Division of Intramural Research

NAEHS Council Update

February, 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.

NEW HIRES IN DIR

Earl Stadtman Tenure-Track Investigator in Epidemiology
Dr. Chandra L. Jackson, a Research Associate at the Harvard Catalyst Clinical and Translational Science Center and Harvard T.H. Chan School of Public Health, has accepted a position as an Earl Stadtman tenure-track investigator in the Epidemiology Branch at NIEHS. She started January 9, 2017.

Tenure-Track Investigator in Epidemiology
Dr. Anne Marie Jukic, Assistant Professor of Epidemiology at School of Public Health, Yale University, has accepted a position as tenure-track investigator at NIEHS. She will have her primary appointment in the Epidemiology Branch and a secondary appointment in the Reproductive and Developmental Biology Laboratory. She is scheduled to start in the Spring/Summer of 2017.

Staff Scientist in Biostatistics
Dr. Yufeng Li, Full Professor in the Division of Preventive Medicine, Department of Medicine, and Director of Biostatistics and Bioinformatics Core for the Breast Specialized Program of Research Excellence (SPORE), Comprehensive Cancer Center, University of Alabama at Birmingham, AL, has accepted a position as a Staff Scientist in the Biostatistics and Computational Biology Branch to replace Dr. Grace Kissling upon her retirement. Dr. Li is scheduled to start in the Winter of 2017.

SCIENTIFIC UPDATE BY A DIR PRINCIPAL INVESTIGATOR

Molecular Mechanisms of Protein-DNA Crosslink Reversal

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

DNA strand breaks occur continuously as our cells duplicate their chromosomes, and as a consequence of oxidation or environmental exposure to chemical mutagens and DNA-damaging radiation. Inflammation, cellular respiration, routine DNA metabolism, and xenobiotics including pharmaceutical drugs all lead to the production of cytotoxic DNA strand breaks. Akin to splintered wood, DNA breaks are not "clean." Rather, DNA breaks typically lack DNA 5′-phosphate and/or 3′-hydroxyl moieties required for DNA synthesis and ligation. Failure to resolve damage at DNA ends can lead to abnormal DNA replication and repair, and is associated with genomic instability, mutagenesis, neurological disease, aging and carcinogenesis. A key class of adducted strand breaks are protein-DNA crosslinks linked to metabolic topoisomerase reactions. Eukaryotic Topoisomerase 2 (TOP2) enzymes are vital for life and facilitate key DNA transactions including DNA replication, transcription, and recombination, but also pose unique threats to genomic integrity. Pre-existing DNA damage, environmental toxicants and chemotherapeutic drugs poison these enzymes, generating highly genotoxic 5′-TOP2 adducted DNA double strand breaks. This cellular Achilles heel underpins the potency of front-line TOP2-targeting chemotherapeutic agents, and unrepaired TOP2 lesions are linked neurological pathologies. Understanding how TOP2 protein-DNA adducts are resolved is critical not only for unveiling the molecular basis of TOP2-mediated genome organization and dynamics and its impact on neural development and function, but also for driving discovery of new chemotherapeutic approaches and understanding emerging mechanisms of chemotherapy resistance. We have been studying the cellular TOP2 DNA damage clearance apparatus. Our work establishes a novel paradigm for the direct resolution of TOP2-DNA protein crosslinks by the Tyrosyl-DNA phosphodiesterase complex, with implications for cancer therapy chemoresistance. Recent progress in this area will be discussed.

BSC REVIEW OF THE BIOSTATISTICS AND COMPUTATIONAL BIOLOGY BRANCH

The NIEHS DIR Board of Scientific Counselors reviewed the Biostatistics and Computational Biology Branch, November 13-15, 2015.

Members of the Board of Scientific Counselors that Attended:

- Christopher I. Amos, Ph.D., Professor, Dept. of Community and Family Medicine, Geisel School of Medicine at Dartmouth, Hanover, NH
- Juan C. Celedón, M.D., Dr.P.H., [BSC Acting Chair] Niels K. Jerne Professor of Pediatrics, Dept. of Pediatrics, Children's Hospital of Pittsburgh of UPMC, University of Pittsburgh, Pittsburgh, PA
- Monica J. Justice, Ph.D., Head and Senior Scientist, Genetics & Genome Biology Program, SickKids Research Institute, The Peter Gilgan Centre for Research and Learning, Toronto, ON, Canada
- Carol A. Lange, Ph.D., Professor, Departments of Medicine and Pharmacology, University of Minnesota, Minneapolis MN
- Donald P. McDonnell, Ph.D., Glaxo-Wellcome Professor and Chairman of Pharmacology and Cancer Biology, Duke University School of Medicine, Durham, NC
- Ivan Rusyn, M.D., Ph.D., Professor, Department of Veterinary Integrative Biosciences, Texas A&M University College of Veterinary Medicine & Biomedical Sciences, College Station, TX
- Daniel O. Stram, Ph.D., Professor, Division of Biostatistics and Genetic Epidemiology, Department of Preventive Medicine, University of Southern California Keck School of Medicine, Los Angeles, CA
- Karen M. Vasquez, Ph.D., Professor, Division of Pharmacology and Toxicology, Dell Pediatric Research Institute, The University of Texas at Austin, Austin, TX
- Roland A. Owens, Ph.D., Ex-Officio BSC Member, Assistant Director, Office of Intramural Research, NIH, Bethesda, MD

Ad Hoc Reviewers that Attended:

- Paul Albert, Ph.D., Chief, Biostatistics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD
- A. John Bailer, Ph.D., University Distinguished Professor and Chair, Department of Statistics, Miami University, Oxford, OH
- Inanc Birol, Ph.D., Scientist, Michael Smith Genome Sciences Center, British Columbia Cancer Agency, Vancouver, BC, Canada
- Michael P. Jones, Ph.D., Professor, Department of Statistics and Actuarial Science, University of Iowa, Iowa City, IA
- Eden R. Martin, Ph.D., Director, Center for Genetic Epidemiology and Statistical Genetics, John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL
- Bhramar Mukherjee, Ph.D., John D. Kalbfleisch Collegiate Professor of Biostatistics, Associate Chair of Biostatistics, Professor of Epidemiology, Department of Biostatistics, School of Public Health, University of Michigan, Ann Arbor, MI

- Dan Nettleton, Ph.D., Laurence H. Baker Endowed Chair, Distinguished Professor, Department of Statistics, Iowa State University, Ames, Iowa
- Ivan Ovcharenko, Ph.D., Senior Investigator, National Library of Medicine, National Center for Biotechnology Information, Bethesda, MD
- Janet Sinsheimer, Ph.D., Professor of Biostatistics, Biomethematics, and Human Genetics, Interim Chair, Department of Biomathematics, UCLA David Geffen School of Medicine, Los Angeles, CA
- Ying Zhang, Ph.D., Professor and Director of Education, Department of Biostatistics, Indiana University Fairbanks School of Public Health, Indianapolis, IN

Agenda:

Sunday, November 13 – Doubletree by Hilton
Closed Evening Session

7:00 – 8:00 p.m.	Welcome and Discussion of Past Board Reviews, Drs. Darryl Zeldin and Shyamal Peddada
8:00 – end	BSC Discussion Review, Dr. Juan Celedon and panel

Monday, November 14 - NIEHS Rodbell Conference Rooms 101 ABC
Morning Session

8:30 – 8:45 a.m.	Welcome, Dr. Linda Birnbaum
8:45 – 9:05	Overview, Biostatistics and Computational Biology Branch
9:05 – 9:55	Statistical Methods with Applications to Environmental Health Sciences, Shyamal Peddada
9:55 - 10:10	COFFEE BREAK
10:10 – 11:00	Bioinformatics Group, Leping Li, Ph.D.
11:00 – 11:50	Statistical Genetic Approaches for Mapping Human Diseases, Dmitri Zaykin, Ph.D.
11:50 – 12:35	Closed 1:1 Sessions with Investigators, Drs. Peddada, Li and Zaykin
12:35 – 1:30	Closed Working Lunch, 101ABC

Afternoon Session

1:30 – 3:00 pm	Poster Session—Fellows and Staff Scientists, Rodbell Lobby
3:00 – 3:30	Closed Sessions with Fellows and Staff Scientists, 101ABC
3:30 – 3:45	COFFEE BREAK
3:45 – 4:35	Statistical Methods in Disease Risk Assessment and Prediction, Shanshan Zhao, Ph.D.
4:35 – 5:25	Methods and Applications in Epidemiology, Clarice Weinberg, Ph.D.
5:25 – 5:55	Closed 1:1 Sessions with Investigators, Drs. Zhao and Weinberg
5:55 – 6:35	Closed 1:1 Sessions with Programmatic Staff Scientists, Drs. Bushel, Kissling, Shockley and Umbach
6:45	Return to Doubletree Hotel

Closed Evening Session

7:00 – end	BSC Discussion and completion of individual review assignments by each member, All BSC reviewers at hotel

Tuesday November 15- NIEHS Rodbell Conference Rooms 101 ABC
Closed Morning Session

8:30 – 10:00 am	Debriefing to NIEHS/DIR Leadership, 101ABC
10:00 – 10:15	COFFEE BREAK
10:15 – 12:00	Standing BSC Membership consideration of Scientific Directors Award for Outstanding Intramural Research
12:00	Adjourn

NIEHS SCIENCE DAYS

As part of the NIEHDS 50th Anniversary Celebration, the Fourteenth Annual NIEHS Science Days were held on November 3-4, 2014, at the Rall Building on the NIEHS Campus to celebrate the achievements of NIEHS scientists. The event was open to the public and more than 250 attendees from universities and research institutions in the Triangle Area attended. NIEHS Science Days consisted of a mini-symposium on Nuclear Receptors: Mediators of Environmental Impacts on Physiology and Disease in which presentations were given by scientists in DIR and DNTP and a DERT grantee, a presentation by a former NIEHS trainee, 9 oral presentations given by fellows, students, and technicians, 98 poster presentations and an Awards Ceremony. Judging for the awards was done by Extramural Scientists from universities and research organizations in the Triangle Area, Intramural Scientists and the NIEHS Trainees Assembly.

Mentor of the Year: Humphrey H-C. Yao, Ph.D., Reproductive and Developmental Biology Laboratory
Fellow of the Year: Katie O'Brien, Ph.D., Biostatistics and Computational Biology Branch
Best Poster Presentation:

1. Matthew A. Quinn, Ph.D., Signal Transduction Laboratory, "Loss of Ovarian Function Results in Metabolic Syndrome and Steatosis via a Glucocorticoid Receptor dependent mechanism."
2. Matthew J. Schellenberg, Ph.D., Genome Integrity and Structural Biology Laboratory, "ZATT SUMO Ligase Licenses Direct Reversal of Topoisomerase 2 DNA-protein crosslinks by Tdp2."
3. Ashutosh Kumar, Ph.D., Immunity, Inflammation and Disease Laboratory, "Cytochrome c as a peroxidase plays a role in α-synuclein radical formation: Implications of α-synuclein in alterations of biological pathways and neuronal death in Maneb- and paraquat-induced model of Parkinson's disease."
4. Shannon L. Farris, Ph.D., Neurobiology Laboratory, "Transcriptome profiling in hippocampal dendrites reveals a role for mitochondria in CA2 physiology and function."
5. Fei Zhao, Ph.D., Reproductive and Developmental Biology Laboratory, "Wolffian duct regression in the female embryo is the result of COUP-TFII action, not a lack of androgen action."
6. Douglas Ganini da Silva, Ph.D., Immunity, Inflammation and Disease Laboratory, "Fluorescent proteins such as eGFP catalytically generate superoxide anion free radical and H2O2 in the presence of NAD(P)H."
7. Christopher G. Duncan, Ph.D., Epigenetics and Stem Cell Biology Laboratory, "DNA methylation landscape of the X chromosome in mouse liver."
8. Pishun Li, Ph.D., Epigenetics and Stem Cell Biology Laboratory, "Rif1-dependent Repressive Chromatin Modifications are Required for Endogenous Retrotransposons Silencing in Embryonic stem cell."
9. Kathryn McClelland, Ph.D., Reproductive and Developmental Biology Laboratory, "Loss of COUP-TFII (NR2F2) in Different Interstitial Cell Populations Has Varying Effects on Fetal Testicular Development."

Best Oral Presentation: Mahita Kadmiel, Ph.D., Signal Transduction Laboratory, "Glucocorticoid Actions at the Window of the Eye."

DIR PAPERS OF THE YEAR FOR 2016

Whirledge SD, Oakley RH, Myers PH, Lydon JP, DeMayo F, Cidlowski JA. Uterine glucocorticoid receptors are critical for fertility in mice through control of embryo implantation and decidualization. *Proc. Natl. Acad. Sci. U.S.A.,* **112:**15166-15171, 2015

> In addition to the well-characterized role of the sex steroid receptors in fertility and reproduction, organs of the female reproductive tract are also regulated by the hypothalamic-pituitary-adrenal axis. These endocrine organs are sensitive to stress-mediated actions of glucocorticoids, and the mouse uterus contains high levels of the glucocorticoid receptor (GR). Although the presence of GR in the uterus is well established, uterine glucocorticoid signaling has been largely ignored in terms of its reproductive and/or immunomodulatory functions on fertility. To define the direct in vivo function of glucocorticoid signaling in adult uterine physiology, we generated a uterine-specific GR knockout (uterine GR KO) mouse using the PR^{cre} mouse model. The uterine GR KO mice display a profound subfertile phenotype, including a significant delay to first litter and decreased pups per litter. Early defects in pregnancy are evident as reduced blastocyst implantation and subsequent defects in stromal cell decidualization, including decreased proliferation, aberrant apoptosis, and altered gene expression. The deficiency in uterine GR signaling resulted in an exaggerated inflammatory response to induced decidualization, including altered immune cell recruitment. These results demonstrate that GR is required to establish the necessary cellular context for maintaining normal uterine biology and fertility through the regulation of uterine-specific actions.

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

> The hippocampus supports a cognitive map of space and is critical for encoding declarative memory (who, what, when and where). Recent studies have implicated hippocampal subfield CA2 in social and contextual memory but how it does so remains unknown. Here we find that in adult male rats, presentation of a social stimulus (novel or familiar rat) or a novel object induces global remapping of place fields in CA2 with no effect on neuronal firing rate or immediate early gene expression. This remapping did not occur in CA1, suggesting this effect is specific for CA2. Thus, modification of existing spatial representations might be a potential mechanism by which CA2 encodes social and novel contextual information.

Joubert BR, den Dekker HT, Felix JF, Bohlin J, Ligthart S, Beckett E, Tiemeier H, van Meurs JB, Uitterlinden AG, Hofman A, Håberg SE, Reese SE, Peters MJ, Andreassen BK, Steegers EA, Nilsen RM, Vollset SE, Midttun Ø, Ueland PM, Franco OH, Dehghan A, de Jongste JC, Wu MC, Wang T, Peddada SD, Jaddoe VW, Nystad W, Duijts L, London SJ. Maternal plasma folate impacts differential DNA methylation in an epigenome-wide meta-analysis of newborns. *Nat. Commun.,* **7:**10577, 2016.

> Folate is vital for fetal development. Periconceptional folic acid supplementation and food fortification are recommended to prevent neural tube defects. Mechanisms whereby

periconceptional folate influences normal development and disease are poorly understood: epigenetics may be involved. We examine the association between maternal plasma folate during pregnancy and epigenome-wide DNA methylation using Illumina's HumanMethyl450 Beadchip in 1,988 newborns from two European cohorts. Here we report the combined covariate-adjusted results using meta-analysis and employ pathway and gene expression analyses. Four-hundred forty-three CpGs (320 genes) are significantly associated with maternal plasma folate levels during pregnancy (false discovery rate 5%); 48 are significant after Bonferroni correction. Most genes are not known for folate biology, including APC2, GRM8, SLC16A12, OPCML, PRPH, LHX1, KLK4 and PRSS21. Some relate to birth defects other than neural tube defects, neurological functions or varied aspects of embryonic development. These findings may inform how maternal folate impacts the developing epigenome and health outcomes in offspring.

Patial S, Curtis AD 2nd, Lai WS, Stumpo DJ, Hill GD, Flake GP, Mannie MD, Blackshear PJ. Enhanced stability of tristetraprolin mRNA protects mice against immune-mediated inflammatory pathologies. *Proc. Natl. Acad. Sci. U.S.A.*, **113:**1865-1870, 2016.

Tristetraprolin (TTP) is an inducible, tandem zinc-finger mRNA binding protein that binds to adenylate-uridylate-rich elements (AREs) in the 3'-untranslated regions (3'UTRs) of specific mRNAs, such as that encoding TNF, and increases their rates of deadenylation and turnover. Stabilization of Tnf mRNA and other cytokine transcripts in TTP-deficient mice results in the development of a profound, chronic inflammatory syndrome characterized by polyarticular arthritis, dermatitis, myeloid hyperplasia, and autoimmunity. To address the hypothesis that increasing endogenous levels of TTP in an intact animal might be beneficial in the treatment of inflammatory diseases, we generated a mouse model (TTPΔARE) in which a 136-base instability motif in the 3'UTR of TTP mRNA was deleted in the endogenous genetic locus. These mice appeared normal, but cultured fibroblasts and macrophages derived from them exhibited increased stability of the otherwise highly labile TTP mRNA. This resulted in increased TTP protein expression in LPS-stimulated macrophages and increased levels of TTP protein in mouse tissues. TTPΔARE mice were protected from collagen antibody-induced arthritis, exhibited significantly reduced inflammation in imiquimod-induced dermatitis, and were resistant to induction of experimental autoimmune encephalomyelitis, presumably by dampening the excessive production of proinflammatory mediators in all cases. These data suggest that increased systemic levels of TTP, secondary to increased stability of its mRNA throughout the body, can be protective against inflammatory disease in certain models and might be viewed as an attractive therapeutic target for the treatment of human inflammatory diseases.

Chen LY, Willis WD, Eddy EM. Targeting the Gdnf Gene in peritubular myoid cells disrupts undifferentiated spermatogonial cell development. *Proc. Natl. Acad. Sci. U.S.A.*, **113:**1829-1834, 2016.

Spermatogonial stem cells (SSCs) are a subpopulation of undifferentiated spermatogonia located in a niche at the base of the seminiferous epithelium delimited by Sertoli cells and peritubular myoid (PM) cells. SSCs self-renew or differentiate into spermatogonia that proliferate to give rise to spermatocytes and maintain spermatogenesis. Glial cell line-

derived neurotrophic factor (GDNF) is essential for this process. Sertoli cells produce GDNF and other growth factors and are commonly thought to be responsible for regulating SSC development, but limited attention has been paid to the role of PM cells in this process. A conditional knockout (cKO) of the androgen receptor gene in PM cells resulted in male infertility. We found that testosterone (T) induces GDNF expression in mouse PM cells in vitro and neonatal spermatogonia (including SSCs) co-cultured with T-treated PM cells were able to colonize testes of germ cell-depleted mice after transplantation. This strongly suggested that T-regulated production of GDNF by PM cells is required for spermatogonial development, but PM cells might produce other factors in vitro that are responsible. In this study, we tested the hypothesis that production of GDNF by PM cells is essential for spermatogonial development by generating mice with a cKO of the Gdnf gene in PM cells. The cKO males sired up to two litters but became infertile due to collapse of spermatogenesis and loss of undifferentiated spermatogonia. These studies show for the first time, to our knowledge, that the production of GDNF by PM cells is essential for undifferentiated spermatogonial cell development in vivo.

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

BACKGROUND: Transcription factor-dependent cellular reprogramming is integral to normal development and is central to production of induced pluripotent stem cells. This process typically requires pioneer transcription factors (TFs) to induce de novo formation of enhancers at previously closed chromatin. Mechanistic information on this process is currently sparse.
RESULTS: Here we explore the mechanistic basis by which GATA3 functions as a pioneer TF in a cellular reprogramming event relevant to breast cancer, the mesenchymal to epithelial transition (MET). In some instances, GATA3 binds previously inaccessible chromatin, characterized by stable, positioned nucleosomes where it induces nucleosome eviction, alters local histone modifications, and remodels local chromatin architecture. At other loci, GATA3 binding induces nucleosome sliding without concomitant generation of accessible chromatin. Deletion of the transactivation domain retains the chromatin binding ability of GATA3 but cripples chromatin reprogramming ability, resulting in failure to induce MET.
CONCLUSIONS: These data provide mechanistic insights into GATA3-mediated chromatin reprogramming during MET, and suggest unexpected complexity to TF pioneering. Successful reprogramming requires stable binding to a nucleosomal site; activation domain-dependent recruitment of co-factors including BRG1, the ATPase subunit of the SWI/SNF chromatin remodeling complex; and appropriate genomic context. The resulting model provides a new conceptual framework for de novo enhancer establishment by a pioneer TF.

Joubert BR, Felix JF, Yousefi P, Bakulski KM, Just AC, Breton C, Reese SE, Markunas CA, Richmond RC, Xu CJ, Küpers LK, Oh SS, Hoyo C, Gruzieva O, Söderhäll C, Salas LA, Baïz N, Zhang H, Lepeule J, Ruiz C, Ligthart S, Wang T, Taylor JA, Duijts L, Sharp GC, Jankipersadsing SA, Nilsen RM, Vaez A, Fallin MD, Hu D, Litonjua AA, Fuemmeler BF, Huen K, Kere J, Kull I, Munthe-Kaas MC, Gehring U, Bustamante M, Saurel-Coubizolles MJ,

Quraishi BM, Ren J, Tost J, Gonzalez JR, Peters MJ, Håberg SE, Xu Z, van Meurs JB, Gaunt TR, Kerkhof M, Corpeleijn E, Feinberg AP, Eng C, Baccarelli AA, Benjamin Neelon SE, Bradman A, Merid SK, Bergström A, Herceg Z, Hernandez-Vargas H, Brunekreef B, Pinart M, Heude B, Ewart S, Yao J, Lemonnier N, Franco OH, Wu MC, Hofman A, McArdle W, Van der Vlies P, Falahi F, Gillman MW, Barcellos LF, Kumar A, Wickman M, Guerra S, Charles MA, Holloway J, Auffray C, Tiemeier HW, Smith GD, Postma D, Hivert MF, Eskenazi B, Vrijheid M, Arshad H, Antó JM, Dehghan A, Karmaus W, Annesi-Maesano I, Sunyer J, Ghantous A, Pershagen G, Holland N, Murphy SK, DeMeo DL, Burchard EG, Ladd-Acosta C, Snieder H, Nystad W, Koppelman GH, Relton CL, Jaddoe VW, Wilcox A, Melén E, London SJ. DNA Methylation in Newborns and Maternal Smoking in Pregnancy: Genome-wide Consortium Meta-analysis. *Am. J. Hum. Genet.*, **98:**680-696, 2016.

Epigenetic modifications, including DNA methylation, represent a potential mechanism for environmental impacts on human disease. Maternal smoking in pregnancy remains an important public health problem that impacts child health in a myriad of ways and has potential lifelong consequences. The mechanisms are largely unknown, but epigenetics most likely plays a role. We formed the Pregnancy And Childhood Epigenetics (PACE) consortium and meta-analyzed, across 13 cohorts (n = 6,685), the association between maternal smoking in pregnancy and newborn blood DNA methylation at over 450,000 CpG sites (CpGs) by using the Illumina 450K BeadChip. Over 6,000 CpGs were differentially methylated in relation to maternal smoking at genome-wide statistical significance (false discovery rate, 5%), including 2,965 CpGs corresponding to 2,017 genes not previously related to smoking and methylation in either newborns or adults. Several genes are relevant to diseases that can be caused by maternal smoking (e.g., orofacial clefts and asthma) or adult smoking (e.g., certain cancers). A number of differentially methylated CpGs were associated with gene expression. We observed enrichment in pathways and processes critical to development. In older children (5 cohorts, n = 3,187), 100% of CpGs gave at least nominal levels of significance, far more than expected by chance (p value < 2.2 × 10(-16)). Results were robust to different normalization methods used across studies and cell type adjustment. In this large scale meta-analysis of methylation data, we identified numerous loci involved in response to maternal smoking in pregnancy with persistence into later childhood and provide insights into mechanisms underlying effects of this important exposure.

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

Defects in clearance of dying cells have been proposed to underlie the pathogenesis of systemic lupus erythematosus (SLE). Mice lacking molecules associated with dying cell clearance develop SLE-like disease, and phagocytes from patients with SLE often display defective clearance and increased inflammatory cytokine production when exposed to dying cells in vitro. Previously, we and others described a form of noncanonical autophagy known as LC3-associated phagocytosis (LAP), in which phagosomes containing engulfed particles, including dying cells, recruit elements of the autophagy pathway to facilitate maturation of phagosomes and digestion of their contents. Genome-wide association studies have

identified polymorphisms in the Atg5 (ref. 8) and possibly Atg7 (ref. 9) genes, involved in both canonical autophagy and LAP, as markers of a predisposition for SLE. Here we describe the consequences of defective LAP in vivo. Mice lacking any of several components of the LAP pathway show increased serum levels of inflammatory cytokines and autoantibodies, glomerular immune complex deposition, and evidence of kidney damage. When dying cells are injected into LAP-deficient mice, they are engulfed but not efficiently degraded and trigger acute elevation of pro-inflammatory cytokines but not anti-inflammatory interleukin (IL)-10. Repeated injection of dying cells into LAP-deficient, but not LAP-sufficient, mice accelerated the development of SLE-like disease, including increased serum levels of autoantibodies. By contrast, mice deficient in genes required for canonical autophagy but not LAP do not display defective dying cell clearance, inflammatory cytokine production, or SLE-like disease, and, like wild-type mice, produce IL-10 in response to dying cells. Therefore, defects in LAP, rather than canonical autophagy, can cause SLE-like phenomena, and may contribute to the pathogenesis of SLE.

Zhou B, Wang L, Zhang S, Bennett BD, He F, Zhang Y, Xiong C, Han L, Diao L, Li P, Fargo DC, Cox AD, Hu G. INO80 governs superenhancer-mediated oncogenic transcription and tumor growth in melanoma. *Genes Dev.,* **30:**1440-1453, 2016.

Superenhancers (SEs) are large genomic regions with a high density of enhancer marks. In cancer, SEs are found near oncogenes and dictate cancer gene expression. However, how oncogenic SEs are regulated remains poorly understood. Here, we show that INO80, a chromatin remodeling complex, is required for SE-mediated oncogenic transcription and tumor growth in melanoma. The expression of Ino80, the SWI/SNF ATPase, is elevated in melanoma cells and patient melanomas compared with normal melanocytes and benign nevi. Furthermore, Ino80 silencing selectively inhibits melanoma cell proliferation, anchorage-independent growth, tumorigenesis, and tumor maintenance in mouse xenografts. Mechanistically, Ino80 occupies >90% of SEs, and its occupancy is dependent on transcription factors such as MITF and Sox9. Ino80 binding reduces nucleosome occupancy and facilitates Mediator recruitment, thus promoting oncogenic transcription. Consistently, genes co-occupied by Ino80 and Med1 are selectively expressed in melanomas compared with melanocytes. Together, our results reveal an essential role of INO80-dependent chromatin remodeling in SE function and suggest a novel strategy for disrupting SEs in cancer treatment.

Hussain S, Ji Z, Taylor AJ, DeGraff LM, George M, Tucker CJ, Chang CH, Li R, Bonner JC, Garantziotis S. Multiwalled Carbon Nanotube Functionalization with High Molecular Weight Hyaluronan Significantly Reduces Pulmonary Injury. *ACS Nano.,* **10:**7675-7688, 2016.

Commercialization of multiwalled carbon nanotubes (MWCNT)-based applications has been hampered by concerns regarding their lung toxicity potential. Hyaluronic acid (HA) is a ubiquitously found polysaccharide, which is anti-inflammatory in its native high molecular weight form. HA-functionalized smart MWCNTs have shown promise as tumor-targeting drug delivery agents and can enhance bone repair and regeneration. However, it is unclear whether HA functionalization could reduce the pulmonary toxicity potential of MWCNTs. Using in vivo and in vitro approaches, we investigated the effectiveness of MWCNT

functionalization with HA in increasing nanotube biocompatibility and reducing lung inflammatory and fibrotic effects. We utilized three-dimensional cultures of differentiated primary human bronchial epithelia to translate findings from rodent assays to humans. We found that HA functionalization increased stability and dispersion of MWCNTs and reduced postexposure lung inflammation, fibrosis, and mucus cell metaplasia compared with nonfunctionalized MWCNTs. Cocultures of fully differentiated bronchial epithelial cells (cultivated at air-liquid interface) and human lung fibroblasts (submerged) displayed significant reduction in injury, oxidative stress, as well as pro-inflammatory gene and protein expression after exposure to HA-functionalized MWCNTs compared with MWCNTs alone. In contrast, neither type of nanotubes stimulated cytokine production in primary human alveolar macrophages. In aggregate, our results demonstrate the effectiveness of HA functionalization as a safer design approach to eliminate MWCNT-induced lung injury and suggest that HA functionalization works by reducing MWCNT-induced epithelial injury.

Zhang J, McCann KL, Qiu C, Gonzalez LE, Baserga SJ, Hall TM. Nop9 is a PUF-like protein that prevents premature cleavage to correctly process pre-18S rRNA. *Nat Commun.,* **7:**13085, 2016.

Numerous factors direct eukaryotic ribosome biogenesis, and defects in a single ribosome assembly factor may be lethal or produce tissue-specific human ribosomopathies. Pre-ribosomal RNAs (pre-rRNAs) must be processed stepwise and at the correct subcellular locations to produce the mature rRNAs. Nop9 is a conserved small ribosomal subunit biogenesis factor, essential in yeast. Here we report a 2.1-Å crystal structure of Nop9 and a small-angle X-ray-scattering model of a Nop9:RNA complex that reveals a 'C'-shaped fold formed from 11 Pumilio repeats. We show that Nop9 recognizes sequence and structural features of the 20S pre-rRNA near the cleavage site of the nuclease, Nob1. We further demonstrate that Nop9 inhibits Nob1 cleavage, the final processing step to produce mature small ribosomal subunit 18S rRNA. Together, our results suggest that Nop9 is critical for timely cleavage of the 20S pre-rRNA. Moreover, the Nop9 structure exemplifies a new class of Pumilio repeat proteins.

Parr CL, Magnus MC, Karlstad Ø, Haugen M, Refsum H, Ueland PM, McCann A, Nafstad P, Håberg SE, Nystad W, London SJ. Maternal Folate Intake During Pregnancy and Childhood Asthma in a Population Based Cohort. *Am. J. Respir. Crit. Care Med.,***195:**221-228, 2017.

RATIONALE: A potential adverse effect of high folate intake during pregnancy on children's asthma development remains controversial.
OBJECTIVES: To prospectively investigate folate intake from both food and supplements during pregnancy and asthma at age seven years when the diagnosis is more reliable than at preschool age.
METHODS: This study included eligible children born 2002-2006 from the Norwegian Mother and Child Cohort Study, a population-based pregnancy cohort, linked to the Norwegian Prescription Database. Current asthma at age seven was defined by asthma medications dispensed at least twice in the year (1,901 cases, n=39,846) or by maternal questionnaire report (1,624 cases, n=28,872). Maternal folate intake was assessed with a

food frequency questionnaire validated against plasma folate. We used log-binomial and multinomial regression to calculate adjusted relative risks with 95% confidence intervals.
MEASUREMENTS AND MAIN RESULTS:
Risk of asthma was increased in the highest vs. lowest quintile of total folate intake with an adjusted relative risk of 1.23 (95% confidence interval 1.06 to 1.44) that was similar for maternally reported asthma. Mothers in the highest quintile had a relatively high intake of food folate (median 308, interquartile range 241-366 μg/day) and nearly all took at least 400 μg/day of supplemental folic acid (median 500, interquartile range 400-600).
CONCLUSIONS:
In this large prospective population based cohort with essentially complete follow-up, pregnant women taking supplemental folic-acid at or above the recommended dose, combined with a diet rich in folate, reach a total folate intake level that may slightly increase risk of asthma in children.

AWARDS AND HONORS

Scientific Awards

Dr. Franco DeMayo (Reproductive & Developmental Biology Laboratory) received 2016 research award from the Campion Society.

Dr. Janet E. Hall (Clinical Director, Clinical Research Branch) received the Endocrine Society Laureate Award – Sidney H. Ingbar Award for Distinguished Service – to be awarded March 2017.

Dr. Thomas Kunkel (Genome Integrity & Structural Biology Laboratory) received the Champion of Environmental Health Research Award, NIEHS 50th Anniversary, 2016.

Dr. Masahiko Negishi (Reproductive & Developmental Biology Laboratory) received the 2016 Bernard B. Brodie Award in Drug Metabolism from the American Society for Pharmacology and Experimental Therapeutics.

Dr. Lisa Rider (Clinical Research Branch) received the 2016 PhRMA Research and Hope Award for Government Research in Autoimmune Disease.

Dr. Allen Wilcox (Epidemiology Branch) was a Finalist for the Samuel J. Heyman Service to America Medal, Career Achievement in Federal Service, 2016; received the National Institutes of Health Director's Award, 2016 ("For pioneering epidemiologic research in human reproduction that has defined the field and led to improved understanding of fertility and pregnancy"); and received the Champion of Environmental Health Research Award, NIEHS 50th Anniversary, 2016.

Dr. Samuel H. Wilson (Genome Integrity & Structural Biology Laboratory) received the Champion of Environmental Health Research Award, NIEHS 50th Anniversary, 2016.

Named Professorships/Lectures

Dr. Franco DeMayo (Reproductive & Developmental Biology Laboratory) presented the NIH Distinguished Lecture at the International Federation of Placental Associations in Portland, OR. September 13, 2016.

Dr. E. Mitch Eddy (Reproductive & Developmental Biology Laboratory) presented the Keynote address at the Gordon Research Conference on Mammalian Reproduction, August 21-26, 2016, Waterville Valley Resort, NH.

Dr. Steven Kleeberger (Immunity, Inflammation & Disease Laboratory) was the International Meeting on Respiratory Syncytial Virus in Bariloche, Argentina, September 28 to October 1, 2016.

Dr. Thomas Kunkel (Genome Integrity & Structural Biology Laboratory) presented the Keynote address at the Gordon Conference on Mechanisms of Mutagenesis and Genome Alterations, Girona, Spain, 2016.

Dr. Stephanie J. London (Epidemiology Branch) presented the plenary talk (Title: In utero exposures and DNA methylation profiles in offspring: maternal smoking as a test case) at the Conference on "Application of Big Data in Environmental Medicine", Karolinska Institute, Stockholm Sweden, October 20, 2016.

Dr. James W. Putney (Signal Transduction Laboratory) presented the Michael J. Berridge Lecturer at the 14th Meeting of the European Calcium Society, Valladolid, Spain, 2016.

Dr. Samuel H. Wilson (Genome Integrity & Structural Biology Laboratory) presented the Keynote Lecturer at the 18th Midwest DNA Repair Meeting, Columbus, Ohio, 2016; and will present the Keynote Address at the 48th Annual Meeting of the Environmental Mutagenesis and Genomics Society, 2017.

Advisory/Editorial Boards

Dr. Zhenglin Gu (Neurobiology Laboratory) served on the Editorial Board of the journal *Health Care: Current Reviews*.

Dr. Janet E. Hall (Clinical Director, Clinical Research Branch) serves as Associate Editor of *Endocrine Reviews*.

Dr. Stephanie J. London (Epidemiology Branch) was invited to serve on the Editorial board of the journal *Epigenomics*.

Dr. Jennifer Martinez (Immunity, Inflammation & Disease Laboratory) was selected to be Lead Guest Editor for a Special Issue of *Mediators of Inflammation*.

Dr. Lisa Rider (Clinical Research Branch) served on the Editorial Boards of *Annals of Pediatric Rheumatology*, *Journal of Neuromuscular Diseases*, and the *International Journal of Rheumatology*.

Dr. Roel Schaaper (Genome Integrity & Structural Biology Laboratory) serves on the Editorial Board of the journal *Mutation Research Fundamental and Mechanisms of Mutagenesis.*

Dr. Darryl Zeldin (Immunity Inflammation and Disease Laboratory) serves on the Editorial Board of the *Journal of Biological Chemistry*, *American Journal of Physiology: Lung Cellular and Molecular Biology*, *American Journal of Respiratory Cell and Molecular Biology*, *Prostaglandins and Other Lipid Mediators*, *Molecular and Cellular Pharmacology* and is Associate Editor of *Pharmacology and Therapeutics*.

TRAINING AND MENTORING

NIEHS Trainee Alumni

DIR has recently analyzed where recent postdoctoral trainees have gone upon completing their training, what they are doing and the level of the positions they took. Below is a summary of the analysis of 40 postdoctoral trainees that left NIEHS from January 1, 2016 through December 31, 2016.

What are they doing?

Additional postdoctoral training	**4**
Internship	**0**
Additional advanced degree	**2**
Primarily teaching	**1**
Primarily basic research	**10**
Primarily clinical research	**0**
Primarily clinical practice	**0**
Primarily applied research	**9**
Primarily patient care	**0**
Regulatory affairs	**1**
Science administration/project management	**1**
Intellectual property/ licensing and patenting	**0**
Consulting	**1**
Public policy	**0**
Science writing or communications	**1**
Grants management	**0**
Business development or Operations	**2**
Computation/informatics	**2**
Sales/marketing	**0**
Technical/customer support	**0**
Unknown or Undecided	**5**
Other	**1**
Deceased	**0**
TOTAL	**40**

Where did they go?	
Academic institution	20
Government agency	0
For-profit company	12
Non-profit organization	2
Private medical practice	0
Independent/self-employed	1
Unknown or Undecided	5
Deceased	0
TOTAL	40

What is the level of their position?	
Tenure track faculty	9
Non-tenure track faculty	2
Professional Staff	14
Support staff	0
Management	4
Trainee	6
Unknown or Undecided	5
Deceased	0
TOTAL	40

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.

Shannon Farris, Ph.D., received a K99/R00 award from the National Institute of Mental Health (NIMH). The title of the award is "Cell-type specific regulation of dendritic mRNA in the hippocampus". Dr. Farris will train in the Neurobiology Laboratory under the mentorship of Serena M. Dudek, Ph.D.