an extremely rare and severe birth defect, and through next generation DNA sequencing, implicated missense mutations in SMCHD1 as the primary genetic cause of this disorder. This finding was perplexing because 1) SMCHD1 is an epigenetic repressor (turns other genes off during development) with no known role in craniofacial development, and 2) similar (and in a few cases, identical) mutations in SMCHD1 cause a rare form of late-onset muscular dystrophy (FSHD2). Functional studies in patient blood cells and animal models both suggested that these mutations impede SMCHD1 function, akin to what has been reported in FSHD2.

Shaw ND, Brand H, Kupchinsky ZA, Bengani H, Plummer L, Jones TI, Erdin S,Williamson KA, Rainger J, Stortchevoi A, Samocha K, Currall BB, Dunican DS, Collins RL, Willer JR, Lek A, Lek M, Nassan M, Pereira S, Kammin T, Lucente D, Silva A, Seabra CM, Chiang C, An Y, Ansari M, Rainger JK, Joss S, Smith JC, Lippincott MF, Singh SS, Patel N, Jing JW, Law JR, Ferraro N, Verloes A, Rauch A, Steindl K, Zweier M, Scheer I, Sato D, Okamoto N, Jacobsen C, Tryggestad J, Chernausek S, Schimmenti LA, Brasseur B, Cesaretti C, García-Ortiz JE, Buitrago TP, Silva OP, Hoffman JD, Mühlbauer W, Ruprecht KW, Loeys BL, Shino M, Kaindl AM, Cho CH, Morton CC, Meehan RR, van Heyningen V, Liao EC, Balasubramanian R, Hall JE, Seminara SB, Macarthur D, Moore SA, Yoshiura KI, Gusella JF, Marsh JA, Graham JM Jr, Lin AE, Katsanis N, Jones PL, Crowley WF Jr, Davis EE, FitzPatrick DR, Talkowski ME. SMCHD1 mutations associated with a rare muscular dystrophy can also cause isolated arhinia and Bosma arhinia microphthalmia syndrome. *Nat. Genet.*, 49:238-248, 2017

Female mouse embryos actively remove male reproductive systems via a local factor

NIEHS scientists have found that a protein called COUP-TFII determines whether a mammalian embryo develops male reproductive tracts. The discovery changes the long-standing view that a mammalian embryo will automatically become female unless androgens in the embryo make it male. The work demonstrated that female embryos actively promote the elimination of the male tract through the action COUP-TFII, challenging the paradigm that the pattern of female reproductive system arises by default.

Zhao F, Franco HL, Rodriguez KF, Brown PR, Tsai MJ, Tsai SY, Yao HHC. Elimination of the male reproductive tract in the female embryo is actively promoted by COUP-TFII. *Science*, 357:717-720, 2017.

Why TNF blockade improves lung function in only some patients with asthma

Asthma is associated with exposure to a wide variety of allergens and adjuvants. The extent to which overlap exists between the cellular and molecular mechanisms triggered by these various agents is poorly understood, but might explain the differential responsiveness of patients to specific therapies. In particular, it is unclear why some, but not all, patients benefit from blockade of TNF. Here, investigators showed that mice sensitized to an innocuous protein using

Toll-like receptor ligands or house dust extracts as adjuvants developed airway inflammation and airway hyperresponsiveness and that these responses were dependent on the cytokine, TNF. Mice sensitized to the same allergen using a protease as the adjuvant developed similar allergic responses, but they were not dependent on TNF. These findings might help to explain why TNF blockade improves lung function in only some patients with asthma.

Whitehead GS, Thomas SY, Shalaby KH, Nakano K, Moran TP, Ward JM, Flake GP, Nakano H, Cook, DN. TNF induction is required for TLR ligand–mediated but not protease-mediated allergic airway inflammation. *J. Clin. Invest.,* epub ahead of print, doi: 10.1172/JCI90890.

Potential health effects from the Deepwater Horizon Oil Spill being studied
The Gulf Long-term Follow-up Study (GuLF STUDY, www.gulfstudy.nih.gov) was initiated by NIEHS researchers in response to the April 2010 explosion of the Deepwater Horizon drilling rig and resulting oil spill in the Gulf of Mexico, the largest marine oil spill in U.S. history. It is the largest study ever conducted on the potential health effects associated with an oil spill, with nearly 33,000 participants and is focused on both physical and mental health effects related to the oil spill. The GuLF STUDY is collecting information that can be used by individuals, communities and governments to better understand the consequences of oil spills and plan for future disasters.

Kwok RK, Engel LS, Miller AK, Blair A, Curry MD, Jackson WB, Stewart PA, Stenzel MR, Birnbaum LS, Sandler DP; GuLF STUDY Research Team. The GuLF STUDY: A Prospective Study of Persons Involved in the Deepwater Horizon Oil Spill Response and Clean-Up. *Environ. Health Perspect.,* 125:570-578, 2017

Engel LS, Kwok RK, Miller AK, Blair A, Curry MD, McGrath JA, Sandler DP. The Gulf Long-Term Follow-Up Study (GuLF STUDY): Biospecimen collection at enrollment. *J. Toxicol. Environ. Health A.*, 80:218-229, 2017.

Phytoestrogens in soy formula induce DNA methylation changes in infant girls
Utilizing vaginal cell data from the Infant Feeding and Early Development Study, NIEHS researchers demonstrated that infant girls fed soy formula exhibited increased DNA methylation in three CpG sites of the proline rich 5 like (PRR5L) gene. Analogous results were found in mice exposed to genistein, the principal phytoestrogen found in soy. Since phytoestrogens affect the development of rodent and human reproductive systems, this work has important implications in the study of early life exposures. Recent epidemiologic studies have shown that soy formula feeding is associated with alteration in reproductive tract structure and function, including occurrence of uterine fibroids, endometriosis, and early age at menarche. Findings from the new study are consistent with the ability of genistein, the principal phytoestrogen in soy formula to act as an estrogen and to produce epigenetic alterations in animal models, but do not yet constitute a clear contraindication for soy formula use for those infants where its use is indicated.

Harlid S, Adgent M, Jefferson WN, Panduri V, Umbach DM, Xu Z, Stallings VA, Williams CJ, Rogan WJ, Taylor JA. Soy Formula and Epigenetic Modifications: Analysis of

Vaginal Epithelial Cells from Infant Girls in the IFED Study. *Environ. Health Perspect.*, 125:447-452, 2017.

Vitamin D may help protect women from breast cancer.
Vitamin D is an essential nutrient that is derived both from diet and from sunlight-induced processes in the skin. In an analysis based on a case-control comparison nested within the NIEHS Sister Study, women with blood vitamin D levels in the highest quartile experienced an estimated reduction of about 21% in their risk of being diagnosed with breast cancer within the subsequent five years. Blood measurements were not available for the whole cohort, but among the more than 50,000 who were followed up, those who had reported that they took supplements that included vitamin D were also at significantly reduced risk. These associations were even stronger in premenopausal women.

O'Brien KM, Taylor J, Sandler DP, Weinberg CR. Serum vitamin D and breast cancer within five years. *Environ. Health Perspect.,* 125:077004, 2017.

Rheumatoid arthritis linked to use of some pesticides
Previous studies have reported associations between farming and rheumatoid arthritis (RA), a disabling chronic autoimmune disease involving the joints. Because RA is more common in women, few studies have focused on men, but farm-related exposures are higher among men who are licensed to apply pesticides. Researchers studied self-reported rheumatoid arthritis (confirmed by medication use or physician report) in relation to pesticide use in the Agricultural Health Study, a prospective study of licensed pesticide applicators that has been followed since enrollment in 1993-1997. Among more than 26,000 male licensed pesticide applicators followed for a median 18 years, 220 new cases of RA were diagnosed. Incident RA was significantly associated with ever use of fonofos, carbaryl and chlorimuron ethyl, with odds ratios of 1.4 to 1.7. A significant dose-response relationship was seen for lifetime days of use of atrazine (with a 60% increase in risk for RA in the highest tertile of days of use) and trends were suggestive for fonofos and carbaryl. These results represent novel but potentially important associations between exposure to some pesticides and RA in male farmers.

Meyer A, Sandler DP, Beane-Freeman LE, Hofmann JN, Parks CG. Pesticide exposure and risk of rheumatoid arthritis among licensed male pesticide applicators in the Agricultural Health Study. *Environ. Health Perspect.*, 125:077010, 2017.

Indoor air pollution from wood-burning stoves and fireplaces is associated with small increase in breast cancer risk
Indoor wood and gas burning in the home can result in exposure to carcinogens such as polycyclic aromatic hydrocarbons (PAHs), benzene and other compounds at levels that are comparable to those from ambient urban air pollution. Investigators used data from the Sister Study, a prospective cohort study of over 50,000 US women who are at enhanced risk for breast cancer because of their family history, to evaluate the risk of developing breast cancer in women who reported using wood-stoves or fireplaces in their longest residence prior to enrolling in the Sister Study. A total of 2,416 women developed invasive or *in-situ* breast cancer after an average follow-up of 6.4 years. We found that women who used a wood stove or fireplace more than once a week had a 17% in breast cancer risk (Hazard Ratio 1.17, 95% confidence interval

1.02 – 1.34), with risk increasing with increasing frequency of use. Associations were seen for burning wood or natural gas but not fire logs. Findings corroborate the one other study on this topic, a retrospective study comparing women with breast cancer to those without.

White AJ, Sandler DP. Indoor wood-burning stove and fireplace use and breast cancer in a prospective cohort study. *Environ. Health Perspect.*, 125:077011, 2017.

Smoking leaves a long-lasting signature on the human genome
Smoking remains the leading preventable cause of death worldwide. Even decades after stopping, former smokers have increased risk of many diseases. Mechanisms remain unclear. The researchers examined genome-wide signatures of methylation, a type of modification of the genetic code in over 16,000 people from 16 studies and found a widespread impact across the genome at about 1/3 of human genes. Many of these marks persist long after quitting smoking.

Joehanes R, Just AC, Marioni RE, Pilling LC, Reynolds LM, Mandaviya PR, Guan W, Xu T, Elks CE, Aslibekyan S, Moreno-Macias H, Smith JA, Brody JA, Dhingra R, Yousefi P, Pankow JS, Kunze S, Shah SH, McRae AF, Lohman K, Sha J, Absher DM, Ferrucci L, Zhao W, Demerath EW, Bressler J, Grove ML, Huan T, Liu C, Mendelson MM, Yao C, Kiel DP, Peters A, Wang-Sattler R, Visscher PM, Wray NR, Starr JM, Ding J, Rodriguez CJ, Wareham NJ, Irvin MR, Zhi D, Barrdahl M, Vineis P, Ambatipudi S, Uitterlinden AG, Hofman A, Schwartz J, Colicino E, Hou L, Vokonas PS, Hernandez DG, Singleton AB, Bandinelli S, Turner ST, Ware EB, Smith AK, Klengel T, Binder EB, Psaty BM, Taylor KD, Gharib SA, Swenson BR, Liang L, DeMeo DL, O'Connor GT, Herceg Z, Ressler KJ, Conneely KN, Sotoodehnia N, Kardia SL, Melzer D, Baccarelli AA, van Meurs JB, Romieu I, Arnett DK, Ong KK, Liu Y, Waldenberger M, Deary IJ, Fornage M, Levy D, London SJ. Epigenetic Signatures of Cigarette Smoking. *Circ. Cardiovasc. Genet*., 9:436-447, 2016

Season of conception predicts risk of the pregnancy complication, preeclampsia.
Preeclampsia is a poorly-understood and dangerous pregnancy complication associated with high blood pressure and increased risk of seizures. Though diagnosed later in pregnancy, it is thought to originate from abnormal formation of the placenta. An effect of season on risk would suggest a potentially modifiable seasonally-varying environmental contribution to this syndrome; we found substantial variation in risk with season of conception, based on the extensive data gathered by the Medical Birth Registry of Norway.

Weinberg CR, Shi M, Basso O, DeRoo LA, Harmon Q, Wilcox AJ, Skjærven R. Season of Conception, Smoking, and Preeclampsia in Norway. *Environ. Health Perspect.*, 125:067022, 2017.

Gestational diabetes may lead to increased breast cancer risk
Gestational diabetes – diabetes that develops during pregnancy and usually resolves – is associated with poor pregnancy outcomes and long term adverse health effects, including later adult onset type 2 diabetes. Some studies have found associations between diabetes and breast cancer and there are many commonalities in risk factors across the two conditions. Women with

multiple pregnancies with gestational diabetes may represent women with recurring exposure to episodes of clinical and subclinical insulin resistance that could enhance their risk for developing breast cancer. In an analysis of 39,000 parous women enrolled in the Sister Study who did not have diabetes prior to pregnancy and were free of cancer of any kind at enrollment, it was found that having 2 or more pregnancies with gestational diabetes was associated with a nearly 70% increase in breast cancer risk (Hazard Ratio 1.68, 95% confidence interval 1.15 – 2.44) and with estrogen receptor positive (ER+) breast cancer (Hazard Ratio 1.81, 95% confidence interval 1.10-2.98). These results suggest that women who have had gestational diabetes might benefit from increased breast cancer surveillance.

Park YM, O'Brien KM, Zhao S, Weinberg CR, Baird DD, Sandler DP. Gestational diabetes mellitus may be associated with an increased risk of breast cancer. *Br. J. Cancer*, 116:960-963, 2017.

Early Life Farm Exposures Protects Against Allergies in Adults

Previous studies have suggested that childhood farm animal exposures and consumption of unpasteurized milk reduces the risk of childhood asthma and allergies. But what about early-life farm exposures and adult allergies? In a study of over 3000 farmers and their spouses, the investigators found that exposure to a farming environment, especially farm animals, when still in the womb, was related with decreased risk of allergies to common environmental allergens, measured objectively by blood tests. Childhood exposure to farm animals was also protective. This study builds upon previous research supporting "the hygiene hypothesis," that is, exposures to diverse types of organisms early in life influences immune development in a way that can reduce risk of allergies throughout life.

House JS, Wyss AB, Hoppin JA, Richards M, Long S, Umbach DM, Henneberger PK, Beane Freeman LE, Sandler DP, Long O'Connell E, Barker-Cummings C, London SJ. Early-life farm exposures and adult asthma and atopy in the Agricultural Lung Health Study. *J. Allergy Clin. Immunol.*, 140:249-256.e14, 2017.

Endotoxin Exposure and WBC Count

The peripheral leukocyte count is a biomarker of inflammation and is associated with human mortality. This study examined the relationship between house dust endotoxin concentration and peripheral blood leukocyte counts in 6,254 human subjects enrolled in the National Health and Nutrition Examination Survey (NHANES) and 1,708 subjects enrolled in the Agricultural Lung Health Study (ALHS). NIEHS investigators found a statistically significant, positive association between endotoxin concentration and total leukocytes, monocytes, lymphocytes, and neutrophils in the NHANES. Similar positive associations were found in the ALHS. For total leukocytes, there was evidence in the ALHS of a gene by environment interaction for minor allele carrier status at the TLR4 haplotype. This is, to our knowledge, the first report of an association between house dust endotoxin and leukocyte count in a national survey.

Fessler MB, Carnes MU, Salo PM, Wilkerson J, Cohn RD, King D, Hoppin JA, Sandler DP, Travlos G, London SJ, Thorne PS, Zeldin DC. House Dust Endotoxin and Peripheral Leukocyte Counts: Results from Two Large Epidemiologic Studies. *Environ. Health Perspect.*, in press.

Structural requirements for human DNA polymerase (pol) β

Accurate replication and repair of the genome is vital to genome integrity in all cells, and DNA polymerases provide many of the DNA synthesis and other functions that are essential in the replication and repair processes. To understand features that control the speed and accuracy of DNA polymerases, NIEHS scientists are applying time-lapse crystallography, NMR spectroscopy, various kinetic assays and computational techniques to study human DNA polymerase (pol) β, considered a model enzyme for understanding nucleotidyl transfer reactions by polymerases. The crystallography approach provides novel snapshots of structural intermediates of pol β and its substrates and products as they pass through the catalytic cycle. There is an "open to closed" conformational transition in pol β that hastens correct, and deters incorrect, nucleotide insertion into DNA, and this can be visualized through time-lapse crystallography. The structures are being subjected to computational analyses to gain a better understanding of conformational dynamics as the enzyme passes from one step to the next. Importantly, after correct nucleotide insertion, the enzyme remains in the closed conformation, and the active site has a new divalent metal ion-binding site, termed the "product metal." Thus, a divalent metal ion is associated with the pyrophosphate product, and the enzyme is in the closed conformation and is poised for nucleotidyl transfer. The implications of these findings toward understanding the nucleotidyl transferase reaction are under investigation. In other studies of pol β, it was discovered the mechanism of discrimination against mismatched primer extension and special features of the enzyme's use of two divalent metals in the active site.

Batra VK, Beard WA, Pedersen LC, Wilson SH. Structures of DNA Polymerase Mispaired DNA Termini Transitioning to Pre-catalytic Complexes Support an Induced-Fit Fidelity Mechanism. *Structure*, 24:1863-1875, 2016.

Shock DD, Freudenthal BD, Beard WA, Wilson SH. Modulating the DNA polymerase reaction equilibrium through chemical biology. *Nat. Chem. Biol.*, epub ahead of print, doi: 10.1038/NCHEMBIO.2450.

Perera L, Beard WA, Pedersen LG, Wilson SH. The Bimetallic Magnesium Covalent Bond in Enzyme Active Sites. *Inorg. Chem.*, 56:313-320, 2017.

Perera L, Freudenthal BD, Beard WA, Pedersen LG, Wilson SH. Revealing the role of the product metal in DNA polymerase β catalysis. *Nucleic Acids Res.*, 45:2736-2745, 2017.

Jamsen JA, Beard WA, Pedersen LC, Shock DD, Moon AF, Krahn JM, Bebenek K, Kunkel TA, Wilson SH. Time-Lapse Crystallography Snapshots of A Double-Strand Break Repair Polymerase In Action. *Nat. Commun*. in press

Bedroom Allergen Exposure in the U.S.

Bedroom allergen exposures contribute to allergic disease morbidity because people spend considerable time in bedrooms, having close contact with allergen reservoirs. Scientists investigated participant and housing characteristics, including sociodemographic, regional and climatic factors, associated with bedroom allergen exposures in a nationally representative sample of the US population enrolled in the National Health and Nutrition Examination Survey (NHANES). Almost all participants (>99%) had at least one and 74% had 3-6 allergens detected. Over 2/3 of participants (73%) had at least one allergen and 18% had ≥3 allergens exceeding elevated levels. Although exposure variability showed significant racial/ethnic and regional differences, high exposure burden to multiple allergens was most consistently associated with the

presence of pets and pests, living in mobile homes/trailers, older and rental homes, and in non-metropolitan areas. Exposure to multiple allergens is common. Despite highly variable exposures, bedroom allergen burden is strongly associated with the presence of pets and pests.

Salo PM, Wilkerson J, Rose KM, Cohn RD, Calatroni A, Mitchell HE, Sever ML, Gergen PJ, Thorne PS, Zeldin DC. Bedroom Allergen Exposures in U.S. Households. *J. Allergy Clin. Immunol.,* in press.

Proteins involved in cell death have immune specific role in neurons.
Recent work has implicated additional roles for a member of the cell death machinery, RIPK3, in inflammatory signaling independent of cell death. $Ripk3^{-/-}$ mice were more susceptible to West Nile Virus, resulting from decreased central nervous system (CNS) recruitment of T lymphocytes and inflammatory myeloid cells. These data identify pleiotropic functions for RIPK3 in the restriction of viral pathogenesis and implicate RIPK3 as a key coordinator of immune responses within the CNS.

Daniels BP, Snyder AG, Olsen TM, Orozco S, Oguin TH 3rd, Tait SW, Martinez J, Gale M Jr, Loo YM, Oberst A. RIPK3 Restricts Viral Pathogenesis via Cell Death-Independent Neuroinflammation. *Cell*, 169:301-313.e11, 2017.

Zinc finger domains critical for DNA damage processing and signaling
NIEHS investigators reported discovery and characterization of a novel DNA binding domain in the apurinic/apyrimidc endonuclease 2 (APE2) that is critical for activating DNA damage response (DDR) pathways following oxidative stress. The domain is a highly conserved and widely distributed zinc finger module, the Zf-GRF, which in APE2 serves to activate its nuclease activity to reverse DNA damage at single strand DNA breaks. This work adds to the growing body of knowledge about how the body deals with the genomic instability that occurs with oxidative stress and its possible links to human disease. When the APE2 complex recognizes genomic lesions, it acts to generate ssDNA, a process critical for activation of the cellular DNA damage checkpoint, an alarm that is signaled in response to DNA damage.

Wallace BD, Berman Z, Mueller GA, Lin Y, Chang T, Andres SN, Wojtaszek JL, DeRose EF, Appel CD, London RE, Yan S, Williams RS. APE2 Zf-GRF facilitates 3'-5' resection of DNA damage following oxidative stress. *Proc. Natl. Acad. Sci. USA*., 114:304-309, 2017.

Grc3 Programs Las1 for Cleavage
Ribonucleases are molecular scissors that catalyze the cleavage of RNA phosphodiester bonds and play essential roles in RNA processing and maturation. Precursor ribosomal RNA (rRNA) must be processed by several ribonucleases, including the endonuclease Las1, in a carefully orchestrated manner to generate the mature ribosomal subunits. Las1 is essential for cell viability and mutations in the mammalian gene have been linked with human disease underscoring the importance of this enzyme. NIEHS researches have shown that on its own Las1 has weak nuclease activity, however when associated with its binding partner, the poly-nucleotide kinase Grc3, Las1 is programmed to specifically cleave pre-rRNA at the C2 site. Together Grc3 and

Las1 assemble into a higher-order complex exquisitely primed for cleavage and phosphorylation of RNA.

Pillon MC, Sobhany M, Borgnia MJ, Williams JG, Stanley RE. Grc3 programs the essential endoribonuclease Las1 for specific RNA cleavage. *Proc. Natl. Acad. Sci. USA.,* 114: E5530-E5538, 2017.

Oxidized nucleotide insertion by human DNA polymerase β

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 investigators 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 (both dNTP and rNTP).

Sassa A, Çağlayan M, Rodriguez Y, Beard WA, Wilson SH, Nohmi T, Honma M, Yasui M. Impact of Ribonucleotide Backbone on Translesion Synthesis and Repair of 7,8-Dihydro-8-oxoguanine. *J. Biol. Chem.*, 291:24314-24323, 2016.

Kim T, Freudenthal BD, Beard WA, Wilson SH, Schlick T. Insertion of oxidized nucleotide triggers rapid DNA polymerase opening. *Nucleic Acids Res.*, 44:4409-4424, 2016.

Cilli P, Ventura I, Minoprio A, Meccia E, Martire A, Wilson SH, Bignami M, Mazzei F. Oxidized dNTPs and the OGG1 and MUTYH DNA glycosylases combine to induce CAG/CTG repeat instability. *Nucleic Acids Res.*, 44:5190-5203, 2016.

Çağlayan M, Horton JK, Dai DP, Stefanick DF, Wilson SH. Oxidized nucleotide insertion by pol β confounds ligation during base excision repair. *Nat. Commun.*, 8:14045, 2017.

Caglayan M, Wilson SH. Role of DNA ligase in poisoning cells after pol β insertion of oxidized nucleotide. *J. Radiat. Res.*, epub ahead of print, doi: 10.1093/jrr/rrx027.

Genome-Wide Somatic Mutagenesis Over Human Lifetime

NIEHS investigators developed a new approach to measure genome-wide mutation load that accumulates during the lifetime of healthy individuals. The results demonstrated that the amount of DNA damage in skin cells caused by ultraviolet (UV) light was similar to the amount due to naturally occurring processes in the body. Analysis of mutation spectra and genome-wide distribution suggested that the mechanisms involved in cancer development also occur in healthy cells. The research might help scientists design better preventative and therapeutic strategies to address cancer and other genetic diseases.

Saini N, Roberts SA, Klimczak LJ, Chan K, Grimm SA, Dai S, Fargo DC, Boyer JC, Kaufman WK, Taylor JA, Lee E, Cortes-Ciriano I, Park PJ, Schurman SH, Malc EP, Mieczkowski PA, Gordenin DA. 2016. The impact of environmental and endogenous damage on somatic mutation load in human skin fibroblasts. *PLoS Genet.,* 12:e1006385, 2016.

Gene product identified that drives cancer
Investigators at the NIEHS/NIH recently identified a gene that, when turned on in a specific way, promotes the survival and growth of cancer cells. The gene, TNFAIP8, had previously been noted to be turned on in human tumors, but how it is turned on and what it does had not been defined. In their report, the NIEHS investigators now define for the first time that a specific variant, or version, of the TNFAIP8 gene is turned on in tumors, and show that blocking this variant causes cancer cells to stop growing and to become more susceptible to chemotherapeutic drugs. These findings suggest the possibility that TNFAIP8 may represent a novel target for drug development against cancer.

Lowe JM, Nguyen TA, Grimm SA, Gabor KA, Peddada SD, Li L, Anderson CW, Resnick MA, Menendez D, Fessler MB. The novel p53 target TNFAIP8 variant 2 is increased in cancer and offsets p53-dependent tumor suppression. *Cell. Death Differ*., 24:181-191, 2017.

Intestinal SIRT1 age-dependently regulates intestinal inflammation through modulation of gut microbiota
Intestinal epithelial homeostasis is maintained through complex interplays between epithelial cells, commensal gut microorganisms, and immune cells. Disruption of this homeostasis is associated with inflammatory bowel disease (IBD), however, the underlying molecular mechanisms remain poorly defined. In this study, researchers show that SIRT1, the most conserved mammalian NAD^+-dependent protein deacetylase, is vital in maintenance of intestinal tissue integrity through modulation of gut microbiota. Deletion of intestinal epithelial SIRT1 in mice age-dependently elicits NF-κB signaling and other stress response pathways, and enhances spontaneous inflammation. Intestinal epithelial SIRT1 deficiency also alters the commensal gut microbial composition through altered bile acid metabolism and increases susceptibility to DSS-induced colitis. Consistently, the expression level of human SIRT1 is significantly reduced in human ulcerative colitis patients. However, remarkably, these SIRT1 deficiency-associated impairments are largely eliminated upon depletion of gut microbiota. Our findings define a crucial role of intestinal SIRT1 in mediating host-microbe interactions, and suggest that SIRT1-activating compounds may be therapeutically beneficial for the treatment of human IBD.

Wellman AS, Metukuri MR, Kazgan N, Xu X, Xu Q, Ren NSX, Czopik A, Shanahan MT, Kang A, Chen W, Azcarate-Peril MA, Gulati AS, Fargo DC, Guarente L, Li X. Intestinal Epithelial Sirtuin 1 Regulates Intestinal Inflammation during Aging in Mice by Altering the Intestinal Microbiota. *Gastroenterology*, epub ahead of print, doi: 10.1053/j.gastro.2017.05.022.

Estrogen Receptor β activation by endocrine-disrupting chemicals
Estrogen signaling is transmitted by estrogen receptors (ER) alpha and beta. NIEHS scientists found that mouse has a specific unique form of ER-beta that reduces ER beta dependent transcription activity. This would suggest and informs researchers that the estrogenic activities of some compounds, such as endocrine-disrupting chemicals (EDCs) could be underestimated as to their biological activities in mouse studies compared to humans who do not express this isoform of ER-beta.

Donoghue LJ, Neufeld TI, Li Y, Arao Y, Coons LA, Korach KS. Differential activation of a mouse estrogen receptor β isoform (mERβ2) with endocrine-disrupting chemicals (EDCs). *Environ. Health Perspect.*, 125: 634-642, 2017.

Bacterial secretion systems direct host proteins to phagosome to mediate immune response
Many invasive bacteria establish pathogen-containing vacuoles (PVs) as intracellular niches for microbial growth. Immunity to these infections is dependent on the ability of host cells to recognize PVs as targets for host defense. The researchers show that the presence of bacterial secretion systems directs Galectin-3 to PVs, which in turn mediates the delivery of antimicrobial GBP1 and GBP2 as part of a coordinated host defense program.

Feeley EM, Pilla-Moffett DM, Zwack EE, Piro AS, Finethy R, Kolb JP, Martinez J, Brodsky IE, Coers J. Galectin-3 directs antimicrobial guanylate binding proteins to vacuoles furnished with bacterial secretion systems. *Proc. Natl. Acad. Sci. USA.*, 114:E1698-E1706, 2017.

Autophagy protein VPS34 is required for antigen presentation
The class III PI3K Vacuolar protein sorting 34 (Vps34) plays a role in both canonical and noncanonical autophagy, key processes that control the presentation of antigens by dendritic cells (DCs) to naive T lymphocytes. The investigators observed that DC-specific Vps34-deficient mice displayed a defect in the capacity of DC to cross-present cell corpse-associated antigens to MHC class I-restricted T cells. This defect also resulted in increased metastases in response to challenge with melanoma cells. These findings have important implications for the development of small-molecule inhibitors of Vps34 for therapeutic purposes.

Parekh VV, Pabbisetty SK, Wu L, Sebzda E, Martinez J, Zhang J, Van Kaer L. Autophagy-related protein Vps34 controls the homeostasis and function of antigen cross-presenting CD8α+ dendritic cells. *Proc. Natl. Acad. Sci. USA.*, 114:E6371-E6380, 2017.

Glorund is a multifunctional RNA regulatory protein
NIEHS researchers and collaborators revealed that an RNA-binding protein called Glorund uses more than one RNA-binding mode to recognize and regulate mRNA transcripts. This study shows how a single protein can regulate diverse target RNAs. The scientists demonstrated that Glorund not only interacts with translational control elements that contain structured RNA motifs, but also interacts with single-stranded RNAs that are enriched for guanine bases. The researchers also demonstrated that Glorund uses both recognition modes for its functions in organisms.

Tamayo JV, Teramoto T, Chatterjee S, Hall TMT, Gavis ER. The Drosophila hnRNP F/H Homolog Glorund Uses Two Distinct RNA-Binding Modes to Diversify Target Recognition. *Cell Rep.*, 19:150-161, 2017.

CNOT3-Dependent mRNA Deadenylation Safeguards the Pluripotent State
In this study, the authors showed that CNOT3, a component of the Ccr4-Not deadenylase complex, is required for mouse embryonic development. They further showed that CNOT3 promotes differentiation gene mRNA deadenylation and degradation to support the pluripotent

state of the epiblast cells in the embryo. Their study identified poly(A) tail-length regulation as a critical post-transcriptional mechanism that controls pluripotency and early development.

Zheng X, Yang P, Lackford B, Bennett BD, Wang L, Li H, Wang Y, Miao Y, Foley JF, Fargo DC, Jin Y, Williams CJ, Jothi R, Hu G. CNOT3-Dependent mRNA Deadenylation Safeguards the Pluripotent State. *Stem Cell Reports*, 7:897-910, 2016.

SIRT1 dose-dependently regulates tumor cell metabolism and cancer development

SIRT1, the most conserved mammalian NAD^+-dependent protein deacetylase, plays a vital role in the regulation of metabolism, stress responses, and genome stability. However, the role of SIRT1 in the multi-step process leading to transformation and/or tumorigenesis, as either a tumor suppressor or tumor promoter, is complex and maybe dependent upon the context in which SIRT1 activity is altered, and the role of SIRT1 in tumor metabolism is unknown. This study demonstrates that SIRT1 dose-dependently regulates cellular glutamine metabolism and apoptosis, which in turn differentially impact cell proliferation and cancer development. Heterozygous deletion of *Sirt1* induces c-Myc expression, enhancing glutamine metabolism and subsequent proliferation, autophagy, stress resistance and cancer formation. In contrast, homozygous deletion of *Sirt1* triggers cellular apoptotic pathways, increases cell death, diminishes autophagy, and reduces cancer formation. Consistent with the observed dose-dependence in cells, intestine-specific *Sirt1* heterozygous mice have enhanced intestinal tumor formation, whereas intestine-specific *Sirt1* homozygous knockout mice have reduced development of colon cancer. Furthermore, *SIRT1* reduction but not deletion is associated with human colorectal tumors, and colorectal cancer patients with low protein expression of SIRT1 have a poor prognosis. Taken together, these findings indicate that the dose-dependent regulation of tumor metabolism and possibly apoptosis by SIRT1 mechanistically contributes to the observed dual roles of SIRT1 in tumorigenesis. This study highlights the importance of maintenance of a suitable SIRT1 dosage for metabolic and tissue homeostasis, which will have important implications in SIRT1 small molecule activators/inhibitors based therapeutic strategies for cancers.

Ren NS, Ji M, Tokar EJ, Busch EL, Xu X, Lewis D, Li X, Jin A, Zhang Y, Wu WK, Huang W, Li L, Fargo DC, Keku TO, Sandler RS, Li X. Haploinsufficiency of SIRT1 Enhances Glutamine Metabolism and Promotes Cancer Development. *Curr. Biol.*, 27:483-494, 2017.

The mammalian base excision repair (BER) pathway

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

Prasad R, Poltoratsky V, Hou EW, Wilson SH. Rev1 is a base excision repair enzyme with 5'-deoxyribose phosphate lyase activity. *Nucleic Acids Res.*, 44:10824-10833, 2016.
Horton JK, Seddon HJ, Zhao ML, Gassman NR, Janoshazi AK, Stefanick DF, Wilson SH. Role of the oxidized form of XRCC1 in protection against extreme oxidative stress. *Free Radic. Biol. Med.*, 107:292-300, 2017.
Howard MJ, Rodriguez Y, Wilson SH. DNA polymerase β uses its lyase domain in a processive search for DNA damage. *Nucleic Acids Res.*, 45:3822-3832, 2017.
Rodriguez Y, Howard M, Cuneo M, Prasad R, Wilson SH. Unencumbered Pol β Lyase Activity in Nucleosome Core Particles. Nucleic Acids Res., epub ahead of print, doi: 10.1093/nar/gkx593.
Çağlayan, M., Prasad R., Krasich R., Longley M.J., Kadod M., Tsuda M., Sasanuma H., Takeda S., Tano. K., Copeland W.C., and Wilson S.H. Complementation of aprataxin deficiency by base excision repair enzymes in mitochondrial extracts. *Nucleic Acids Res.,* doi:10.1093/nar/gkx654.
Jamsen JA, Beard WA, Pedersen LC, Shock DD, Moon AF, Krahn JM, Bebenek K, Kunkel TA, Wilson SH. Time-Lapse Crystallography Snapshots of A Double-Strand Break Repair Polymerase In Action. *Nat. Commun*. in press
Howard MJ, Wilson SH. Variable processive searching ability of the gap-filling DNA polymerase X family. *J. Biol. Chem.* in press

DNA polymerase β contains a functional nuclear localization signal at its N-terminus.
The human DNA repair system provides essential protection against the toxicity of environmental agents. Efficient DNA repair is dependent on the intranuclear assembly of damage-dependent repair complexes. Since proteins are generally not synthesized in the nucleus, DNA repair therefore requires nuclear transport of these repair proteins. Most transported proteins contain a nuclear localization signal (NLS) that allows them to interact with the nuclear membrane proteins that act as transporters. Among the most important repair polymerases is DNA polymerase β (pol β), which participates in thousands of repairs per cell per day. It had been widely assumed that due to its relatively small size, pol β was able to enter the nucleus through a nuclear pore and did not require an NLS. However, evaluation of the binding of pol β with the nuclear transport protein Importin α, revealed that there was significant binding. The authors then quantified this interaction, and identified the N-terminal sequence as having an unusual NLS that supports an interaction with a minor site binding pocket on the Importin α. The group further showed that if a modified form of pol β lacking its N-terminal NLS was substituted into cells, the enzyme equilibrates between the cytosol and the nucleus. It was proposed that nuclear localization signals are important even for small proteins, since they readily leak out of the nucleus as well as getting in.

Kirby TW, Gassman NR, Smith CE, Zhao ML, Horton JK, Wilson SH, London RE. DNA polymerase β contains a functional nuclear localization signal at its N-terminus. *Nucleic Acids Res*., 45:1958-1970, 2017.

Binge drinking modifies the association between lifetime alcohol intake and breast cancer risk.
The prevalence of heavy drinking and binge drinking has increased sharply in the United State, especially among women. Heavy drinking increases the risk for a number of chronic diseases.

Drinking 2 or more alcoholic beverages a day is a long-established risk factor for breast cancer, though the relative risks for moderate to heavy drinkers are usually found to be less than 2-fold. Researchers followed 50,000 women in the Sister Study to explore the impact of episodic heavy drinking on breast cancer risk. The researchers identified about 1,800 new cases of invasive breast cancer after an average follow-up of 6.4 years in this cohort of mostly low to moderate drinkers. Higher lifetime alcohol intake was associated with a 35% increase in breast cancer risk (comparing those drinking >230/drinks per year to those drinking <60 drinks per year. Relative to low-level drinkers, those reporting ever binge drinking or blacking out after drinking had about a 30% increase in breast cancer risk. It was also observed that moderate drinkers (those having between 60 and 229 drinks per year on average) who also reported binge drinking had higher breast cancer risk than low-level drinkers with no binge drinking. These findings support the established association between lifetime alcohol intake and breast cancer and underscore the potential for adverse health impacts of binge drinking.

White AJ, DeRoo LA, Weinberg C, Sandler DP. Binge drinking modifies the association between lifetime alcohol intake and breast cancer risk in moderate drinkers. *Am. J. Epidemiol.*, epub ahead of print, doi: 10.1093/aje/kwx118.

New Response Criteria for Clinical Trials Developed for Juvenile and Adult Dermatomyositis and Polymyositis

To develop response criteria for juvenile dermatomyositis (DM) and adult DM and polymyositis (PM), researchers analyzed the performance of more than 300 candidate response criteria that used core set activity measures from either the International Myositis Assessment and Clinical Studies Group (IMACS) or the Paediatric Rheumatology International Trials Organisation (PRINTO) and were derived from natural history data and a conjoint analysis survey. These were further validated using data from the PRINTO trial of prednisone alone compared to prednisone with methotrexate or cyclosporine and the Rituximab in Myositis trial. At a consensus conference, myositis experts considered top candidate criteria based on their performance characteristics and clinical face validity. Consensus was reached for a conjoint analysis-based continuous model, using absolute per cent change in core set measures to determine minimal, moderate, and major improvement. The same criteria were chosen for adult DM and PM, with differing thresholds for improvement. These new response criteria had excellent performance characteristics in existing datasets and should be sensitive criteria that will provide a uniform approach for assessing treatment responses in future therapeutic and preventive clinical trials.

Rider LG, Aggarwal R, Pistorio A, Bayat N, Erman B, Feldman BM, Huber AM, Cimaz R, Cuttica RJ, de Oliveira SK, Lindsley CB, Pilkington CA, Punaro M, Ravelli A, Reed AM, Rouster-Stevens K, van Royen-Kerkhof A, Dressler F, Magalhaes CS, Constantin T, Davidson JE, Magnusson B, Russo R, Villa L, Rinaldi M, Rockette H, Lachenbruch PA, Miller FW, Vencovsky J, Ruperto N; International Myositis Assessment and Clinical Studies Group and the Paediatric Rheumatology International Trials Organisation. 2016 American College of Rheumatology/European League Against Rheumatism Criteria for Minimal, Moderate, and Major Clinical Response in Juvenile Dermatomyositis: An International Myositis Assessment and Clinical Studies Group/Paediatric Rheumatology International Trials Organisation Collaborative Initiative. *Arthritis Rheumatol.*, 69:911-923, 2017; *Ann. Rheum. Dis.*, 76:782-791, 2017.

Aggarwal R, Rider LG, Ruperto N, Bayat N, Erman B, Feldman BM, Oddis CV, Amato AA, Chinoy H, Cooper RG, Dastmalchi M, Fiorentino D, Isenberg D, Katz JD, Mammen A, de Visser M, Ytterberg SR, Lundberg IE, Chung L, Danko K, García-De la Torre I, Song YW, Villa L, Rinaldi M, Rockette H, Lachenbruch PA, Miller FW, Vencovsky J; International Myositis Assessment and Clinical Studies Group and the Paediatric Rheumatology International Trials Organisation. 2016 American College of Rheumatology/European League Against Rheumatism criteria for minimal, moderate, and major clinical response in adult dermatomyositis and polymyositis: An International Myositis Assessment and Clinical Studies Group/Paediatric Rheumatology International Trials Organisation Collaborative Initiative. *Ann. Rheum. Dis.*, 76:792-801, 2017.

Rider LG, Ruperto N, Pistorio A, Erman B, Bayat N, Lachenbruch PA, Rockette H, Feldman BM, Huber AM, Hansen P, Oddis CV, Lundberg IE, Amato AA, Chinoy H, Cooper RG, Chung L, Danko K, Fiorentino D, Garcia de la Torre I, Reed AM, Song YW, Cimaz R, Cuttica RJ, Pilkington CA, Martini A, van der Net J, Maillard S, Miller FW, Vencovsky J, Aggarwal R, for the International Myositis Assessment and Clinical Studies Group (IMACS) and the Paediatric Rheumatology International Trials Organisation (PRINTO). 2016 Development of Adult Dermatomyositis and Polymyositis and Juvenile Dermatomyositis Response Criteria—Methodological Aspects: An American College of Rheumatology/European League Against Rheumatism/International Myositis Assessment and Clinical Studies Group/Paediatric Rheumatology International Trials Organisation Collaborative Initiative. *Rheumatology*, in press.

Rapid effects of air pollution exposure on inflammation biomarkers may be mediated through epigenetic changes.

Particulate air pollution is known to have acute health effects and some of this is thought to be due to inflammatory responses. In a "panel" study of healthy graduate students in Shanghai, China, scientists measured personal exposures to air pollution over time and clinically tracked inflammatory responses and changes in DNA epigenetics that regulate the expression of the measured inflammation-related biomarkers. In response to increases in particulate air pollution, changes in epigenetics and corresponding increases in inflammatory biomarkers were measurable within the first 24 hours.

Wang C, Chen R, Shi M, Cai J, Shi J, Yang C, Li H, Lin Z, Meng X, Liu C, Niu Y, Xia Y, Zhao Z, Kan H, Weinberg CR. Acute inflammation effects of personal exposures to fine-particulate air pollution may be mediated by methylation. *Am. J. Epidemiol.*, in press.

Genital Chlamydia Is Not Associated with Increased Risk of Uterine Fibroids

Since the 1930s researchers have hypothesized that reproductive tract infections could increase the risk of uterine fibroid development, but there have been few studies testing this hypothesis. A history of genital Chlamydia, the most frequently reported bacterial reproductive tract infection in the U.S., can be assesses by examination of serum antibodies which develop in response to the infection. This marker of past infection was not associated with a higher risk of fibroids in a study of nearly 1600 young African-American women, 22% of whom had ultrasound evidence of fibroids.

Moore KR, Smith JS, Cole SR, Dittmer DP, Schoenbach VJ, Baird DD. Chlamydia trachomatis seroprevalence and ultrasound diagnosed uterine fibroids in a large population of young African-American Women. *Am. J. Epidemiol.,* epub ahead of print, doi: 10.1093/aje/kwx231.

Pet Allergen Exposure and Asthma

While pets are found in more than 50% of U.S. homes, the effect of pet allergen exposure on asthma morbidity in the United States population is not well documented. Investigators examined the effect of dog and cat allergen exposures on asthma morbidity in the U.S. population in the National Health and Nutrition Examination Survey (NHANES). The prevalence of allergic sensitization in the NHANES population was similar for dog and cat with both being approximately 12%. Among those who were sensitized, elevated exposure to pet allergens was associated with an increased prevalence of asthma and asthma attacks. Indeed, 44% of the asthma attacks were attributable to exposure to high levels of dog allergen in the bedroom among asthmatics sensitive to dog and 30% attributable to cat allergen exposure among the comparable cat sensitive-exposed group. Projecting these results to the U.S. population, indicates more than 1 million increased asthma attacks each year for the dog sensitive-exposed group and more than 500,000 increased asthma attacks for the cat sensitive-exposed population of asthmatics. Elevated exposure to dog and cat allergens among those sensitized individuals with asthma is associated with excess asthma attacks. Reducing pet allergen exposures has the potential for a significant decrease in asthma morbidity.

Gergen PJ, Mitchell HE, Calatroni A, Sever ML, Cohn RD, Salo PM, Thorne PS, Zeldin DC. Sensitization and Exposure to Pets: The Effect on Asthma Morbidity in the United States Population. *J. Allergy Clin. Immunol. – In Pract*., epub ahead of print, doi: 10.1016/j.jaip.2017.05.019.

Dust mite allergens are more stable and more abundant

NIEHS scientists and colleagues from Wright State University and Duke University performed a large scale study to better understand the properties that define allergens from the many different proteins to which people are exposed. The scientists measured the abundance and stability of over 600 proteins from dust mite extract and concluded that the 19 allergens were statistically different in both of these properties from other mite proteins. While these properties have been studied on a limited basis before, this is the first study to provide an unbiased and large scale comparison in order to get superior statistics. It is proposed that the more abundant and stable proteins survive longer in dust and pollen, generating increased exposure, and increasing the possibility of an allergic response.

Ogburn RN, Randall TA, Xu Y, Roberts JH, Mebrahtu B, Karnuta JM, Rider SD, Kissling GE, London RE, Pomes A, Arlian L, Fitzgerald MC, Mueller GA. Are dust mite allergens more abundant and/or more stable than other Dermatophagoides pteronyssinus proteins? *J. Allergy Clin. Immunol.,* 139:1030-1032; 2017.

Environmental Factors Identified for Disease Flare in Dermatomyositis

The objectives of this investigation were to assess factors associated with increases of disease activity (called disease flares) in a group of rare systemic autoimmune diseases called juvenile

and adult dermatomyositis, in which the immune system attacks skeletal muscle, skin and other organ systems. Through a web-based questionnaire study, 210 patients participated, including 64% who experienced a disease flare within two years of the survey. Subjects more often reported disease flare after sun exposure, although use of photoprotective measures did not differ between those with and without a flare. Urinary tract infections and gastroenteritis were more frequent in the preceding 6 months in those who flared. Subjects who flared reported recently using NSAIDS, blood pressure medicines or medication for depression or mood changes. Moving to a new house was more common in those who flared. Only sun exposure and NSAIDs were significant factors in multivariable analysis. This is the first study to identify environmental factors to be associated with disease flares in patients with myositis. In conclusion, certain classes of environmental agents that have been associated with the initiation of dermatomyositis, including sun exposure and medications, may also play a role in disease flares.

Mamyrova G, Rider LG, Ehrlich A, Jones O, Pachman LM, Nickeson R, Criscione-Schreiber LG, Jung LK, Miller FW, Katz JD. Environmental Factors Associated with Disease Flare in Juvenile and Adult Dermatomyositis. *Rheumatology (Oxford)*, epub ahead of print, doi: 10.1093/rheumatology/kex162.

The transcription factor Foxa2 is critical for the regulation of uterine receptivity independent of uterine gland development.

NIEHS researchers have previously demonstrated that the transcription factor FOxa2 is critical for uterine gland development and mouse fertility. As part of a collaboration with scientists at the University of Missouri it was shown that ablation of Foxa2 in the mouse uterus after the development of uterine glands results in infertility. This infertility can be rescued by the administration of the cytokine Leukemia inhibitory factor, Lif. This shows that Foxa2 regulates genes directly involved in implantation and is not just responsible for gland development. Foxa2 is regulated by estrogens and may be susceptible to endocrine disruptors in the environment.

Kelleher AM, Peng W, Pru JK, Pru CA, DeMayo FJ, Spencer TE. Forkhead box a2 (FOXA2) is essential for uterine function and fertility. *Proc. Natl. Acad. Sci. USA.,* 114: E1018-E1026, 2017**.**

Structural Analysis of a Ribosome Assembly Protein

Through multiple structural analysis NIEHS researchers have discovered important functions of the ribosome assembly factor Nsa1 from *Saccharomyces cerevisiae*. Ribosomes are large macromolecular machines required for making proteins. Assembling these machines is a hard task for cells to undertake and requires the aid of hundreds of helpers, known as ribosome assembly factors. Dysfunction in ribosome production and deregulation of ribosome assembly pathway have been linked to many human diseases, highlighting the need to study this pathway. Through a combination of X-ray crystallography and small angle X-ray scattering NIEHS researchers were able to resolve the entire structure of Nsa1. Nsa1 is composed of an N-terminal seven-bladed WD40 domain followed by a flexible lysine-rich C terminus that is required for nucleolar localization. The WD40 domain of Nsa1 helps to recruit Rix7, a type II double ring AAA-ATPases to pre-60S particles to drive the subsequent release of Nsa1 from pre-60S particles**.**

Lo YH, Romes EM, Pillon MC, Sobhany M, Stanley RE. Structural Analysis Reveals Features of Ribosome Assembly Factor Nsa1/WDR74 Important for Localization and Interaction with Rix7/NVL2. *Structure*, 25:762-772.e4, 2017.

Cnot3 enhances human embryonic cardiomyocyte proliferation by promoting cell cycle inhibitor mRNA degradation.
In this study, the authors found that CNOT3, a component of the Ccr4-Not deadenylase complex, can enhance the proliferation of cardiomyocytes both in culture and in infarcted mouse hearts, likely by promoting cell cycle inhibitor mRNA degradation. Their study revealed a previously unrecognized role of mRNA degradation in cardiomyocyte growth, and suggested a potential strategy to control cardiac cell fates in development and disease.

Zhou B, Liu J, Ren Z, Yao F, Ma J, Song J, Bennett B, Zhen Y, Wang L, Hu G, Hu S. Cnot3 enhances human embryonic cardiomyocyte proliferation by promoting cell cycle inhibitor mRNA degradation. *Sci. Rep.*, 7:1500, 2017.

BRG1 regulated genes involved in the development of the lymphatic system
Temporal deletion of BRG1 at different developmental stages of the mouse results in edema, hemorrhage, and embryonic death with malformed lymphatic vessels. The lymphatic system regulates tissue fluids to maintain tissue homeostasis and when dysregulated it results in diseases like lymphedema and also contributes to cancer metastasis. BRG1 is mutated in several cancers and the findings from this study could explain its role in some of the cancers of the lymphatic system.

Singh AP, Foley J, Tandon A, Phadke D, Kinyamu HK, Archer TK. A role for BRG1 in the regulation of genes required for development of the lymphatic system. *Oncotarget*, epub ahead of print, doi: 10.18632/oncotarget.18976.

GLIS3: a new and critical regulator in postnatal spermatogenesis.
Spermatogonial stem cells have an extensive self-renewal capacity and are critical in maintaining the balance between differentiation and proliferation in order to provide a life-long source for the generation of mature germ cells. In this study, NIEHS investigators demonstrate that the transcriptional regulator Glis3 is indispensable for the differentiation and proliferation of spermatogonial stem and progenitor cells. Deficiency Glis3 in mouse leads to male germ cell depletion. The Glis3 signaling pathway might provide new therapeutic strategies for infertility.

Kang HS, Chen LY, Lichti-Kaiser K, Liao G, Gerrish K, Bortner CD, Yao HH, Eddy EM, Jetten AM. Transcription factor Glis3: a new and critical regulator of postnatal stages of mouse spermatogenesis. *Stem Cells*, 34: 2772-2783, 2016.

The transcription factor GATA2 is a critical regulator or uterine hormone sensitivity.
Ablation of the transcription factor Gata2 in the mouse results in infertility due to alterations in the ability of the uterus to respond to steroid hormones as well as the differentiation of the uterus. Loss of Gata 2 results in a significant decrease in the progesterone receptor. It also results in a change in the cell type of the uterine epithelium. Bioinformatic analysis shows these pathways

are conserved in the human uterus. The progesterone receptor controls the uterine response to estrogens and understanding progesterone actions can be used to understand how endocrine disruptors in the environment impact reproductive health.

Rubel CA, Wu SP, Lin L, Wang T, Lanz RB, Li X, Kommagani R, Franco HL, Camper SA, Tong Q, Jeong JW, Lydon JP, DeMayo FJ. A Gata2-Dependent Transcription Network Regulates Uterine Progesterone Responsiveness and Endometrial Function. *Cell Rep.*, 17: 1414-1425, 2016.

Identification of drivers for the metamorphic transition of HIV-1 reverse transcriptase
HIV-1 reverse transcriptase (RT) remains a very important target for the treatment of patients infected with the AIDS virus. The most effective therapies currently available generally involve a combination of drugs that target two different sites on RT. In many patients, it periodically becomes necessary to alter the drug combinations due to the emergence of drug-resistant RT variants or to development of undesirable side effects. The NIEHS group has been working on an alternative strategy that is based on the observation that formation of the active reverse transcriptase involves a complex structural maturation pathway that may be susceptible to drug interference. Research directed at characterization of this pathway has shown that it relies on a metamorphic protein, i.e. a protein that is able to adopt either of two alternate forms, but it has been unclear what drives the transition from one form to the other. The group previously suggested that the initial form of the protein is a "spring-loaded" structure, so that after the connection domain dissociates, the separate parts or subdomains, rapidly change forms. During the past year, the NIEHS group demonstrated that this transition is at least in part driven by the tendency of a helix in the palm subdomain to change from having a bent structure in one form of the protein, to adopting a straighter form in the other. In addition, a segment of the protein that is constrained to be partially disordered in the monomer exhibits an intrinsic tendency to adopt a β-sheet in the other. These structural changes of the individual protein segments help to drive the structural transition between the two alternate forms of RT.

Zheng X, Mueller GA, Kim K, Perera L, DeRose EF, London RE. Identification of drivers for the metamorphic transition of HIV-1 reverse transcriptase. *Biochem. J.*, in press.

Membrane protein orchestrates dialogue between fetus and mother
Placental blood vessels, which are formed early in pregnancy, provide nutrition from the mother to the growing fetus. Using a mouse model, investigators found that epithelial membrane protein 2 (EMP2) regulates placental development. Placentas lacking EMP2 from the mouse fetus have abnormal blood vessels, and some of these fetuses are lost during pregnancy. In humans, EMP2 expression was reduced in placentas obtained from pregnancies with growth-restricted babies. These findings suggest EMP2 as a critical regulator of human placentation.

Williams CJ, Chu A, Jefferson WN, Casero D, Sudhakar D, Khurana N, Hogue CP, Aryasomayajula C, Patel P, Sullivan P, Padilla-Banks E, Mohandessi S, Janzen C, Wadehra M. Epithelial membrane protein 2 (EMP2) deficiency alters placental angiogenesis mimicking features of human intrauterine growth restriction. *J. Pathol.,* 242: 246-259, 2017.

Centrality of α-synuclein in Pesticide related Parkinson's disease
NIEHS scientist used several lines of evidence to show that peroxidase activity of cytochrome c contributes to α-synuclein radical formation and oligomerization, and that α-synuclein, through its co-localization with cytochrome c or on its own, affects several biological pathways which contribute to increased neuronal death in an Maneb- and Paraquat -induced model of Parkinson's Disease. Based on this study which shows centrality of α-synuclein, it is suggested that experimental approaches which rely on scavenging α-synuclein radical or decreasing α-synuclein levels, can possibly lead to development of potential therapeutics for Parkinson's Disease.

Kumar A, Ganini D, Mason RP. Role of cytochrome c in α-synuclein radical formation: implications of α-synuclein in neuronal death in Maneb- and paraquat-induced model of Parkinson's disease. *Mol. Neurodegener.*, 11:70, 2016.

Molecular mechanisms of dendritic cell precursor's trafficking were elucidated.
Precursors of dendritic cells (pre-DCs) arise in the bone marrow (BM), egress to the blood, and finally migrate to peripheral tissue, where they differentiate to dendritic cells (DCs). Upon their activation, antigen-bearing DCs migrate from peripheral tissue to regional lymph nodes. To maintain immune homeostasis, these departing DCs must be replenished by new DCs that develop from pre-DCs, but the molecular mechanisms that direct pre-DC trafficking from the BM to the blood and peripheral tissues had been poorly understood. This study found that multiple chemokine receptors, CXCR4, CCR2 and CX3CR1, cooperate in a step-wise fashion to coordinate the trafficking of pre-DCs from the BM to the circulation and peripheral tissues.

Nakano H, Lyons-Cohen MR, Whitehead GS, Nakano K, Cook DN. Distinct functions of CXCR4, CCR2, and CX3CR1 direct dendritic cell precursors from the bone marrow to the lung. *J. Leukoc. Biol.*, 101:1143-1153, 2017.

Vitamin D3 metabolites can bind to RORα and RORγ
NIEHS investigators reported that several vitamin D3 metabolites can bind to RORα and RORγ and function as inverse agonists for these nuclear receptors, thereby providing an alternative mechanism of action for vitamin D3.

Slominski AT, Zmijewski MA, Jetten AM. RORα is not a receptor for melatonin. *BioEssays,* 38: 1193-1194, 2016.

Slominski AT, Kim TK, Hobrath JV, Oak ASW, Tang EKY, Tieu EW, Li W, Tuckey RC, Jetten AM. Endogenously produced nonclassical vitamin D hydroxy-metabolites act as "biased" agonists on VDR and inverse agonists on RORα and RORγ. *J. Steroid Biochem. Mol. Biol.,* epub ahead of print, doi: 10.1016/j.jsbmb.2016.09.024.

Slominski AT, Brożyna AA, Zmijewski MA, Jóźwicki W, Jetten AM, Mason RS, Tuckey RC, Elmets CA. Vitamin D signaling and melanoma: Role of vitamin D and its receptors in melanoma progression and management. *Lab. Invest.* 97: 706-724, 2017.

Slominski AT, Brożyna AA, Skobowiat C, Zmijewski MA, Kim TK, Janjetovic Z, Oak AS, Jozwicki W, Jetten AM, Mason RS, Elmets C, Li W, Hoffman RM, Tuckey RC. On the role of classical and novel forms of vitamin D in melanoma progression and management. *J. Steroid Biochem. Mol. Biol.*, epub ahead of print, doi: 10.1016/j.jsbmb.2017.06.013.

Interaction between growth factor and estrogen effects in uterine tissue.
Estrogen and insulin-like growth factor 1 (IGF1) are both important to the function of the uterus, but estrogen works through the estrogen receptor that is bound to DNA in the cell nucleus and IGF1 works through the IGF1 receptor on the outside surface of cells. Our experiments showed that IGF1 changes the expression levels of many of the same genes as estrogen does and in some, but not all cases, utilizes the DNA-bound estrogen receptor. This means that exposures that impact growth factor signaling may perturb estrogen receptor mediated outcomes leading to effects on human health and wildlife.

Hewitt SC, Winuthayanon W, Lierz SL, Hamilton KJ, Donoghue LJ, Ramsey JT, Grimm SA, Arao Y, Korach KS. Role of ERalpha in mediating female uterine transcriptional responses to IGF1. *Endocrinology*, 158 2427-2435, 2017.

Pathogenic mechanism due to a pair of disease mutations in the gene for the mitochondrial DNA polymerase.
Human mitochondrial DNA (mtDNA) polymerase γ (Pol γ) is the only polymerase known to replicate the mitochondrial genome. The Pol γ holoenzyme consists of the p140 catalytic subunit (POLG) and the p55 homodimeric accessory subunit (POLG2), which enhances binding of Pol γ to DNA and promotes processivity of the holoenzyme. Mutations within POLG impede maintenance of mtDNA and cause mitochondrial diseases. Two common POLG mutations, T251I and P587L, are usually found in the same POLG gene copy and cause with progressive external ophthalmoplegia. Researchers at the NIEHS sought to determine whether T251I or P587L is the primary pathogenic allele and found that both mutations are important and act synergistically to cause disease by decreasing the stability and catalytic efficiency of the polymerase.

DeBalsi KL, Longley MJ, Hoff KE, Copeland WC. Synergistic effects of the *in cis* T251I and P587L DNA polymerase γ (*POLG*) disease mutation. *J. Biol. Chem.,* 292:4198-4209, 2017.

Tissue-specific functions of SIRT1 phosphorylation in metabolism
SIRT1 is an important metabolic regulator that plays a central role in the regulation of transcriptional networks in various critical metabolic processes in multiple tissues. However, the mechanisms by which SIRT1 is regulated *in vivo* remain elusive, and the functional/ physiological consequences of disruption of its regulation is still unclear. These researchers have previously reported that SIRT1 can be phosphorylated at T522 by two anti-apoptotic members of the dual specificity tyrosine phosphorylation-regulated kinase (DYRK) in response to acute environmental stresses, and this modification activates its deacetylase activity independently of the cellular NAD^+ level through preventing the formation of less-active SIRT1 oligomers/ aggregates. In this study, utilizing two transgenic mouse models in which the wild type SIRT1 gene is replaced by a T522E (phosphorylation mimic) or a T522A (dephosphorylation mimic) mutation, the researchers show that phosphorylation modification of T522 on SIRT1 is crucial for tissue-specific regulation of SIRT1 activity in mice. Dephosphorylation of T522 is critical for repression of its activity during adipogenesis. The phospho-T522 level is reduced during adipogenesis. Knocking-in a constitutive T522 phosphorylation mimic activates the β-

catenin/GATA3 pathway, repressing PPARγ signaling, impairing differentiation of white adipocytes, and ameliorating high-fat diet induced dyslipidemia in mice. In contrast, phosphorylation of T522 is crucial for activation of hepatic SIRT1 in response to over-nutrition. Hepatic SIRT1 is hyperphosphorylated at T522 upon high-fat diet feeding. Knocking-in a SIRT1 mutant defective in T522 phosphorylation disrupts hepatic fatty acid oxidation, resulting in hepatic steatosis after high-fat diet feeding. In addition, the T522 dephosphorylation mimic impairs systemic energy metabolism. These findings unveil an important link between environmental cues, SIRT1 phosphorylation, and energy homeostasis, and demonstrate that the phosphorylation of T522 is a critical element in tissue-specific regulation of SIRT1 activity *in vivo*.

Lu J, Xu Q, Ji M, Guo X, Xu X, Fargo DC, Li X. The phosphorylation status of T522 modulates tissue-specific functions of SIRT1 in energy metabolism in mice. *EMBO Rep.*, 18:841-857, 2017.

Noradrenergic neurons of the locus coeruleus complex are heterogeneous with respect to dorsoventral origin

Scientists used an intersectional genetic fate mapping strategy in mice to investigate the embryonic origins of locus coeruleus (LC) complex noradrenergic neurons. Consistent with prior studies, the authors found that the majority of LC complex neurons originate in the dorsal neural tube, as defined by expression of Pax7. Surprisingly, however, they also identified a subpopulation of the LC complex that arises from outside the Pax7 expression domain. Compared to the broader LC complex, this newly identified subpopulation has very sparse axonal projections to thalamic nuclei, suggestive of distinct functions. This developmental genetic analysis opens new avenues of investigation into the functional diversity of the LC complex.

Plummer NW, Scappini EL, Smith KG, Tucker CJ, Jensen P. Two subpopulations of noradrenergic neurons in the locus coeruleus complex distinguished by expression of the dorsal neural tube marker Pax7. *Front. Neuroanat.*, 11:60, 2017.

Characterization of EPHX3 Null Mice

Cytochrome P450 epoxygenases metabolize arachidonic acid into epoxyeicosatrienoic acids (EETs), which play an important role in blood pressure regulation, protection against ischemia-reperfusion injury, angiogenesis, and inflammation. Epoxide hydrolases metabolize EETs to their corresponding diols which are biologically less active. Microsomal epoxide hydrolase (EPHX1) and soluble epoxide hydrolase (EPHX2) were identified >30 years ago and are capable of hydrolyzing EETs to DHETs. A novel epoxide hydrolase, EPHX3, was recently identified and also exhibits epoxide hydrolase activity with a substrate preference 11,12-EET. EPHX3 is highly expressed in the skin, lung, stomach, and esophagus; however, its endogenous function is unknown. Scientists investigated the impact of genetic disruption of Ephx3 on fatty acid epoxide hydrolysis and EET-related physiology in mice. LC-MS/MS analysis of Ephx3 null heart, lung, and skin lysates revealed no differences in endogenous epoxide:diol ratios compared to wild type. Ephx3 null mice also exhibited no change in plasma levels of fatty acid epoxides and diols. Incubations of cytosolic and microsomal fractions prepared from Ephx3 null and wild type tissues with synthetic EETs revealed no significant differences in rates of fatty acid diol

formation. Ephx3 null hearts had similar functional recovery compared to wild type hearts following ischemia/reperfusion injury and were not different from wild type in terms of lung inflammation after lipopolysaccharide exposure. Thus, genetic disruption of Ephx3 does not result in an overt phenotype and has no significant effects on the metabolism of EETs in vivo.

Hoopes, SL, Gruzdev A, Edin ML, Graves JP, Bradbury JA, Flake GP, Lih FB, DeGraff LM, Zeldin DC. Generation and Characterization of Epoxide Hydrolase 3 (EPHX3)-Deficient Mice. *PLoS One*. 12: e0175347, 2017.

Hormone signaling and fatty liver in females
Estrogen is an important factor for controlling lipid metabolism which related to lipid metabolism disorder in postmenopausal women, including hepatic lipid accumulation. This report suggested that the balance between non-hepatic estrogen signaling and hepatic or non-hepatic testosterone action controls hepatic lipid accumulation.

Hart-Unger S, Arao Y, Hamilton KJ, Lierz SL, Malarkey DE, Hewitt SC, Freemark M, Korach KS. Hormone signaling and fatty liver in females: analysis of estrogen receptor α mutant mice. *Int. J. Obes.*, 41:945-954, 2017.

An intracellular mechanism for sensing extracellular nutrient supply.
Phosphate has multiple functions that direct the survival of all living organisms: in its organic form, phosphate is a component of genomic material, it serves as an energy currency, and it is ubiquitous in cell signaling. Thus, the sensing and regulation of phosphate supply is essential to life, but the mechanisms by which this occurs in humans are largely unknown. NIEHS investigators have discovered a mechanism by which, in intestinal cells, the intracellular activity of a cell-signaling enzyme fluctuates in response to changes in dietary phosphate levels – this is a key advance in our understanding of the body's interaction with a vital environmental component - a vital nutrient**.**

Gu C, Nguyen HN, Hofer A, Jessen HJ, Dai X, Wang H, Shears SB. The Significance of the Bifunctional Kinase/Phosphatase Activities of Diphosphoinositol Pentakisphosphate Kinases (PPIP5Ks) for Coupling Inositol Pyrophosphate Cell Signaling to Cellular Phosphate Homeostasis. *J. Biol. Chem.*, 292:4544-4555, 2017.

Genome instability results from ribonucleotide removal from DNA
Researchers studying replication fidelity discovered that misincorporation of ribonucleotides occurs frequently and can cause genome instability. In addition to mutations, one of the most lethal form of DNA damage, a DNA double-strand break (DSB), can be generated upon removal of a ribonucleotide by the Topoisomerase I enzyme. These discoveries may have implications for human diseases that include cancer and autoimmune disorders.

Huang SN, Williams JS, Arana ME, Kunkel TA, Pommier Y. Topoisomerase I-mediated cleavage at unrepaired ribonucleotides generates DNA double-strand breaks. *EMBO J.*, 36: 361-373, 2017.

Novel mutation in the human mitochondrial DNA polymerase accessory subunit that causes adult-onset syndromic sensory neuropathy, ataxia and parkinsonism.
Mitochondrial dysfunction plays a key role in the pathophysiology of neurodegenerative disorders such as ataxia and Parkinson's disease. Investigators describe an extended Belgian pedigree where seven individuals presented with adult-onset cerebellar ataxia, axonal peripheral ataxic neuropathy, and tremor, in variable combination with parkinsonism, seizures, cognitive decline, and ophthalmoplegia. Researchers sought to identify the underlying molecular etiology and characterize the mitochondrial pathophysiology of this neurological syndrome and identified a splice acceptor variant in POLG2, c.970-1G>C, segregating with disease in this family and associated with a concomitant decrease in levels of POLG2 protein in patient cells. This work extends the clinical spectrum of POLG2 deficiency to include an overwhelming, adult-onset neurological syndrome that includes cerebellar syndrome, peripheral neuropathy, tremor, and parkinsonism.

Van Maldergem L, Besse A, De Paepe B, Blakely EL, Appadurai V, Humble MM, Piard J, Craig K, He L, Hella P, Debray FG, Martin JJ, Gaussen M, Laloux P, Stevanin G, Van Coster R, Taylor RW, Copeland WC, Mormont E, Bonnen PE. POLG2 deficiency causes adult-onset syndromic sensory neuropathy, ataxia and parkinsonism. *Ann. Clin. Transl. Neurol.,* 4:4-14, 2016.

Homozygous POLG2 gene mutation causing mitochondrial disease, hepatic failure, and mitochondrial DNA depletion.
Mitochondrial DNA (mtDNA) depletion syndrome manifests as diverse early-onset diseases that affect skeletal muscle, brain and liver function. Mutations in several nuclear DNA-encoded genes cause mtDNA depletion. Prior to this report, mutation in the POLG2 gene for the accessory subunit of the mitochondrial DNA polymerase has caused a late onset mtDNA deletions. Researches at the NIEHS, and Columbia University report on a patient, a 3-month-old boy who presented with hepatic failure, and was found to have severe mtDNA depletion in liver and muscle. Whole-exome sequencing identified a homozygous gene mutation (c.544C > T, p.R182W) in the accessory subunit of mitochondrial DNA polymerase gamma (POLG2), which is required for mitochondrial DNA replication. This is the first report of a patient with a homozygous mutation in POLG2 and with a clinical presentation of severe hepatic failure and mitochondrial depletion.

Varma H, Faust PL, Iglesias AD, Lagana SM, Wou K, Hirano M, DiMauro S, Mansukani MM, Hoff KE, Nagy PL, Copeland WC, Naini AB. Whole exome sequencing identifies a homozygous POLG2 missense variant in an infant with fulminant hepatic failure and mitochondrial DNA depletion. *Eur. J. Med. Genet.*, 59:540-545, 2016.

How cells achieve high accuracy of chromosomal DNA replication
The accuracy of DNA replication is a crucial factor for the mechanisms by which cells and organisms produce mutations. To gain understanding in this area researchers 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 has been studied 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. These researchers have also demonstrated the important role of the 5'-deoxynucleoside-triphosphates (dNTPs), which are the building blocks used by the polymerases for synthesizing DNA.

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

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

Babu VMP, Itsko M, Baxter JC, Schaaper RM, Sutton MD. Insufficient levels of the nrdAB-encoded ribonucleotide reductase underlie the severe growth defect of the Δhda E. coli strain. *Mol. Microbiol.,* 104:377-399, 2017.

Itsko M, Schaaper RM. Suppressors of dGTP Starvation in Escherichia coli. *J. Bacteriol.*, 199: e00142-17, 2017.

Synaptic weakening is a required step in the process of neuronal activity-dependent synapse pruning during brain development.

There is compelling evidence that shrinkage of neuronal structures called dendritic spines coincides with weakening of synaptic effectiveness (called long-term depression; LTD), and that LTD is accompanied by synapse loss, however, whether LTD is a required step in the progression toward synapse pruning is still unknown. Using repeated induction of LTD in dissociated neuronal cultures, NIEHS investigators found that synapse density, as measured by colocalization of fluorescent markers for pre- and postsynaptic structures, was decreased. Supporting a model by which LTD is required for synapse loss, the effect of LTD on fluorescence colocalization was prevented by inhibitors of this particular type of plasticity. These data support the idea that synaptic plasticity in the form of LTD is a required step in synapse pruning and contribute to our understanding of the basic mechanisms of this developmental process in the brain.

Henson MA, Tucker CJ, Zhao M, Dudek SM. Long-term depression-associated signaling is required for an in vitro model of NMDA receptor-dependent synapse pruning. *Neurobiol. Learn. Mem.,* 138:39-53, 2017.

Antidepressant may enhance delivery of medication to the brain

Scientists at NIEHS reported that by pairing the antidepressant amitriptyline with drugs designed to treat central nervous system diseases, delivery of medication to the brain of rats is enhanced, due to inhibiting the blood-brain barrier. The blood-brain barrier serves as a natural, protective boundary, preventing most medicine from entering the brain. Researchers cautioned that more studies are needed to determine whether people will benefit from the discovery. Even so, the new finding has the potential to revolutionize treatment for a whole host of brain-centered conditions, including epilepsy, stroke, human amyotrophic lateral sclerosis (ALS), depression, and others.

Banks DB, Chan GNY, Evans RA, Miller DS, Cannon RE. Lysophosphatidic acid and amitriptyline signal through LPA1R to reduce p-glycoprotein transport at the blood-brain-barrier. *J. Cereb. Blood Flow Metab.*, epub ahead of print, doi: 10.1177/0271678X17705786.

A new role in lung disease for an ancient cell organelle
Primary cilia (PC) are solitary cellular organelles that play critical roles in development, homeostasis, and disease pathogenesis by modulating key signaling pathways such as sonic hedgehog and calcium flux. The antenna-like shape of PC enables them also to facilitate sensing of extracellular and mechanical stimuli into the cell. However, nothing was known about the role of PC in airway smooth muscle cells (ASMC) in the context of airway remodeling (scarring) which occurs in chronic lung disease like asthma and COPD. Investigators found that PC are expressed on ASMC in asthmatic lungs. Using pharmacological and genetic methods they demonstrated that PC are necessary for ASMC contraction both in the absence of external stimulus and in response to the extracellular component hyaluronan. Mechanistically, the scientists demonstrated that the effect of PC on ASMC contraction is to a small extent due to their effect on sonic hedgehog signaling, and to a larger extent due to their effect on calcium influx and membrane depolarization. In conclusion, PC are necessary for the development of airway remodeling by mediating calcium flux and sonic hedgehog signaling.

Trempus CS, Song W, Lazrak A, Yu Z, Creighton JR, Young BM, Heise RL, Yu YR, Ingram JL, Tighe RM, Matalon S, Garantziotis S. A novel role for primary cilia in airway remodeling. *Am. J. Physiol. Lung Cell. Mol. Physiol*., 313: L328-L338, 2017.

Tight control of the progesterone receptor is critical for embryo implantation.
Loss of the progesterone receptor in the uterine epithelium is given as a sign of embryo receptivity. Here investigators generated a genetically engineered mouse model that expresses the progesterone in the uterine epithelium through the implantation window. The investigators show that continued expression of the progesterone receptor alters key pathways that are important for the regulation of receptivity. The progesterone receptor controls the uterine response to estrogens and understanding progesterone actions can be used to understand how endocrine disruptors in the environment impact reproductive health.

Wetendorf M, Wu SP, Wang X, Creighton CJ, Wang T, Lanz RB, Blok L, Tsai SY, Tsai MJ, Lydon JP, DeMayo FJ. Decreased epithelial progesterone receptor A at the window of receptivity is required for preparation of the endometrium for embryo attachment. Biol. Reprod., 96: 313-326, 2017.

A Comprehensive Pan-cancer Classification
In this study, scientists were able to identify many sets of 20 genes that could correctly classify more than 90% of the samples from 31 different tumor types using gene expression data generated by The Cancer Genome Atlas. This accuracy is remarkable given the number of the tumor types and the total number of samples involved. The scientists also identified a few genes that might play a role in sexual dimorphism in certain cancers.

Li Y, Kang K, Krahn JM, Croutwater N, Lee K, Umbach DM, Li L. A comprehensive genomic pan-cancer classification using The Cancer Genome Atlas gene expression data. *BMC Genomics*, 18:508, 2017.

The Cytidine Deaminase APOBEC3 Family Is Subject to Stress-Induced p53 Transcriptional Regulation

The APOBEC3 (A3) family of proteins are DNA cytidine deaminases that act as sentinels in the innate immune response against retroviral infections that recently were identified as potent enzymatic sources of mutations in several human cancers. Using human cancer cells and lymphocytes, the authors show that under stress conditions and immune challenges, all A3 genes are direct transcriptional targets of the tumor suppressor p53. In addition, they demonstrated that p53 could enhance IFN type-I induction of A3 genes. Activated p53 can integrate chromosomal stresses and immune responses through its influence on expression of APOBEC3 genes, which are key components of the innate immune system that also influence genomic stability.

Menendez D, Nguyen TA, Snipe J, Resnick MA. The Cytidine Deaminase APOBEC3 Family Is Subject to Transcriptional Regulation by p53. *Mol. Cancer Res.,* 15:735-743, 2017.

Unknown regulation mechanism of a pivotal receptor for lipid adsorption revealed

A receptor called Farnesoid X Receptor (FXR) that regulates production of bile acid, a key substance for lipid adsorption, was shown to be regulated by an unknown mechanism. Activated FXR modified by newly identified phosphorylation becomes unstable and is degraded in the cells.

Hashiguchi T, Arakawa S, Takahashi S, Gonzalez FJ, Sueyoshi T, Negishi M. Phosphorylation of Farnesoid X Receptor at Serine 154 Links Ligand Activation With Degradation. *Mol. Endocrinol.,* 30:1070-1080, 2016.

Crystal Structure of 6-O-sulfotransferase reveals substrate specificity for this enzyme involved in a key modification of Heparan sulfate

Heparan Sulfates (HS) are polysaccharides involved in physiological and pathophysiological functions such as infection, inflammatory responses, blood coagulation, cancer, and embryonic development. In this study, we solved the structure of the 6-O-sulfotransferase in complex with a polysaccharide substrate to reveal the specificity requirements of this enzyme. Investigators used this information to generate mutations that changed the specificity. This information is valuable to the technique of chemoenzymatic synthesis where these enzymes are being used to synthesize HS for drug development.

Xu Y, Moon AF, Xu S, Krahn JM, Liu J, Pedersen LC. Structure Based Substrate Specificity Analysis of Heparan Sulfate 6-O-Sulfotransferases. *ACS Chem. Biol.*, 12:73-82, 2017.

Valid Method of Measuring Thyroid Hormone Concentrations Found for Samples from Large Birth Cohort

Using a procedure for handling specimens that mimicked the one used in a large birth cohort study, the authors showed that use of the T3 uptake test combined with total thyroid hormone concentration gave valid results for free thyroid hormone, even though the subjects were 17 weeks pregnant at the time of blood draw, and the samples were shipped at ambient temperature.

Villanger GD, Learner E, Longnecker MP, Ask H, Aase H, Zoeller RT, Knudsen GP, Reichborn-Kjennerud T, Zeiner P, Engel SM. Effects of Sample Handling and Analytical Procedures on Thyroid Hormone Concentrations in Pregnant Women's Plasma. *Epidemiology*, 28:365-369, 2017.

New qPCR Methods to Detect CYP2C Subfamily P450s

The CYP2C subfamily of the cytochrome P450 gene superfamily encode heme-thiolate proteins which have a myriad of biological functions. CYP2C proteins detoxify xenobiotics and metabolize endogenous lipids such as arachidonic acid to bioactive eicosanoids. NIEHS investigators reported new methods for the quantitative polymerase reaction (qPCR) analysis for the 15 members of the mouse Cyp2c subfamily. Commercially available TaqMan primer/probe assays were compared to developed SYBR Green primer sets for specificity toward the mouse Cyp2c cDNAs and analysis of their tissue distribution.

Graves JP, Gruzdev A, Bradbury JA, DeGraff LM, Edin ML, Zeldin DC. Quantitative Polymerase Chain Reaction Analysis of the Mouse Cyp2c Subfamily and Characterization of their Tissue Distribution. *Drug Metab. Dispos*., 45:807-816, 2017.

The more you test, the more you find: The smallest P-values become increasingly enriched with real findings as more tests are conducted

NIEHS investigators show that an increase in the number of tested hypotheses increases the proportion of true signals among epidemiological predictors with the smallest P-values. This happens regardless of whether multiple testing corrections were applied and contradicts a common belief that more testing leads to spurious findings.

Vsevolozhskaya OA, Kuo CL, Diatchenko L, Ruiz G, Zaykin DV. The more you test, the more you find: The smallest P-values become increasingly enriched with real findings as more tests are conducted. *Genet. Epidemiol.*, in press

New Statistical Method Developed for Epidemiologic Studies

Building on their previous work that combines subject selection strategies with new statistical techniques, the authors have taken this approach a step further by extending the method to accommodate sampling and analyses in the setting of related outcomes being studied simultaneously.

Lu TS, Longnecker MP, Zhou H. Statistical inferences for data from studies conducted with an aggregated multivariate outcome-dependent sample design. *Stat. Med.*, 36:985-997, 2017.