Division of Intramural Research

NAEHS Council Update

September 2020

DIR RECRUITMENTS

Tenure-Track Investigator in the Clinical Research Branch
The National Institute of Environmental Health Sciences (NIEHS) is recruiting outstanding candidates for a Tenure-Track Investigator position in the Clinical Research Branch within the Division of Intramural Research at the NIEHS in Research Triangle Park, NC. The individual selected for this position will have a strong record of participation and publications in patient-oriented research defined as research that requires direct interactions with human subjects and may include the development of new technologies, understanding mechanisms of human disease, therapeutic interventions and/or clinical trials. The Clinical Research Branch is interested in candidates with expertise in areas such as endocrinology, neuroendocrinology, metabolism, exercise, sleep, immune-mediated diseases, pulmonology and human genetics, among others. Applicants should have an M.D. or equivalent doctoral degree with direct clinical research experience, with three or more years of research training in clinical research and publications and other evidence of the ability to design and carry out original, innovative patient-oriented research. Dr. Alison Motsinger-Reif, Chief of the Biostatistics and Computational Biology Branch serves as chair of the search committee which was launched on July 8, 2019.

Scientific Information Officer
The Division of Intramural Research is seeking an accomplished scientist in information technology to take on a leadership position as the NIEHS Scientific Information Officer (SIO). This individual will head the Office of Scientific Computing (OSC) and will lead a team that is directly focused on scientific information technology. The ideal candidate will have a record of accomplishment in information technology support to a scientific enterprise as well as application to basic and clinical research. Applicants should have a Ph.D., M.D., or equivalent advanced degree. Dr. Charles Schmitt, Director of the Office of Data Science serves as chair of the search committee which was launched on July 11, 2019.

Chief of the Comparative Medicine Branch
The National Institute of Environmental Health Sciences is searching for an Animal Program Director, Attending Veterinarian and Chief of the Comparative Medicine Branch (CMB). CMB provides a broad range of services and collaborative support for NIEHS intramural research programs. The incumbent will be responsible for an AAALAC accredited animal care and use program and for support of NIEHS animal research programs that engage in molecular, reproductive, neurological and immunological research as well as studying the effects of environmental agents in order to develop methods of disease prevention and treatment.
The incumbent actively support the NIEHS mission, participate in ongoing planning and management discussions to successfully resolve pertinent issues and challenges, participate in long range strategic planning processes to develop and implement effective goals and directions for the animal care program and provide information and recommendations to the Scientific Director and will conduct/collaborate in research relative to the mission of CMB and the institute. Applicants must have a DVM/VMD degree from an AVMA-accredited or approved college, a current license to practice veterinary medicine in any state in the United States and board certification by the American College of Laboratory Animal Medicine. Dr. Donald Cook, Senior Investigator in the Immunity, Inflammation and Disease Laboratory and Chair of the

NIEHS Animal Care and Use Committee serves as chair of the search committee which was launched on August 21, 2020.

Assistant Scientific Director
The Office of the Scientific Director in the Division of Intramural Research of NIEHS is seeking an accomplished scientist and science administrator to serve as the Assistant Scientific Director. This individual will function as a senior scientific advisor and provide leadership support in such areas as coordination of scientific activities, policy formulation and execution, programmatic review and will serve as a liaison to other NIEHS Divisions and across NIH on matters of special interest to the Scientific Director. Dr. Paul Doetsch, Deputy Scientific Director serves as chair of the advisory committee. The position was announced on August 11, 2020.

Recruitment of 2019-20 NIH Earl Stadtman Investigator Finalists
In addition to targeted recruitment, DIR is actively seeking outstanding scientists through the central NIH Stadtman recruitment mechanism. Seven outstanding candidates from a range of disciplines central to the NIEHS mission were interviewed in January and February 2020. Two candidates were offered positions at NIEHS with one declining and the second pending.

NEW HIRES AND CHANGES IN DIR LEADERSHIP

Chief of the Signal Transduction Laboratory
Dr. Anant Parekh has accepted an offer to become the Chief of the Signal Transduction Laboratory (STL) in DIR. He was appointed as Senior Investigator and Deputy Chief of STL in December 2019 after arriving from the University of Oxford (UK) where he was Professor in the Department of Physiology, Anatomy and Genetics and Director of the Centre for Integrative Physiology. At NIEHS, Dr. Parekh continues his research program focused on defining molecular mechanisms that control intracellular calcium signaling through plasma membrane store-operated Ca2+ channels and how these calcium signals are altered in human disease. Dr. Parekh will assume the role of Chief in October 2020.

New Tenure-Track Investigators

Dr. Jason Watts from the Life Sciences Institute at the University of Michigan has accepted an offer to join the Epigenetics & Stem Cell Biology Laboratory as an Earl Stadtman Tenure Track Investigator and was also selected to participated in the NIH Distinguished Scholars Program. Dr. Watts arrived at NIEHS on August 31, 2020 and will initiate an independent research program focused on understanding the mechanism of RNA polymerase pausing and its role in disease.

Dr. Stavros Garantziotis the Medical Director of the Clinical Research Unit at NIEHS and head of the Matrix Biology Group has accepted an offer to join the Immunity, Inflammation and Disease Laboratory as a Tenure Track Investigator. Dr. Garantziotis will continue and expand his independent research program focused on extracellular matrix biology, innate immunity, lung inflammatory diseases, and airway remodeling triggered by the environment. He is expected to start as a Tenure Track Investigator in 2021.

Metabolomics Core Leader
Dr. Alan Jarmusch from the University of California, San Diego has accepted an offer to join the Immunity, Inflammation and Disease Laboratory and the Mass Spectrometry Research and Support Group to establish and lead a new Metabolomics Core in DIR and further support the role of NIEHS in a trans-NIH Metabolomics and Lipidomics Consortium. Dr. Jarmusch is expected to arrive at NIEHS on September 14, 2020.

TRAINING AND MENTORING

The Fellows Award for Research Excellence "FARE"

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 $1500 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. NIEHS trainees were very successful in the FARE competition this year with the fourth highest number of awardees among all ICs.

The NIEHS Division of Intramural Research had 15 FARE award winners:

FARE Awardee	Mentor	Laboratory/Branch
Alicia Chi, Ph.D.	Francesco DeMayo, Ph.D.	Reproductive & Developmental Biology Laboratory
Nicholas Dietrich, Ph.D.	Trevor Archer, Ph.D.	Epigenetics & Stem Cell Biology Laboratory
Alexander C. Foo, Ph.D.	Geoff Mueller, Ph.D.	Genome Integrity & Structural Biology Laboratory
Ming Ji, Ph.D.	Xiaoling Li, Ph.D.	Signal Transduction Laboratory
Jacob Kresovich, Ph.D.	Jack Taylor, M.D., Ph.D.	Epidemiology Branch
Kaitlyn G. Lawrence, Ph.D.	Dale Sandler, Ph.D.	Epidemiology Branch
Xingyao Li, Ph.D.	Stephen Shears, Ph.D.	Signal Transduction Laboratory
Wan-chi Lin, Ph.D.	Michael Fessler, M.D.	Immunity, Inflammation & Disease Laboratory
Christopher Mazzone, Ph.D.	Guohong Cui, M.D., Ph.D.	Neurobiology Laboratory
Prashant Rai, Ph.D.	Michael Fessler, M.D.	Immunity, Inflammation & Disease Laboratory
Saniya Rattan, Ph.D.	Humphrey Yao, Ph.D.	Reproductive & Developmental Biology Laboratory
Yosuke Sakamachi, Ph.D.	Stavros Garantziotis, M.D.	Immunity, Inflammation & Disease Laboratory

Chitrangda Srivastava, Ph.D.	Anton Jetten, Ph.D.	Immunity, Inflammation & Disease Laboratory
Barrett M. Welch, Ph.D., M.P.H.	Kelly Ferguson, Ph.D., M.P.H.	Epidemiology Branch
Jingheng Zhou, Ph.D.	Guohong Cui, Ph.D.	Neurobiology 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 R00 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.

Yesenia Rodriguez, Ph.D. was awarded a K99/R00 from NIEHS and will be mentored by Dr. Samuel Wilson in the Genome Integrity and Structural Biology Laboratory.

DIR RESEARCH ACCOMPLISHMENTS FOR FY 2020

Bacteria boost host NAD synthesis by engaging an alternative pathway

Nicotinamide adenine dinucleotide (NAD), a cofactor for hundreds of metabolic reactions in all cell types, plays an essential role in metabolism, DNA repair, and aging. Cellular levels of NAD are tightly balanced by consumption and biosynthesis, and disruption of this homeostasis is associated with decreased cellular NAD levels in aging yet an elevated NAD synthesis-breakdown cycle in cancer cells. However, how NAD metabolism is impacted by the environment remains unclear. In this study, we report an unexpected bacteria contribution to host NAD biosynthesis. We found that different bacteria, including mycoplasma and *E.coli*, confer resistance to inhibitors of NAMPT, the rate limiting enzyme in a presumably dominate cellular NAD recycle pathway, in human cancer cells and xenograft tumors. We further show that a microbial enzyme (PncA) that converts nicotinamide to nicotinic acid, a precursor in the alternative NAD synthesis pathway, is necessary and sufficient for this protective effect. Using stable isotope tracing and microbiota-depleted mice, we demonstrate that this bacteria-mediated reaction contributes substantially to the NAD-boosting effect of oral nicotinamide and nicotinamide riboside supplementation in several tissues. Collectively, our findings reveal an important role of bacteria-enabled NAD biosynthesis pathway in host NAD metabolism.

Shats I, Williams JG, Liu J, Makarov MV, Wu X, Lih FB, Deterding LJ, Lim C, Xu X, Randall TA, Lee E, Li W, Fan W, Li JL, Sokolsky M, Kabanov AV, Li L, Migaud ME, Locasale JW, Li X. Bacteria Boost Mammalian Host NAD Metabolism by Engaging the Deamidated Biosynthesis Pathway. *Cell Metab*. 2020 Mar 3;31(3):564-579.e7. doi: 10.1016/j.cmet.2020.02.001. PMID: 32130883; PMCID: PMC7194078.

Largest study of genital powder use and risk of ovarian cancer

In a pooled analysis led by NIEHS Sister Study researchers, we observed a small, positive, but not statistically significant association between self-reported use of powder on the genital area and risk of ovarian cancer. Altogether, the study included 252,745 women from 4 large observational studies, 2168 of whom developed ovarian cancer. The observed positive association may be limited to women with intact reproductive tracts (i.e., women who have not had a hysterectomy or tubal ligation). We did not observe an association between duration or frequency of genital powder use and ovarian cancer risk. Though the results are not definitive, this is the largest study of the topic to date and it improves upon some of the design limitations of earlier retrospective studies.

O'Brien KM, Tworoger SS, Harris HR, Anderson GL, Weinberg CR, Trabert B, Kaunitz AM, D'Aloisio AA, Sandler DP, Wentzensen N. Association of Powder Use in the Genital Area With Risk of Ovarian Cancer. *JAMA*. 2020 Jan 7;323(1):49-59. doi: 10.1001/jama.2019.20079. PMID: 31910280; PMCID: PMC6990816.

NIH Scientists identify neural circuits contributing to the hardships of dieting

A collaborative effort led by researchers at the National Institute of Environmental Health Sciences (NIEHS) and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) characterized the impact of high-fat diets on neural circuits regulating hunger and reward. Mice provided unlimited access to a palatable high-fat food pellet would forego consumption of their standard chow even after the high-fat food was removed from their cages. To identify the neural

underpinnings of this behavior, the researchers recorded the activity of agouti-related peptide (AgRP) neurons of the hypothalamus and dopamine neurons of the ventral tegmental area (VTA) that mediate hunger and reward, respectively. They found that high-fat food exposure dramatically reduced how AgRP and dopamine neurons responded to their standard food pellet and affected the functional ability of these neural populations to drive standard chow intake when artificially stimulated. These findings underscore profound influences of diet on multiple brain circuits underlying the difficulties of dieting and making healthy eating choices.

Mazzone CM, Liang-Guallpa J, Li C, Wolcott NS, Boone MH, Southern M, Kobzar NP, Salgado IA, Reddy DM, Sun F, Zhang Y, Li Y, Cui G, Krashes MJ. High-fat food biases hypothalamic and mesolimbic expression of consummatory drives. *Nat Neurosci*. 2020 Aug 3. doi: 10.1038/s41593-020-0684-9. Epub ahead of print. PMID: 32747789.

A master regulator of liver function dictates sensitivity to methionine restriction in liver cancer

Methionine restriction, a dietary regimen that protects against metabolic diseases and aging, has been shown to repress cancer growth and improve cancer therapy. However, the response of different cancer cells to this nutritional manipulation is highly variable, and the molecular determinants of this heterogeneity remain poorly understood. In this study we report that hepatocyte nuclear factor 4α (HNF4α), a transcriptional factor that is critical for maintenance of hepatocyte identity and specification of hepatic functions, dictates the sensitivity of liver cancer to methionine restriction. We show that hepatic sulfur amino acid (SAA) metabolism, including methionine metabolism, is under transcriptional control of HNF4α. Depletion of HNF4α or SAA enzymes in HNF4α-positive epithelial liver cancer lines impairs SAA metabolism, increases resistance to methionine restriction or sorafenib, promotes epithelial-mesenchymal transition, and induces cell migration. Conversely, genetic or metabolic restoration of the transsulfuration pathway in SAA metabolism significantly alleviates the outcomes induced by HNF4α deficiency in liver cancer cells. Our study identifies HNF4α as a novel regulator of hepatic SAA metabolism that regulates the sensitivity of liver cancer to methionine restriction.

Xu Q, Li Y, Gao X, Kang K, Williams JG, Tong L, Liu J, Ji M, Deterding LJ, Tong X, Locasale JW, Li L, Shats I, Li X. HNF4α regulates sulfur amino acid metabolism and confers sensitivity to methionine restriction in liver cancer. *Nat Commun*. 2020 Aug 7;11(1):3978. doi: 10.1038/s41467-020-17818-w. PMID: 32770044; PMCID: PMC7414133.

Discovering genetic components of disease by combining decorrelated association statistics

The majority of statistical association methods have been designed assuming availability of SNP-level information. Genetic sequence data present new challenges to access and sharing of genotype-phenotype datasets. On the other hand, analysis based on aggregation of association statistics can be as efficient as analysis of raw data and has some advantages, including ease of transfer, accessibility, and simplified extraction from publications. We propose a powerful method based on decorrelating association scores. The new method gains power in situations that are most commonly encountered in practice; namely, under heterogeneity of association strengths and high variability of correlation between genetic variants.

Vsevolozhskaya OA, Shi M, Hu F, Zaykin DV. DOT: Gene-set analysis by combining decorrelated association statistics. *PLoS Comput Biol*. 2020 Apr 14;16(4):e1007819. doi: 10.1371/journal.pcbi.1007819. PMID: 32287273; PMCID: PMC7182280.

DNA 3' blocks reversed by the APE2 nuclease are vulnerabilities for BRCA1 and BRCA2 deficiency

We report novel DNA repair functions for APE2 protein in resolution of Topoisomerase 1 (TOP1) generated DNA damage. We discovered that APE2 is a key human enzyme reverting endogenous DNA 3' blocks, problematic DNA lesions that preclude synthesis of DNA in cells. Furthermore, DNA damage created by TOP1 conversion of genomic ribonucleotides into 3' blocked lesions underlies an APE2-BRCA1/2 synthetic lethality phenotype. Together our results suggest that targeting the APE2 DNA damage response pathway is a tractable vulnerability of homologous recombination deficient BRCA1/2 breast cancer tumor cells.

Álvarez-Quilón A, Wojtaszek JL, Mathieu MC, Patel T, Appel CD, Hustedt N, Rossi SE, Wallace BD, Setiaputra D, Adam S, Ohashi Y, Melo H, Cho T, Gervais C, Muñoz IM, Grazzini E, Young JTF, Rouse J, Zinda M, Williams RS, Durocher D. Endogenous DNA 3' Blocks Are Vulnerabilities for BRCA1 and BRCA2 Deficiency and Are Reversed by the APE2 Nuclease. *Mol Cell*. 2020 Jun 18;78(6):1152-1165.e8. doi: 10.1016/j.molcel.2020.05.021. Epub 2020 Jun 8. PMID: 32516598; PMCID: PMC7340272.

Active Site Coordination Within a Multienzyme Complex

Precursor ribosomal RNA (rRNA) must be processed by many trans-acting enzymes including the endonuclease Las1 and the kinase Grc3, in a highly coordinated 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. Through a series of atomic resolution cryo-EM structures the Stanley Lab revealed that the Las1 nuclease and Grc3 kinase assemble into a butterfly-like structure harboring a composite nuclease active site flanked by discrete RNA kinase sites. Las1 and Grc3 harbor molecular switches that coordinate the enzymatic functions of the complex and promote ribosome production.

Pillon MC, Hsu AL, Krahn JM, Williams JG, Goslen KH, Sobhany M, Borgnia MJ, Stanley RE. Cryo-EM reveals active site coordination within a multienzyme pre-rRNA processing complex. *Nat Struct Mol Biol*. 2019 Sep;26(9):830-839. doi: 10.1038/s41594-019-0289-8. Epub 2019 Sep 5. PMID: 31488907; PMCID: PMC6733591.

A novel epitranscriptomic process: environmental influences upon mRNA structure and stability.

IP7 is an intracellular signal with a license to ameliorate environmental impacts upon cellular bioenergetic health, through dynamic control over uptake, storage and utilization of various metabolic fuels. We have discovered a new aspect to IP7's actions by showing it stabilizes the structure of key mRNAs, both by inhibiting removal of a protective 'cap' structure, and by elevating the cell's storage capacity for stabilized mRNAs inside biomolecular condensates. These mRNAs are translationally silenced, but they can rapidly be returned to the translating pool in response to other environmental stimuli.

Sahu S, Wang Z, Jiao X, Gu C, Jork N, Wittwer C, Li X, Hostachy S, Fiedler D, Wang H, Jessen HJ, Kiledjian M, Shears SB. InsP7 is a small-molecule regulator of NUDT3-mediated mRNA decapping and processing-body dynamics. *Proc Natl Acad Sci U S A*. 2020 Aug 11;117(32):19245-19253. doi: 10.1073/pnas.1922284117. Epub 2020 Jul 29. PMID: 32727897; PMCID: PMC7431097.

Higher vitamin D is associated with higher conception rates.
We measured blood levels of a biomarker of vitamin D status (25-hydroxyvitamin D) and we looked at whether those levels were associated with the amount of time it took the couples in our study to get pregnant. We found that women with the lowest levels of vitamin D took the longest to get pregnant, and women with the highest levels got pregnant the fastest (Jukic et al. 2020). All of the women in our study were over age 30. Our study suggests that vitamin D supplementation may improve conception rates in women over 30. We followed up on these results by examining vitamin D levels and reproductive hormones, and we found lower estrogen levels in women who had low vitamin D levels (Harmon, Kissell, Jukic, et al. 2020). We summarized the research surrounding vitamin D and reproduction in a journal commentary (Jukic and Harmon, 2020).

Jukic AMZ, Baird DD, Weinberg CR, Wilcox AJ, McConnaughey DR, Steiner AZ. Pre-conception 25-hydroxyvitamin D (25(OH)D) and fecundability. *Hum Reprod*. 2019 Nov 1;34(11):2163-2172. doi: 10.1093/humrep/dez170. PMID: 31665286; PMCID: PMC7209776.

Harmon QE, Kissell K, Jukic AMZ, Kim K, Sjaarda L, Perkins NJ, Umbach DM, Schisterman EF, Baird DD, Mumford SL. Vitamin D and Reproductive Hormones Across the Menstrual Cycle. *Hum Reprod*. 2020 Feb 29;35(2):413-423. doi: 10.1093/humrep/dez283. PMID: 32068843.

Jukic AMZ, Harmon QE. Accumulating evidence for vitamin D and conception. *Fertil Steril.* 2020 Feb;113(2):330-331. doi: 10.1016/j.fertnstert.2019.10.025. PMID: 32106980.

Scientists developed a new fluorescent sensors to measure dopamine levels in the brain.
In a collaborative study led by researchers at Peking University, NIH/NIEHS and New York University, scientists have developed a new generation of genetically encoded green and red fluorescent sensors that can be used to monitor dopamine levels in the brain of live animals. These new sensors provide scientists with powerful new tools to study the function of dopamine in healthy animals and in animal models of psychiatric and neurological disorders.

Sun F, Zhou J, Dai B, Qian T, Zeng J, Li X, Zhuo Y, Zhang Y, Wang Y, Qian C, Tan K, Feng J, Dong H, Lin D, Cui G, Li Y. Next-generation GRAB sensors for monitoring dopaminergic activity in vivo. *Nat Methods.* 2020, *in press*.

Smokers' immune cells show signs of aging and immune dysfunction
Exposure to tobacco smoke is associated with impaired immunity and increased risk of inflammatory diseases. Martos et al. compared thousands of individual immune cells from the blood of healthy smokers and nonsmokers to find that smokers' cells exhibited characteristics typically observed in older individuals. CD8 T cells, which have an effector role in adaptive

immunity, showed signs of senescence combined with high cytolytic potential in smokers. These findings may provide a link between immune dysfunction and smoking-mediated diseases.

Martos, S. N., Campbell, M. R., Lozoya, O. A., Wang, X., Bennett, B. D., Thompson, I. J. B., Wan, M., Pittman, G. S., Bell, D. A. (2020). Single-Cell Analyses Identify Dysfunctional CD16+ CD8 T Cells in Smokers. *Cell Reports Medicine*, 1(4). doi:10.1016/j.xcrm.2020.100054

Exposures Early in Life May Set the Stage for Breast Cancer

Research based on the NIEHS Sister Study suggests that women who were gestated in a pregnancy complicated by pre-eclampsia may be at increased risk for breast cancer. In related work also based on the Sister Study, we found evidence that the risk of breast cancer increases at ages near the age at which an older sister had earlier been diagnosed with breast cancer, even if that onset was late in life. This correlation in the timing of onset suggests that early life shared exposures, perhaps acting jointly with genetic factors, may set a specific risk trajectory for breast cancer.

Diaz-Santana MV, O'Brien KM, D'Aloisio AA, Regalado G, Sandler DP, Weinberg CR. Perinatal and postnatal exposures and risk of young-onset breast cancer. *Breast Cancer Res.* 2020 Aug 13;22(1):88. doi: 10.1186/s13058-020-01317-3. PMID: 32791983; PMCID: PMC7427289.

Von Holle, A., O'Brien, K.M., Sandler, D.P. and Weinberg, C.R. Familial clustering in breast cancer age at onset. *Int J Epidemiol. 2020, in press*.

Single-cell analysis of Lung Cancer Development: Toward Precision Medicine

Cancer cells are heterogeneous and interact with their environment during tumor progression changing their phenotypic states. This challenges the field of precision medicine which is currently not optimized for the individual patient. We now have the ability to obtain highly resolved molecular phenotypes directly from individual cells from patient samples that can be used to define cell states, understand cell circuitry, and study cellular responses to drugs or chemicals. We recently developed and published a machine learning model denoted as PHENOtypic STAte MaP (PHENOSTAMP) with the potential to phenotypically characterize clinical samples in the context of in vitro studies. For example, we showed that induction of a reversible and significant process like Epithelial Mesenchymal Transition EMT-MET, using a stimulus like TGFβ could help assess clinical relevance of EMT in lung cancer and drug resistance.

Karacosta LG, Anchang B, Ignatiadis N, Kimmey SC, Benson JA, Shrager JB, Tibshirani R, Bendall SC, Plevritis SK. Mapping lung cancer epithelial-mesenchymal transition states and trajectories with single-cell resolution. *Nat Commun*. 2019 Dec 6;10(1):5587. doi: 10.1038/s41467-019-13441-6. PMID: 31811131; PMCID: PMC6898514.

DNA repair preferentially going reverse with the ligand imidodiphosphate

In the presence of metal ions such as magnesium, DNA replication and repair reactions consume deoxyribonucleotide triphosphates (dNTP) as substrates to add a deoxyribonucleotide monophosphate with a loss of a pyrophosphate group. Under same conditions, this reaction is reversed by reattaching the newly formed pyrophosphate and thereby regenerating dNTP. The overall equilibrium favors the DNA synthesis over the pyrophosphorolysis with the natural substrate and this chemical reaction catalyzed by DNA polymerase □ has been structurally and kinetically characterized by employing natural and various chemically modified substrates. When the bridging oxygen between P□ and P□ of dNTP was replaced by an imido-moiety (NH), overall enzymatic activity was observed to be drastically decreased while the chemical equilibrium strongly favoring the reverse pyrophosphorolysis reaction. Employing quantum mechanical/molecular mechanical calculations in conjunction with the utilization of quantum mechanically derived atomic charges, we examined the chemical foundation of the altered equilibrium with this central biological reaction.

Perera L, Beard WA, Pedersen LG, Shock DD, Wilson SH. Preferential DNA Polymerase β Reverse Reaction with Imidodiphosphate. *ACS Omega*. 2020 Jun 19;5(25):15317-15324. doi: 10.1021/acsomega.0c01345. PMID: 32637805; PMCID: PMC7331038.

Stress hormone receptors determine a neuronal phenotype

Mineralocorticoid receptors (MRs), a type of steroid hormone receptor, are highly enriched in the CA2 region of both mouse and human hippocampus. The authors therefore investigated the effects of MR deletion on CA2 neuron gene expression, synaptic properties, and functions. Mice with neuronal- or CA2-targeted deletion of MRs had a complete loss of CA2 molecular markers, disrupted social behavior, and altered responses to novel objects. The findings reveal essential roles of MRs in the development and maintenance of CA2 neuron phenotype, as well as on CA2-related behaviors. In addition, the study provides insights into how this receptor, encoded by the human NR3C2 gene, plays a role in one form of syndromic autism and several psychiatric disorders that are exacerbated by stress.

McCann KE, Lustberg DJ, Shaughnessy EK, Carstens KE, Farris S, Alexander GM, Radzicki D, Zhao M, Dudek SM. Novel role for mineralocorticoid receptors in control of a neuronal phenotype. *Mol Psychiatry*. 2019 Nov 19:10.1038/s41380-019-0598-7. doi: 10.1038/s41380-019-0598-7. Epub ahead of print. PMID: 31745235; PMCID: PMC7234915.

Novel viral-genetic method for tracing axon collaterals of broadly projecting neurons

Understanding how broadly projecting neurons in the mammalian brain coordinate complex behaviors requires comprehensive knowledge of the distribution and targets of their axon collaterals. To address this need, NIEHS researchers led by Patricia Jensen, Ph.D., developed a new method for mapping axon collaterals of genetically defined neuronal subtypes in mice. As a proof of concept, they have applied this method to analysis of the nucleus locus coeruleus (LC), a small cluster of brainstem noradrenergic neurons that project to almost every part of the brain, revealing differences in the pattern of axon collateralization of two LC subpopulations defined by projection to different regions of the cerebral cortex.

Plummer NW, Chandler DJ, Powell JM, Scappini EL, Waterhouse BD, Jensen P. An Intersectional Viral-Genetic Method for Fluorescent Tracing of Axon Collaterals Reveals Details of Noradrenergic Locus Coeruleus Structure. *eNeuro*. 2020 May 15;7(3):ENEURO.0010-20.2020. doi: 10.1523/ENEURO.0010-20.2020. PMID: 32354756; PMCID: PMC7294462.

Discovery of a novel backup DNA repair pathway for sunlight-induced DNA damage.
In a long-term collaboration with the group of Shunichi Takeda in Japan, we uncovered a novel back-up DNA repair pathway (Topoisomerase I-initiated Long Patch Base Excision Repair-LP BER) for removal of sunlight-induced DNA damage in genomic DNA. Sunlight exposure induced base-base dimers between adjacent nucleotides in a strand of genomic DNA. The main repair pathway addressing this type of DNA damage was discovered many years ago and is termed Nucleotide Excision Repair (NER). Deficiencies in NER factors have been linked to cancer and neurological disorders. Modifiers of the penetrance of disease have been observed but are largely unexplained. Our discovery of the LP BER backup repair pathway in NER-deficient mammalian cells explains one of the possible mechanisms of the observed differences in disease penetrance. The findings have clinical implications because the adverse effects of NER deficiency may be complemented by boosting the backup LP BER pathway, leading to a strategy for experiments toward clinical applications.

Saha LK, Wakasugi M, Akter S, Prasad R, Wilson SH, Shimizu N, Sasanuma H, Huang SN, Agama K, Pommier Y, Matsunaga T, Hirota K, Iwai S, Nakazawa Y, Ogi T, Takeda S. Topoisomerase I-driven repair of UV-induced damage in NER-deficient cells. *Proc Natl Acad Sci U S A*. 2020 Jun 23;117(25):14412-14420. doi: 10.1073/pnas.1920165117. Epub 2020 Jun 8. PMID: 32513688; PMCID: PMC7321995.

Discovery of a surprising role for a well-known nucleotide excision repair factor in the base excision repair pathway.
In a collaborative project with the group lead by Bennett Van Houten at the University of Pittsburgh, we discovered that the nucleotide excision repair (NER) factor UV-DDB can recognize base excision repair (BER) lesions in DNA and boost the efficiency of the BER pathway.
These results have uncovered the presence of a “cross-talk” between the NER and BER pathways, where lesion recognition by the NER pathway enhances BER lesion repair.

Jang S, Kumar N, Beckwitt EC, Kong M, Fouquerel E, Rapić-Otrin V, Prasad R, Watkins SC, Khuu C, Majumdar C, David SS, Wilson SH, Bruchez MP, Opresko PL, Van Houten B. Damage sensor role of UV-DDB during base excision repair. *Nat Struct Mol Biol*. 2019 Aug;26(8):695-703. doi: 10.1038/s41594-019-0261-7. Epub 2019 Jul 22. PMID: 31332353; PMCID: PMC6684372.

Abasic sites in RNA were characterized and linked to R-loop formation and repair.
In a collaborative project with the group lead by Vivian Cheung at the University of Michigan, we observed abasic sites in cellular RNA molecules (messenger RNA, ribosomal RNA and transfer RNA). Abasic sites are found in nascent messenger RNA, and these abasic sites could be involved in regulating gene transcription. Therefore, it is important to understand the repair mechanism for RNA abasic sites and especially those RNA abasic sites in R-loops. We revealed that the base

excision repair DNA repair factors AP endonuclease 1 and methyl purine DNA glycosylase are able to remove a base from RNA in an R-loop, creating an R-loop abasic site, and that incision the abasic site can be performed by AP endonuclease 1.

Liu Y, Rodriguez Y, Ross RL, Zhao R, Watts JA, Grunseich C, Bruzel A, Li D, Burdick JT, Prasad R, Crouch RJ, Limbach PA, Wilson SH, Cheung VG. RNA abasic sites in yeast and human cells. *Proc Natl Acad Sci U S A*. 2020 Aug 25;117(34):20689-20695. doi: 10.1073/pnas.2011511117. Epub 2020 Aug 11. PMID: 32788345.

Elevation in cellular formaldehyde level triggers mitochondrial DNA damage and attendant cell signaling

Maintaining genome stability involves coordination between different subcellular compartments providing eukaryotic cells with DNA repair systems that safeguard against environmental and endogenous stresses. Living organisms produce the chemically reactive molecule formaldehyde (FA) as a normal product of 1-carbon metabolism, and a role of FA as a signaling molecule is emerging. Cells have developed sophisticated systems to restrict endogenous levels of FA under physiological conditions, and this prevents genotoxicity, among other adverse effects on macromolecules. Deregulation of FA has been associated with several diseases, such as progeria, autism, cancer and neurodegenerative disorders, and cause and effect relationships are under study. In our present work, we found that FA deregulation leading to increased FA levels in cells induces strand breaks in mitochondrial DNA. In addition, we were surprised to find that nuclear double strand break repair factors accumulate at strand breaks in mitochondrial DNA. This finding suggests a robust signaling mechanism is at play mobilizing nuclear double strand break repair factors to leave the nucleus and accumulate at mitochondrial DNA damage.

Nadalutti CA, Stefanick DF, Zhao ML, Horton JK, Prasad R, Brooks AM, Griffith JD, Wilson SH. Mitochondrial dysfunction and DNA damage accompany enhanced levels of formaldehyde in cultured primary human fibroblasts. *Sci Rep*. 2020 Mar 27;10(1):5575. doi: 10.1038/s41598-020-61477-2. PMID: 32221313; PMCID: PMC7101401.

Revealing dynamics of two base excision repair factors in living mammalian cells: AP endonuclease 1 and poly(ADP ribose) polymerase1

Fluorescently-tagged repair proteins have been used historically to probe their recruitment to micro-irradiation-induced nuclear DNA damage in living cells. We described new methodology that, for the first time, allows quantification of APE1 dynamics after micro-irradiation-induced DNA damage. Conditions were standardized for induction of the oxidatively-induced 8-oxoG DNA lesion, since repair of this lesion involves production of the AP-site base excision repair intermediate, which is a substrate for both APE1 and PARP1. Time-lapse analysis of APE1-GFP illustrated very rapid recruitment to the DNA damage, with a half-time of less than 1 s. In cells co-transfected with APE1-GFP and PARP1-mCherry, the half-time of recruitment of PARP1 was much slower than that of APE1, indicating APE1 is the first responder to the AP-site repair intermediate. Although APE1 recruitment was unchanged, dissociation from micro-irradiation-induced DNA damage was slower with double transfection with PARP1, indicating a type of coordination between these two repair factors. In additional experiments in DNA polymerase beta null cells, APE1 dissociation from micro-irradiation-induced DNA damage was completely

blocked. These results support the hypothesis that coordination of the individual enzymatic steps in the base excision repair process is a fundamental requirement in the DNA repair pathway.

Janoshazi AK, Horton JK, Zhao ML, Prasad R, Scappini EL, Tucker CJ, Wilson SH. Shining light on the response to repair intermediates in DNA of living cells. *DNA Repair* (Amst). 2020 Jan;85:102749. doi: 10.1016/j.dnarep.2019.102749. Epub 2019 Nov 12. PMID: 31790865.

Uncovering a DNA substrate localization process by DNA polymerase β and other X-family DNA polymerases

DNA polymerase (pol) β catalyzes two repair reactions in small DNA gaps generated during base excision repair, gap-filling DNA synthesis and 5' end deoxyribosephosphate (dRP) lyase removal. Polβ must locate these DNA gaps that are generated throughout the genome in a robust manner. To provide insight into the mechanism of substrate search and recognition, we compared DNA binding affinities of purified pol β and several variants, for 1-nt gap containing DNA and undamaged control DNA. Surprisingly, this analysis revealed that mutation of 3 lysine residues in the lyase active site of pol β lead to tighter non-specific DNA binding affinity, i.e., for un-damaged DNA, but little change in specific DNA binding. Due to the tighter non-specific DNA binding, the lysine mutant was deficient in processive searching. We also found that non-specific DNA binding affinity was negatively correlated with processive searching ability among the other two pol x-family members, pols lambda and mu. Using in vivo experiments with mouse fibroblasts, we found that the processive searching-deficient mutant of pol β was reduced in its ability to localize to sites of laser-induced DNA damage, as compared to wild-type pol β. These data suggested that destabilization of the non-specifically DNA bound pol β complex promotes micro-dissociations and that this manifests in enhanced DNA scanning. These results are consistent with the hypothesis that DNA scanning is an important function during pol β substrate localization both *in vitro* and *in vivo*.

Howard, M.J., Zhao, M.-L., Horton, J.K. and Wilson, S. H. An imbalance between specific and non-specific DNA binding is important for productive DNA scanning among gap-filling X-family DNA polymerases. *J Biol Chem.* 2020, 295:12181-12187.

Understanding DNA polymerase mediated pro-mutagenic DNA synthesis during double-strand break repair

DNA polymerase (pol) μ is a DNA-dependent polymerase that incorporates nucleotides during gap-filling synthesis in the non-homologous end-joining pathway of double-strand break repair. Previous structural characterization of pre- and post-catalytic complexes of pol m indicated the enzyme is rigid, failing to undergo large conformational adjustments during catalysis, in contrast to many other pols. Here we employed time-lapse X-ray crystallography to visualize catalytic events during gap-filling DNA synthesis by pol μ. Unique catalytic intermediates and active site conformational changes that underlie catalysis were uncovered, and a transient product manganese ion was observed in the product metal site. This product metal (manganese) coordinates phosphate oxygens of both products (the inserted nucleotide and PPi). Single-turnover kinetic analyses indicated that manganese strongly increases dNTP insertion and emphasizes the likely product stabilization role of the manganese product metal in pol μ. These results are an important advance

in our understanding of how pol μ functions. The observations also provide insight on structural attributes of the X-family double-strand break repair polymerases that impact their biological function in maintenance of genomic integrity.

Jamsen, J.A., Sassa, A., Shock, D.D., Beard, W.A., and Wilson, S.H. Structural basis for proficient oxidized deoxynucleotide Insertion by double strand break repair polymerase μ revealed by time-lapse crystallography. *Nat Commun*. 2020, *in press.*

Neutrophil Dysregulation is Pathogenic in Myositis

The myositis syndromes are systemic autoimmune diseases with characteristic muscle inflammation and weakness, myositis-specific autoantibodies (MSAs), and organ damage. Neutrophils have recently been found to play important roles in several autoimmune diseases, through their enhanced ability to form neutrophil extracellular traps (NETs), which are lattices outside of the cell that are extruded in response to danger signals and may be important sources of modified autoantigens and molecules that stimulate the immune system. In this study, we found pathogenic neutrophil subsets (low-density granulocytes [LDGs]) and NETs were elevated in the blood of patients with myositis. LDGs from myositis patients had an enhanced ability to form NETs. LDGs and NETs correlated with myositis disease activity and muscle damage. The serum autoantibody anti-MDA5 correlated with circulating and tissue NETs, and directly enhanced NET formation. An enhanced neutrophil gene signature was present in the affected muscle tissue from myositis patients and was associated with muscle injury in the test tube and interferon gene expression, an important immune pathway activated in patients with dermatomyositis and polymyositis. These data suggest that dysregulated neutrophil pathways may have a role in the disease process in myositis patients through their ability to directly injure muscle cells and other affected tissues and activate the immune system.

Seto N, Torres-Ruiz JJ, Carmona-Rivera C, Pinal-Fernandez I, Pak K, Purmalek MM, Hosono Y, Fernandes-Cerqueira C, Gowda P, Arnett N, Gorbach A, Benveniste O, Gómez-Martín D, Selva-O'Callaghan A, Milisenda JC, Grau-Junyent JM, Christopher-Stine L, Miller FW, Lundberg IE, Kahlenberg JM, Schiffenbauer AI, Mammen A, Rider LG, Kaplan MJ. Neutrophil dysregulation is pathogenic in idiopathic inflammatory myopathies. *JCI Insight*. 2020 Feb 13;5(3):e134189. doi: 10.1172/jci.insight.134189. PMID: 31945019; PMCID: PMC7098779.

Evaluating Toxicity of Legacy and Emerging Flame Retardants

Flame retardants have been used in response to fire safety regulations since the last half of the 20th century. Exposure to these chemicals can occur at home or in the workplace. The toxicities of three legacy and six emerging brominated flame retardants were compared in male rats following exposure to each of nine chemicals at various concentrations. Increases in liver weights were found following exposure to PBDE-47, HBCD and HCDBCO flame retardants. In the liver, gene expression changes related to liver disease and/or metabolic changes after exposure to PBDE-47, decaBDE, and HBCD while fewer gene changes were identified in response to the other flame retardants (TBB, TBPH, TBBPA-DBPE, BTBPE, DBDPE, or HCDBCO).

Shockley KR, Cora MC, Malarkey DE, Jackson-Humbles D, Vallant M, Collins BJ, Mutlu E, Robinson VG, Waidyanatha S, Zmarowski A, Machesky N, Richey J, Harbo S, Cheng E, Patton K, Sparrow B, Dunnick JK. Comparative toxicity and liver transcriptomics of

legacy and emerging brominated flame retardants following 5-day exposure in the rat. *Toxicol Lett*. 2020 Oct 10;332:222-234. doi: 10.1016/j.toxlet.2020.07.016. Epub 2020 Jul 15. PMID: 32679240.

ORSO provides a web-based tool for enhancing connections between scientists and next-generation sequencing data.

Online Resource for Social Omics (ORSO) has been developed to improve the accessibility and reuse of the vast and rapidly growing quantity of public NGS data. The NCBI Gene Expression Omnibus currently hosts data from several million samples. However relevant or meaningful next-generation sequencing (NGS) data may not be easy to find. Annotations in data repositories are frequently incomplete and inconsistent, as data provenance, interoperability, and reusability are often secondary concerns in publication-driven research. ORSO helps improve accessibility by combining data and metadata similarity with aspects of the connective experiences most often found in social networks.

Lavender CA, Shapiro AJ, Day FS, Fargo DC. ORSO (Online Resource for Social Omics): A data-driven social network connecting scientists to genomics datasets. *PLoS Comput Biol*. 2020 Jan 24;16(1):e1007571. doi: 10.1371/journal.pcbi.1007571. PMID: 31978042; PMCID: PMC7001987.

LostArc, an ultrasensitive mitochondrial DNA deletion detection system developed at the NIEHS, reveals millions of mtDNA deletions and links mitochondrial DNA replication to disease and aging.

In collaboration with researchers at Newcastle University in the UK, research lead by Copeland and colleagues in the Genome Integrity and Structural Biology Laboratory at the NIEHS have developed an ultrasensitive approach, termed LostArc, to detect mitochondrial DNA deletions in human samples. Acquired human mitochondrial genome (mtDNA) deletions are symptoms and drivers of focal mitochondrial respiratory deficiency, a pathological hallmark of aging and late-onset mitochondrial disease. To decipher connections between these processes, NIEHS used LostArc, and identified revealed 35 million deletions (~470,000 unique spans) in skeletal muscle from 22 individuals with and 19 individuals without pathogenic variants in POLG, the nuclear gene for the catalytic subunit of replicative mitochondrial DNA Polymerase γ. Ablation, the deleted mtDNA fraction, suffices to explain skeletal muscle phenotypes of aging and POLG-derived disease. Unsupervised bioinformatic analyses reveal distinct age- and disease-correlated deletion patterns. These patterns implicate replication by DNA Polymerase γ as the deletion driver and suggest little purifying selection against mtDNA deletions by mitophagy in postmitotic muscle fibers. Observed deletion patterns are best modeled as mtDNA deletions initiated by replication fork stalling during strand displacement mtDNA synthesis.

Lujan SA, Longley MJ, Humble MH, Lavender CA, Burkholder AB, Blakely EL, Alston CL, Gorman GS, Turnbull DM, McFarland R, Taylor RW, Kunkel TA, Copeland WC. Ultrasensitive detection of mtDNA deletions in POLG patients elucidates the mechanism of mtDNA replication. *Genome Biology*. 2020, *In press*

Single molecule imaging reveals the dynamic assembly of the mitochondrial DNA helicase

A collaboration between scientists at NIEHS and North Carolina State University demonstrated that the mitochondrial replicative DNA helicase, Twinkle, can self-assemble into hexamers from individual Twinkle monomers, in the presence of DNA. They employed atomic force microscopy imaging to visualize ring assembly, DNA binding, and unwinding activity of individual Twinkle hexamers at the single-molecule level. They also revealed that closed-ring conformers bind and unwind several hundred base pairs of duplex DNA at an average rate of ~240 bp/min. and that the addition of mitochondrial single-stranded (ss) DNA-binding protein both influences the ways Twinkle loads onto defined DNA substrates and stabilizes the unwound ssDNA product, resulting in a ~5-fold stimulation of the apparent DNA-unwinding rate. The strategies used in this work provide a new platform to examine Twinkle disease variants and the core mtDNA replication machinery. They also offer an enhanced framework to investigate molecular mechanisms underlying deletion and depletion of the mitochondrial genome as observed in mitochondrial diseases.

Kaur P, Longley MJ, Pan H, Wang W, Countryman P, Wang H, Copeland WC. Single-molecule level structural dynamics of DNA unwinding by human mitochondrial Twinkle helicase. *J Biol Chem*. 2020 Apr 24;295(17):5564-5576. doi: 10.1074/jbc.RA120.012795. Epub 2020 Mar 25. PMID: 32213598; PMCID: PMC7186178.

Study finds that reduced lung function in oil spill cleanup works following the Deepwater Horizon disaster not long-lasting

NIEHS researchers previously reported reduced lung function in oil spill cleanup workers 1-3 years after the Deepwater Horizon disaster in the Gulf of Mexico. In a follow-up study, participants who completed two spirometry test tests 1-3 years and 4-6 years after the spill (N = 1,838) were evaluated to examine changes in forced expiratory volume in 1 second (FEV1; ml), forced vital capacity (FVC; ml), and ratio (FEV1/FVC; %) over time. Despite reduced lung function at 1-3 years, at the 4-6-year exam workers with total hydrocarbon (THC) exposure 1-2.99 ppm and ≥3 ppm compared to those with ≤0.29 ppm exhibited higher FEV1 (β: 108 ml, 95% CI: 17, 198) and (β: 118 ml, 95% CI: 5, 232), respectively. Lung function decrements seen shortly after the spill were no longer apparent 4-6 years later, with the greatest improvement among those with the highest exposures.

Lawrence KG, Keil AP, Garantziotis S, Umbach DM, Stewart PA, Stenzel MR, McGrath JA, Jackson WB, Kwok RK, Curry MD, Engel LS, Sandler DP. Lung function in oil spill responders 4-6 years after the Deepwater Horizon disaster. *J Toxicol Environ Health A*. 2020 Mar 18;83(6):233-248. doi: 10.1080/15287394.2020.1745111. Epub 2020 Apr 5. PMID: 32249687.

Recognition and processing of transfer RNA, mediators of the genetic code

NIEHS researchers and their collaborators discovered how precursor RNAs are recognized by an enzyme that generates mature transfer RNAs (tRNAs), critical molecules that physically convert the genetic code to proteins. Although there are many different tRNAs that carry amino acids for protein synthesis, the 'elbow' of the L-shaped tRNA is similar in many tRNAs. This study illustrates at the atomic level how the enzyme recognizes the conserved tRNA 'elbow' for correct generation of mature tRNAs. The findings also demonstrate that peptide motifs in this enzyme and functional RNAs in other enzymes have converged on a similar solution for recognizing the tRNA 'elbow.'

Teramoto T, Kaitany KJ, Kakuta Y, Kimura M, Fierke CA, Hall TMT. Pentatricopeptide repeats of protein-only RNase P use a distinct mode to recognize conserved bases and structural elements of pre-tRNA. *Nucleic Acids Res*. 2020 Jul 28:gkaa627. doi: 10.1093/nar/gkaa627. Epub ahead of print. PMID: 32719843.

Small nucleolar RNA levels dynamically change during stem cell differentiation

NIEHS researchers developed a new method and discovered that the levels of small nucleolar RNAs (snoRNAs) are dynamic during stem cell differentiation. Different cell types express distinct profiles of these small RNAs that guide modification of ribosomal RNA, and expression levels of a subset of snoRNAs change as stem cells differentiate. Decreasing the level of one of these small RNAs decreases modification at its target site in the ribosomal RNA, which could influence protein synthesis by the ribosome in stem cells versus differentiated cells.

McCann KL, Kavari SL, Burkholder AB, Phillips BT, Hall TMT. H/ACA snoRNA levels are regulated during stem cell differentiation. *Nucleic Acids Res*. 2020 Jul 25:gkaa612. doi: 10.1093/nar/gkaa612. Epub ahead of print. PMID: 32710630.

Early embryos need a molecule called tankyrase to develop successfully

Embryonic genome activation (EGA) is orchestrated by an intrinsic developmental program initiated during oocyte maturation. We show that tankyrase, a poly(ADP-ribosyl) polymerase that regulates β-catenin levels, undergoes programmed translation during oocyte maturation and serves an essential role in mouse EGA by promoting the generation of ribosomes that synthesize new proteins required for embryo development. Our findings indicate that post-transcriptional regulation of tankyrase serves as a developmental mechanism for post-translational β-catenin activation and is required to complete EGA.

Gambini A, Stein P, Savy V, Grow EJ, Papas BN, Zhang Y, Kenan AC, Padilla-Banks E, Cairns BR, Williams CJ. Developmentally Programmed Tankyrase Activity Upregulates β-Catenin and Licenses Progression of Embryonic Genome Activation. *Dev Cell*. 2020 Jun 8;53(5):545-560.e7. doi: 10.1016/j.devcel.2020.04.018. Epub 2020 May 21. PMID: 32442396; PMCID: PMC7335218.

Brain nicotine-binding protein receptors regulate brain theta wave production

Brain alpha7 nicotinic acetylcholine receptors are a subtype of acetylcholine receptors that can bind to nicotine. Brain theta waves are synchronized brain activities in brain subregion hippocampus and hippocamps-associated brain subregions and are involved in many higher brain functions especially spatial learning and memory. By infusing selective nicotinic receptor antagonist to the hippocampus and knocking out the receptors in neuronal subpopulations and recording hippocampal electrical activities in freely-moving mice, the authors show that these nicotinic receptors play a crucial role in regulating normal theta waves production and corresponding behavioral performance that dependent on intact spatial memory.

Gu Z, Smith KG, Alexander GM, Guerreiro I, Dudek SM, Gutkin B, Jensen P, Yakel JL. Hippocampal Interneuronal α7 nAChRs Modulate Theta Oscillations in Freely Moving Mice. *Cell Rep*. 2020 Jun 9;31(10):107740. doi: 10.1016/j.celrep.2020.107740. PMID: 32521265; PMCID: PMC7333865.

NIEHS Scientists Discover a Protein in the Lung that Supports the Entry of White Blood Cells into the Airspace During Pneumonia

Neutrophils, an abundant type of white blood cell, play a crucial role in combatting infections in the lung, but they can also cause bystander damage to lung cells. Although the mechanisms controlling the recruitment of neutrophils from the bloodstream to the lung have been studied for decades, many questions remain. This year, NIEHS scientists reported their discovery that Epithelial Membrane Protein 2 (EMP2), a protein in the outer membrane of the epithelial cells that line the lung's airspaces, plays a critical role in supporting the transit of neutrophils from the bloodstream into the airspace lumen (the air-conducting interior passages of the lung). Interestingly, EMP2-deficient animals were found to have reduced numbers of neutrophils in their lungs during bacterial pneumonia, but this was associated with attenuated lung injury and an improved outcome. These exciting findings suggest that targeting EMP2 with drugs or other therapeutics may be a promising treatment strategy in humans with pneumonia or other forms of lung disease.

Lin WC, Gowdy KM, Madenspacher JH, Zemans RL, Yamamoto K, Lyons-Cohen M, Nakano H, Janardhan K, Williams CJ, Cook DN, Mizgerd JP, Fessler MB. Epithelial membrane protein 2 governs transepithelial migration of neutrophils into the airspace. *J Clin Invest*. 2020 Jan 2;130(1):157-170. doi: 10.1172/JCI127144. PMID: 31550239; PMCID: PMC6934223.

It Takes Two to Make an Ovary.

Abnormal reproductive functions or infertility in adult female are often the results of impaired development of the ovaries during fetal life. Principal investigator Humphrey Hung-Chang Yao, along with the Staff Scientist Barbara Nicol and collaborators, identified a new gene, Runx1, that promotes the development of the mouse fetal ovary through an interplay with another factor called Foxl2. Together, these two factors prevent the fetal ovary from becoming a testis, therefore ensuring the female embryo develop a functional ovary. This study fills a critical void in our understanding of the genetic mechanisms behind ovarian development and provides a new candidate gene, Runx1, for the study of disorders and pathology of the ovary.

Nicol B, Grimm SA, Chalmel F, Lecluze E, Pannetier M, Pailhoux E, Dupin-De-Beyssat E, Guiguen Y, Capel B, Yao HH. RUNX1 maintains the identity of the fetal ovary through an interplay with FOXL2. *Nat Commun*. 2019 Nov 11;10(1):5116. doi: 10.1038/s41467-019-13060-1. PMID: 31712577; PMCID: PMC6848188.

Mutation signatures of alkylating agents revealed

Utilizing yeast strains engineered for large-scale production of single stranded DNA, we probed the substrate specificity, mutation spectra and signatures associated with DNA alkylating agents. The spectra and signatures derived from yeast were detectable in lung cancers, head and neck cancers and tumors from patients exposed to a subclass of alkylating chemicals. The estimates of mutation loads associated with this alkylation signature were higher in lung tumors from smokers

than never-smokers, pointing towards the mutagenic activity of alkylating carcinogens in cigarettes. In summary, our analysis of mutations in yeast strains treated with alkylating agents, as well as in whole-exome and whole-genome sequenced tumors identified signatures highly specific to alkylation mutagenesis and indicate the pervasive nature of alkylation-induced mutagenesis in cancers.

Saini N, Sterling JF, Sakofsky CJ, Giacobone CK, Klimczak LJ, Burkholder AB, Malc EP, Mieczkowski PA, Gordenin DA. Mutation signatures specific to DNA alkylating agents in yeast and cancers. *Nucleic Acids Res*. 2020 Apr 17;48(7):3692-3707. doi: 10.1093/nar/gkaa150. PMID: 32133535; PMCID: PMC7144945.

How cells achieve high accuracy of DNA replication

The accuracy of DNA replication is a crucial factor for the mechanisms by which cells and organisms produce mutations. To gain understanding in this area we are studying the accuracy (fidelity) of DNA replication in the bacterium Escherichia coli, which is a useful model system for these questions. The bacterial chromosome is replicated by the DNA polymerase III holoenzyme (HE), whose accuracy we have studies in detail. In particular, we have discovered that the two DNA strands are not replicated with the same accuracy. Specifically, the lagging strand is replicated more accurately than the leading strand. We have also deciphered the entire genome sequence of an E. coli strain important for biotechnical applications.

Kozmin SG, Rogozin IB, Moore EA, Abney M, Schaaper RM, Pavlov YI. Comment on "A commensal strain of Staphylococcus epidermidis protects against skin neoplasia" by Nakatsuji et al. *Sci Adv*. 2019 Sep 11;5(9):eaaw3915. doi: 10.1126/sciadv.aaw3915. PMID: 31535021; PMCID: PMC6739109.

Makiela-Dzbenska K, Maslowska KH, Kuban W, Gawel D, Jonczyk P, Schaaper RM, Fijalkowska IJ. Replication fidelity in E. coli: Differential leading and lagging strand effects for dnaE antimutator alleles. *DNA Repair* (Amst). 2019 Nov;83:102643. doi: 10.1016/j.dnarep.2019.102643. Epub 2019 Jul 4. PMID: 31324532; PMCID: PMC6801068.

Bhawsinghka N, Glenn KF, Schaaper RM. Complete Genome Sequence of Escherichia coli BL21-AI. *Microbiol Resour Announc*. 2020 Mar 5;9(10):e00009-20. doi: 10.1128/MRA.00009-20. PMID: 32139577; PMCID: PMC7171200.

Deep learning methods differentiated pollen species using chemical signatures.

The daily pollen count provides allergic patients with valuable data to avoid aggravating their symptoms. However, current methods to measure the pollen count are labor intensive and expensive. This study demonstrated that the chemical signature, or metabolome, of various pollens could be used to differentiate the taxa, using NMR chemical analysis and automated deep learning algorithms. The use of the metabolome and NMR to identify the component pollens, instead of visual identification, is novel. This may be developed into an automated method to measure the daily pollen count.

Klimczak LJ, von Eschenbach CE, Thompson PM, Buters JTM, Mueller GA. Mixture Analyses of Air-sampled Pollen Extracts Can Accurately Differentiate Pollen Taxa. *Atmos Environ*. 2020, *In press*

NIEHS scientists determine the gene expression profile of the human breast during puberty

These RNA-seq studies represent the first interrogation of the human breast at the molecular level during late puberty/young adulthood. They demonstrate that in girls, obesity is associated with increased expression of pro-inflammatory genes which may increase estrogen action in the immature breast environment. These findings add to a growing body of literature from animal models suggesting that adolescence and puberty are in fact critical "windows of susceptibility" to breast cancer in adulthood.

Burkholder A, Akrobetu D, Pandiri AR, Ton K, Kim S, Labow BI, Nuzzi LC, Firriolo JM, Schneider SS, Fenton SE, Shaw ND. Investigation of the adolescent female breast transcriptome and the impact of obesity. *Breast Cancer Res.* 2020 May 11;22(1):44. doi: 10.1186/s13058-020-01279-6. PMID: 32393308; PMCID: PMC7216667.

Two HEPN Nuclease Motifs are Required to cleave RNA

HEPN (higher eukaryotes and prokaryotes nucleotide binding) domains are found in endoribonucleases involved in many different biological processes such as bacterial defense systems, the unfolded protein response, and ribosome assembly. Las1 is an essential, HEPN nuclease required for regulating early stages of ribosome assembly. In order to cleave pre-ribosomal RNA Las1 assembles into a higher-ordered complex containing two juxtaposed Las1 HEPN nuclease domains. Through a combination of in vivo and in vitro studies the Stanley Lab revealed that both the active site motifs from both Las1 HEPN nuclease domains are required for Las1 nuclease activity and fidelity.

Pillon MC, Goslen KH, Gordon J, Wells ML, Williams JG, Stanley RE. It takes two (Las1 HEPN endoribonuclease domains) to cut RNA correctly. *J Biol Chem*. 2020 May 1;295(18):5857-5870. doi: 10.1074/jbc.RA119.011193. Epub 2020 Mar 27. PMID: 32220933; PMCID: PMC7196650.

Regulation of tRNA Splicing by an RNA Kinase

Transfer RNA (tRNA) plays a critical role in decoding the genetic code during protein translation. Before tRNAs can participate in translation they undergo a series of processing and modifications steps, such as the excision or splicing of tRNA introns. In mammals tRNA splicing is facilitated by the tRNA splicing endonuclease (TSEN) complex, which co-purifies with the RNA kinase Clp1, however the role of Clp1 in tRNA splicing is unclear. Mutations in the TSEN complex and Clp1 cause neurodegenerative disorders, underscoring the need to understand the mechanisms of intron removal. Through in vitro reconstitution and vivo work in flies, the Stanley Lab was able to determine that the Clp1 RNA kinase is a critical regulator of tRNA splicing by negatively modulating the ligation of tRNA exons.

Hayne CK, Schmidt CA, Haque MI, Matera AG, Stanley RE. Reconstitution of the human tRNA splicing endonuclease complex: insight into the regulation of pre-tRNA cleavage.

Nucleic Acids Res. 2020 Aug 20;48(14):7609-7622. doi: 10.1093/nar/gkaa438. PMID: 32476018.

Cooperation between immune receptors leads to enhanced inflammation after exposure to pollution

Immune cells in the lung help guard against infections. On the surface of these cells are proteins called TLR receptors that recognize dangerous molecules from disease-causing microbes such as bacteria. When the immune cells detect these invaders, the TLR receptors spring into action and trigger an inflammatory response to destroy the microbes. This inflammation usually helps the lung clear infections. But it can also be harmful and damage the lung, for example when inflammation is caused by non-infectious substances such as pollutants in the atmosphere. There are several TLR receptors that each recognize a specific molecule. In 2010, researchers showed that the receptor TLR4 is responsible for causing inflammation in the lung after exposure to pollution. Another receptor called TLR5 also helps activate the immune response in the lung. But it was unclear whether this receptor also plays a role in pollution-linked lung damage. We investigated the role of TLR5 in immune cells from the lungs of humans and mice. The experiments showed that TLR5 works together with TLR4 and helps trigger an inflammatory response to both pollutants and bacteria. We found that people lacking a working TLR5 receptor (approx. 3–10% of the population) are less likely to experience lung inflammation when exposed to pollution or bacterial proteins that activate TLR4. These findings suggest that people without TLR5 may be protected from pollution-induced lung injury. Further research into the role of TLR5 will help develop genetic tests for identifying people who are more sensitive to damage from pollution. This information could then be used to determine the likelihood of a patient experiencing certain lung diseases.

Hussain S, Johnson CG, Sciurba J, Meng X, Stober VP, Liu C, Cyphert-Daly JM, Bulek K, Qian W, Solis A, Sakamachi Y, Trempus CS, Aloor JJ, Gowdy KM, Foster WM, Hollingsworth JW, Tighe RM, Li X, Fessler MB, Garantziotis S. TLR5 participates in the TLR4 receptor complex and promotes MyD88-dependent signaling in environmental lung injury. *Elife*. 2020 Jan 28;9:e50458. doi: 10.7554/eLife.50458. PMID: 31989925; PMCID: PMC7032926.

Revealing the enzymology of a specialized form of DNA replication

We have found that only one of three major DNA replicases for the eukaryotic nuclear genome, DNA polymerase delta, is required for a highly specialized form of DNA replication at the ends of chromosomes celled break-induced replication.

Donnianni RA, Zhou ZX, Lujan SA, Al-Zain A, Garcia V, Glancy E, Burkholder AB, Kunkel TA, Symington LS. DNA Polymerase Delta Synthesizes Both Strands during Break-Induced Replication. *Mol Cell*. 2019 Nov 7;76(3):371-381.e4. doi: 10.1016/j.molcel.2019.07.033. Epub 2019 Sep 5. PMID: 31495565; PMCID: PMC6862718.

Describing the enzymology of eukaryotic nuclear genome replication.

We have discovered that DNA polymerase delta participates in both initiating and termination DNA replication in budding yeast.

Zhou ZX, Lujan SA, Burkholder AB, Garbacz MA, Kunkel TA. Roles for DNA polymerase δ in initiating and terminating leading strand DNA replication. *Nat Commun*. 2019 Sep 5;10(1):3992. doi: 10.1038/s41467-019-11995-z. PMID: 31488849; PMCID: PMC6728351.

Cytochrome P450 Expression and Oxylipin Levels during LPS-Induced Inflammation
Inflammatory stimuli, such as bacterial LPS, alter the expression of many cytochromes P450. CYP2C and CYP2J subfamily members actively metabolize fatty acids to bioactive eicosanoids, which exhibit potent anti-inflammatory effects. We examined mRNA levels of the 15 mouse Cyp2c and 7 mouse Cyp2j isoforms in liver, kidney, duodenum, and brain over a 96-h time course of LPS-induced inflammation and resolution and measured plasma and liver eicosanoid levels by liquid chromatography with tandem mass spectrometry. Expression changes in Cyp2c and Cyp2j isoforms were both isoform and tissue specific. Plasma eicosanoids transiently increased 3-6 h after administration of LPS whereas in liver, esterified oxylipin levels decreased during acute inflammation and before recovering. The biphasic suppression and recovery of mouse Cyp2c and Cyp2j isoforms and associated changes in eicosanoid levels during LPS-induced inflammation and resolution may have important physiologic consequences.

Graves JP, Bradbury JA, Gruzdev A, Li H, Duval C, Lih FB, Edin ML, Zeldin DC. Expression of Cyp2c/Cyp2j subfamily members and oxylipin levels during LPS-induced inflammation and resolution in mice. *FASEB J*. 2019 Dec;33(12):14784-14797. doi: 10.1096/fj.201901872R. Epub 2019 Nov 5. PMID: 31690125; PMCID: PMC6894073.

Adherence to National Asthma Guidelines by Allergists and Pulmonologists
Little is known about specialist-specific variations in guideline agreement and adoption. To assess similarities and differences between allergists and pulmonologists in adherence to cornerstone components of the National Asthma Education and Prevention Program's Third Expert Panel Report, we examined self-reported guideline agreement, self-efficacy, and adherence in the 2012 National Asthma Survey of Physicians. Multivariate models were used to assess if physician and practice characteristics explained bivariate associations between specialty and adhering to recommendations. Allergists and pulmonologists reported high guideline self-efficacy and moderate guideline agreement. Both groups almost always assessed asthma control, assessed school/work asthma triggers, and endorsed inhaled corticosteroids use. Repeated assessment of the inhaler technique, use of asthma action/treatment plans, and spirometry were lower. Compared with pulmonologists, more allergists almost always performed spirometry, asked about nighttime awakening and emergency department visits, assessed home triggers, and performed allergy testing. Overall, allergists and pulmonologists adhere to the asthma guidelines with notable exceptions, including asthma action plan use and inhaler technique assessment. Recommendations with low implementation offer opportunities for further exploration and could serve as targets for increasing guideline uptake.

Cloutier MM, Akinbami LJ, Salo PM, Schatz M, Simoneau T, Wilkerson JC, Diette G, Elward KS, Fuhlbrigge A, Mazurek JM, Feinstein L, Williams S, Zeldin DC. Use of National Asthma Guidelines by Allergists and Pulmonologists: A National Survey. *J Allergy Clin Immunol Pract*. 2020 Apr 25:S2213-2198(20)30377-9. doi: 10.1016/j.jaip.2020.04.026. Epub ahead of print. PMID: 32344187.

Association of Urinary Bisphenol Levels with Asthma and Hay Fever
Bisphenols F (BPF) and S (BPS) are bisphenol A (BPA) analogs used as substitutes in consumer products. Despite previous reports of BPA's association with asthma, no studies have examined its structural analogs in relation to asthma and allergy outcomes. We examined the association of urinary BPF, BPS, and BPA with asthma and hay fever in a U.S. representative sample of 3,538 participants aged 12 years or older in the 2013-2016 National Health and Nutrition Examination Survey (NHANES). BPF, BPS, and BPA were detected in 57.1%, 88.4%, and 94.8% of the urine samples, respectively. Urinary BPF detection was positively associated with current asthma and hay fever. Urinary BPS was associated with increased odds of current asthma in men and urinary BPA was associated with increased odds of asthma without hay fever in children aged 6-11 years. Our nationally-representative findings document that BPF and BPS exposure is common in the U.S. and that exposure to these BPA analogs is associated with asthma and/or hay fever. Our results suggest that BPF and BPS may not be safe alternatives to BPA; however, prospective studies should be conducted to confirm these results.

Mendy A, Salo PM, Wilkerson J, Feinstein L, Ferguson KK, Fessler MB, Thorne PS, Zeldin DC. Association of urinary levels of bisphenols F and S used as bisphenol A substitutes with asthma and hay fever outcomes. *Environ Res*. 2020 Apr;183:108944. doi: 10.1016/j.envres.2019.108944. Epub 2019 Nov 22. PMID: 31911000; PMCID: PMC7167336.

Questionnaire-based Definitions for COPD
Various questionnaire-based definitions of chronic obstructive pulmonary disease (COPD) have been applied using the U.S.-representative National Health and Nutrition Examination Survey (NHANES), but few have been validated against objective lung function data. We validated two prior definitions that incorporated self-reported physician diagnosis, respiratory symptoms, and/or smoking. We also validated a new definition that we developed empirically using gradient boosting, an ensemble machine learning method. The spirometry-based COPD prevalence was 26% for smokers and 8% for never smokers. Among smokers, using questionnaire-based definitions resulted in a COPD prevalence ranging from 11-16%, sensitivity ranging from 18-35%, and specificity ranging from 88-92%. The new definition classified participants based on age, bronchodilator use, BMI, smoking pack-years, and occupational organic dust exposure, and resulted in the highest sensitivity (35%) and specificity (92%) among smokers. Among never smokers, the COPD prevalence ranged from 4-5%, and good specificity was attained (96%) at the expense of sensitivity (9-10%). Our results can be used to parametrize misclassification assumptions for quantitative bias analysis when pulmonary function data are unavailable.

Feinstein L, Wilkerson J, Salo PM, MacNell N, Bridge MF, Fessler MB, Thorne PS, Mendy A, Cohn RD, Curry MD, Zeldin DC. Validation of Questionnaire-based Case Definitions for Chronic Obstructive Pulmonary Disease. *Epidemiology*. 2020 May;31(3):459-466. doi: 10.1097/EDE.0000000000001176. PMID: 32028323; PMCID: PMC7138734.

Breast cancer related DNA methylation changes in blood happened long before clinical diagnosis
In a large DNA methylation association study of 2776 samples from Sister Study cohort, we identified 9601 CpG markers associated with invasive breast cancer, and 2095 of these CpGs

were replicated in an independent dataset. The magnitude of DNA methylation change is associated with time to diagnosis. We found these changes are more likely a consequence rather than a cause of breast cancer.

Xu Z, Sandler DP, Taylor JA. Blood DNA Methylation and Breast Cancer: A Prospective Case-Cohort Analysis in the Sister Study. *J Natl Cancer Inst.* 2020 Jan 1;112(1):87-94. doi: 10.1093/jnci/djz065. PMID: 30989176.

Autoimmunity may be increasing in the U.S.

The prevalence of ANA in the US increased from 11.0% in 1988-1991, to 11.5% in 1999-2004, to 15.9% in 2011-2012 (P for trend < 0.0001), which corresponds to ~22 million, ~27 million, and ~41 million affected individuals, respectively. Increases were greatest among adolescents aged 12-19 years, men, adults ≥50 years, and non-Hispanic whites. Additional studies to determine factors underlying these increases in ANA prevalence could elucidate causes of autoimmunity and enable the development of preventative measures.

Dinse GE, Parks CG, Weinberg CR, Co CA, Wilkerson J, Zeldin DC, Chan EKL, Miller FW. Increasing Prevalence of Antinuclear Antibodies in the United States. *Arthritis Rheumatol*. 2020 Jun;72(6):1026-1035. doi: 10.1002/art.41214. Epub 2020 Apr 30. PMID: 32266792; PMCID: PMC7255943.

Permanent Hair Dye and Chemical Straightener Use Associated with Higher Risk of Breast Cancer

Hair products such as hair dyes and chemical straighteners contain many different chemicals that may act as carcinogens or endocrine disruptors and thus may be important for breast cancer risk. Women who used permanent hair dye and chemical straighteners were at a higher risk of breast cancer compared to women who did not use those products. The association with permanent hair dye differed by race; black women had a higher risk associated with the use of permanent hair dye compared to the risk for white women. These findings suggest that women should consider their use of hair products in light of the fact that the chemicals in hair dye and chemical straighteners may influence their risk of developing breast cancer.

Eberle CE, Sandler DP, Taylor KW, White AJ. Hair dye and chemical straightener use and breast cancer risk in a large US population of black and white women. *Int J Cancer*. 2020 Jul 15;147(2):383-391. doi: 10.1002/ijc.32738. Epub 2019 Dec 3. PMID: 31797377; PMCID: PMC7246134.

Altered expression of FOXL2 impairs reproduction in the mouse

FOXL2 is a transcription factor those expression is altered in diseases of the female reproductive tract such as endometriosis. A mouse model was generated which deregulated the expression of FOXL2 in the pituitary, ovary and uterus. The mice were sterile and FOXL2 impaired uterine morphology with increased fibrosis and altered uterine gland development demonstrating the potential role of this transcription factor in diseases of the female reproductive tract.

Li R, Wu SP, Zhou L, Nicol B, Lydon JP, Yao HHC, DeMayo FJ. Increased FOXL2 Expression Alters Uterine Structures and Functions. *Biol Reprod*. 2020, *In press*

The progesterone receptor can drive ovarian tumorigenesis

Mice were generated which overexpressed either the SA or B isoforms of the progesterone receptor, PGRA and PGRB respectively). Mice expression PGRB developed ovarian tumors which had a molecular signature found in human ovarian cancers. This work shows the pathways in cancer that are regulated by altered PGR expression.

Wetendorf M, Li R, Wu SP, Liu J, Creighton CJ, Wang T, Janardhan KS, Willson CJ, Lanz RB, Murphy BD, Lydon JP, DeMayo FJ. Constitutive Expression of the Progesterone Receptor Isoforms Promotes Hormone-Dependent Development of Ovarian Neoplasms. *Sci Signal*. 2020, *In press*

Identification of the pathways regulated by the progesterone in the human myometrium during pregnancy

This manuscript identified the role of the progesterone in regulating the biology of the smooth muscle of the uterus. PGR binding sites were identified in term pregnant human myometrium. The comparison of genes altered between non pregnant human myometrium and term myometrium identified the process directly regulated by PGR during pregnancy in women.

Wu SP, Anderson ML, Wang T, Zhou L, Emery OM, Li X, DeMayo FJ. Dynamic transcriptome, accessible genome, and PGR cistrome profiles in the human myometrium. *FASEB J*. 2020 Feb;34(2):2252-2268. doi: 10.1096/fj.201902654R. Epub 2019 Dec 12. PMID: 31908010.