Division of Intramural Research

NAEHS Council Update

February, 2016

DIR RECRUITMENTS

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

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

NEW HIRES AND CHANGES IN DIR LEADERSHIP

Scientific Information Officer
Dr. David Fargo the acting Scientific Information Officer and the Director of Integrative Bioinformatics at NIEHS has accepted the position of Scientific Information Officer (SIO). Dr. Fargo will have an appointment in the Epigenetics and Stem Cell Biology Laboratory. As SIO, Dr. Fargo will lead a new office with a broad mission to advance NIEHS scientific Information Technology (IT) and research computing. These efforts will include direct scientific IT support, policy implementation, governance, and strategic innovation across a number of critical areas including laboratory IT, scientific high-performance computing, data and knowledge management, and IT infrastructure modernization. Dr. Fargo started his position as NIEHS SIO on November 30, 2015.

Tenure Track Investigator in Epidemiology
Dr. Kelly Ferguson from the University of Michigan School of Public Health has accepted a position as a Tenure Track Investigator in the Epidemiology Branch with a joint appointment in the Reproduction and Developmental Biology Laboratory. Dr. Ferguson investigates the impact of endocrine disrupting compounds on women's reproductive health and birth outcomes. She proposes to examine the effect of mixtures of chemical exposures and psychosocial stressors on timing of delivery; and the role of oxidative stress as an underlying mechanism connecting environmental exposures and reproductive disease. Dr. Ferguson started her position on January 10, 2016.

Branch Chief in Biostatistics & Computational Biology
After more than 19 years as the Chief of the Biostatistics Branch (now called Biostatistics and Computational Biology Branch, BCBB), Dr. Clare Weinberg has decided to step down for personal reasons. She will remain a Principal Investigator in the BCBB and continue to lead her own research group. Dr. Shyamal Peddada has agreed to assume the duties of Acting Chief, BCBB beginning on February 1, 2016.

NEWLY TENURED DIR PRINCIPAL INVESTIGATOR

At the October 5, 2015 meeting of the NIH Central Tenure Committee held in Bethesda **Dr. Raja Jothi** of the Epigenetics & Stem Cell Biology Laboratory was awarded tenure.

Research Summary
Raja Jothi, Ph.D.
Systems Biology Group
Epigenetics & Stem Cell Biology Laboratory
DIR, NIEHS

The long-term goal of the Systems Biology Group is to understand how transcription regulators and epigenetic modifications regulate gene expression programs controlling key cell fate decisions during cellular development, differentiation, and pathogenesis. To this end, we use integrative interdisciplinary approaches—merging systems biology, functional genomics, and biochemistry—to reconstruct and characterize developmentally- and environmentally-responsive gene networks in embryonic stem (ESCs). Research within the group is largely data-driven, through computational analyses of published and in-house-generated high-throughput genomic and proteomic datasets, with the goal of generating testable hypotheses. The laboratory component provides the means to not only test some of the hypotheses that come out of computational analyses but also to perform traditional biochemical experiments to gain mechanistic insights. Over the years, we have not only shed light on many genes and pathways with previously unknown roles in ESC biology but also help connect the dots on gene networks controlling the pluripotent state. Our ongoing studies on signaling networks will build on these findings and contribute to the comprehensive understanding of how signaling cascades instruct epigenetic and/or transcriptional programs controlling cell fate decisions. Collectively, our studies will provide a foundation for defining the mechanism and scope of developmentally- and environmentally-responsive gene networks for a better understanding of how ESCs can be used as effective model systems for regenerative medicine, disease modeling, and toxicity/drug testing.

BSC REVIEW OF THE IMMUNITY, INFLAMMATION AND DISEASE LABORATORY

The NIEHS DIR Board of Scientific Counselors reviewed the Immunity, Inflammation and Disease Laboratory November 15-17, 2015.

Members of the Board of Scientific Counselors that Attended:

- Kenneth B. Adler, Ph.D., [BSC Chair], Professor, Dept. of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC
- 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., Niels K. Jerne Professor of Pediatrics, Dept. of Pediatrics, Children's Hospital of Pittsburgh of UPMC, University of Pittsburgh, Pittsburgh, PA
- 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
- Ann M. Reed, M.D., Professor and Chair, Department of Pediatrics, Physician-in-Chief, Duke Children's Hospital, Duke University Medical Center, 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:

- Veena B. Antony, M.D., Professor of Medicine, Engineering and Environmental Health Sciences, Director, Program for Environmental and Translational Medicine, University of Alabama at Birmingham, Birmingham, AL
- David B. Corry, M.D., Professor of Medicine and Immunology, Baylor College of Medicine, Houston, TX
- Gregory P. Downey, M.D., Executive Vice President for Academic Affairs, National Jewish Health, Denver, CO
- Andrew P. Fontenot, M.D., Henry N. Claman Professor of Medicine, Division Head, Allergy and Clinical Immunology, University of Colorado, Aurora, CO
- Kai Ge, Ph.D., Senior Investigator, Section Chief, Adipocyte Biology and Gene Regulation, NIDDK, NIH, Bethesda, MD
- Steve N. Georas, M.D., Professor of Medicine, University of Rochester Medical Center, Rochester, NY

- Terry Gordon, Ph.D., Professor, Environmental Medicine, New York University, Tuxedo, NY
- Cheryl B. Knudson, Ph.D., Professor and Chair, Department of Anatomy And Cell Biology, East Carolina University, Brody School of Medicine, Greenville, NC
- George Leikauf, Ph.D., Professor, Environmental and Occupation Health, University of Pittsburgh, Pittsburgh, PA
- Rama K. Mallampalli, M.D., Division Chief, PACCM, UPMC Endowed Professor and Director, Acute Lung Injury Center of Excellence, Department of Medicine, University of Pittsburgh Medical Center (UPMC), Pittsburgh, PA
- Gokhan Mutlu, M.D., Chief, Section of Pulmonary and Critical Care Medicine, The University of Chicago School of Medicine, Chicago, IL
- Noa Noy, Ph.D., Professor of Pharmacology, Case Western Reserve University, Cleveland, OH
- Ray S. Peebles, Jr., M.D., Elizabeth and John Murray Professor of Medicine, Vanderbilt University Medical School, Nashville, TN
- Larry Schlesinger, M.D., Samuel Saslaw Professor of Medicine, Chair, Dept. of Microbial Infection and Immunity, Director, Center for Microbial Interface Biology, Ohio State University, Columbus, OH
- Thomas Sisson, M.D., Professor of Internal Medicine, University of Michigan, Ann Arbor, MI

Agenda:

Sunday, November 15 – Doubletree by Hilton
Closed Evening Session

7:00 – 8:00 p.m.	Welcome and Discussion of Past Board Reviews, Drs. Linda Birnbaum, Darryl Zeldin and Anton Jetten
8:00 – end	BSC Discussion Review, Dr. Ken Adler and panel

Monday, November 16 - NIEHS Rodbell Conference Rooms 101 ABC
Morning Session

8:30 – 8:45 a.m.	Welcome, Dr. Kenneth Adler
8:45 – 9:05	Overview, Immunity, Inflammation and Disease Laboratory, Dr. Anton Jetten
9:05 – 9:55	Cell Biology Group, Anton M. Jetten, Ph.D.
9:55 - 10:10	COFFEE BREAK
10:10 – 11:00	Clinical Investigation of Host Defense Group, Michael B. Fessler, M.D.
11:00 – 11:50	Immunogenetics Group, Donald Cook, Ph.D.
11:50 – 12:35	Closed 1:1 Sessions with Investigators, Drs. Jetten, Fessler & Cook
12:35 – 1:30	Closed Working Lunch, 101ABC

Afternoon Session

1:30 – 3:00 pm	Poster Session—IIDL 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	Matrix Biology Group, Stavros Garantziotis, M.D.

4:35 – 5:25	Environmental Genetics Group, Steven Kleeberger, Ph.D.
5:25 – 5:55	Closed 1:1 Sessions with Investigators, Drs. Garantziotis & Kleeberger
6:00	Return to Doubletree Hotel

Closed Evening Session

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

Tuesday November 17- NIEHS Rodbell Conference Rooms 101 ABC

Morning Session

8:30 – 9:20 am	Inflammation & Autoimmunity Group, Jennifer A. Martinez, Ph.D.
9:20 – 10:10	Environmental Cardiopulmonary Disease Group, Darryl C. Zeldin, M.D.
10:10 – 10:40	Closed 1:1 Sessions with Investigators, Drs. Martinez & Zeldin
10:40 – 10:55	COFFEE BREAK
10:55 - 12:00	BSC Discussion and completion of individual review assignments by each member/ Lunch Optional
12:00 – 1:30	Closed Session and Debriefing to NIEHS/DIR Leadership, 101ABC
1:30	Adjourn

NIEHS SCIENCE DAYS

The Thirteenth Annual NIEHS Science Days were held on November 5-6, 2015, 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 Inflammation, the Environment 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, 92 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: Carmen J. Williams, M.D., Ph.D, Reproductive & Developmental Biology Laboratory
Fellow of the Year: Miranda Bernhardt, Ph.D., Reproductive & Developmental Biology Laboratory
Best Poster Presentation:

1. Telmo F. Henriques, Ph.D., Epigenetics & Stem Cell Biology Laboratory, "Probing co-transcriptional RNA processing"
2. Sonika Patial, Ph.D., Signal Transduction Laboratory, "Genetic deletion of an instability motif in the 3'-untranslated region of Tristetraprolin mRNA increases TTP mRNA stability and protein expression and protects against immune-mediated inflammatory diseases"
3. David Chen, Ph.D., Neurobiology Laboratory, "Reactive microgliosis is essential in driving chronic neuroinflammation-related neurodegeneration: role of the MAC1-NOX2 signaling pathway"
4. Joanne C. Damborsky, Ph.D., Neurobiology Laboratory, "Interplay between cholinergic and galaninergic modulation of GABA release in the basal forebrain"
5. Douglas Ganini Da Silva, Ph.D., Immunity, Inflammation & Disease Laboratory, "Human mitochondrial SOD2 and bacterial SOD A incorporated with iron become prooxidant peroxidases"
6. Samantha L. Hoopes, Ph.D., Immunity, Inflammation & Disease Laboratory, "Transgenic mice expressing CYP4F2 in endothelial cells exhibit altered retinal angiogenesis in vivo"
7. Julie Lowe, Ph.D., Immunity, Inflammation & Disease Laboratory, "The novel p53 target TNFAIP8 variant 2 is increased in cancer and offsets p53-dependent tumor suppression"
8. Ngome L. Makia, Ph.D., National Toxicology Program Laboratory, "Cadmium and arsenic transformed human peripheral lung cells expressing CD34 display stem cell-like and malignant properties"
9. Dan Su, Ph.D., Genome Integrity & Structural Biology Laboratory, "Tobacco-smoke associated DNA methylation and gene transcription in human blood cell lineages"

Best Oral Presentation: Motoki Takaku, Ph.D., Epigenetics & Stem Cell Biology Laboratory, "A stepwise mechanism of chromatin reprogramming by the pioneer transcription factor GATA3"

DIR PAPERS OF THE YEAR FOR 2015

Young MT, Sandler DP, DeRoo LA, Vedal S, Kaufman JD, London SJ. Ambient air pollution exposure and incident adult asthma in a nationwide cohort of U.S. women. *Am. J. Respir. Crit. Care. Med.*, **190**: 914-21, 2014.

RATIONALE: Limited prior data suggest an association between traffic-related air pollution and incident asthma in adults. No published studies assess the effect of long-term exposures to particulate matter less than 2.5 μm in diameter (PM2.5) on adult incident asthma.
OBJECTIVES: To estimate the association between ambient air pollution exposures (PM2.5 and nitrogen dioxide, NO2) and development of asthma and incident respiratory symptoms.
METHODS: The Sister Study is a U.S. cohort study of risk factors for breast cancer and other health outcomes (n = 50,884) in sisters of women with breast cancer (enrollment, 2003-2009). Annual average (2006) ambient PM2.5 and NO2 concentrations were estimated at participants' addresses, using a national land-use/kriging model incorporating roadway information. Outcomes at follow-up (2008-2012) included incident self-reported wheeze, chronic cough, and doctor-diagnosed asthma in women without baseline symptoms.
MEASUREMENTS AND MAIN RESULTS: Adjusted analyses included 254 incident cases of asthma, 1,023 of wheeze, and 1,559 of chronic cough. For an interquartile range (IQR) difference (3.6 μg/m(3)) in estimated PM2.5 exposure, the adjusted odds ratio (aOR) was 1.20 (95% confidence interval [CI] = 0.99-1.46, P = 0.063) for incident asthma and 1.14 (95% CI = 1.04-1.26, P = 0.008) for incident wheeze. For NO2, there was evidence for an association with incident wheeze (aOR = 1.08, 95% CI = 1.00-1.17, P = 0.048 per IQR of 5.8 ppb). Neither pollutant was significantly associated with incident cough (PM2.5: aOR = 0.95, 95% CI = 0.88-1.03, P = 0.194; NO2: aOR = 1.00, 95% CI = 0.93-1.07, P = 0.939).
CONCLUSIONS: Results suggest that PM2.5 exposure increases the risk of developing asthma and that PM2.5 and NO2 increase the risk of developing wheeze, the cardinal symptom of asthma, in adult women.

Nichols HB, DeRoo LA, Scharf DR, Sandler DP. Risk-benefit profiles of women using tamoxifen for chemoprevention. *J. Natl. Cancer Inst.*, **107**: 354, 2015

BACKGROUND: Tamoxifen has been US Food and Drug Administration-approved for primary prevention of breast cancer since 1998 but has not been widely adopted, in part because of increased risk of serious side effects. Little is known about the risk-benefit profiles of women who use chemoprevention outside of a clinical trial. We examined characteristics associated with initiation and discontinuation of tamoxifen for primary prevention of breast cancer within a large cohort of women with a first-degree family history of breast cancer.
METHODS: This research was conducted within The Sister Study, a cohort of 50884 US and Puerto Rican women age 35 to 74 years enrolled from 2003 to 2009. Eligible women were breast cancer-free at enrollment and had a sister who had been diagnosed with breast cancer. Participants reported tamoxifen use, ages started and stopped taking tamoxifen, and total duration of use at enrollment. We identified 788 tamoxifen users and 3131 nonusers matched on age and year of enrollment who had no history of contraindicating factors (stroke, transient ischemic attack, cataract, endometrial or uterine cancer). Characteristics associated

with tamoxifen initiation were evaluated with multivariable conditional logistic regression. All statistical tests were two-sided.
RESULTS: Based on published risk-benefit indices, 20% of women who used tamoxifen had insufficient evidence that the benefits of tamoxifen outweigh the risk of serious side effects. After 4.5 years, 46% of women had discontinued tamoxifen.
CONCLUSIONS: While the majority of women who used tamoxifen for primary prevention of breast cancer were likely to benefit, substantial discontinuation of tamoxifen before five years and use by women at risk of serious side effects may attenuate benefits for breast cancer prevention.

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

Oxidative stress promotes genomic instability and human diseases. A common oxidized nucleoside is 8-oxo-7,8-dihydro-2′-deoxyguanosine, which is found both in DNA (8-oxo-G) and as a free nucleotide (8-oxo-dGTP). Nucleotide pools are especially vulnerable to oxidative damage. Therefore cells encode an enzyme (MutT/MTH1) that removes free oxidized nucleotides. This cleansing function is required for cancer cell survival and to modulate Escherichia coli antibiotic sensitivity in a DNA polymerase (pol)-dependent manner. How polymerases discriminate between damaged and non-damaged nucleotides is not well understood. This analysis is essential given the role of oxidized nucleotides in mutagenesis, cancer therapeutics, and bacterial antibiotics. Even with cellular sanitizing activities, nucleotide pools contain enough 8-oxo-dGTP to promote mutagenesis. This arises from the dual coding potential where 8-oxo-dGTP(anti) base pairs with cytosine and 8-oxo-dGTP(syn) uses its Hoogsteen edge to base pair with adenine. Here we use time-lapse crystallography to follow 8-oxo-dGTP insertion opposite adenine or cytosine with human pol β, to reveal that insertion is accommodated in either the syn- or anti-conformation, respectively. For 8-oxo-dGTP(anti) insertion, a novel divalent metal relieves repulsive interactions between the adducted guanine base and the triphosphate of the oxidized nucleotide. With either templating base, hydrogen-bonding interactions between the bases are lost as the enzyme reopens after catalysis, leading to a cytotoxic nicked DNA repair intermediate. Combining structural snapshots with kinetic and computational analysis reveals how 8-oxo-dGTP uses charge modulation during insertion that can lead to a blocked DNA repair intermediate.

Caballero MT, Serra ME, Acosta PL, Marzec J, Gibbons L, Salim M, Rodriguez A, Reynaldi A, Garcia A, Bado D, Buchholz UJ, Hijano DR, Coviello S, Newcomb D, Bellabarba M, Ferolla FM, Libster R, Berenstein A, Siniawaski S, Blumetti V, Echavarria M, Pinto L, Lawrence A, Ossorio MF, Grosman A, Mateu CG, Bayle C, Dericco A, Pellegrini M, Igarza I, Repetto HA, Grimaldi LA, Gudapati P, Polack NR, Althabe F, Shi M, Ferrero F, Bergel E, Stein RT, Peebles RS, Boothby M, Kleeberger SR, Polack FP. TLR4 genotype and environmental LPS mediate RSV bronchiolitis through Th2 polarization. *J. Clin. Invest.*, **125**: 571-582, 2015.

While 30%-70% of RSV-infected infants develop bronchiolitis, 2% require hospitalization. It is not clear why disease severity differs among healthy, full-term infants; however, virus titers, inflammation, and Th2 bias are proposed explanations. While TLR4 is associated with

these disease phenotypes, the role of this receptor in respiratory syncytial virus (RSV) pathogenesis is controversial. Here, we evaluated the interaction between TLR4 and environmental factors in RSV disease and defined the immune mediators associated with severe illness. Two independent populations of infants with RSV bronchiolitis revealed that the severity of RSV infection is determined by the TLR4 genotype of the individual and by environmental exposure to LPS. RSV-infected infants with severe disease exhibited a high GATA3/T-bet ratio, which manifested as a high IL-4/IFN-γ ratio in respiratory secretions. The IL-4/IFN-γ ratio present in infants with severe RSV is indicative of Th2 polarization. Murine models of RSV infection confirmed that LPS exposure, Tlr4 genotype, and Th2 polarization influence disease phenotypes. Together, the results of this study identify environmental and genetic factors that influence RSV pathogenesis and reveal that a high IL-4/IFN-γ ratio is associated with severe disease. Moreover, these molecules should be explored as potential targets for therapeutic intervention.

Andres SN, Appel CD, Westmoreland JW, Williams JS, Nguyen Y, Robertson PD, Resnick MA, Williams RS. Tetrameric Ctp1 coordinates DNA binding and DNA bridging in DNA double-strand-break repair. *Nat. Struct. Mol. Biol.*, **22**: 158-66, 2015.

Ctp1 (also known as CtIP or Sae2) collaborates with Mre11-Rad50-Nbs1 to initiate repair of DNA double-strand breaks (DSBs), but its functions remain enigmatic. We report that tetrameric Schizosaccharomyces pombe Ctp1 contains multivalent DNA-binding and DNA-bridging activities. Through structural and biophysical analyses of the Ctp1 tetramer, we define the salient features of Ctp1 architecture: an N-terminal interlocking tetrameric helical dimer-of-dimers (THDD) domain and a central intrinsically disordered region (IDR) linked to C-terminal 'RHR' DNA-interaction motifs. The THDD, IDR and RHR are required for Ctp1 DNA-bridging activity in vitro, and both the THDD and RHR are required for efficient DSB repair in S. pombe. Our results establish non-nucleolytic roles of Ctp1 in binding and coordination of DSB-repair intermediates and suggest that ablation of human CtIP DNA binding by truncating mutations underlie the CtIP-linked Seckel and Jawad syndromes.

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

Ribonucleotides are frequently incorporated into DNA during replication in eukaryotes. Here we map genome-wide distribution of these ribonucleotides as markers of replication enzymology in budding yeast, using a new 5' DNA end-mapping method, hydrolytic end sequencing (HydEn-seq). HydEn-seq of DNA from ribonucleotide excision repair-deficient strains reveals replicase- and strand-specific patterns of ribonucleotides in the nuclear genome. These patterns support the roles of DNA polymerases α and δ in lagging-strand replication and of DNA polymerase ε in leading-strand replication. They identify replication origins, termination zones and variations in ribonucleotide incorporation frequency across the genome that exceed three orders of magnitude. HydEn-seq also reveals strand-specific 5' DNA ends at mitochondrial replication origins, thus suggesting unidirectional replication of a circular genome. Given the conservation of enzymes that incorporate and process

ribonucleotides in DNA, HydEn-seq can be used to track replication enzymology in other organisms.

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

Organogenesis of the ovary is a highly orchestrated process involving multiple lineage determination of ovarian surface epithelium, granulosa cells and theca cells. Although the sources of ovarian surface epithelium and granulosa cells are known, the origin(s) of theca progenitor cells have not been definitively identified. Here we show that theca cells derive from two sources: $Wt1^+$ cells indigenous to the ovary and $Gli1^+$ mesenchymal cells that migrate from the mesonephros. These progenitors acquire theca lineage marker Gli1 in response to paracrine signals Desert hedgehog (Dhh) and Indian hedgehog (Ihh) from granulosa cells. Ovaries lacking Dhh/Ihh exhibit theca layer loss, blunted steroid production, arrested folliculogenesis and failure to form corpora lutea. Production of Dhh/Ihh in granulosa cells requires growth differentiation factor 9 (GDF9) from the oocyte. Our studies provide the first genetic evidence for the origins of theca cells and reveal a multicellular interaction critical for the formation of a functional theca.

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

The nourishment of neonates by nursing is the defining characteristic of mammals. However, despite considerable research into the neural control of lactation, an understanding of the signaling mechanisms underlying the production and expulsion of milk by mammary epithelial cells during lactation remains largely unknown. Here we demonstrate that a store-operated $Ca2^+$ channel subunit, Orai1, is required for both optimal $Ca2^+$ transport into milk and for milk ejection. Using a novel, 3D imaging strategy, we visualized live oxytocin-induced alveolar unit contractions in the mammary gland, and we demonstrated that in this model milk is ejected by way of pulsatile contractions of these alveolar units. In mammary glands of Orai1 knockout mice, these contractions are infrequent and poorly coordinated. We reveal that oxytocin also induces a large transient release of stored $Ca2^+$ in mammary myoepithelial cells followed by slow, irregular $Ca2^+$ oscillations. These oscillations, and not the initial $Ca2^+$ transient, are mediated exclusively by Orai1 and are absolutely required for milk ejection and pup survival, an observation that redefines the signaling processes responsible for milk ejection. These findings clearly demonstrate that $Ca2^+$ is not just a substrate for nutritional enrichment in mammals but is also a master regulator of the spatiotemporal signaling events underpinning mammary alveolar unit contraction. Orai1-dependent $Ca2^+$ oscillations may represent a conserved language in myoepithelial cells of other secretory epithelia, such as sweat glands, potentially shedding light on other Orai1 channelopathies, including anhidrosis (an inability to sweat).

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

OBJECTIVE: Autonomic dysfunction frequently occurs in the context of Parkinson disease (PD) and may precede onset of motor symptoms. Limited data exist on the prospective association of heart rate variability (HRV), a marker of autonomic function, with PD risk.
METHODS: We included 12,162 participants of the Atherosclerosis Risk in Communities study, a community-based cohort, without a diagnosis of PD at baseline (1987-1989) and with available HRV data (mean age = 54 years, 57% women). A 2-minute electrocardiogram was used to measure HRV. Incident PD was identified through 2008 from multiple sources, and adjudicated. Multivariable Cox models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) of PD by quartiles of HRV measurements.
RESULTS: During a mean follow-up of 18 years, we identified 78 incident PD cases. Lower values of the root mean square of successive differences in normal-to-normal R-R intervals (rMSSD) and standard deviation of normal-to-normal R-R intervals (SDNN), markers of parasympathetic activity and total variability, respectively, were associated with higher PD risk during follow-up. In multivariate models, the HR (95% CI) of PD in the bottom quartiles of rMSSD and SDNN compared to the top quartiles were 2.1 (1.0-4.3) and 2.9 (1.4-6.1), respectively. Other measures of cardiac autonomic function, including mean R-R interval and frequency-domain measurements, were not associated with PD risk.
INTERPRETATION: In this prospective cohort, decreased HRV was associated with an increased risk of PD. Assessment of cardiac autonomic function may help identify individuals at risk for PD.

Scruggs BS, Gilchrist DA, Nechaev S, Muse GW, Burkholder A, Fargo DC, Adelman K. Bidirectional Transcription Arises from Two Distinct Hubs of Transcription Factor Binding and Active Chromatin. *Mol. Cell.*, **58**: 1101-1112, 2015.

Anti-sense transcription originating upstream of mammalian protein-coding genes is a well-documented phenomenon, but remarkably little is known about the regulation or function of anti-sense promoters and the non-coding RNAs they generate. Here we define at nucleotide resolution the divergent transcription start sites (TSSs) near mouse mRNA genes. We find that coupled sense and anti-sense TSSs precisely define the boundaries of a nucleosome-depleted region (NDR) that is highly enriched in transcription factor (TF) motifs. Notably, as the distance between sense and anti-sense TSSs increases, so does the size of the NDR, the level of signal-dependent TF binding, and gene activation. We further discover a group of anti-sense TSSs in macrophages with an enhancer-like chromatin signature. Interestingly, this signature identifies divergent promoters that are activated during immune challenge. We propose that anti-sense promoters serve as platforms for TF binding and establishment of active chromatin to further regulate or enhance sense-strand mRNA.

Chan K, Roberts SA, Klimczak LJ, Sterling JF, Saini N, Malc EP, Kim J, Kwiatkowski DJ, Fargo DC, Mieczkowski PA, Getz G, Gordenin DA. An APOBEC3A hypermutation signature is distinguishable from the signature of background mutagenesis by APOBEC3B in human cancers. *Nat. Genet.*, **47**: 1067-1072, 2015.

Elucidation of mutagenic processes shaping cancer genomes is a fundamental problem whose solution promises insights into new treatment, diagnostic and prevention strategies. Single-strand DNA-specific APOBEC cytidine deaminase(s) are major source(s) of mutation in several cancer types. Previous indirect evidence implicated APOBEC3B as the more likely major mutator deaminase, whereas the role of APOBEC3A is not established. Using yeast models enabling the controlled generation of long single-strand genomic DNA substrates, we show that the mutation signatures of APOBEC3A and APOBEC3B are statistically distinguishable. We then apply three complementary approaches to identify cancer samples with mutation signatures resembling either APOBEC. Strikingly, APOBEC3A-like samples have over tenfold more APOBEC-signature mutations than APOBEC3B-like samples. We propose that APOBEC3A-mediated mutagenesis is much more frequent because APOBEC3A itself is highly proficient at generating DNA breaks, whose repair can trigger the formation of single-strand hypermutation substrates.

Mueller GA, Pedersen LC, Glesner J, Edwards LL, Zakzuk J, London RE, Arruda LK, Chapman MD, Caraballo L, Pomés A. Analysis of glutathione S-transferase allergen cross-reactivity in a North American population: Relevance for molecular diagnosis. *J. Allergy Clin. Immunol.*, **136**: 1369-1377, 2015.

BACKGROUND: It is not clear whether cross-reactivity or cosensitization to glutathione S-transferases (GSTs) occurs in tropical and subtropical environments. In the United States, Bla g 5 is the most important GST allergen and lack of coexposure to GSTs from certain species allows a better assessment of cross-reactivity.
OBJECTIVES: To examine the molecular structure of GST allergens from cockroach (Bla g 5), dust mites (Der p 8 and Blo t 8), and helminth (Asc s 13) for potential cross-reactive sites, and to assess the IgE cross-reactivity of sensitized patients from a temperate climate for these allergens for molecular diagnostic purposes.
METHODS: Four crystal structures were determined. Sera from patients allergic to cockroach and mite were tested for IgE reactivity to these GSTs. A panel of 6 murine anti-Bla g 5 mAb was assessed for cross-reactivity with the other 3 GSTs using antibody binding assays.
RESULTS: Comparisons of the allergen structures, formed by 2-domain monomers that dimerize, revealed few contiguous regions of similar exposed residues, rendering cross-reactivity unlikely. Accordingly, anti-Bla g 5 or anti-Der p 8 IgE from North American patients did not recognize Der p 8 or Bla g 5, respectively, and neither showed binding to Blo t 8 or Asc s 13. A weaker binding of anti-Bla g 5 IgE to Der p 8 versus Bla g 5 (~100-fold) was observed by inhibition assays, similar to a weak recognition of Der p 8 by anti-Bla g 5 mAb. Patients from tropical Colombia had IgE to all 4 GSTs.
CONCLUSIONS: The lack of significant IgE cross-reactivity among the 4 GSTs is in agreement with the low shared amino acid identity at the molecular surface. Each GST is needed for accurate molecular diagnosis in different geographic areas.

AWARDS AND HONORS

Scientific Awards

Dr. Pierre Bushel (Biostatistics & Computational Biology Branch) received the Distinguished Alumni Award from The University of Massachusetts at Amherst, Massachusetts for his research in bioinformatics, computational biology and toxicogenomics.

Dr. Hye-Youn Cho (Immunity, Inflammation & Disease Laboratory) received the Presidential Award from the 7th International Congress of Asian Society of Toxicology.

Dr. David Kurtz (Comparative Medicine Branch) received the 3rd place award for best poster in the Laboratory Investigation category at the 2015 National Meeting of the American Association of Laboratory Animal Science (AALAS) held in Phoenix, AZ.

Dr. Ronald Mason (Immunity, Inflammation & Disease Laboratory) received the 2015 Discovery Award from the Society for Free Radical Biology and Medicine.

Dr. Walter J. Rogan (Epidemiology Branch) received the Children's Environmental Health Network: "Child Health Advocate, Science" award from the Milken Institute School of Public Health, George Washington University.

Dr. Dale Sandler (Chief, Epidemiology Branch) received the 2015 Nathan Davis Award for Outstanding Government Service from the American Medical Association.

Dr. Jack Taylor (Epidemiology Branch) was elected into the American Society of Epidemiology.

Dr. Allen Wilcox (Epidemiology Branch) received the 2015 Mentor of the Year Award from the Society for Pediatric and Perinatal Research.

Dr. Samuel H. Wilson (Genome Integrity & Structural Biology Laboratory) received the 2015 NIH Director's Award (the Ruth L. Kirschstein Mentoring Award).

Dr. Darryl C. Zeldin (Scientific Director and Immunity, Inflammation & Disease Laboratory) was elected into the American Association of Physicians (AAP).

Named Professorships/Lectures

Dr. Karen Adelman (Epigenetics & Stem Cell Biology Laboratory) has been invited to present one of three keynote lectures at RECOMB (Research in Computational Molecular Biology). RECOMB is one of the premier conferences in Computational Biology, with a special focus on algorithmic problems.

Dr. Janet Hall (Clinical Research Branch) delivered the Keynote Address at the Women in Endocrinology 2015 Annual National Meeting.

Dr. Kenneth Korach received the Neena Schwartz Lecture Award from Northwestern University Medical School for characterizing a spectrum of estrogen responses by developing a variety of estrogen receptor mutant mouse models. In addition, Dr. Korach presented the Plenary Lectures at the International VI Ovarian Club Congress and the Asian Ovarian Congress; presented the International Plenary Lecturer at the 55th Annual Meeting of the Endocrine Society of Australia; was the Keynote International Speaker at the 88th Annual Meeting of the Japanese Endocrine Society; and was the Plenary Speaker at the IFFS/JSRM International Conference and the 15th World Congress on Human Reproduction.

Dr. Thomas A. Kunkel (Genome Integrity & Structural Biology Laboratory) delivered the Plenary Lecture at the International Conference on Yeast Genetics and Molecular Biology, Trento, Italy.

Dr. Stephanie J. London (Epidemiology Branch) presented the Langmuir Lecture at the American Epidemiological Society. Berkeley CA, March 26, 2015.

Dr. James Putney (Signal Transduction Laboratory) has been invited to present the Michael Berridge Lecture at the European Calcium Society meeting in Valladolid, Spain.

Dr. Darryl C. Zeldin (Scientific Director and Immunity, Inflammation & Disease Laboratory) was the Keynote Speaker, Nanjing Medical University, School of Public Health, Nanjing, China: "A Vision for Environmental Health: Overview of the NIEHS;" and was the Keynote Speaker, First Tongji Hospital International Medical Science Summit: "Soluble Epoxide Hydrolase Regulates Macrophage Phagocytosis and Lung Clearance of S. Pneumoniae."

Advisory/Editorial Boards

Dr. Karen Adelman (Epigenetics & Stem Cell Biology Laboratory) serves of the Editorial Boards of *RNA Biology, METHODS, Molecular Cell, eLife, Genes and Development*, and *Trends in Biochemical Sciences*.

Dr. Perry Blackshear (Signal Transduction Laboratory) serves on the Editorial Board of *Molecular and Cellular Biology*.

Dr. Stavros Garantziotis (Immunity, Inflammation & Disease Laboratory) serves as the academic editor at the journal *PLoS ONE*.

Dr. Dmitry A. Gordenin (Genome Integrity & Structural Biology Laboratory) served on Board of Editors of *Mutation Research Fundamental and Molecular Mechanisms of Mutagenesis*.

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

Dr. Janet Hall (Clinical Research Branch) was appointed to the Editorial Board of the journal *Endocrine Reviews.*

Dr. Kathy Laber (Chief, Comparative Medicine Branch) serves as 'Council Member Emeritus' for Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

Dr. Hideki Nakano (Immunity, Inflammation & Disease Laboratory) serves on the Editorial Board of the journal *Frontiers in Immunology*.

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

Dr. Samuel H. Wilson (Genome Integrity & Structural Biology Laboratory) served as Editor-in-Chief of the journal *DNA Repair*.

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 47 postdoctoral trainees that left NIEHS from January 1, 2015 through December 31, 2015.

What are they doing?

Additional postdoctoral training	5
Internship	0
Additional advanced degree [1 RN]	1
Primarily teaching	1
Primarily basic research	14
Primarily clinical research	2
Primarily clinical practice	0
Primarily applied research	9
Primarily patient care	0
Regulatory affairs	1
Science administration/project management	2
Intellectual property/ licensing and patenting	0
Consulting	1
Public policy	0
Science writing or communications	2
Grants management	2
Business development or Operations	0
Computation/informatics	3
Sales/marketing	0
Technical/customer support	1
Unknown or Undecided	3
Other (Finance Administration)	0
Deceased	0
TOTAL	47

Where did they go?	
Academic institution	25
Government agency	3
For-profit company	15
Non-profit organization	1
Private medical practice	0
Independent/self-employed	0
Unknown or Undecided	3
Deceased	0
TOTAL	47

What is the level of their position?	
Tenure track faculty	11
Non-tenure track faculty	8
Professional Staff	17
Support staff	1
Management	0
Trainee	7
Unknown or Undecided	3
Deceased	0
TOTAL	47

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.

Melike Caglayan, Ph.D., received a K99/R00 grant for his proposal entitled, "Oxidant and environmental toxicant-induced effects compromise ligation in DNA repair" Dr. Caglayan will train in the Genome Integrity & Structural Biology Laboratory under the mentorship of Samuel H. Wilson, M.D.