National Heart, Lung, and Blood Institute
Division of Intramural Research

Twelfth Annual NHLBI DIR Research Day

Photo courtesy of NIH Almanac

June 9, 2014
Natcher Conference Center
Bethesda, MD

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Special thanks to the Foundation for Advanced Education in Sciences (FAES) for providing lunch for Research Day.

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The DIR Office of Education
Office of the Scientific Director
NHLBI Cores and Offices

Twelfth Annual NHLBI DIR Research Day
June 9, 2014

8:15 – 8:30	**Arrival, Registration, Check-In**
8:30 – 8:50	**Introduction & Welcome** – *Auditorium* Chair: Cindy St. Hilaire, Ph.D. Herbert M. Geller, Ph.D., Director, Office of Education, NHLBI Robert S. Balaban, Ph.D., Scientific Director, NHLBI
8:50 – 10:10	**Research Highlights, Session I** – *Auditorium* Chair: Robert Gahl, Ph.D. Brian Busser, Ph.D., Staff Scientist, Laboratory of Developmental Systems Biology, *A Multi-Species Epigenetic Signature of Differentiating Embryonic Stem Cells Uncovers Regulators of Cardiogenesis* Zhiyun Ge, Ph.D., Postdoctoral Fellow, Laboratory of Ribonucleoprotein Biochemistry, *A conserved mechanism of cellular and retroviral mRNA evasion of NMD* John Hatch, Postbaccalaureate Fellow, Laboratory of Stem Cell and Neuro-Vascular Biology, *Temporal Mapping of Enteric Neurovascular Development and Villus Morphogenesis* Cynthia St. Hilaire, Ph.D., Research Fellow, Laboratory of Cardiovascular Regenerative Medicine, *Development of in vitro and in vivo human disease models to study mechanisms regulating vascular calcification*
10:10 – 10:20	BREAK
10:20– 12:00	**Research Highlights, Session II** – *Auditorium* Chair: Jacob Bitterman, Ph.D. Elena Cherkasova, Ph.D., Research Fellow, Laboratory of Transplantation Immunotherapy, *T-cell Mediated Graft-vs-tumor Effect against Kidney Cancer after Allogeneic Stem Cell Transplant Identifies an Immunogenic Human Endogenous Retrovirus Type-E (HERV-E) with Selective Expression in Clear Cell Kidney Cancer* Natalia Dmitrieva, Ph. D., Biologist, Renal Cellular and Molecular Biology Section, *Stimulation of Thrombogenic and Inflammatory Signaling in Endothelial Cells by Elevated Extracellular Sodium: Possible Role in Blood Hypercoagulability, Atherosclerosis and Cardiovascular Health.*

Ashlea Morgan, Postbaccalaureate Fellow, Developmental Neurobiology Section, *Differential Binding of Receptor Protein Tyrosine Phosphatase Type IIa Family (RPTPσ/RPTPδ/LAR) to Glycosaminoglycans*

Moonjung Jung, Ph.D., Clinical Fellow, Molecular Hematopoiesis Section, *Induced Pluripotent Stem Cells from a Patient with GATA2 Deficiency Display a Block in Hematopoietic Differentiation*

12:00-12:10 **Introduction to FAES**
Christina Farias, Executive Director, FAES

12:10 –12:20 **Group Picture** - *Auditorium*

12:20-1:30 **Lunch**, **Poster Session I** – *Atrium*

1:30 – 2:30 **Research Highlights, Session III** – *Auditorium*
Chair: Chad Williamson, Ph.D.

Colleen Skau, Ph.D., Postdoctoral Fellow, Laboratory of Cell and Tissue Morphodynamics, *A Novel Actin-Adhesion Structure Involved in Nuclear Positioning Requires the Formin FMN2*

Nathan Baird, Ph.D., Postdoctoral Fellow, RNA Biophysics and Cellular Physiology, *Uncovering Hidden RNA-drug Interactions: When Ligand Binding Both Promotes and Disrupts Folding*

Julian Haimovich, Postbaccalaureate Fellow, Laboratory of Developmental Systems Biology, *Machine-learning Identifies Predictive Signatures of Enhancer Activity in Individual Cardiac Cells*

2:30 – 3:30 **Poster Session II** – *Atrium*

3:30 – 4:50 **Research Highlights, Session IV** – *Auditorium*
Chair: Juliane Caviston, Ph.D.

Brian Galletta, Ph. D., Research Fellow, Laboratory of Molecular Machines and Tissue Architecture, *A Detailed Centrosome Interactome Reveals a Novel Role for Asterless/CEP152 in Controlling Centriole Length Via Cep97*

Elizabeth Mushaben, Ph.D., Postdoctoral Fellow, Laboratory of Asthma and Lung Inflammation, *The Macrophage Scavenger Receptor CD163 Suppresses Airway Hyperreactivity in a Murine Model of Experimental Cockroach-induced Asthma.*

Carsten Niemann, M.D., Ph.D., Special Volunteer, Laboratory of Lymphoid Malignancies, *Cytokine and T Cell Changes Upon in vivo Ibrutinib Therapy for CLL –Targeting CLL and Microenvironment*

Seth Thacker, Ph.D., Postdoctoral Fellow, Lipoprotein Metabolism Section, *High Density Lipoprotein suppresses inflammasome activation in response cholesterol crystals and other particulates*

4:50 – 5:00 **Conclusion** – *Auditorium*
Chair: Herbert Geller, Ph.D.
Susan Shurin, M.D., Deputy Director, NHLBI

5:00 **Happy Hour**
Rock Bottom Restaurant & Brewery - Bethesda, MD

Animal MRI Core Facility
Stasia A. Anderson, Ph.D., Director
Building 10, Room B1D49C, E-mail: andersos1@nhlbi.nih.gov
Phone: (301) 402-0908; Web: https://intranet.nhlbi.nih.gov/laboratories-and-core-facilities/animal-mri-core

The Animal MRI Core performs magnetic resonance imaging of small animal models in the NHLBI. We provide imaging expertise, experimental design, and data interpretation for investigators interested in incorporating imaging studies in NHLBI research. We are a teaching resource, and investigators and fellows can learn to perform MRI studies.

Examples of imaging studies we perform:
- Cardiac imaging for heart function and size: ejection fraction, ventricle size and wall thickness
- High resolution imaging of myocardium for identification of infarct and scars
- Imaging blood vessels; dimensions of the aorta and pulmonary artery; angiography; vessel wall
- Atherosclerotic plaque imaging
- Perfusion of skeletal muscle or tumors
- Whole body imaging for identification of non-cardiac defects in mouse models
- High resolution microimaging of embryos and fixed tissue

We work with investigators on the best approaches for the research model and goals. We can incorporate additional imaging modalities such as computed tomography, ultrasound and bioluminescence. Core imaging studies are performed in the NIH Mouse Imaging Facility.

Animal Surgery & Resources (ASR)
Randall R. Clevenger, B.S., LATG, 14E Surgical Facility Manager,
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Phone: (301) 496-0405, Fax: (301) 402-0170

Joni Taylor, B.S., LATG, Large Animal Resources Manager
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Timothy Hunt, B.S., LATG, CRC Surgery Manager
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James “Buster” Hawkins, DVM, MS, DACLAM
Animal Program Director
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Web: http://www.nhlbi.nih.gov/research/intramural/researchers/core/animal-surgery-and-resources-core/index.html

The Animal Surgery & Resources (ASR) Core provides veterinary medical care and technical services for NHLBI research animals used in both basic and preclinical research. These support services include: anesthesia, veterinary medical care, surgery, surgical support, training in surgical and microsurgical techniques, post-operative care, purchasing (large animals), and health monitoring. In addition, ASR provides technical services such as blood & tissue collection, and tail vein injections.

The ASR staff also provides NHLBI investigators with collaborative research support services such as developing animal models and new surgical procedures, assistance with research design, as well as animal protocol development and execution. Our surgical support equipment includes anesthesia machines with mechanical ventilation for species ranging from rodents to nonhuman primates, radiography, digital fluoroscopy, Faxitron, ultrasound, laser Doppler, and operating microscopes.

ASR supports a wide range of animal models for DIR investigators. Some of the animal models we provide surgical support for include xenotransplantation (involving baboons and genetically engineered pigs), stem cell models (rats, mice and pigs) myocardial infarction with and without reperfusion (rats, mice, rabbits, dogs, and nonhuman primates), hind limbischemia (rats, mice, rabbits), gene vector delivery to liver (mice, rabbits), plethysmography (mice) and support for MRI and other imaging procedures. We can also performcardiac function testing on rats and mice including invasive blood pressure, left ventricular pressure, and pressure-volumeloops. Surgical and perioperative support services are provided in the Building 14E Surgical Facility, the B-2 level of the CRC and the NMR Center. We provide support services to all NHLBI animals housed in NIH facilities.

Our goal is to facilitate getting the research accomplished expeditiously using the best, most humane methods. We will work with each investigator in developing their respective animal model and then either train them in all procedures to enable them to work independently, perform the procedures for them, or work in concert to expedite the animal data generated.

Biochemistry Core Facility
Duck-Yeon Lee, Ph.D., Director
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Web: http://www.nhlbi.nih.gov/research/intramural/researchers/core/biochemistry-core/index.html

Mission of the Biochemistry Core is to provide service and consultation with expertise in biochemical enzyme/protein purification and assay to NHLBI researchers. The Core currently features 1) ESI-LC/MS spectrometers to detect the accurate mass of intact protein and small molecules, 2) HPLCs equipped with radiochemical detector and fluorescence detector that allow a purification of enzyme or protein labeled with radioisotope or fluorescence probe, 3) Atomic absorption spectrometer to measure metal content and 4) Amino acid analysis.

Bioinformatics and Systems Biology
Xujing Wang, Ph.D., Director
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Phone: (301) 451-2862
Web: http://www.nhlbi.nih.gov/research/intramural/researchers/core/bioinformatics-and-systems-biology/index.html

The BioInformatics and Systems Biology (BISB) Core aims to assist PIs to overcome the technical challenges in utilizing bioinformatics and systems biology techniques. In addition, the core is interested to collaborate with PIs to incorporate systems biology approaches synergistically and develop transformative and translational research. It currently maintains a Linux server that contains 12 computational nodes of ~600 cores and 250TB storage.

Some examples of services that we provide include: the Analysis of high throughput sequencing, microarray, proteomic data; Experimental design assistance for OMICS studies; Integrated analysis of transcriptome, miRNA, epigenome and proteomics data; Functional Genomics analysis of data including pathway and functional enrichment analysis; Systems biology analysis of data to identify potential therapeutic targets or biomarkers; Development of predictors for diagnosis and prognosis of disease; Data and text mining from Public databases and literature; Personalized consulting; Customized data analysis and interpretation.

Biophysics Facility
Grzegorz (Greg) Piszczek, Ph.D., Director
Building 50, Room 3124; E-mail: piszczek@nih.gov
Phone: (301) 435 8082; Web: http://www.nhlbi.nih.gov/research/intramural/researchers/core/biophysics-core/index.html

The mission of the Biophysics Core Facility is to provide state of the art equipment and training to assist investigators within the NHLBI in studies of macromolecular interactions, dynamics and stability. The Biophysics Core Facility currently has resources to study oligomeric state of biomolecular assemblies, perform measurements of affinity, stoichiometry, kinetics and thermodynamics of interactions between proteins, DNA, RNA and their cognate ligands. Biophysical characterization capabilities include measurements of molecular weight, shape, and conformation of biological macromolecules. Oligomeric state of biomolecules as well as their hydrodynamic size and shape, can be studied using both analytical ultracentrifugation (Beckman XLI-Proteomelab) and light scattering techniques (Dynamic Light Scattering; DLS and Multi Angle Static Light Scattering; SEC-MALS).

Most physical or chemical processes have an associated heat effect that can be used as basis for a number of analytical techniques. Microcalorimetry is now the biophysical method of choice for label-free analysis of biomolecular interactions and stability. The Biophysics Facility has several Isothermal Titration and Differential Scanning Calorimeters (ITC and DSC) that can be applied in these studies. Surface Plasmon Resonance (SPR) is a complimentary method for studying macromolecular interactions that can also provide information on binding kinetics and the Biophysics Core is equipped with the Biacore 3000 and ForteBio Octet RED 96 systems. Molecular interactions can also be studied by micro-scale thermophoresis (MST) using the Nanotemper Monolith NT.115 instrument. Additionally, Facility users can take advantage of several optical spectroscopy methods, including steady-state and time resolved fluorescence, fluorescence anisotropy and circular dichroism (CD).

DNA Sequencing and Genomics Core Facility
Jun Zhu, Ph.D., Director
Phone: (301) 443-7927 E-mail: zhuj4@nhlbi.nih.gov
Web: http://www.nhlbi.nih.gov/research/intramural/researchers/core/dna-sequencing-and-genomics-core/index.html

The DNA Sequencing Core and Genomics core (DSGC) strives to provide the state-of-art next generation sequencing (NGS) services to NHLBI investigators in a cost-effect manner. Equipped with Illumina sequencers (Miseq and Hiseq), the core supports diverse NGS applications, including not but limited to genome/exome sequencing, RNA-seq, ChIP-seq and small RNA sequencing.

Our integrated services consist of experimental design and consultation, library QA/QC, data acquisition and in-depth data analysis. In addition, the DGSC also offers trainings on library preparation and computational tools for a wide range of NGS applications. Additional assistance will also be provided in a collaborative manner for projects that require extensive protocol optimization and software development.

Electron Microscopy Core Facility
Chris Brantner, Ph.D., Acting Director
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Web: http://www.nhlbi.nih.gov/research/intramural/researchers/core/electron-microscopy-core/index.html

The NHLBI Electron Microscopy Core Facility offers consultation, specimen preparation services, transmission and scanning electron microscope imaging of samples, results discussion, figure preparation, training and use of the equipment to all investigators in the NHLBI/DIR. We have collaborated with many NHLBI investigators in the past, and hope to work with many more in the future as we roll out new protocols. Our technical capabilities include: 1) Transmission electron microscopy (including electron tomography) and scanning electron microscopy of tissues, cells and other biological or material samples. 2) Immunocytochemistry at the electron microscopic level including immuno-gold labeling. 3) Negative staining and rotary shadowing of macromolecular preparations (nucleic acids or proteins) as well as other small structures such as viruses, protein polymers and liposomes. Training is offered in the use of the transmission electron microscope and the scanning electron microscope as well as all preparatory equipment. We will assist in the experimental planning as well as data interpretation. We will also produce figures for your manuscripts. Stop by and chat with us about how EM can fit into your projects or ask us about our future plans for the lab.

Flow Cytometry Core Facility
J. Philip McCoy, Jr., Ph.D., Director
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Phone: (301) 594-6950, Fax: (301) 480-4774
Web: http://www.nhlbi.nih.gov/research/intramural/researchers/core/flow-cytometry-core/index.html

The mission of the NHLBI Flow Cytometry Core Facility is to provide investigators at the NHLBI access to state-of the-art flow cytometry, including cell sorting, high dimensional cell analysis, cytokine quantification, and imaging flow cytometry. This is done by having cytometers and software available in the core facility and by providing consultation to investigators. Investigators are responsible for specimen preparation and staining. The staff of the flow cytometry laboratory will gladly assist you in designing your experiments and in developing optimal preparation and staining procedures. For analytical experiments, data will be provided as either hard copies or on appropriate media as listmode files. Software and computer workstations are available for "offline" analysis of these files. For sorting experiments, each investigator is responsible for bringing appropriate media and test tubes. In addition to cell sorting and analytical cytometry, the core facility also provides multiplex bead array expertise for analysis of extracellular cytokines, and imaging flow cytometry where the staining patterns of fluorochromes can be visualized.

Gene-targeting and Animal Model Unit, NHLBI iPSC and Genome Engineering Core
Chengyu Liu, Ph.D., Head
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The NHLBI Gene-targeting and Animal Model Unit was established in October, 2012. It resulted from the merging of the Transgenic Core with the iPSC (induced pluripotent stem cell) Core. It is now a semi-independent unit under the newly integrated core that has now been renamed as the NHLBI iPSC and Genome Engineering Core. Our unit's major responsibilities include: 1) assisting NHLBI scientists in generating genetically engineered mice, including transgenic and knockout/knockin mouse models; 2) assisting NIH scientists in deriving and characterizing non-human ESCs (embryonic stem cells) and iPSCs; 3) modifying the genome of ESCs and iPSCs through gene-targeting and other genetic engineering methods; 4) developing animal models to facilitate studies on stem cell transplantation and tissue engineering.

Our expertise in precisely modifying the genome of ESCs and microinjecting early embryos has traditionally enabled us to generate transgenic and knockout mouse models. In recent years, these skills have been increasingly used

to support ESC/iPSC research, including editing the genome of iPSC lines and assessing stem cell's differentiation potential in vivo.

Conversely, some of the new technologies developed primarily for stem cell research, including ZFN, Talen, and CRISPR methods, have substantially shorten the timeline for creating sophisticated mouse models as well as greatly broadened the spectrum of animal species, in which gene-targeted models can be efficiently created. Our unit is aimed at taking advantage of the synergies between the animal model field and the stem cell field to develop capabilities that complement the expertise possessed by the other members of the iPSC and Genome Engineering Core. This should allow the NHLBI iPSC and Genome Engineering Core to provide a comprehensive list of capabilities to support translational, clinical, as well as basic research.

The Imaging Probe Development Center
Murali Cherukuri, James Mitchell , Acting Directors
Web: http://www.nhlbi.nih.gov/research/intramural/researchers/programs/imaging-probe-development-center/

The Imaging Probe Development Center (IPDC) was founded with the goal of providing fundamental synthetic chemistry support needed to advance molecular imaging technologies for interdisciplinary NIH research community. The IPDC laboratories are located in Rockville, MD with state-of-the-art equipment and the new PET facility to open in March 2014 at NIH main campus. The IPDC has a rolling solicitation system and NIH scientists are welcome to contact and submit a proposal with the request for synthesis of a particular probe in which they are interested. Probes can be intended for all types of imaging modalities, such as optical fluorescence, PET/SPECT and MRI. IPDC scientists can synthesize requested probes that are published in literature but commercially unavailable, or are completely novel. Examples of molecular imaging probes we have produced range from low molecular weight entities to high molecular weight conjugates and include fluorescent dyes and their analogs, lanthanide complexes, fluorogenic enzyme substrates, caged dyes which become fluorescent upon irradiation, radio and fluorescent labeled peptides, proteins and antibodies, gold and iron oxide nanoparticles, dendrimers and liposomes.

iPSC and Genome Engineering Core Facility
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Web: http://www.nhlbi.nih.gov/research/intramural/researchers/core/ipsc-and-genome-engineering-core/index.html

The mission of the Human iPSC Core Facility is to provide investigators consultation, technical services and training in human pluripotent stem cell technology in the NHLBI/DIR. Our technical capabilities include: 1) Complete culture facility for human ES and iPS cells. 2) Cell culture quality controls. We offer training in the derivation of human iPS cells, and encourage fellows and students to perform stem cell studies in our facility. We will also collaborate with investigators to validate new technologies, and establish standard procedures for specific project.

Light Microscopy Core Facility
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Xufeng Wu, Ph.D., Deputy Director
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Web: http://www.nhlbi.nih.gov/research/intramural/researchers/core/light-microscopy-core/index.html

We are a state-of-the-art microscopy facility that has been helping researchers in the NHLBI-DIR since 2000. The mission of this facility is to provide state of the art equipment, training, experimental design, and image processing capabilities to assist researchers within the NHLBI-DIR in experiments involving light microscopy. We have twenty-one microscopes in six locations in Bldgs. 10 and 50. These microscopes provide a wide array of techniques including wide-field fluorescence and transmitted light imaging, confocal, two-photon, total internal reflection fluorescence microscopy (TIRF) and super-resolution microscopy. Drs. Malide, Wu and Combs are available for help with all facets of your imaging experiments including consultation, training, image analysis, and help with data acquisition. We place a large emphasis on training to allow researchers to independently use LMF resources. Once you have been trained you may book our microscopes online using iLabs software. We also have a broad array of image processing software for quantification of

image intensities, morphological analysis, co-localization analysis, particle tracking, 3D volume reconstruction, etc. We are available to help all NHLBI-DIR researchers.

Murine Phenotyping Core
Danielle Springer, VMD, DACLAM, Director
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Phone: (301) 594-6171; Fax: (301)480-7576
Web: http://www.nhlbi.nih.gov/research/intramural/researchers/core/murine-phenotyping-core/index.html

The NHLBI phenotyping core's central mission is development of a comprehensive in-depth knowledge of murine phenotyping methodologies in order to assist investigators with design, research applications, experimental methodologies, data acquisition and interpretation of murine cardiovascular, metabolic, neuromuscular and pulmonary platforms. We seek to provide investigators with high quality scientific and technical support as well as centralized access to state of the art murine phenotyping equipment for the characterization of genetically engineered mouse models. We are currently developing Standard Operating Procedures for our specialized equipment in order to provide high quality, reproducible and reliable data.

We provide consultation on appropriate methodologies to acquire cardiovascular, metabolic, neuromuscular, or pulmonary data from your mouse model. The lab assists with experimental design, data collection and acquisition, and data analysis. As most platforms require technical expertise, well developed standard operating procedures, and consistent and refined technique we recommend using our laboratory to collect your data for you. We also are happy to train any interested NHLBI scientist on any SOP, technical skill, equipment operation, etc. that you would like to acquire knowledge on.

NHLBI Safety Committee
Ilsa I. Rovira, M.S., Chair, E-mail: rovirai@nih.gov
Shruti Mulki, Occupational Health and Safety Specialist, E-mail: mulkis@mail.nih.gov
The NHLBI Safety Committee was formed to promote a safer working environment by encouraging more staff involvement, improved communication of common safety hazards and a more proactive attitude to safety issues. The members were chosen from laboratory areas to benefit from their collective experience. We intend to gather more practical input of common safety problems. We strive to become a resource for all staff regarding safety questions that come up regularly in laboratories and welcome input from the community to improve safety practices in the Institute.

Office of Biostatistics Research (OBR)
Nancy L. Geller, Ph.D., Director
Rockledge 2, Room 9202; E-mail: gellern@nhlbi.nih.gov
Phone: (301) 435-0434

The OBR collaborates in the planning, design, implementation, monitoring and analyses of studies funded by NHLBI. OBR also provides statistical consultation to any NHLBI investigator who requests advice. OBR has expertise in study design and collaborates in data management and analysis of many studies sponsored by the NHLBI DIR. OBR's primary responsibility is to provide objective, statistically sound, and medically relevant solutions to problems that are presented. When a question raised requires new methodology, the OBR is expected to obtain a new and valid statistical solution. OBR has a broad research mission and the professional staff is often asked to serve on in-house administrative committees as well as advisory committees for other Institutes within NIH and other agencies within DHHS.

Office of Technology Transfer and Development (OTTAD)
Alan H. Deutch, Ph.D., Director
Denise Crooks, Ph.D., Technology Development Specialist
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The OTTAD provides a complete array of services to support the National Heart, Lung, and Blood Institute's technology development activities. To ensure that these activities comply with Federal statutes, regulations and the policies of the National Institutes of Health, a large part of OTTAD's responsibilities includes the day-to-day negotiations of transactional agreements between the NHLBI and outside parties, including universities, pharmaceutical and biotechnology companies. These agreements provide for:

- The exchange of research materials under the Simple Letter of Agreement (SLA) or the Material Transfer Agreement (MTA);
- Collaborative research conducted under cooperative research and development agreements (CRADAs);
- Clinical studies of the safety and efficacy of new pharmaceuticals under clinical trial agreements (CTAs); and

- Exchange of confidential information under confidential disclosure agreements (CDAs)

The OTTAD also reviews employee invention reports, and provides instructions to the NIH's Office of Technology Transfer (OTT) concerning filing of domestic and foreign patent applications. The OTTAD participates in the marketing of NHLBI technologies as well as provides educational presentations and brochures related to technology transfer for NHLBI scientists. Additionally, the OTTAD advises NHLBI scientists on patent rights, policies, and procedures related to technology transfer. The NHLBI OTTAD staff participates in meetings, discussions and conferences, as appropriate, to stay apprised of and monitor the scientists' needs.

Pathology Core Facility
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Web: http://www.nhlbi.nih.gov/research/intramural/researchers/core/pathology-core/index.html

The Pathology Core provides morphologic services for the studies of experimental pathology (animal models) and optimizes the uses of supplies and equipment for histology to all NHLBI DIR scientists. Services include standard histological and tissue preparation, embedding, sectioning and routine histology staining; frozen tissue section, immunohistochemistry and diagnostic pathology. Ongoing interaction of Pathology Core personnel with each investigator facilitates communication regarding histology products, morphologic findings, histopathological interpretation, and new technical developments, thus increasing the efficiency of the research projects. Staff members are well-trained, extremely experienced technicians, and the laboratory has a wide repertoire of specialized techniques. The research pathology and immunohistochemistry are subsequently operating using standard operating procedures based on good lab practice guidelines.

Proteomics Core Facility
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Web: http://www.nhlbi.nih.gov/research/intramural/researchers/core/proteomics-core/index.html

The mission of the NHLBI Proteomics Core Facility is to provide investigators at the NHLBI access to mass spectrometry and gel based proteomics for identification and quantitation of proteins and their posttranslational modifications (PTM). We have state-of-the-art equipment, including latest additions of Orbitrap Velos and 5800 MALDI TOF/TOF. Our workflows for relative protein quantitation are based on DIGE, label-free and iTRAQ approaches. We can also help you identify and quantify protein posttranslational modifications, including phosporylation, nitrosylation, acetylation, etc. We provide training in proper sample preparation and lead the researchers through mass spectrometric analysis to data searching
and interpretation. Users have access to a variety of proteomics software platforms (Sequest, Mascot, Proteome Discoverer, Scaffold, Protein Pilot) for re-searching the data or viewing the results. In addition to helping the NHLBI investigators, we develop new approaches for PTM characterization and absolute protein quantitation.

Poster Session Titles and Assignments

Session I: Odd numbered posters

Session II: Even numbered posters

**Regardless of your session, please mount your poster upon arrival so it may be viewed for the entire retreat.*

1. **Endotoxin Contamination of Apolipoprotein A1: Effect on Macrophage Proliferation – A Cautionary Tale.** X. Jin[1], Q. Xu[1], K. Champion[2], H.S. Kruth[1]. [1]Lab of Experimental Atherosclerosis. [2]BioDtech Inc., Birmingham, AL.

2. **Uncovering Hidden RNA-drug Interactions: When Ligand Binding Both Promotes and Disrupts Folding**. N.J. Baird[1], R. Macarthur[2], J. Inglese[2], and A.R. Ferré-D'Amaré[1]; [1]RNA Biophysics and Cellular Physiology Lab, [2]Assay Development and Screening Technology Lab, NCATS.

3. **Antimicrobial Peptides Piscidins King at a Central Glycine to Maximize their Hydrophobic Moments.** B. S. Perrin Jr[1], R. Fu[2], R. M. Venable[1], E. Mihailescu[3], C. Grant[4], Y. Tian[4], S. Opella[4], R. W. Pastor[1], M. Cotten[5]. [1]NHLBI/NIH, Rockville, MD; [2]National High Magnetic Field Laboratory, Tallahassee, FL; [3]IBBR, University of Maryland, Gaithersburg, MD; [4] Dept. of Chemistry and Biochemistry, UCSD, La Jolla, CA; [5]Hamilton College, Clinton, NY.

4. **Effects of Heterologous Expression of Human Cyclic Nucleotide Phosphodiesterase 3A (hPDE3A) on Redox Regulation in Yeast**. DK Rhee, JC Lim, S Hockman, F Ahmad, YW Chung, S Liu, A Hockman and V Manganiello; Cardiovascular Pulmonary Branch.

5. **Parkin Ameliorates ER Stress in Countering Cardiac Maladaptation to Pressure Overload.** K. Han[1], T.T. Nguyen[2], M.V. Stevens[1], S. Hassanzadeh[1], H. San[3], S. Anderson[4], E. Murphy[2], M.N. Sack[1]; [1]Cardiovascular and Pulmonary Branch, [2]Systems Biology Center, [3]Animal Surgery Program, [4]MRI Imaging Core.

6. **Disabled-1 Attenuates House Dust Mite-induced Airway Inflammationby Suppressing the Recruitment of Intraepithelial Mast Cells**. X. Yao, M. Gao, K. S. Meyer, K. J. Keeran, G. Z. Nugent, K. R. Jeffries, X. Qu, Z. X. Yu, Y. Yang, N. Raghavachari, and S. J. Levine; Laboratory of Asthma and Lung Inflammation, Cardiovascular and Pulmonary Branch.

7. **Alternating-Access Mechanism of the Proton-Drug Antiporter AcrB**. C. Anselmi[1], W. Zhou[1], K. Martin Pos[2] and J.D. Faraldo-Gómez[1]. [1]Theoretical Molecular Biophysics Section; [2]Goethe University, Frankfurt, Germany.

8. **Cytokine and T Cell Changes Upon *in vivo* Ibrutinib Therapy for CLL –Targeting CLL and Microenvironment.** CU Niemann, A Biancotto, B Chang, J Buggy, M Farooqui, and A Wiestner Laboratory of Lymphoid Malignancies.

9. **Molecular Details of α-Synuclein Membrane Association Revealed by Photons**. S. K. Hess, Z. Jiang, J.C. Lee; Laboratory of Molecular Biophysics.

10. **The Macrophage Scavenger Receptor CD163 Suppresses Airway Hyperreactivity in a Murine Model of Experimental Cockroach-induced Asthma**. E.M. Mushaben[1], C. Dai[1], X. Yao[1], G.Z. Nugent[2], K.M. Keeran[2], Z. Yu[3], A. Aponte[4], M. Gucek[4] and S.J. Levine[1]. Laboratory of Asthma and Lung Inflammation, Cardiovascular and Pulmonary Branch[1]; Laboratory of Animal Surgery and Resources Core Facility[2]; Pathology Core Facility[3]; and Proteomics Core Facility[4].

11. **In the Clutches of Maxwell's Demon: Below-Equilibrium DNA Topology Simplification by Type II Topoisomerases**. T.R. Litwin, S.K. Sarkar, A.H. Hardin, I.J. Holt, K.C. Neuman. Laboratory of Molecular Biophysics.

12. **Stimulation of Thrombogenic and Inflammatory Signaling in Endothelial Cells by Elevated Extracellular Sodium: Possible Role in Blood Hypercoagulability, Atherosclerosis and Cardiovascular Health.** N. I. Dmitrieva, M. B. Burg; Renal Cellular and Molecular Biology Section.

13. **Novel IgM-derived antibody-drug conjugate targeting Fcμ-receptor selectively kills chronic lymphocytic leukemia through intracellular cytotoxic payload delivery.** Martin Skarzynski[1], Bérengère Vire[1], Joshua D Thomas[2], Christopher G Nelson[2], Alexandre David[3], GeorgAue[1], Terrence R Burke[2], Christoph Rader[4,5], Adrian Wiestner[1]. [1]Hematology Branch. [2]Chemical Biology Laboratory, Molecular Discovery Program, Frederick National Laboratory for Cancer Research, CCR, NCI, [3]Laboratory of Viral Diseases, NIAID, [4]Experimental Transplantation and Immunology Branch, CCR, NCI, [5]Department of Cancer Biology and Department of Molecular Therapeutics, The Scripps Research Institute, Scripps Florida, Jupiter, FL.

14. **Polo-like kinase 2 activates an antioxidant pathway to promote the survival of cells with mitochondrial dysfunction**. Jie Li, Ping-yuan Wang and Paul M. Hwang. Center of Molecular Medicine.

15. **Whole Exome Sequencing to Establish a Genetic Etiology for Pentalogy of Cantrell: In Rare Diseases the Code can be Difficult to Decipher**. M. A. Markowitz[1], X. Ma[1], L. Leatherbury[2], Y. Yang[3], J. Zhu[3], J. Chong[4], M. J. Bamshad[4], R. S. Adelstein[1], E.K. Grant[1, 2]; [1]NHLBI Laboratory of Molecular Cardiology, [2]Children's National Medical Center, [3]NHLBI Genomics Core Facility and [4]University of Washington Centers for Mendelian Genomics.

16. **The Relationship Between Sleep and Evolutionary Fitness in *Drosophila melanogaster*.** A.S. Lobell, S.T. Harbison; Laboratory of Systems Genetics.

17. **Cardiometabolic risk factors predict vascular disease beyond Framingham risk score in the systemic inflammatory disorder psoriasis.** S. Rose, M.D., Ph.D.[1,2], P. Krishnamoorthy, M.D.[3] J. Dave, M.S.[1], H. Naik, M.D.[4], M. P. Playford, Ph.D.[1], N. N. Mehta M.D., M.S.C.E[1]. [1]Section of Inflammation and Cardiometabolic Diseases, [2] NIAMS, [3] Englewood Hospital, Englewood, NJ. [4]NCI.

18. **A conserved mechanism of cellular and retroviral mRNA evasion of NMD** Z. Ge, J. Hogg; Laboratory of Ribonucleoprotein Biochemistry.

19. **TGF-ß signaling positively regulates lymphatic endothelial cell sprouting in vitro**. B. Shepherd, J. James, Y. Mukouyama. Laboratory of Stem Cell and Neuro-Vascular Biology. Genetics and Developmental Biology Center.

20. **Temporal Mapping of Enteric Neurovascular Development and Villus Morphogenesis**. J. Hatch and Y. Mukouyama. Laboratory of Stem Cell & Neuro-Vascular Biology.

21. **Arf Guanine Nucleotide-exchange Factors BIG1 and BIG2 Regulate β-catenin Signaling via Direct Interaction and cAMP/PKA Signaling**. K. Le, CC. Li, J. Moss, M. Vaughan; Cardiovascular and Pulmonary Branch.

22. **Regulation of UPF1 binding to 3'UTRs**. T. Nguyen, S.L. Baker, J.R. Hogg; Laboratory of Ribonucleoprotein Biochemistry

23. **Inhibition of nonsense-mediated mRNA decay by translational readthrough**. S. Baker and J.R. Hogg.

24. **Collagen: Order, Disorder, and Dynamics at Super-Resolution.** A. Dittmore, S.K. Sarkar, B. Marmer, G.Goldberg, K.C. Neuman; Laboratory of Molecular Biophysics.

25. **ATP Binding Induced Conformational Change in RECQ Helicase**. M. Mills, K. Neuman; Laboratory of Molecular Biophysics.

26. **Conformational Rearrangements in Bax as Apoptosis is Initiated: Inner-Workings of a Cellular Death Switch**. R. F. Gahl, N. Tjandra. Laboratory of Molecular Biophysics, Biochemistry and Biophysics Center.

27. **Role of CD73 in osteogenic differentiation.** J.M. Davaine[1,2], J. Hui[1], C. St Hilaire[1], G. Chen[1], M. Boehm[1]. 1- Laboratory of Cardiovascular Regenerative Medicine, NHLBI**.** 2- Unité Inserm UMR-957, Nantes, France.

28. **A Multi-Species Epigenetic Signature of Differentiating Embryonic Stem Cells Uncovers Regulators of Cardiogenesis**. B. W. Busser[1], G. Chen[2], A. M. Michelson[1]; [1]Laboratory of Developmental Systems Biology, [2]NHLBI iPSC and Genome Engineering Core.

29. **Identification of Readers of the H3.3 Histone Variant Code**. D.C. Kraushaar, S. Patel, G. Wang, Q. Tang, M. Gucek, K. Zhao. Systems Biology Center, Laboratory of Epigenome Biology.

30. **Cardiomyocyte differentiation from human pluripotent stem cells in chemically defined E8 medium.** Y. Lin, K. Linask, W. Xie, Y. Du, J. Beers, and G. Chen. iPSC and Genome Engineering Core Facility.

31. **Amyloid Triangles, Squares, and Loops of Apolipoprotein C-III**. M. de Messieres, R. K. Huang, Y. He, J. C. Lee; Laboratory of Molecular Biophysics, Protein Expression Facility, and Laboratory of Structural Biology Research.

32. **Facile fluorine-18 labeling of serum albumins via the coupling of [^{18}F]fluoronicotinic acid tetrafluorophenyl ester with albumin amine groups.** F. Basuli[1], O. Vasalatiy, B. Xu[1], G. L. Griffiths[1], E.

Jagoda[2], M. Green[2], J. Seidel[2], P. Choyke[2], R. Swenson[1]. [1]Imaging Probe Development Center, [2]Molecular Imaging Program, NCI.

33. **Synthesis and Characterization of ^{153}Gd radiolabeled Eovist for Prostate Cancer Studies**. V. Coble[1], O. Vasalatiy[1], P. Choyke[2], R. Swenson[1]. 1Imaging Probe Development Center, 2Molecular Imaging Program, NCI.

34. **LIM Domains as Cytoskeletal Strain Sensors**. R.C. Cail, G.M. Alushin; Laboratory of Macromolecular Interactions.

35. **Neuro-vascular wiring in obese mice**. T. Yamazaki, W. Li, Y. Mukouyama. Laboratory of Stem Cell and Neuro-Vascular Biology.

36. **A mitochondrial protein GCN5L1 regulates hepatic glucose production and insulin signaling pathway**. L. Wang, I. Scott, L. Zhu, K. Han, M. Sack; Cardiovascular and Pulmonary Branch.

37. **Membrane Remodeling by α-Synuclein and Effects on Amyloid Formation.** Z. Jiang, M. de Messieres, and J.C. Lee. Laboratory of Molecular Biophysics.

38. **Rbfox3-regulated Alternative Splicing of Numb Promotes Neuronal Differentiation during Development.** K.K. Kim[1], J. Nam[2], Y. Mukouyama[2] and S. Kawamoto[1]**.** [1]Laboratory of Molecular Cardiology, [2]Laboratory of Stem Cell and Neuro-Vascular Biology.

39. **Metabolomic Profiling in Psoriasis Patients May Provide a Link between Inflammation and Cardiometabolic Diseases**. E. Weiner[1], S. Rose[1], J. Doveikis[1], P. Krishnamoorthy[2], M. P. Playford[1], J. M. Gelfand[3], N. N. Mehta[1]. [1]Section of Inflammation and Cardiometabolic Diseases, [2]Englewood Hospital, Englewood, NJ. [3]University of Pennsylvania, Philadelphia, PA.

40. **How does the metabolic microenvironment trigger the vascular patterning factor Cxcl12 and arterial differentiation factor VEGF-A in the nerve-mediated arterial branching?** W. Li, A. Sajja, Y. Mukouyama. Laboratory of Stem Cell and Neuro-Vascular Biology.

41. **In PDE3B KO Mice, White Epididymal Adipose Tissue (WAT) Exhibits Phenotypic Characteristics of "Fat-burning" Brown Adipose Tissue (BAT), with reduced expression of pro-inflammatory markers, including the NLRP3 Inflammasome**. F.A. Khan[1], Y.W. Chung[1] ,Y. Tang[1], S.C. Hockman[1],K. Berger[2], E.M. Billings, O. Gavrilova[3], E. Guirguis[1],S. Liu[1], Y. Khan[1], K. Huo[1], D.M. Schimel[4], A.M. Aponte[5], E. Degerman[2], V.C. Manganiello[1]. [1]Cardiovascular Pulmonary Branch, [2]Department of Experimental Medical Science, Lund University, Sweden. [3]Mouse Metabolism Core, NIDDK, [4]NIH MRI Research Facility. [5]Proteomics Core Facility.

42. **Sirt5 functions as a desuccinylase in cardiac mitochondria**. JA. Boylston[1], Y. Chen[2], M. Gucek[2], MN. Sack[3], E. Murphy[1]; [1]Systems Biology Center, [2]Proteomics Core Facility, [3]Center for Molecular Medicine.

43. **CD73 Regulates Inflammation during Vascular Remodeling**. J. D. Lanzer, C. St. Hilaire, R. Schwartzbeck, H. San, F. Fang, M. Boehm. Laboratory of regenerative cardiovascular medicine.

44. **A Detailed Centrosome Interactome Reveals a Novel Role for Asterless/CEP152 in Controlling Centriole Length Via Cep97**. B. J. Galletta[1], K.C. Jacobs[1], C. J. Fagerstrom[1], K. C. Slep[2], N. M. Rusan[1]; [1]Cell Biology and Physiology Center, [2]Department of Biology, University of North Carolina, Chapel Hill, NC.

45. **Identification and Analysis of Cardiogenic Subnetworks Regulated by Forkhead (Fox/Fkh) Domain Transcription Factors.** S. M. Ahmad[1], P. Bhattachryya[1], N. Jeffries[2], S. S. Gisselbrecht[3], A. M. Michelson[1]; [1]Laboratory of Developmental Systems Biology, [2]Office of Biostatistics Research, [3]Division of Genetics, Department of Medicine, Brigham and Women's Hospital.

46. **Developing Assays of Angiogenesis in Cyanotic Congenital Heart Disease Patients with Aortopulmonary Collateral Vessels**. N. Sandeep, Y. Mukoyama; Laboratory of Stem Cell and Neuro-Vascular Biology.

47. **Calmodulin Regulates the Behavior of Abnormal Spindle to Coordinate Mitotic Spindle Dynamics**. T. Schoborg, B. J. Galletta, N. M. Rusan; Laboratory of Molecular Machines & Tissue Architecture.

48. **Opposing actions of IL-2 and IL-21 on Th9 differentiation inversely correlate with BCL6 expression in CD4+ T cells.** W. Liao, R. Spolski, P. Li, N. Du, E. E. West, M. Ren, S. Mitra, and W. J. Leonard. Laboratory of Molecular Immunology and the Immunology Center.

49. **Zinc finger RNA binding protein (ZFR) protects macroH2A mRNA from degradation**. N. Haque and J.R. Hogg. Laboratory of Biochemistry.

50. **Vascular Inflammation by FDG PET-CT is Associated with Aortic Wall Properties by MRI**. A Sadek, J Dave, J Hasan, P Krishnamoorthy, S. Rose, H. Naik, E. Weiner, M.A. Ahlman, D.A. Bluemke, N.N. Mehta; Section of Inflammation and Cardiometabolic Diseases.

51. **Ischemic preconditioning preferentially increases protein S-nitrosylation in subsarcolemmal but not interfibrillar mitochondria.** J. Sun[1], T. Nguyen[1], A. Aponte[2], E. Murphy[1]; [1]Laborotary of Cardiac Physiology, Systems Biology Center, [2]Proteomic Core Facility.

52. **Monocyte Subsets Predict Vascular Inflammation Beyond Traditional Risk Factors of Cardiovascular Disease in Psoriasis**. E Stansky, S Rose, P Krishnamoorthy, E Weiner, J Dave, N.N. Mehta; Section of Inflammation and Cardiometabolic Diseases; FACS Core.

53. **Semaphorin3G-Neuropilinp2 signaling influences arterio-venous alignment in the skin**. Y. Uchida, Y. Mukouyama; Laboratory of Stem Cell and Neuro-Vascular Biology.

54. **Regulators of MT Dynamics in the Spindle**. A.L. Zajac, C.J. Fagerstrom, N.M. Rusan; Laboratory of Molecular Machines and Tissue Architecture.

55. **Novel Pericentrin structures orchestrate the PCM in rapidly dividing embryos**. D.A. Lerit[1], J.S. Poulton[2], H.A. Jordan[1], M. Peifer[2], and N.M. Rusan[1]; [1] Laboratory of Molecular Machines and Tissue Architechture, [2]Department of Biology University of North Carolina Chapel Hill.

56. **Cancer cell migration, blebbing and the actin cortex**. J.S. Logue, R.S. Chadwick[2], C.M. Waterman[1], . [1]Laboratory of Cell and Tissue Morphodynamics. [2]Section on Auditory Mechanics, Laboratory of Cellular Biology, NIDCD.

57. **Structural Analysis of the 5´-UTR of the HIV-1 Genome.** C. P. Jones and A. R. Ferré-D'Amaré**.** Laboratory of RNA Biophysics and Cellular Physiology.

58. **Development of in vitro and in vivo human disease models to study mechanisms regulating vascular calcification**. C. St. Hilaire, H. Jin, J-M. Davaine, G. Chen and M. Boehm. NHLBI, Laboratory of Cardiovascular Regeneration.

59. **Effects of Rosuvastatin on the Lipoprotein Subfractions, Protein Composition and Function***.* S.M. Gordon, G. Kemeh, A.T. Remaley. Lipoprotein Metabolism Section/Cardiovascular Pulmonary Branch.

60. **Emerging Evidence of Lysosomal Degradation of α-Synuclein by Cysteine Cathepsins**. R. P. McGlinchey, J. C. Lee; Laboratory of Molecular Biophysics.

61. **Directly Measured Inflammation in Psoriasis Skin Associates with Vascular Inflammation Beyond Traditional Risk Factors.** H.B. Naik, P Krishnamoorthy, J. Dave, S. Rose, N.N. Mehta; Section of Inflammation and Cardiometabolic Diseases.

62. **Imaging Flow Cytometry in the NHLBI Flow Cytometry Core Facility: Combining Statistical Power with High Resolution Single Cell Imaging**. L. Samsel, J. P. McCoy; NHLBI Flow Cytometry Core Facility.

63. **Machine-learning Identifies Predictive Signatures of Enhancer Activity in Individual Cardiac Cells**. J.S. Haimovich, B.W. Busser, D. Huang, I. Ovcharenko, A.M. Michelson; Laboratory Developmental Systems Biology.

64. **A Point Mutation in Nonmuscle Myosin II-A Disrupts Mouse Spermatogenesis**. C.B. Lerma Cervantes,[1] K. Tokuhiro,[2] Y. Zhang,[1] M.A. Conti,[1] D. H. Bae,[3] M.P. Daniels,[3] J. Dean,[2] R.S. Adelstein,[1]; [1]Laboratory of Molecular

Cardiology, [2]Laboratory of Cellular and Developmental Biology, [3]Electron Microscopy Core. Nonmuscle Myosin II Isoforms Coassemble in Living Cells

65. **Aberrant Resting State Brain Connectivity in Sickle Cell Patients with Frequent pain.** DS. Darbari, JG. Taylor, J. Hampson, E. Ichesco, N. Kadom, I. Evangelou, G. Vezina, D. Clauw, R. Harris. Children's National Medical Center, George Washington University, Washington, DC, MD University of Michigan, Ann Arbor, MI.

66. **A Novel Actin-Adhesion Structure Involved in Nuclear Positioning Requires the Formin FMN2**. C.T. Skau, C. M. Waterman. Laboratory of Cell and Tissue Morphodynamics.

67. **Programmed Death-1 Receptor and Myeloid-Derived Suppressor Cells Have Protective Roles in Drug-Induced Allergic Hepatitis in Mice.** K. M. Semple[1], M. Chakraborty[1], A. Fullerton[1], D. E. Kleiner[2], L. R. Pohl[1]. [1]Molecular and Cellular Toxicology Section, LMI, [2]Laboratory of Pathology, NCI.

68. **High-resolution analysis of genome-wide long-distance chromatin interactions using sureHi-C**. G. Ren, W. Jin, K. Cui, Q. Tang, K. Zhao, Systems Biology Center.

69. **Cardiac prolyl hydroxylation influences protein degradation and stability**. A. Stoehr, A.M. Evangelista, S. Menazza, E. Murphy.

70. **Sirt3 modulates nutrient-dependent NLRP3 inflammasome regulation**. J. Traba[1], T. Okoli[1], M. Pelletier[2], K. Han[1], R.M. Siegel[2], M.N. Sack[1]. [1]Laboratory of Mitochondrial Biology in Cardiometabolic Syndromes, Cardiovascular and Pulmonary Branch, [2]NIAMS.

71. **Molecular modeling of membrane curvature induction by a peptide**. A. Sodt, R. Pastor. Laboratory of Computational Biology.

72. **Induced Pluripotent Stem Cells from a Patient with *GATA2* Deficiency Display a Block in Hematopoietic Differentiation**. M. Jung, T. Winkler, F. Donaires, S. Hong, A. Hsu, D. Hickstein, S. Holland and C. Dunbar. Molecular Hematopoiesis Section, Hematology Branch.

73. **KMT2D histone methyltransferase is a positive regulator of T cell development.** K. Placek, K. Cui, G. Hu, W. Jin, K. Zhao, Systems Biology Center.

74. **CD146 expression on T cells identifies Th17 and Tc17 subsets in psoriatic blood and skin.** P.K. Dagur[1], E. Stansky[1], S. Rose[3], A. Biancotto[2], H.B. Naik[4], M. Playford[3], N.N. Mehta[3], J.P. McCoy, Jr[1,2]. [1]Hematology Branch, [2]Center for Human Immunology, Autoimmunity and Inflammation, [3]Section of Inflammation and Cardiometabolic Diseases, [4]Dermatology Branch, NCI.

75. **Proteome-Wide Identification of PRG-3-Interacting Proteins Reveals a Functional Interaction among PRG Family Members.** P. Yu[1], C. Agbaegbu[1, 2], D.A. Malide[3], Y. Katagiri[1], H.M. Geller[1]. [1]Developmental Neurobiology Section, [3]Light Microscopy Core Facility, [2]Department of Physics, Georgetown University.

76. **Low-dose Phase-Contrast Imaging of a Mammography Phantom Using Talbot Interferometry**. K. Harmon[1], H. Miao[1], A. Gomella[1], E. Bennett[1], P. Bhandarkar[2], H. Wen[1]. [1] Imaging Physics Laboratory, [2] Breast Imaging Center, Walter Reed Army Medical Center.

77. **High Density Lipoprotein suppresses inflammasome activation in response cholesterol crystals and other particulates**. S. G. Thacker, D. O. Sviridov, S. Demosky, A. T. Remaley.

78. **Clonal Tracking of Rhesus Macaque Hematopoiesis Highlights a Distinct Lineage Origin for Natural Killer Cells**. S.J. Koelle, C. Wu, B. Li, R. Lu, Y. Yang, A. Jares, A.E. Krouse, M. Metzger, F. Liang, K. Loré, C.O. Wu, R.E. Donahue, I.S. Chen, I. Weissman, C.E. Dunbar.; Molecular Hematopoiesis Section, Hematology Branch.

79. **Functional niche competition between normal and leukemic stem cells**. C. Glait-Santar; X. Feng; J. Chen; R. Desmond; B. Mizukawa; J. Mulloy; N. Young; C.E. Dunbar. Molecular Hematopoiesis section.

80. **Path to the clinic: Assessment of iPSC-based cell therapies *in vivo* in a non-human primate model.** S.G. Hong[1], T. Winkler[1], C. Wu[1], V. Guo[1], S. Pittaluga[2], A. Nicolae[2], R.E. Donahue[1], M.E. Metzger[1], S.D. Price[1],

N.Uchida[3], S.A. Kuznetsov[4], T.Kilts[4], L. Li[4], P.G. Robey[4], and C.E. Dunbar[1]. [1]Hematology Branch, [2]Laboratory of Pathology, CCR, NCI, [3]Molecular and Clinical Hematology Branch, NIDDK, [4]Craniofacial and Skeletal Diseases Branch, NIDCR.

81. **Differential Binding of Receptor Protein Tyrosine Phosphatase Type IIa Family (RPTPσ/RPTPδ/LAR) to Glycosaminoglycans**. Y. Katagiri, A.A. Morgan, P. Yu, N.J. Bangayan, R. Junka, and H.M. Geller. Developmental Neurobiology Section, Cell Biology and Physiology Center.

82. **Identifying transcriptional targets of vasopressin using combined ChIP-seq and RNA-seq analysis of mpkCCD cells.** J. S. Claxton, P. C. Sandoval, J. W. Lee, J. D. Hoffert, F. Saeed, M. A. Knepper.

83. **Identification of H2A.Z-interacting Proteins Using a High-throughput in Vivo Protein-protein Interaction Assay**. Y. Zhang[1,2], W. Jin[1], Q. Tang[1], B. Ni[2], K. Zhao[1], [1]Systems Biology Center, [2]Third Military Medical University, China.

84. **Aquaporin-2 interactome in rat inner medullary collecting duct**. C.L. Chou, D. Hageman, M. A. Knepper. Epithelial Systems Biology Laboratory.

85. **An Alternative Mechanism for Actin Arc Formation at the Immunological Synapse of Jurkat T cells**. S. Murugesan, J. Yi, D. Li, L. Shao, E. Betzig, X. Wu, J.A. Hammer; Lab of Cell Biology.

86. **Selective Propagation of Functional mtDNA During Oogenesis Restricts the Transmission of A Deleterious Mitochondrial Variant**. J. H. Hill, Z Chen, and H Xu; Laboratory of Molecular Genetics.

87. **Exploring myosin-5 elasticity under strain**. A. Lopata[1,2], A. Nagy[2], N. Billington[2], M. Peckham[1], P. Knight[1], J. Sellers[2]; [1]University of Leeds, UK; [2]Laboratory of Molecular Physiology.

88. **T-cell Mediated Graft-vs-tumor Effect against Kidney Cancer after Allogeneic Stem Cell Transplant Identifies an Immunogenic Human Endogenous Retrovirus Type-E (HERV-E) with Selective Expression in Clear Cell Kidney Cancer.** E. Cherkasova, P. Scrivani, N. Harashima, S. Kagigaya, Y. Takahashi,and R. W. Childs; Hematology Branch.

89. **Generation of HERV-E specific CD8+ T-cells for immunotherapy of renal cell carcinoma.** C. Scrivani, E. Cherkasova, and R.W. Childs. Hematology Branch.

90. **Experimental Pain Phenotyping in Adults with Sickle Cell Anemia and Normal Volunteers**. K. Vaughan, D. Darbari, M. Quinn, C.A. Seamon, G. Wallen, I. Belfer, J.G. Taylor VI. Genomic Medicine Section, Hematology Branch, University of Pittsburgh and Nursing Department, Clinical Center, Division of Blood Diseases, Children's National Medical Center.

91. **Structural Basis of the Vinculin–F-actin Interaction.** K.Y. Kim[1], P.M. Thompson[2], S. L. Campbell[2], G.M. Alushin[1], [1]Laboratory of Macromolecular Interactions [2]Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill.

92. **Characterization of Endocytosis in T Cells.** D. Johnson[1, 2], J. Wilson[1], J. G. Donaldson[2], [1]Cellular and Molecular Medicine, University of Arizona, [2]Cell Biology and Physiology Center.

Abstracts in Order of First Author

Identification and Analysis of Cardiogenic Subnetworks Regulated by Forkhead (Fox/Fkh) Domain Transcription Factors. S. M. Ahmad[1], P. Bhattachryya[1], N. Jeffries[2], S. S. Gisselbrecht[3], A. M. Michelson[1]; [1]Laboratory of Developmental Systems Biology, [2]Office of Biostatistics Research, [3]Division of Genetics, Department of Medicine, Brigham and Women's Hospital.

Cardiogenesis involves the integration of multiple cellular specification and differentiation processes. A detailed understanding of heart development thus requires the identification and functional analyses of the cardiogenic subnetworks (comprising both upstream regulators and downstream effectors) that mediate each of these developmental processes. Multiple Forkhead (Fox/Fkh) domain transcription factors (TFs) regulate heart development in mammals. In *Drosophila*, which exhibits remarkable conservation of mammalian cardiac subnetworks, two Fkh genes, *jumu* and *CHES-1-like*, also participate in cardiogenesis. We compared genome-wide transcription expression profiles of flow cytometry-sorted mesodermal cells from wild-type, *jumu* loss-of-function, *jumu* overexpressing, *CHES-1-like* loss-of-function, and *CHES-1-like* overexpressing embryos to identify the Fkh-regulated target genes comprising these subnetworks. Significant overrepresentation of Gene Ontology attributes of these targets suggests that both Fkh TFs mediate subnetworks involved in processes as diverse as cardiac progenitor cell division, heart lumen formation, cell adhesion involved in heart morphogenesis, and myofibril formation. Ongoing functional analyses involve RNA-interference-based knockdown-screening and classical genetic studies of candidate Fkh targets to search for cardiac phenotypes. As an example, we present preliminary results involving phenotypic analyses, synergistic genetic interactions, ChIP binding data, and enhancer activity to suggest that the Fkh TFs govern cardiac mesoderm fate specification by regulating the fibroblast growth factor receptor Heartless. Genome-wide ChIP-seq binding data for these Fkh TFs are being used to distinguish direct from indirect transcriptional targets, and also utilized in computational approaches to identify the enhancers that drive the cardiac expression of the corresponding genes. Subsequent regulatory analyses of these enhancers combined with functional experiments will provide a detailed systems-level understanding of Fkh-regulated cardiogenic subnetworks.

Alternating-Access Mechanism of the Proton-Drug Antiporter AcrB. C. Anselmi(1), W. Zhou(1), K. Martin Pos(2) and J.D. Faraldo-Gómez(1). (1) Theoretical Molecular Biophysics Section; (2) Goethe University, Frankfurt, Germany.

The AcrA/AcrB/TolC multidrug efflux pump confers Escherichia coli with antibiotic resistance by sequestering toxic compounds found within the periplasm and inner membrane and extruding them into the extracellular space. The AcrB trimer is the central component of this efflux complex. Anchored in the inner membrane, it forms an asymmetric assembly that undergoes a conformational cycle in which each protomer adopts three different structures (L, T and O states). Importantly, the conformational cycle within AcrB is driven by the translocation of protons down the electrochemical gradient sustained by the inner membrane, through a mechanism that has not been characterized so far. Here, we investigate this microscopic mechanism through structural modeling, electrostatic calculations and molecular dynamics simulations based upon novel high-resolution structural data.

Our results show how reversible protonation of key sites within the transmembrane domain translate into structural changes that are transduced to the periplasmic domain, thus coupling proton and drug transport. These conformational changes can be understood in terms of semirigid roto-translations of two topologically equivalent 5-helix repeats. Importantly, we show that these changes result in the opening and closing of water-filled pathways that reach from either side of the membrane into the proton binding site, which is found at the interface between repeats, half-way through the membrane. More specifically, the transition between the L T, and O states of the functional cycle represents a change from inward to outward to inward-facing conformations. Interestingly, our analysis also shows that the structural changes associated with this alternating-access mechanism differ in the proton-free (L to T) and proton-loaded (T to O) portions of the cycle, a feature that might be shared by other ion-coupled secondary transporters. Finally, we propose a mechanism of strict coupling between transmembrane and periplasmic domains, and thus between proton-import and drug-efflux.

Uncovering Hidden RNA-drug Interactions: When Ligand Binding Both Promotes and Disrupts Folding. N.J. Baird[1], R. Macarthur[2], J. Inglese[2], and A.R. Ferré-D'Amaré[1]; [1]RNA Biophysics and Cellular Physiology Lab, and [2]Assay Development and Screening Technology Lab, NCATS.

Stabilization of RNA tertiary structures is achieved through the binding of cations, small molecules, and proteins. These ligands function by neutralizing the polyanionic backbone or promoting specific structural interactions. Conformational changes in response to varying cellular ligand concentrations regulate RNA function, as observed in bacterial riboswitches. Equilibrium RNA folding studies are used to examine conformational changes in response to various ligands. These studies routinely employ titrations of individual ligands one at a time. Here we employ a novel RNA folding FRET methodology to obtain multi-dimensional data probing the role of cations, ligands, and temperature in a single experiment. For the cyclic-di-GMP (c-di-GMP) riboswitch, the FRET efficiency (E_{FRET}) resulting from a 2D titration with varying magnesium and c-di-GMP concentrations exhibits a response that can be described by a smooth three-dimensional surface. Both Mg^{2+} and c-di-GMP promote higher E_{FRET}, characteristic of the folded conformation. Unsurprisingly, increased Mg^{2+} concentrations result in higher c-di-GMP binding affinity. In contrast, 2D titrations varying Mg^{2+} and aminoglycoside concentrations exhibit complex surfaces with multiple, distinct E_{FRET} populations. For example, at a single Mg^{2+} concentration, addition of Kanamycin B results in both a decrease and an increase in E_{FRET} as a function of

ligand concentration. Temperature melt analysis of this complex multi-dimensional surface reveals that the various E_{FRET} populations represent distinct RNA conformations, including an off-pathway conformation. These data indicate that binding of the cyclic-di-GMP riboswitch by Kanamycin B is specific and is not due to simple electrostatic charge neutralization. Overall our data demonstrate the importance of probing the interaction of multiple solution parameters when investigating the binding of ligands to RNA. Since our multi-dimensional analysis can identify otherwise hidden interactions, it is likely to be a powerful new high-throughput tool guiding the discovery of novel RNA ligands.

Inhibition of nonsense-mediated mRNA decay by translational readthrough. S. Baker and J.R. Hogg.

The nonsense-mediated mRNA decay (NMD) pathway plays an important role in modulating the severity of many genetic diseases by recognizing and degrading mRNAs containing premature termination codons. The selective decay of aberrant mRNAs is carried out by the RNA helicase Upf1 and several additional NMD factors.

Previously, we have found that retroviral recoding elements promoting translational frameshifting or readthrough antagonize NMD at two distinct steps. Relatively frequent stop codon bypass can reduce steady-state accumulation of Upf1 in mRNPs, disrupting its ability to monitor 3'UTR length. In addition, experiments using variants of the Moloney murine leukemia virus (M-MLV) recoding pseudoknot show that less frequent readthrough events permit recovery of Upf1 binding to mRNPs but remain able to inhibit degradation of mRNAs containing intronless long 3'UTRs. These findings point to a two-step model for Upf1-dependent recognition and decay of long 3'UTR-containing transcripts. In this model, Upf1 accumulation in mRNPs is a prerequisite for decay but is not sufficient to induce transcript destruction. Instead, the initiation of decay requires the completion of one or more additional rate-limiting steps susceptible to disruption by translational readthrough events.

We are currently investigating the mechanism by which rare translational readthrough events prevent commitment to RNA decay despite efficient recruitment of Upf1. As part of these studies, we have constructed a dual-fluorescent protein reporter that can be used to simultaneously assay mRNA decay, translational readthrough, and the protein composition of mRNPs. With this system, we are comparing the ability of distinct readthrough-promoting elements to inhibit NMD and analyzing the effects of readthrough on recruitment of Upf1 and other NMD proteins to mRNPs.

Facile fluorine-18 labeling of serum albumins via the coupling of [^{18}F]fluoronicotinic acid tetrafluorophenyl ester with albumin amine groups. F. Basuli[1], O. Vasalatiy, B. Xu[1], G. L. Griffiths[1], E. Jagoda[2], M. Green[2], J. Seidel[2], P. Choyke[2], R. Swenson[1]. [1]Imaging Probe Development Center. [2]Molecular Imaging Program, NCI.

Our objective is to develop an improved, optimized, simple, reproducible and clinically translatable method to label serum albumins with fluorine-18 for use as a blood pool imaging agent. Fluorine-18 labeling of serum albumin was achieved by an indirect method. Fluorine-18 radiolabeled fluoronicotinic acid-2,3,5,6-tetrafluorophenyl ester was prepared first by the reaction of its quaternary ammonium triflate precursor with [^{18}F]TBAF according to a peptide F-18 radiolabeling literature method, with modifications. Final conjugation of the serum albumin with [^{18}F] fluoronicotinic acid-2,3,5,6-tetrafluorophenyl ester in phosphate buffer, pH 9, for 20 min produced fluorine-18-radiolabeled albumins (rat & human). Purification of the products was done using a mini-PD MiniTrap G-25 column. The conjugation yield was 92-98% within 20 min at 37-40 ^{o}C. The overall radiochemical yield of the reaction was 18-35% (n= 30, uncorrected) in a 90 minute radiolabeling time. Fluorine-18 labeling of serum albumins was successfully accomplished with a moderate overall radiochemical yield in a 90 minute synthesis time. No time consuming HPLC purifications of intermediates or products are required with this method. Preclinical imaging and testing will be done primarily on rat [^{18}F]-albumin prior to planned clinical development of human [^{18}F]-albumin.

Nonmuscle Myosin II Isoforms Coassemble in Living Cells. J. R. Beach, L. Shao, K. Remmert, D. Li, E. Betzig, J. A. Hammer 3rd; Cell Biology and Physiology Center.

Nonmuscle myosin II (NM II) powers myriad developmental and cellular processes, including embryogenesis, cell migration, and cytokinesis. To exert its functions, monomers of NM II assemble into bipolar filaments that produce a contractile force on the actin cytoskeleton. Mammalian cells express up to three isoforms of NM II (NM IIA, IIB, and IIC), each of which possesses distinct biophysical properties and supports unique as well as redundant cellular functions. Despite previous efforts, it remains unclear whether NM II isoforms assemble in living cells to produce mixed (heterotypic) bipolar filaments or whether filaments consist entirely of a single isoform (homotypic). We addressed this question using fluorescently tagged versions of NM IIA, IIB, and IIC, isoform-specific immunostaining of the endogenous proteins, and two-color total internal reflection fluorescence structured-illumination microscopy, or TIRF-SIM, to visualize individual myosin II bipolar filaments inside cells. We show that NM II isoforms coassemble into heterotypic filaments in a variety of settings, including various types of stress fibers, individual filaments throughout the cell, and the contractile ring. We also show that the differential distribution of NM IIA and NM IIB typically seen in confocal micrographs of well-polarized cells is reflected in the composition of individual bipolar filaments. Interestingly, this differential distribution is less pronounced in freshly spread cells, arguing for the existence of a sorting mechanism acting over time. Together, our work argues that individual NM II isoforms are potentially performing both isoform-specific and isoform-redundant functions while coassembled with other NM II isoforms.

Sirt5 functions as a desuccinylase in cardiac mitochondria. JA. Boylston[1], Y. Chen[2], M. Gucek[2], MN. Sack[3], E. Murphy[1]; [1]Systems Biology Center, [2]Proteomics Core Facility, [3]Center for Molecular Medicine.

Many studies demonstrate a relationship between diet, nutritional status, and the occurrence of cardiovascu-

lar disease. The Sirtuin protein family includes NAD^+ dependent deacylases that have been strongly conserved by evolution. These proteins are expressed to varying degrees across many tissue types where they modulate metabolic and stress response pathways. Sirtuin dependence on NAD^+ for activity intimately links their function with an organism's nutritional status. Sirtuins (Sirt) 3, 4 and 5 have been shown to localize to the mitochondria. Sirt3 has been shown to function as a deacetylase. It has recently been reported that Sirt5 can act as a desuccinylase in liver mitochondria. Succinylation is the post-translational modification of lysine residues with succinyl moieties, which has been shown to regulate the activity of metabolic enzymes. The goal of this study was to investigate whether Sirt5 acts as a desuccinylase in cardiac mitochondria. We characterized the succinylome of mouse heart mitochondria using proteomic methods and mass spectrometry. We isolated mitochondria from hearts of wild type (WT) mice and mice lacking Sirt5 (Sirt5-KO) and identified 2786 succinylated peptides which map to 493 proteins. A comparative analysis reveals 1467 peptides in WT samples and 2175 in Sirt5-KO. Of these, 611 are unique to WT, 1320 are unique to Sirt5-KO, and 855 overlap between the two datasets. These data demonstrate a large number of succinylated peptides in WT heart mitochondria and consistent with Sirt5 functioning as a desuccinylase, the number of succinylated peptides increases in Sirt5-KO cardiac mitochondria.

A Multi-Species Epigenetic Signature of Differentiating Embryonic Stem Cells Uncovers Regulators of Cardiogenesis. B. W. Busser[1], G. Chen[2], A. M. Michelson[1]; [1]Laboratory of Developmental Systems Biology, [2]NHLBI iPSC and Genome Engineering Core.

Recent studies have characterized the histone mark distribution and expression profiles of differentiating human and mouse embryonic stem cells (ESCs) to uncover the *cis*-regulatory elements and transcript patterns which characterize the mammalian cardiac lineage. Here we used these data sets to generate a predictive signature of candidate genes that control mouse and human cardiomyocyte (CM) differentiation from ESCs. We reasoned that the histone modifications associated with genes critical for CM differentiation will show marks of repression during the ESC state and marks of activation when pluripotent cells become restricted as cardiac precursors (CPs). Consistent with this hypothesis, the expression of such candidate genes is repressed in ESCs and upregulated in CPs. Furthermore, gene ontology analysis reveals an enrichment for genes involved in the regulation of transcription and having cardiac differentiation functions. To evaluate the potential cardiogenic activities of the identified mammalian genes, we undertook a large-scale RNAi-based screen of the corresponding *Drosophila* orthologs. This approach uncovered dozens of novel regulators of fly heart development whose functions are currently being tested in differentiating human ESCs. Finally, we show that the overlapping mouse and human genes are the best candidates for having cardiac regulatory activities as this group contains genes showing greater evolutionary conservation and enrichment for cardiogenic function and relevant gene ontology functional categories. In total, these results show that an epigenetic signature can be used to uncover the genes which characterize the cardiac lineage, and that the integration of orthologous data sets improves the predictive power of epigenetic findings alone.

LIM Domains as Cytoskeletal Strain Sensors. R.C. Cail, G.M. Alushin; Laboratory of Macromolecular Interactions.

The ability of cells to sense and respond to forces ("mechanosense") plays an important role in cellular development, behavior, and motility; stem cells differentiate in part based on the stiffness of their environment, and cancer cells migrate towards stiffer microenvironments. Cells connect to the extracellular matrix (ECM) through integrin-mediated focal adhesions (FAs) and associated contractile actomyosin stress fibers (SFs). Proteomics studies have demonstrated that FAs, which are composed of hundreds of proteins, undergo substantial compositional remodeling in response to inhibiting contractility, mimicking the conditions of a soft environment. Intriguingly, many proteins which are absent from FAs in the low-force regime contain a common element, a promiscuous protein-protein interaction motif known as a LIM domain. One LIM domain protein, zyxin, has previously been shown to localize to sites of acute strain on SFs to recruit structural and repair proteins. We therefore hypothesized that strain in the actin cytoskeleton could act as a signal to recruit LIM domain proteins as a force-response mechanism. We have undertaken a live-cell imaging-based screen of 27 additional LIM domain proteins and identified at least six that localize to strain sites. Interestingly, two paralogous SF proteins, PDLIM1 and PDLIM4, show differential localization. Domain-swapping experiments demonstrate that the PDLIM1 LIM domain is responsible for strain-site localization. PDLIM1 was recently found to be necessary for breast cancer metastasis in a mouse model, whereas PDLIM4 was dispensable. Future studies will focus on the role of PDLIM1's LIM-domain dependent strain-sensing activity in cell migration and mechanosensation.

T-cell Mediated Graft-vs-tumor Effect against Kidney Cancer after Allogeneic Stem Cell Transplant Identifies an Immunogenic Human Endogenous Retrovirus Type-E (HERV-E) with Selective Expression in Clear Cell Kidney Cancer. E. Cherkasova, P. Scrivani, N. Harashima, S. Kagigaya, Y. Takahashi,and R. W. Childs; Hematology Branch.

Recently, we isolated and expanded a CD8+ T-cell clone that killed patient tumor cells *in vitro* from the blood of a patient who had a graft-vs-tumor effect resulting in regression of metastatic kidney tumor following an HSCT. This clone was found to have tumor specific cytotoxicity, recognizing a 10-mer peptide. Two transcripts encoding this antigen were found to be derived from a novel HERV-E located on chr.6q. Both transcripts present splice variants that share a common sequence region encoded in the 5'LTR that is spliced to non-shared regions derived from the protease and polymerase genes. We have now discovered transcripts encoding for the entire envelope gene of this provirus are also expressed in RCC concurrent with the previously identified transcripts. We

found that HERV-E expression is restricted to the clear cell RCC characterized by an inactivation of the *VHL* tumor suppressor gene with subsequent stabilization of HIF transcription factors. We demonstrated that HIF-2α can serve as transcriptional factor for HERV-E by binding with HIF response elements localized in the 5'LTR. Remarkably, the LTR was found to be hypomethylated only in HERV-E-expressing ccRCC while other tumors and normal tissues possessed a hypermethylated LTR preventing proviral expression. These findings provide the first evidence that inactivation of a tumor suppressor gene can result in aberrant proviral expression in a human tumor. Since proviral expression has been detected in ccRCC tumors even at the earliest stages of disease, antigens derived from this HERV-E could serve as targets for T-cell based immunotherapy for kidney cancer.

Aquaporin-2 interactome in rat inner medullary collecting duct. C.L. Chou, D. Hageman, M. A. Knepper. Epithelial Systems Biology Laboratory.

Vasopressin regulates membrane trafficking of the water channel aquaporin-2 (AQP2). We ran LC-MS/MS analysis to identify AQP2 interacting proteins in native IMCD cells. Freshly isolated rat IMCD suspensions were treated with a vasopressin analog (dDAVP) or vehicle followed by in-cell crosslinking using a homobifunctional crosslinker BSOCOES, detergent solubilization, AQP2 immunoprecipitation, SDS-PAGE separation, and quantitative mass spectrometry of trypsin digests of gel pieces. The peptides passing stringent spectral quality thresholds were quantified (label-free) to identify those with high signal:noise (AQP2 antibody/preimmune IgG > 4). The overall analysis identified a total of 140 replicable AQP2-interacting proteins distributed throughout several cellular compartments (based on *Gene Ontology* analysis). 134 were found in dDAVP-treated samples and 136 in vehicle-treated samples (overlap 130). 24 have previously been found to undergo changes in phosphorylation in response to vasopressin including proteins involved in membrane trafficking (Lrba, Vps26b), cytoskeleton proteins (Cttn, Krt8, Krt18, Krt19, Myh9, Myh10, Sept7, Sptbn1), cell-cell adhesion (□-catenin, □ -catenin, periplakin) and transport (Slc14a2, AQP2). Additionally, several distinct Rab proteins were identified (5B,5C,7A,11A,11B,14,17). These findings provide extensive new data for modeling vasopressin-regulated AQP2 trafficking.

Identifying transcriptional targets of vasopressin using combined ChIP-seq and RNA-seq analysis of mpkCCD cells. J. S. Claxton, P. C. Sandoval, J. W. Lee, J. D. Hoffert, F. Saeed, M. A. Knepper. Epithelial Systems Biology Laboratory.

The peptide hormone vasopressin regulates transport in the renal collecting duct in part through regulation of gene expression. To obtain a global profile of genes regulated by vasopressin, we performed ChIP-seq analysis of RNA polymerase II binding sites associated with transcriptionally active genes. Cultured mpkCCD cells were treated with either the vasopressin analog dDAVP (0.1 nM) or vehicle for 24 hours, and then processed for chromatin IP. DNA-protein complexes were immunoprecipitated using a RNA polymerase II antibody. After reversal of crosslinks, DNA fragments were sequenced and mapped to a reference genome. ChIP-seq data analysis allowed for precise mapping of genomic elements that were significantly enriched or de-enriched with dDAVP treatment including exonic, intronic, intergenic, and promoter regions. We correlated these changes at the genomic level with corresponding changes in RNA using RNA-seq analysis of 'Poly(A)'-enriched libraries from mpkCCD cells treated with or without dDAVP. Targets enriched in ChIP-seq and RNA-seq data sets in the presence of dDAVP included the water channel aquaporin-2 (*Aqp2*), B3gnt7, Sptbn2, Sat1, Mt2. A subset of genes, including the gene associated with AQP2 trafficking (*Mal2*), increased in the ChIP-seq data set and decreased in the amount of mRNA transcript produced in response to vasopressin. This is possibly due the phenomenon of 'pausing' in which the RNA polymerase II stalls and does not complete a full transcript. This study presents a wealth of new data needed for modeling the transcriptional response to vasopressin.

Synthesis and Characterization of ^{153}Gd radiolabeled Eovist for Prostate Cancer Studies. V. Coble[1], O. Vasalatiy[1], P. Choyke[2], R. Swenson[1]. [1]Imaging Probe Development Center, [2]Molecular Imaging Program, NCI.

Prostate cancer is the most common cancer in adult men, excluding skin cancer, and is the second most common cause of cancer death in men. Castration-resistant prostate cancer (CRPC) is resistant to androgen deprivation therapy (ADT), the mainstay treatment for prostate cancer. One proposed mechanism of resistance is the de novo expression of the testosterone transporter OATPIB3. An assay of a patient's OATP1B3 expression levels could be used to predict a patient's response to ADT. The MRI contrast agent Eovist is a substrate for OATP1B3 and can be used as a MRI contrast agent in the detection of OATP1B3 positive prostate cancer. In a clinical trial Eovist-enhanced MRI successfully differentiated a patient with metastatic CRPC from patients with localized disease. To compliment these clinical results ^{153}Gd radiolabeled Eovist was synthesized for in vivo kinetic studies of the uptake of Eovist into prostate cancer cells. Here we present the synthesis and characterization of the ligand EOB DTPA and its corresponding ^{153}Gd radiolabeled complex.

CD146 expression on T cells identifies Th17 and Tc17 subsets in psoriatic blood and skin. P.K. Dagur[1], E. Stansky[1], S. Rose[3], A. Biancotto[2], H.B. Naik[4], M. Playford[3], N.N. Mehta[3], J.P. McCoy, Jr[1,2]. [1]Hematology Branch, [2]Center for Human Immunology, [3]Section of Inflammation and Cardiometabolic Diseases, [4]Dermatology Branch, NCI.

IL-17 has been implicated in the pathogenesis of psoriasis (PSO), and is produced by both CD4 T-cells (Th17) and CD8 T-cells (Tc17). CD146 is an endothelial adhesion molecule that facilitates binding of lymphocytes to endothelium. It is present at low levels on circulating T-cells in healthy individuals, yet its expression in psoriasis is unknown. We demonstrate that CD146+T-cell subsets are enriched in the peripheral circulation and skin of psoriasis patients. Mean frequencies of circulating CD146+

total T-cells (5.50+/-0.41 vs 3.55+/-0.21, p <0.0001) and CD4+ T-cells (3.91+/-0.37 vs 2.96+/-0.19, p =0.033), but not CD8+ T-cells, (2.75+/-0.373 vs 2.30+/-0.216) were significantly increased in psoriatic patients (n=47) compared to healthy controls (n=67). Furthermore, CD146+T-cells were abundant at active sites of inflammation in psoriatic lesional skin as compared with unaffected skin. Immunofluorescence microscopy confirmed abundant CD146+T-cells in lesional skin biopsies. Sorted CD146+CD4+T-cells from blood as well as skin biopsy specimens revealed higher mRNA levels of CD146, RORC2, and IL-17A as compared with CD146-T-cells. CD146+T-cells were found to be the primary producers of IL-17A in lesional skin for both CD4+ (67.8 %±9.50, p=0.0047) and CD8+ (70.8%±11.36, p=0.0044) T-cells. Roughly 75% of IL-17A producing T-cells were CD146+ in the CD4+ and CD8+T-cell subsets in lesional psoriatic skin. In contrast, CD146+T-cells accounted for ~20% of IL-17A producers in peripheral blood from both healthy adults and psoriatics. Our data suggest that CD146+T-cells are a major source of IL-17A in PSO both in peripheral circulation as well as in lesional skin.

Aberrant Resting State Brain Connectivity in Sickle Cell Patients with Frequent pain. D.S. Darbari, JG. Taylor, J. Hampson, E. Ichesco, N. Kadom, I. Evangelou, G. Vezina, D. Clauw, R. Harris. Children's National Medical Center, George Washington University, Washington, DC, Ann Arbor, MI.

Pain is the most frequent complication of sickle cell disease (SCD). Factor contributing to chronic pain have not been completely elucidated. The objective of present study was to explore if SCD patients with and without history of frequent pain have differences in connectivity in the brain regions implicated in perception and modulation of pain. Eight pain and 4 non-pain SCD patients underwent resting state functional magnetic resonance imaging of the brain. We used a region of interest (ROI) or "seed" based approach using Statistical Parametric Mapping (SPM8). Five minutes of resting fMRI data were collected using T2 weighted BOLD pulse sequence. Results from the whole brain between group difference maps of intrinsic connectivity network showed that SCD patients in the high pain group displayed greater connectivity between the somatosensory cortex and other pain regions including the bilateral posterior insula cortex. Greater connectivity in this group was also seen between the Default Mode Network (DMN) and the middle frontal gyrus and also between the left frontal control network and pain inhibiting regions such as the subgenual cingulate. In contrast, patients in the low pain group showed increased anti-correlation between the left primary somatosensory cortex and the left thalamus.

We conclude that differences between brain connectivity patterns between the pain phenotypes suggest the role of aberrant brain connectivity in the pathophysiology of high pain burden similar to previous reports of altered connectivity in "central" pain states such as fibromyalgia. Better powered studies are needed to confirm the findings of this pilot study.

Role of CD73 in osteogenic differentiation. J.M. Davaine[1,2], J. Hui[1], C. St Hilaire[1], G. Chen[1], M. Boehm[1]. [1] Laboratory of Cardiovascular Regenerative Medicine, NHLBI. [2]Unité Inserm UMR-957, Nantes, France.

Arterial calcification due to CD73 deficiency (ACDC) is a rare genetic disease recently identified in our lab. Patients develop massive arterial calcification in the lower extremities as well as distal joint calcification due to a mutation in the ecto-5'-nucleotidase (*NT5E)* gene encoding for the CD73 protein, which transforms AMP into adenosine and phosphate. The disease stems from the lack of adenosine signaling. The objective was to develop patient-specific induced pluripotent stem cells (iPSCs) to establish a cellular model and study in-depth the mechanisms of this disease which overlap with more common type of medial arterial calcification seen in diabetes and renal insufficiency.

Fibroblasts from ACDC patients and healthy donors were obtained and reprogrammed into IPS cells expressing Nanog, Oct4, Tra1-60, SSEA-4 and give rise to teratomas when injected into mice. Then, IPS cells were derived into CD90+, CD105+ induced-Mesenchymal Stem Cells (iMSCs). Deficiency in CD73 expression and activity were maintained in the ACDC-derived iMSCs as opposed to control iMSCs. Both ACDC and control iMSCs were able to differentiate into osteoblasts, adipocytes and chondrocytes. However, immunocytochemical and standard coloration analyses showed that ACDC-IPS cells had a higher osteogenic potential and a lower adipogenic potential than controls. In line with the *in vitro* findings, CT-scan analysis showed that ACDC-derived teratomas were significantly more calcified than controls.

IPS cells and iMSCs can be obtained from ACDC fibroblasts and reproduce both *in vitro* and *in vivo* the patients phenotype, proving the accuracy of this model that will be used for drug screening.

Amyloid Triangles, Squares, and Loops of Apolipoprotein C-III. M. de Messieres, R. K. Huang, Y. He, J. C. Lee; Laboratory of Molecular Biophysics (NHLBI), Protein Expression Facility (NHLBI), and Laboratory of Structural Biology Research (NIAMS).

While a significant component of atherosclerotic plaques has been characterized as amyloid, the specific proteins remain to be fully identified. Probable amyloidogenic proteins are apolipoproteins (Apos), which are vital for the formation and function of lipoproteins. ApoCIII is an abundant protein implicated in atherosclerosis, and we show it forms a ribbonlike looped amyloid, strikingly similar to that previously reported for ApoAI and ApoCII. Triangles and squares with a width of ∼50 nm were also observed, which may be a novel form of amyloid or related to previously reported amyloid rings.

Collagen: Order, Disorder, and Dynamics at Super-Resolution. A. Dittmore, S.K. Sarkar, B. Marmer, G.Goldberg, K.C. Neuman; Laboratory of Molecular Biophysics.

Collagen fibrils exemplify self-organization in biological matter and are subject to remodeling within bodily tissues. Here, we present evidence of spatially distributed structural variations that constitute the initiation points for

collagen remodeling. Using precise and high throughput single-molecule fluorescence tracking of collagenase (matrix metalloproteinase I), we detect thermally driven microscopic rearrangements in the highly ordered lattice of a native type I fibril. In the absence of external stimuli, micron-scale periodicity and dynamics emerge through stability fluctuations of the constituent protein elements. Our results have broad implications for tissue remodeling and suggest a mechanism for mechanosensing in the collagen network.

Stimulation of Thrombogenic and Inflammatory Signaling in Endothelial Cells by Elevated Extracellular Sodium: Possible Role in Blood Hypercoagulability, Atherosclerosis and Cardiovascular Health. N. I. Dmitrieva, M. B. Burg; Renal Cellular and Molecular Biology Section.

Cardiovascular diseases (CVDs) are a leading health problem worldwide. Epidemiologic studies link high salt intake and conditions predisposing to dehydration such as low water intake, diabetes and old age to increased risk of CVD. We hypothesized that the elevation of plasma sodium that is a common consequence of these conditions might contribute to CVD through direct effects on the vascular endothelium. Previously, we demonstrated that high extracellular NaCl increases production and secretion of a key initiator of clotting cascade, von Willebrand Factor (vWF), in human umbilical vein endothelial cells (HUVECs). Also, in mice, water restriction that elevates plasma sodium by 5 mmol/l stimulates hypertonic signaling and endothelial production of vWF accompanied by increased thrombogenesis. In the present studies, we show that high NaCl increases expression of the adhesion molecules VCAM1 and E-selectin and the chemoattractant MCP-1 in HUVECs. These molecules are key participants in the leukocyte adhesion and transmigration that play a major role in the inflammation and pathophysiology of CVD, including formation of atherosclerotic plaques. Indeed, high NaCl increases adhesion of mononuclear cells to HUVECs and their transmigration through HUVEC monolayers. Accordingly, water restriction increases VCAM1, E-selectin and MCP-1 mRNA in mouse tissues and accelerates atherosclerotic plaque formation in $ApoE^{-/-}$ mice. In multivariable regression analysis of clinical data from the Atherosclerosis Risk in Communities Study, serum sodium significantly contributes to prediction of the blood level of vWF and the Risk of Stroke. Thus, elevation of extracellular sodium within the physiological range alters the vascular endothelium in a way that facilitates development of CVD.

Conformational Rearrangements in Bax as Apoptosis is Initiated: Inner-Workings of a Cellular Death Switch. R. F. Gahl, N. Tjandra. Laboratory of Molecular Biophysics, Biochemistry and Biophysics Center.

The Bcl-2 family of proteins regulates the intrinsic pathway for the activation of apoptosis. Pro- and anti-apoptotic members of this family keep each other in check until the correct time to commit to apoptosis. The point of no return for this commitment is the permeabilization of the outer-mitochondrial membrane (OMM). Translocation of the pro-apoptotic member, Bax, from the cytosol to the mitochondria coincides with this event. Our study follows intra-molecular conformational changes and inter-molecular contacts in Bax, resolved to individual helices, as this translocation occurs in live cells. A novel method was employed that made it possible for Förster Resonance Energy Transfer (FRET) to be reliably detected between pairs of engineered sites throughout Bax in the heterogeneous environment of live cells. Before Bax translocates and commits to the permeabilization of the OMM, it was observed to transiently contact a membrane environment, presumably the mitochondria. This coincided with the measurement that the C-terminal helix is exposed to the cytosolic environment instead of tucked away in the core of the protein. Cross-linking the C-terminal helix (α9) to helix α4 reduced the occurrence of this transient interaction. After translocation occurs, Bax was observed to engage in inter-molecular contacts at the BH3 domain and the C-terminal helix. We propose, based on this information coupled with additional inter- and intra-molecular FRET efficiencies, these two sites are secondary and tertiary contact points to form homo-oligomers in the mitochondria. The implications for the contacts with other Bcl-2 proteins necessary for regulation are also discussed.

A Detailed Centrosome Interactome Reveals a Novel Role for Asterless/CEP152 in Controlling Centriole Length Via Cep97. B. J. Galletta[1], K.C. Jacobs[1], C. J. Fagerstrom[1], K. C. Slep[2], N. M. Rusan[1]; [1]Cell Biology and Physiology Center, [2]Department of Biology, University of North Carolina, Chapel Hill, NC.

The centrosome is the major microtubule-organizing center (MTOC) of many eukaryotic cells, critical for building and orienting the mitotic spindle. To better understand the function of the proteins most critical for building centrosomes, we performed a directed yeast two-hybrid screen to identify the possible interactions among 22 conserved *Drosophila* centrosome proteins. To provide additional detail about how centrosomal proteins interact, these 22 proteins were subdivided into smaller fragments, keeping known domains and predicted secondary structures intact. In total, 67 full-length proteins and subfragments were tested. We identified more than 200 interactions among these proteins, the majority of which are novel. This has significantly increased our knowledge of the protein-protein interaction network within the centrosome. With this new interaction information, we attempted to better understand the role of Asterless/CEP152. Since Asterless is essential for the duplication of the centrosome, little is understood about the molecular mechanism of its function or if it plays roles beyond centriole duplication. We have studied the loss of *asterless* in the male germline stem cells (mGSCs) of *Drosophila melanogaster* larvae, where we can reliably locate Asterless-free centrioles. These centrioles were initially built during embryogenesis using Asterless protein provided by the larvae's mother, but by this stage in development no longer have any Asterless protein associated with them. Amazingly, in the mGSCs of asterless mutants, the remnant centrioles are 10 to 30 fold longer than in wild-type mGSCs. By examining the Asterless interacting proteins identified in our screen, we have shown that proteins normally found at the proximal end of wild-type centrioles extend the entire length of these Asterless-free centrioles. Furthermore we found that an Asterless-binding protein associated with the distal end of centrioles, Cep97, is missing in the asterless

mutant. By analyzing flies harboring a mutation in *cep97*, we have confirmed that Cep97 is responsible for controlling the length and protein organization of centrioles in a fashion similar to Asterless. These data suggest that in mGSCs, Asterless plays a role in controlling the length of centrioles and regulating the proximal/distal character along their length via the recruitment of Cep97. Interestingly, *cep97* mutant males are sterile, suggesting that proper regulation of centriole length and / or the proximal/distal organization of centriolar proteins may be important for building functional sperm. Experiments to test this hypothesis are underway.

A conserved mechanism of cellular and retroviral mRNA evasion of NMD Z. Ge, J. Hogg; Laboratory of Ribonucleoprotein Biochemistry.

Nonsense-mediated mRNA decay (NMD) is an mRNA quality control pathway in eukaryotes that degrades mRNAs containing premature termination codons. Previous evidence suggests that 3'UTR length is an important determinant of transcript susceptibility to NMD. The key NMD factor Upf1 associates along its target 3'UTRs in a length-dependent acts as a direct sensor of 3'UTR length. However, a large number of potential decay target mRNAs evade NMD, including cellular transcripts containing long 3'UTRs and un-spliced retroviral RNAs. Here we show that a retroviral RNA element exploits an evolutionarily conserved mechanism to protect transcripts from NMD, and, further, that this strategy is employed by human transcripts with long 3'UTRs. The RNA stability element (RSE) of Rous sarcoma virus is a large (~400nt) RNA segment that resides immediately downstream of the viral *gag* termination codon, preventing it from being recognized as premature. Detailed analysis of mRNP composition identified several proteins that are enriched in RSE-containing mRNPs, including PTBP1. Using biochemical and functional assays, we find that the RSE is able to protect synthetic reporter mRNAs containing long 3'UTRs from NMD by recruiting PTBP1 to prevent UPF1 from associating with mRNPs. Mutations that eliminate putative PTBP1 binding sites cause a decrease in PTBP1 association and subsequent loss of the RSE's protective function, suggesting that PTBP1 plays an essential role in the RSE's antagonistic activity against NMD. Finally, we performed RNAseq analysis on cells depleted of PTBP1 or both PTBP1 and UPF1, identifying a large subset of mammalian transcripts with long 3'UTRs that are protected by PTBP1 from NMD.

Functional niche competition between normal and leukemic stem cells. C. Glait-Santar; X. Feng; J. Chen; R. Desmond; B. Mizukawa; J. Mulloy; N. Young; C.E. Dunbar. Molecular Hematopoiesis section.

Hematopoietic stem cells (HSCs) reside in a niche that regulates their proliferative capacity. The functions of HSCs are regulated by both intrinsic signals from the cells and from surrounding bone microenvironment. We used a murine competitive repopulation model and MLL-AF9 myeloid leukemia to investigate whether normal and leukemic stem cells compete for the same niches. We co-transplanted a fixed number of MLL-AF9 cells along with increasing numbers of normal whole bone marrow (WBM) or purified primary hematopoietic progenitor (LSKs) cells, derived from syngeneic or dsRed transgenic mice, into irradiated mice. Survival was increased and leukemic progression delayed proportional to increasing doses of normal cells. MLL-AF9 cells isolated from transplanted recipient marrows were cycling more actively in the presence of increasing doses of WBM, suggesting competitive exclusion from the protective marrow niche. Osteopontin (OPN) is a cytokine produced by niche and promote quiescence of normal and acute lymphoblastic leukemia stem cells. OPN expression levels in BM supernatants collected from femurs correlated with cycling of recovered ALL-MF9 cells. The competitive model suggests that normal and myeloid leukemic stem cells compete for the same functional niche, thus niche manipulation could impact on response to anti-leukemic therapies, and the extent of normal hematopoiesis could impact on outcomes, informing approaches to cell dose in the context of stem cell transplantation.

Effects of Rosuvastatin on the Lipoprotein Subfractions, Protein Composition and Function.
S.M. Gordon, G. Kemeh and A.T. Remaley. Lipoprotein Metabolism Section/Cardiovascular Pulmonary Branch.

Introduction: Low density lipoprotein associated cholesterol (LDL-C) is a well established risk factor for cardiovascular disease (CVD). Statins, by inhibiting HMG-CoA reductase and upregulating hepatic LDL receptors, can effectively reduce plasma LDL-C by as much as 50%, thus reducing future CVD events. However, the physiological effects of statins are diverse and not all of them may be directly to its primary target.

Goal: Our hypothesis is that some of these alternative effects from statins may be driven by compositional changes to lipoproteins distinct from their cholesterol content. We, therefore, performed a small clinical pilot study to assess the effect of statins on lipoprotein associated proteins in healthy individuals.

Methods: Ten subjects with normal LDL-C (<130 mg/dL) were given rosuvastatin (20 mg/day) for 28 days. Plasma samples collected at baseline and after treatment were used for lipid measurement, NMR lipoprotein profiling and lipoprotein proteomics.

Results: Spectral counting was used to compare relative protein detection before and after treatment. Significant protein changes were found in each lipoprotein class. The most dramatic effect of the treatment was a profound 2,438% increase in alpha-1-antirypsin (A1AT) spectral counts associated with large HDL-1 particles.

Summary: Based on these preliminary results statins do appear to significantly change the protein composition of both LDL and HDL. Some of these changes, such as the upregulation of A1AT on HDL, may affect the function of lipoproteins and could possibly contribute to some of the non-lipid lowering effects of statins, which will be investigated in future studies.

Machine-learning Identifies Predictive Signatures of Enhancer Activity in Individual Cardiac Cells. J.S. Haimovich, B.W. Busser, D. Huang, I. Ovcharenko, A.M. Michelson; Laboratory Developmental Systems Biology.

The *Drosophila* heart is composed of multiple cardiac cell subtypes, which differ from each other in morphology, function, and gene expression patterns. The activities of cell-specific gene regulatory sequences known as enhancers contribute to the fate of these cells during cardiogenesis. Previous studies have demonstrated that histone protein modification and transcription factor (TF) binding sites can be used to identify unique epigenetic and genetic signatures of enhancers that are functional in cardiac tissues during embryogenesis. Here, we developed and applied a machine-learning approach to identify unique signatures of enhancers active in individual cardiac cell subtypes during embryogenesis. To begin, we undertook a large-scale screen of *Drosophila* enhancers having activities in cardiac cell subtypes. These enhancers were then used as a training set for construction of a series of cell-specific support vector machine classifiers based on integrated histone protein modifications, in vivo TF binding, and TF motifs. Using these classifiers, we identified unique enhancer signatures and predicted novel pericardial-specific enhancers in the *Drosophila* genome. Hierarchical clustering of classifier weights for TF motifs revealed differentially weighted TFs amongst the cell-specific classifiers. We hypothesize these cell-specific motifs discriminate individual heart cell fates, which we confirm for a subset of motifs using *cis* mutagenesis in transgenic reporters. Taken together, our data demonstrate that cell-specific classification of transcriptional enhancers is a powerful approach for identifying both novel cardiac regulatory activity in *Drosophila* and genetic features that play a role in cell-fate specification.

Parkin Ameliorates ER Stress in Countering Cardiac Maladaptation to Pressure Overload. K. Han[1], T.T. Nguyen[2], M.V. Stevens[1], S. Hassanzadeh[1], H. San[3], S. Anderson[4], E. Murphy[2], M.N. Sack[1]; [1]Cardiovascular and Pulmonary Branch, [2]Systems Biology Center, [3]Animal Surgery Program, [4]MRI Imaging Core.

The E3-ubiquitin ligase Parkin is highly expressed in the heart and protects against cardiac ischemia-reperfusion injury via the modulation of mitophagy. Parkin has been found to function in a canonical cascade downstream of PTEN-inducible kinase Pink1 in the regulation of mitophagy and mitochondrial dynamics. Whether Parkin orchestrates adaptive cardiac effects via its myriad of known substrates and whether it also confers adaptation to pressure-overload is unknown. To investigate the effects on Parkin-regulated contractile function, we subjected Parkin and Pink1 knockout (KO) mice to thoracic aortic banding. Parkin KO mice had an impaired capacity to tolerate acute pressure-overload, were more susceptible to cardiac hypertrophy and contractile dysfunction in association with exaggerated induction of ER stress. ER stress proteins CHOP and ATF4 are induced by pressure-overload in Parkin KO mice. We found that Parkin interacts with CHOP and facilitates its turnover. Interestingly, autophagy appeared to be blunted by pressure-overload in the Parkin KO mice as evident by both the diminished ratio of LC3 II/I and by the accumulation of p62. Also Parkin KO mice showed greater disruption of mitochondrial DNA integrity and excessive redox stress. Pink1 KO mice had a distinct, albeit exaggerated, maladaptive response to pressure overload, although here there was no evidence of perturbations in ER stress in these mice. Taken together these data show that Parkin, via non-canonical signaling, contributes to adaptation to pressure overload, in part via CHOP mediated ER stress signaling.

Zinc finger RNA binding protein (ZFR) protects macroH2A mRNA from degradation. N. Haque and J.R. Hogg. Laboratory of Biochemistry.

Interactions between mRNAs and trans-acting regulatory factors influence every step of gene expression, from transcription to decay. In particular, we are interested in understanding how differential rates of mRNA turnover are used to determine cellular responses to changing physiological conditions. As one part of this effort, we are using a combination of biochemical and functional assays to identify proteins that modulate mRNA degradation to maintain appropriate cellular levels of specific mRNAs.

Here, we developed a purification scheme to isolate protein complexes assembled on reporter RNAs with distinct structural features and translational efficiencies. In this way, we identified the evolutionarily conserved Zinc finger RNA binding protein (ZFR) to be specifically purified with mRNAs that are poorly translated due to a highly structured 5'UTR element. To identify mRNAs regulated by the ZFR protein, we first performed transcriptome-wide RNAseq studies of control cells and cells depleted of ZFR. In these way, we observed that ZFR regulates the abundance of a wide diversity of mRNAs, including the histone-variant macroH2A mRNA, an important regulator of transcription. Further biochemical studies show that ZFR is directly associated with macroH2A mRNA and that macroH2A mRNA becomes highly unstable in the absence of ZFR. Using siRNA-mediated knockdown of a panel of nucleases, we identified two specialized nucleases as candidate effectors in the degradation of macroH2A mRNA in the absence of ZFR. Taken together, these data suggest a novel function of ZFR in protecting macroH2a and other mRNAs from a previously uncharacterized mRNA decay pathway.

Low-dose Phase-Contrast Imaging of a Mammography Phantom Using Talbot Interferometry. K. Harmon[1], H. Miao[1], A. Gomella[1], E. Bennett[1], P. Bhandarkar[2], H. Wen[1], [1] Imaging Physics Laboratory, BBC/, [2] Breast Imaging Center, Walter Reed Army Medical Center.

Current digital mammograms rely on x-ray attenuation to image breast tissue. It has been shown that phase-contrast imaging can provide additional structural information. In this study, phase contrast is obtained via a Talbot interferometer that introduces a periodic intensity modulation to the image. As x-rays pass through a sample, the phase distribution of the wavefront changes and is detected as distortions of the periodic modulation in a motion-less electromagnetic phase stepping process. Here we compare phase-contrast images of a mammography accreditation phantom, obtained using the Talbot interferometer, with a radiograph of the phantom obtained using a GE Senographe scanner. We observed that only some objects were visible using Talbot interferometry. Resolution of the features in the phase contrast images is limited by random noise as well as artifacts produced by the x-ray

detector. Improvements in image processing eliminated most of the detector artifacts. However, intermittent residual artifacts may still obscure the fine features. This study points to the importance of state-of-the-art x-ray detectors in phase contrast imaging. Our current detector will be replaced with a modern one for a repeat study. Nevertheless, the study demonstrates that phase-contrast imaging can be performed at clinical dose levels.

Temporal Mapping of Enteric Neurovascular Development and Villus Morphogenesis. J. Hatch and Y. Mukouyama. Laboratory of Stem Cell & Neuro-Vascular Biology.

In the intestine, correct patterning of nerves and vasculature is critical for normal digestive function. A hierarchical branching pattern of blood vessels allows for efficient nutrient absorption, while the enteric nervous system, the "second brain," coordinates diverse homeostatic functions, including motility, secretion, absorption, and blood flow. Despite the well-recognized significance of these systems, the precise mechanisms by which they develop remains unclear. In mice, the gut tube forms from endoderm by embryonic day 9.5. To better understand the processes of intestinal innervation and vascularization, we have developed a novel whole-mount immunohistochemical protocol to visualize the pattern of murine neurovascular development in the intestine between embryonic day 10 and birth. Processes of particular interest include the development and remodeling of the enteric vascular plexus, the migration and organization of neural crest-derived cells to form the enteric nervous system, and the morphogenesis and innervation of villi at later stages. Our data reveal that primary capillary sprouting precedes neural crest-derived cell migration and neuronal differentiation, suggesting a functional interaction between migrating neural crest-derived cells and primary endothelial cells. Additional observations have identified a subpopulation of mesenchymal cells that may recruit capillaries and axons to developing villi. These observations provide useful descriptive data for future investigations into the functional mechanisms responsible for the observed processes.

Molecular Details of α-Synuclein Membrane Association Revealed by Photons. S. K. Hess, Z. Jiang, J.C. Lee. Laboratory of Molecular Biophysics.

Understanding how environmental factors affect α-synuclein (α-syn) conformation is critical because its misfolding has been implicated in Parkinson's Disease etiology. The role of membranes in α-syn conformation is of particular interest because membranes alter the protein-folding landscape and are ubiquitous *in vivo*. We have employed time-resolved and steady-state fluorescence techniques to examine how the penetration depth of α-syn into the lipid bilayer changes based on membrane composition. At acidic pH, we saw that all sites examined penetrated comparably bilayers composed of equal molar phosphatidylcholine (PC) and phosphatidic acid (PA) and those composed of equal molar PC and phosphatidylserine (PS), with only slightly greater binding to PC/PA bilayers. Time-resolved depth-dependent fluorescence quenching experiments indicated that the N-terminus and end of the hydrophobic NAC region of α-syn bury comparably, contrary to previously reported results. We propose a revision to the putative α-syn helix in which two breaks in the helix could explain the comparably quenching profiles of these two sites. Alternate structural explanations are also discussed in light of steady-state Trp fluorescence data.

Selective Propagation of Functional mtDNA During Oogenesis Restricts the Transmission of A Deleterious Mitochondrial Variant. J. H. Hill, Z Chen, and H Xu; Laboratory of Molecular Genetics.

Though mitochondrial DNA is prone to mutation and few mtDNA repair mechanisms exist, crippling mitochondrial mutations are exceedingly rare. Recent studies demonstrated strong purifying selection in the mouse female germline. However, the mechanisms underlying the positive selection of healthy mitochondria remain to be elucidated. We visualized mtDNA replication during *Drosophila* oogenesis. We found that mtDNA replication commenced prior to oocyte determination during the late germarium stage, and was dependent on mitochondrial fitness. We isolated a temperature-sensitive lethal mtDNA mutation, $mt{:}CoI^{T300I}$, which displayed reduced mtDNA replication in the germarium at the restrictive temperature. Additionally, the frequency of $mt{:}CoI^{T300I}$ in heteroplasmic flies was decreased both through oogenesis and over multiple generations at the restrictive temperature. Furthermore, we determined that selection against $mt{:}CoI^{T300I}$ overlaps with the timing of selective replication of mtDNA in the germarium. These findings establish a previously uncharacterized developmental mechanism for selective amplification of healthy mtDNA, which may be evolutionarily conserved to limit transmission of deleterious mutations.

Path to the clinic: Assessment of iPSC-based cell therapies *in vivo* in a non-human primate model. S.G. Hong[1], T. Winkler[1], C. Wu[1], V. Guo[1], S. Pittaluga[2], A. Nicolae[2], R.E. Donahue[1], M.E. Metzger[1], S.D. Price[1], N.Uchida[3], S.A. Kuznetsov[4], T.Kilts[4], L. Li[4], P.G. Robey[4], and C.E. Dunbar[1]. [1]Hematology Branch, [2]Laboratory of Pathology, CCR, [3]Molecular and Clinical Hematology Branch, NIDDK, [4]Craniofacial and Skeletal Diseases Branch, NIDCR.

Induced pluripotent stem cell (iPSC)-based cell therapies have great potential for regenerative medicine, but are also potentially associated with significant risks. Here, we developed a clinically relevant non-human primate model to assess the tumorigenic potential and *in vivo* efficacy of iPSCs, in the autologous setting without immunosuppression. We used the transgene-free rhesus macaque iPSCs (RhiPSCs) first to develop a clinically-relevant autologous teratoma model, and second to evaluate the potential of clinical use of RhiPSC derived mesodermal stromal-like cells (RhiPS-SCs) for *in vivo* bone regeneration. RhiPSC cells were subcutaneously injected into both the donor autologous rhesus monkeys and immunocompromised NSG mice. Simultaneously, autologous RhiPS-SCs mixed with 3D scaffolds were subcutaneously implanted to investigate *in vivo* bone formation. After 3-14 weeks, all RhiPSC implants at the cell doses used in this study resulted in teratomas in NSG mice. In

autologous monkeys, undifferentiated autologous iPSCs indeed formed teratomas in a dose-dependent manner. However, teratoma formation is less efficient in autologous monkeys than in NSG mice. Teratomas obtained from the autologous monkeys, but not from NSG mice, were accompanied by an inflammatory reaction. In contrast, simultaneously implanted RhiPS-SCs in scaffolds formed well defined bone trabeculae in the autologous monkeys, without evidence of teratoma formation or evident inflammatory reaction. These studies represent the first autologous large-animal teratoma model, and demonstrate the autologous regenerative potential of iPSCs to form functional bone tissue *in vivo.* Studies investigating the potential immunogenicity of iPSC are ongoing and will be presented at the meeting.

Membrane Remodeling by α-Synuclein and Effects on Amyloid Formation. Z. Jiang, M. de Messieres, and J.C. Lee. Laboratory of Molecular Biophysics.

α-Synuclein (α-Syn), an intrinsically disordered protein, is associated with Parkinson's disease. Though molecular pathogenic mechanisms are ill-defined, mounting evidence connects its amyloid forming and membrane binding propensities to disease etiology. Contrary to recent data suggesting that membrane remodeling by α-syn involves anionic phospholipids and helical structure, we discovered that the protein deforms vesicles with no net surface charge (phosphatidylcholine, PC) into tubules (average diameter ~20 nm). No discernible secondary structural changes were detected by circular dichroism spectroscopy upon the addition of vesicles. Notably, membrane remodeling inhibits α-syn amyloid formation affecting both lag and growth phases. Using five single tryptophan variants and time-resolved fluorescence anisotropy measurements, we determined that α-syn influences bilayer structure with surprisingly weak interaction and no site specificity (partition constant, $K_p \sim 300\ M^{-1}$). Vesicle deformation by α-syn under a variety of different lipid/protein conditions is characterized via transmission electron microscopy. As cellular membranes are enriched in PC lipids, these results support possible biological consequences for α-syn induced membrane remodeling related to both function and pathogenesis.

Endotoxin Contamination of Apolipoprotein A1: Effect on Macrophage Proliferation – A Cautionary Tale. X. Jin[1], Q. Xu[1], K. Champion[2], H.S. Kruth[1]. [1]Lab of Experimental Atherosclerosis, and [2]BioDtech Inc., Birmingham, AL.

Incubation of human macrophages with ApoA1 (purified from human plasma) for 1-2 days resulted in decreased cell density that was independent of cell toxicity. Although ApoA1 treatment caused macrophages to become more rounded and clustered, with some macrophages detaching, this did not account for the decrease in cell density. Thus, we considered that ApoA1 decreased macrophage cell proliferation. Using a BrdU incorporation cell proliferation assay, we demonstrated that ApoA1 (20 ug/ml) resulted in a >90% inhibition in macrophage proliferation. Using a recombinant version of ApoA1, we found that the results were dependent on the expression system: ApoA1 expressed in human cells did not inhibit proliferation but ApoA1 expressed in E. coli did. Human plasma-derived ApoA2 did not inhibit proliferation. All apolipoproteins were obtained commercially.

We considered the possibility that endotoxin contamination of the apolipoproteins contributed to the differential inhibition of macrophage cell proliferation. Endotoxin alone very potently inhibited macrophage proliferation (0.1 ng/ml inhibited macrophage proliferation >90%). Measurement of endotoxin levels in the apolipoprotein products, including an analysis of free versus protein-bound endotoxin, suggested endotoxin mediated inhibition of macrophage proliferation. While human cell-derived recombinant ApoA1 and human plasma-derived ApoA1 showed similar levels of total endotoxin contamination (levels that were sufficient to inhibit macrophage proliferation), the levels of free endotoxin in the human cell-derived recombinant ApoA1 were more than 1000-fold less than levels in the human plasma-derived ApoA1. This finding helps explain why the recombinant ApoA1 did not inhibit macrophage proliferation, but the plasma-derived ApoA1 did.

Conclusion: Our findings show that apparent apolipoprotein-mediated cell effects (or effects of any other biological products) can be significantly influenced by endotoxin contamination, especially when these products are tested on highly endotoxin-sensitive cells, such as macrophages. Lastly, endotoxin potently inhibits macrophage proliferation, which may be a significant pathogenic mechanism by which bacteria escape the immune system.

Characterization of Endocytosis in T Cells. D. Johnson[1, 2], J. Wilson[1], J. G. Donaldson[2], [1]Cellular and Molecular Medicine, University of Arizona, [2]Cell Biology and Physiology Center.

We have characterized clathrin independent endocytosis (CIE) in the human Jurkat T cell line and compared it with what has been reported in HeLa cells. MHCI was endocytosed separately from the transferrin receptor that enters cells by clathrin-mediated endocytosis (CME). Then, MHCI passed through EEA1 endosomes prior to trafficking to LAMP1 vesicles for degradation or it was recycled back to the surface. In contrast, CD98 avoided EEA1 endosomes and was rapidly recycled. Plasma membrane proteins that entered the cell through clathrin independent mechanisms co-localized with the G protein Arf6.

We have also shown that cycles of GTP-binding and hydrolysis on Arf6 are important for T cell activation. When Jurkat cells expressing the dominant negative mutant of Arf6 were mixed with activated antigen presenting cell, the T cell did not form a proper immunological synapse. We are working to determine if this is a signaling defect or a cell-spreading defect.

Our results confirm that CIE and subsequent endosomal trafficking is a highly conserved process. The cycle of the G protein Arf6 is also required for proper T cell conjugate formation. Our future goal is to understand how CIE and endosomal trafficking affects T cell signaling and function.

Structural Analysis of the 5´-UTR of the HIV-1 Genome. C. P. Jones and A. R. Ferré-D'Amaré**.** Laboratory of RNA Biophysics and Cellular Physiology.

The highly structured 5´ UTR of the HIV-1 genome is a ~350-nt RNA that contains motifs critical to viral RNA (vRNA) transcription, reverse transcription, and interactions with viral and cellular factors. The first step of reverse transcription is primer $tRNA^{Lys3}$ annealing to the HIV-1 genome at the primer binding site (PBS), which shares extensive complementarity with $tRNA^{Lys3}$. Subsequently, reverse transcription initiates by NTP addition to the 3´ end of $tRNA^{Lys3}$. Human lysyl-tRNA synthetase, which plays a key role in packaging the tRNA primer, specifically binds to a tRNA anticodon-like element in the HIV-1 genome (Jones et al, (2013) *RNA,* 19:219-29). Using small-angle X-ray scattering (SAXS), the overall shape of the PBS region has been shown to mimic the shape of a tRNA (Jones et al, (2014) *PNAS* 111:3395-400). As SAXS has only provided a low-resolution model for the annealed complex, the details of how the tRNA primer interacts with the PBS region are unknown. The PBS region interacts with $tRNA^{Lys3}$ primarily through an 18-bp duplex formed between the 3´ end of $tRNA^{Lys3}$, but the additional interactions that occur are less clear. These include a 6-bp interaction involving the tRNA variable loop and a 4-bp interaction between the anticodon and an A-rich bulge in the vRNA. To better understand how these additional interactions affect the tRNA/PBS complex, we have examined *in vitro* annealed complexes of HIV-1 PBS RNA (~100 nt) with RNAs of different lengths derived from *in vitro* transcribed $tRNA^{Lys3}$. Using size exclusion chromatography to analyze annealed RNA complexes, we show that RNAs containing all three tRNA/vRNA interactions form complexes more readily than RNAs containing only two of the three interactions. Additionally, we show that extended $tRNA^{Lys3}$ species which mimic the first three nucleotide addition steps of reverse transcription anneal at levels similar to unextended $tRNA^{Lys3}$. Taken together, these results suggest that multiple interactions between $tRNA^{Lys3}$ and the vRNA contribute to the formation of stable annealed complexes. Future work aims to uncover how each interaction between $tRNA^{Lys3}$ and vRNA affect the 3D organization of the initiation complex. This work was partly funded by the Intramural Program of the NIH, NHLBI.

Induced Pluripotent Stem Cells from a Patient with *GATA2* Deficiency Display a Block in Hematopoietic Differentiation. M. Jung, T. Winkler, F. Donaires, S. Hong, A. Hsu, D. Hickstein, S. Holland and C. Dunbar. Molecular Hematopoiesis Section, Hematology Branch.

Heterozygous mutations of the transcription factor *GATA2* result in a clinical syndrome GATA2 deficiency. Bone marrows from these patients display a severe deficiency of a multilineage progenitor. To understand the defects in this syndrome, we derived induced pluripotent stem cells (iPSc) from a patient with a mutation in the second zinc finger domain of *GATA2* (R361H) who presented with the classic MonoMAC syndrome and subsequently developed MDS/AML. IPSc were derived from either skin fibroblasts or bone marrow stromal cells by the cre-excisable polycistronic lentiviral vector. The reprogramming factors were excised by transient expression of cre-recombinase. IPSc derived from fibroblasts of a healthy subject served as a control. IPSc were differentiated towards mesodermal then hematopoietic lineages using a previously described Albumin Polyvinylalcohol Essential Lipids medium in monolayer cell culture with the sequential addition of cytokines. Cells were analyzed by flow cytometry at days 0, 7, 13 and 16 for the expression of the CD34 and CD45. Colony-forming unit (CFU) assays were performed on unselected cells from days 13 and 16. CD34+ hemogenic endothelial cells were significantly reduced in *GATA2* R361H iPSc compared to control iPSc at all time points. Subsequently, CD34+/CD45+ hematopoietic cells were also reduced in *GATA2* R361H iPSc at days 13 and 16. Control iPSc exhibited a robust differentiation capacity on CFU assay at days 13 and 16, however, cells differentiated from *GATA2* R361H iPSc did not produce any CFU. IPSc from a patient with *GATA2* R361H mutation displayed severely decreased hematopoietic differentiation potential and absent clonogenic capacity.

Differential Binding of Receptor Protein Tyrosine Phosphatase Type IIa Family (RPTPσ/RPTPδ/LAR) to Glycosaminoglycans. Y. Katagiri, A.A. Morgan, P. Yu, N.J. Bangayan, R. Junka, H.M. Geller. Developmental Neurobiology Section.

Type IIa Receptor protein tyrosine phosphatases (RPTPs) have been shown to modulate neural regeneration and development. All members of this family (RPTPσ, RPTPδ, and LAR) have a cell adhesion molecule-like extracellular domain that includes three N-terminal Ig domains and four to nine fibronectin type III (FNIII) domains, as well as tandem intracellular tyrosine phosphatase domains. Along with protein tyrosine kinases, protein tyrosine phosphatases are crucial to the regulation of cellular protein tyrosine phosphorylation. This family of leukocyte common antigen-related receptors has been shown to influence axonal outgrowth and guidance during neural development. Compelling evidence suggests that both heparan sulfate (HS) and chondroitin sulfate (CS) are the ligands for RPTPσ and LAR, and the Lys-loop located in the first Ig domain is responsible for the ligand binding. However, the functional outputs of HS and CS are promotion and inhibition of axonal growth, respectively. The opposite effects of HS and CS glycosaminoglycans (GAGs) on axonal growth were attributed to the differential oligomeric state of RPTPσ because binding to HS, but not to CS, induces clustering of the extracellular region of RPTPσ. Here we show that all RPTP type IIa members display high affinity binding for heparin. Both CS-E and dermatan sulfate (DS) have similar dissociation constants within the typical nanomolar range, however, there is considerable variation of binding sites on GAGs among RPTPs. We also demonstrate differential contribution of FNIII domains required for high affinity binding to GAGs. In particular, RPTPσ binding to heparin was not completely abolished by the disruption of the Lys-loop. This, along with a decrease in binding with deletion of the fourth FN domain, led us to hypothesize that there is a greater contribution of the FN domain in binding to glycosaminoglycans that previously believed. Preliminary data shows that the fourth fibronectin repeat is able to bind to heparin without contribution of the three Ig domains. Despite 66%

overall shared amino acid identity amongst RPTPs, we conclude the presence of discrete bindings of RPTPs to GAGs. Furthermore, only RPTPσ is clustered upon binding to both heparin and CS-E, but not DS. In addition, our data indicate that clustering of the receptor may not explain the distinct biological functions of HS and CS.

In PDE3B KO Mice, White Epididymal Adipose Tissue (WAT) Exhibits Phenotypic Characteristics of "Fat-burning" Brown Adipose Tissue (BAT), with reduced expression of pro-inflammatory markers, including the NLRP3 Inflammasome. F.A. Khan[1], Y.W. Chung[1] ,Y. Tang[1], S.C. Hockman[1],K. Berger[2], E.M. Billings, O. Gavrilova[3], E. Guirguis[1],S. Liu[1], Y. Khan[1], K. Huo[1], D.M. Schimel[4], A.M. Aponte[5], E. Degerman[2], V.C. Manganiello[1]. [1]Cardiovascular Pulmonary Branch, [2]Department of Experimental Medical Science, Lund University, Sweden. [3]Mouse Metabolism Core, NIDDK, [4]NIH MRI Research Facility. [5]Proteomics Core Facility.

Obesity is a major risk factor for cardiovascular diseases and type 2 diabetes. In EWAT of PDE3B KO mice in SvJ129 background, cAMP/PKA- and AMPK-signaling pathways are activated, assuming phenotypic characteristics of BAT, including alterations in morphology and increased expression of genes, such as PGC-1α, PDRM16, LRP130, and Elovl3, important in mitochondrial biogenesis, induction of the thermogenic program, and differentiation/recruitment of BAT. In KO WAT, there is coordinate regulation of expression of genes, transcriptional regulators, and mitochondrial proteins required for energy dissipation and fatty acid oxidation, such as PPARα, ATGL, perilipin, UCP-1, CIDEA, and several mitochondrial proteins involved in electron transport and fatty acid β-oxidation. UCP-1, a "marker' for BAT usually not present in WAT, is markedly elevated in KO WAT and contribute to a smaller increase in body weight in response to high fat diets, smaller gonadal fat deposits and adipocytes, uncoupled EWAT mitochondrial respiration, increased oxygen consumption, increased fatty acid oxidation, and increased treadmill endurance. In addition, expression of a number of inflammation-related genes was decreased in KO WAT, including some whose reduced expression might reflect reduced monocyte/ macrophage infiltration/differentiation in KO EWAT as well as a reduction in the NLRP3 inflammasome. These results suggest that PDE3B may regulate a cAMP-sensitive "switch" for WAT/BAT phenotypic conversion, regulating downstream effects of cAMP on cAMP/PKA- and AMPK-signaling, mitochondrial biogenesis and function, energy dissipation, and the pro-inflammatory state of WAT. Understanding mechanisms for these changes in KO EWAT is important, since conversion of "fat-storing" EWAT to "fat-burning" BAT, with reduced inflammation, represents a potential strategy in treatment of obesity and diabetes.

Structural Basis of the Vinculin–F-actin Interaction. L.Y. Kim[1], P.M. Thompson[2], S.L. Campbell[2], G.M. Alushin[1]; [1]Laboratory of Macromolecular Interactions [2]Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill.

The essential protein vinculin is a key component of cell–matrix and cell–cell adhesions, acting as a linker between these connection points and the actin cytoskeleton. Accumulation of vinculin is necessary for adhesions to sustain high traction forces, and its direct interaction with F-actin is required for mechanotransduction. Due to its physiological significance, the molecular determinants of this interface have been under scrutiny for some time, yet conflicting structural models are present in the literature, largely because of the absence of high-resolution analysis. To address this issue, we have obtained a subnanometer-resolution cryo-EM reconstruction of vinculin's C-terminal "tail" domain (Vt) bound to F-actin, sufficient for constructing an unambiguous pseudo-atomic model of the interface by docking crystallographic structures of the components. We support our docking studies with a mass-tagging experiment, localizing a C-terminal GFP fusion to orient the pseudo-symmetric Vt helical bundle in the density map. Interestingly, we observe that the N-terminal half of Vt, distal from the actin surface, undergoes a substantial structural rearrangement, which may have implications for licensing additional protein-protein interactions and actin-mediated dimerization. The actin protomer also undergoes subtle remodeling which could influence interactions with additional binding partners.

Rbfox3-regulated Alternative Splicing of Numb Promotes Neuronal Differentiation during Development. K.K. Kim[1], J. Nam[2], Y. Mukouyama[2] and S. Kawamoto[1]. [1]Laboratory of Molecular Cardiology, [2]Laboratory of Stem Cell and Neuro-Vascular Biology.

Alternative pre-mRNA splicing is a major mechanism to generate diversity of gene products. However, the biological roles of alternative splicing during development remain elusive. Here we focus on a neuron-specific RNA binding protein, Rbfox3, recently identified as the antigen of the widely used anti-NeuN antibody. SiRNA-mediated loss-of-function studies using the developing chicken spinal cord revealed that Rbfox3 is required to promote neuronal differentiation of post-mitotic neurons. Numb pre-mRNA encoding a signaling adaptor protein was found to be a target of Rbfox3 action and Rbfox3 represses the inclusion of an alternative exon via binding to the conserved UGCAUG element in the upstream intron. Depleting a specific Numb splice isoform reproduces similar neuronal differentiation defects. Forced expression of the relevant Numb splice isoform is sufficient to rescue, in an isoform-specific manner, post-mitotic neurons from defects in differentiation caused by Rbfox3 depletion. This study provides a novel mechanism by which Rbfox3-dependent Numb alternative splicing regulates progression of neuronal differentiation during vertebrate development.

Clonal Tracking of Rhesus Macaque Hematopoiesis Highlights a Distinct Lineage Origin for Natural Killer Cells. S.J. Koelle, C. Wu, B. Li, R. Lu, Y. Yang, A. Jares, A.E. Krouse, M. Metzger, F. Liang, K. Loré, C.O. Wu, R.E. Donahue, I.S. Chen, I. Weissman, C.E. Dunbar. Molecular Hematopoiesis Section, Hematology Branch.

For the first time in a large animal we have applied a powerful barcoding approach to track output from HSPCs and their progeny, with predictive value for human HSPC behavior. We "barcoded" individual CD34+ HSPC

from 4 rhesus macaques using lentiviral vectors carrying highly diverse oligonucleotides, allowing precise quantitation of clonal contributions via low cycle PCR amplification followed by high-throughput sequencing and computational analysis (Lu et al, Nat Biotech). Following TBI and infusion of autologous barcoded CD34+ cells, we quantitated barcode levels, and thus individual HSPC clonal contributions, over time and between lineages. We established that the relative fraction of barcode reads retrieved via Illumina sequencing accurately represented clonal abundance in a polyclonal setting, via analyzing mixtures of 10 individual barcoded K562 clones. Duplicate samples were analyzed to confirm the reproducibility of quantification of clonal output via this method. Peripheral blood samples from time points up to 15 months post-transplant in four monkeys were FACS sorted into granulocytes, monocytes, T, B, and NK cells, as well as dendritic cells and NK subsets. Following reconstitution of NK, T, B and Myeloid (My) from uni-lineage progenitors at 1-2 months, by 3-4m, as expected from murine and in vitro models, multi-lineage clones began to contribute and dominate, first B/My, then B/T/My. Reconstitution of the NK compartment was clonally distinct from T, B and My lineages. The predominant CD16+ NK cells remained clonally distinct through 9 m, despite overall clonal diversity and marking levels in NK lineage similar to B/T and My.

Identification of Readers of the H3.3 Histone Variant Code. D.C. Kraushaar, S. Patel, G. Wang, Q. Tang, M. Gucek, K. Zhao. Systems Biology Center.

DNA is wrapped around histone proteins to form nucleosome particles that compact eukaryotic genomes while still allowing access for cellular processes such as transcription, replication and DNA repair. Histones exist as different variants that have evolved crucial roles in specialized functions in addition to their fundamental role in packaging DNA. H3.3 – a conserved histone variant that is structurally very close to the canonical histones H3.1 and H3.2 – has been associated with active transcription. Apart from its interaction with critical chaperones, few other downstream effector proteins of H3.3 are known. To identify additional H3.3-interacting factors, we developed a nucleosome purification method to isolate H3.3-containing nucleosomes from mouse embryonic fibroblasts and used epitope-tag affinity purification and mass spectrometry to identify the co-purified nuclear proteins. Using semi-quantitative spectral counting, we identified around 100 novel H3.3 interacting proteins that are involved in transcriptional regulation, chromatin remodeling and maintenance of chromatin architecture. To address the role of post-translationally modified lysine residues for interactions between H3.3 and its effectors, we inserted point mutations at K4, K9, K18, K27 and K36 positions. In this way, we elucidated a critical role for lysine 4 as a docking site for the nucleosome remodeling and histone deacetylase (NuRD) complex. The interaction between the gene repressor NuRD and H3.3 may have important implications for balancing and dosing gene activity.

CD73 Regulates Inflammation during Vascular Remodeling. J. D. Lanzer, C. St. Hilaire, R. Schwartzbeck, H. San, F. Fang, M. Boehm. Laboratory of Regenerative Cardiovascular Medicine.

The loss-of-function mutations in the gene encoding CD73 protein in humans leads to extensive calcifications of the lower extremities arteries (Arterial Calcification due to Deficiency in CD73, ACDC). The membrane bound CD73 enzyme converts extracellular AMP to adenosine and inorganic phosphate and our data suggests that adenosine protects human arteries from calcification. Further, it is well known that adenosine elicits both pro- and anti-inflammatory effects depending on the cell type and the adenosine receptor subtype it binds. In this study we show that CD73 exhibits anti-inflammatory effects in the remodeling of blood vessels. To study the role of CD73 in vascular remodeling, we transplanted murine jugular veins from wild type (WT) and CD73-/- mice into the femoral arteries of WT mice. The grafted vein is exposed to the high blood pressure and shear stress of arterial environment and thereby undergoes a remodeling process that is characterized by the formation of neointima. Strikingly, CD73-/- vein grafts exhibit aggravated medial dysplasia and a drastic increase in inflammatory cell infiltration. To understand the underlying mechanism we will isolate aortic vascular smooth muscle cells (AvSMCs) from WT and CD73-/- mice and treat them with cytokines (e.g. IL-1β and TNF-α) known to promote inflammatory response. We hypothesize that the CD73-/- AvSMCs will exhibit a more pro-inflammatory phenotype which can be rescued by adenosine treatment. Furthermore we modulate adenosine receptor signaling via knock-down and pharmacological tools to delineate their involvement and further characterize the downstream signaling which leads to transcriptional changes responsible for the inflammatory response.

Arf Guanine Nucleotide-exchange Factors BIG1 and BIG2 Regulate β-catenin Signaling via Direct Interaction and cAMP/PKA Signaling. K. Le, CC. Li, J. Moss, M. Vaughan; Cardiovascular and Pulmonary Branch.

BIG1 and BIG2, brefeldin A-inhibited guanine nucleotide-exchange factors (GEFs) that activate ADP-ribosylation factors (Arfs) by accelerating the replacement of bound GDP with GTP, contain A kinase-anchoring protein (AKAP) sequences that act as scaffolds for multimolecular complexes to facilitate and limit intracellular cAMP signaling in both time and space. β-catenin, with its critical roles in cell-cell adhesion and Wnt signaling, was among HeLa cell proteins co-immunoprecipitated by antibodies against BIG1 or BIG2. Direct interaction of BIG1 N-terminal sequence with β-catenin, found in yeast two-hybrid studies, was confirmed using *in vitro*-synthesized proteins. Depletion of BIG1 and/or BIG2 or over-expression of a GEF-inactive mutant, but not wild-type, BIG1 or BIG2 resulted in β-catenin accumulation in perinuclear Golgi structures. As association of protein kinase A (PKA) with β-catenin, BIG1, and BIG2 in HeLa cells, and PKA-catalyzed phosphorylation of β-catenin S675 were diminished after depletion of BIG1 or BIG2, we infer that function of the AKAP sequences in BIG1 and/or BIG2 may be required for PKA-catalyzed β-catenin modification. In addition, transcriptional activity of β-catenin was lower in HeLa cells after BIG1 and/or BIG2 depletion, further

consistent with their roles in regulating β-catenin action via S675 phosphorylation.

Novel Pericentrin structures orchestrate the PCM in rapidly dividing embryos. D.A. Lerit[1], J.S. Poulton[2], H.A. Jordan[1], M. Peifer[2], N.M. Rusan[1]; [1] Laboratory of Molecular Machines and Tissue Architechture, [2]Department of Biology University of North Carolina Chapel Hill.

The first several mitotic divisions of a developing embryo lack gap phases and proceed through fast cycles of DNA replication followed by mitosis. The rapid assembly and disassembly of the bipolar mitotic spindle from one cell cycle to the next is coordinated by centrosomes, which are the primary microtubule-organizing centers (MTOCs) of most eukaryotic cells. Typically, the MTOC activity of centrosomes oscillates with varying levels of pericentriolar material (PCM) and peaks during mitosis. Previous work in the early syncytial Drosophila embryo shows the PCM is a highly dynamic structure that transitions from a compact structure to an elaborate "flare" and back during a single mitosis. How (or why) centrosomes regulate the organization of PCM throughout the cell cycle is a little understood process. We have identified Drosophila Pericentrin-like-protein (PLP) as an important regulator of PCM organization and activity in early embryos. During interphase, we find that PLP localizes to novel, radial extended structures that are distinct from PCM flares. We show these structures appear to delineate the PCM margin and function as a centrosomal corset, as live imaging and clonal mutant analysis indicates that loss of PLP results in highly disorganized PCM and aberrant centrosome separation. Propagation of these defects leads to loss of early cleavage synchrony, DNA damage, and massive nuclear fallout at the syncytial blastoderm stage. Our data indicate that regulation of the PCM structure is critical for normal cell division and early development.

A Point Mutation in Nonmuscle Myosin II-A Disrupts Mouse Spermatogenesis. C.B. Lerma Cervantes,[1] K. Tokuhiro,[2] Y. Zhang,[1] M.A. Conti,[1] D. H. Bae,[3] M.P. Daniels,[3] J. Dean,[2] R.S. Adelstein,[1]; [1]Laboratory of Molecular Cardiology, [2]Laboratory of Cellular and Developmental Biology, [3]Electron Microscopy Core.

Nonmuscle myosin II-A (NMII-A) is a major cytoskeletal protein composed of a pair of heavy chains (230 kDa, encoded by the Myh9 gene in mice) and 2 pairs of light chains (20 kDa and 17kDa). It is ubiquitously expressed in humans and mice, and contributes to cellular processes including cell migration, cell adhesion, and cytokinesis. Humans with mutations in NMII-A have defects in blood cells, and develop glomerulosclerosis, cataracts and deafness. We have generated a mouse line with a mutation frequently found in human MYH9 disease, E1841K. In addition to modeling the above defects, male, but not female, mice homozygous for the mutation were sterile. The male mice demonstrated severe defects in sperm development. Histological analysis of mutant testis document that spermatocytes arrested at the elongated spermatid stage and degenerate spermatids sloughed off into the lumen. Additionally, some of the seminiferous tubules did not develop a lumen or the lumen appeared degenerate with a complete absence of spermatocytes. Electron microscopy analysis showed abnormal acrosome formation in the mutant testis. TUNEL assays revealed an increase in apoptotic germ cells of mutant testis when compared to wild type. Furthermore, caudal epididymis sections contained only a few malformed spermatozoa that were rarely motile. Heterozygous mice showed similar, albeit less severe, defects and were fertile. Collectively, these observations suggest a previously unknown, pivotal role for NMII-A in spermatogenesis and male fertility.

Polo-like kinase 2 activates an antioxidant pathway to promote the survival of cells with mitochondrial dysfunction. J. Li, P. Wang, P. M. Hwang. Center of Molecular Medicine.

We previously reported that increased expression of *Polo-like kinase 2 (PLK2)* is essential for the survival of cells with mitochondrial dysfunction (Matsumoto, T et al PNAS 2009). Here we report the identification of a novel antioxidant signaling pathway mediated by PLK2 that plays a critical role in preventing cell death caused by defective respiration. *PLK2* expression is induced by increased levels of oxidative stress and intracellular calcium, both associated with mitochondrial dysfunction, and we show that PLK2 can directly phosphorylate GSK3β at the Ser-9 residue. The serine phosphorylation of GSK3β inhibits its kinase activity and prevents the phosphorylation of NRF2, major antioxidant transcription factor. Unphosphorylated NRF2 can then translocate into the nucleus and transactivate various antioxidant genes including NQO1, the prototypical antioxidant target gene of NRF2. We further demonstrate that NQO1 can block necrotic cell death coordinated by reactive oxygen species (ROS) and p53 and can promote xenograft tumor growth. Thus, the antioxidant signaling pathway of PLK2 is an essential function required for the survival of cells with defective mitochondria that is associated with cancer as well as neurodegenerative diseases.

How does the metabolic microenvironment trigger the vascular patterning factor Cxcl12 and arterial differentiation factor VEGF-A in the nerve-mediated arterial branching? W. Li, A. Sajja, Y. Mukouyama. Laboratory of Stem Cell and Neuro-Vascular Biology.

Genetic studies provide evidence that intrinsic genetic programming regulates vascular development. However, it has been shown that organ-specific local signals provided by components such as peripheral sensory nerves coordinate vascular branching pattern and differentiation, and environmental factors may function as guidance cues to form a tissue-specific vascular pattern. Studies have revealed that hypoxia-induced genes are important regulators of angiogenesis, although the precise mechanism by which they coordinated vessel sprouting and remodeling remain unclear. We have recently shown that nerve-derived CXCL12 and VEGF-A coordinate nerve-vessel alignment and arterial differentiation. Given that both CXCL12 and VEGF-A are hypoxia-induced genes and the existence of localized hypoxia prior to nerve-vessel alignment, we have applied multiple approaches to examine whether localized hypoxia functions as an environmental signal to trigger these genes expression in the peripheral nerves thus directing arterial pattern-

ing. These experiments have opened up new areas of research about the regulation of branching morphogenesis and patterning by the metabolic microenvironment.

Opposing actions of IL-2 and IL-21 on Th9 differentiation correlate with their differential regulation of BCL6 expression. W. Liao, R. Spolski, P. Li, N. Du, E. E. West, M. Ren, S. Mitra, and W. J. Leonard. Laboratory of Molecular Immunology and the Immunology Center.

Interleukin-9 (IL-9) is a γ_c-family cytokine that is highly produced by T-helper 9 (Th9) cells and regulates a range of immune responses, including allergic inflammation. Here we show that IL-2-JAK3-STAT5 signaling is required for Th9 differentiation, with critical STAT5 binding sites in the *Il9* promoter. IL-2 also inhibited BCL6 expression, and over-expression of BCL6 impaired Th9 differentiation. In contrast to IL-2, IL-21 induced BCL6 and diminished IL-9 expression in wild-type but not *Bcl6*$^{-/-}$ cells, whereas Th9 differentiation was increased in *Il21*$^{-/-}$ or *Il21r*$^{-/-}$ T cells. Interestingly, BCL6 bound in proximity to many STAT5 and STAT6 binding sites, including at the *Il9* promoter. Moreover, there was increased BCL6 and decreased STAT binding at this site in cells treated with blocking antibodies to IL-2 and the IL-2 receptor, suggesting a possible BCL6-STAT5 binding competition that influences IL-9 production. BCL6 binding was also increased when cells were Th9-differentiated in the presence of IL-21. Thus, our data reveal not only direct IL-2 effects via STAT5 at the *Il9* gene, but also opposing actions of IL-2 and IL-21 on BCL6 expression, with increased BCL6 expression inhibiting IL-9 production. These data suggest a model in which increasing BCL6 expression decreases efficient Th9 differentiation, indicating possible new approaches for controlling this process.

Cardiomyocyte differentiation from human pluripotent stem cells in chemically defined E8 medium. Y. Lin, K. Linask, W. Xie, Y. Du, J. Beers, G. Chen. iPSC and Genome Engineering Core Facility.

Given the unique capacity to generate any cell type of the human body, human pluripotent stem cells (hPSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), hold great promise for drug discovery and future cellular therapy. To provide an effective tool to facilitate research use and satisfy the clinical demands of hPSCs, we previously developed a simplified chemically defined medium (E8 medium) for hPSCs derivation and maintenance, which is completely free of human and animal products including serum and albumin. In this study, we developed a protocol for the differentiation of clinical-grade cardiomyocytes from hPSCs on the defined E8 medium platform. By modulation of exogenous and endogenous cytokines and hormones, such as Wnt, BMP, TGF beta, FGF2, and insulin, we demonstrate that beating cardiomyocytes can be efficiently induced from hPSCs (ESCs and iPSCs) using E8 based medium. This protocol can be successfully applied in both 2D monolayer and 3D embryoid body (EB) culture. Additionally, we further demonstrated that the hPSC-derived cardiomyocytes could be successfully applied in high throughput screening system on 384-well plate format for drug screening and cardiotoxicity assay. In summary, our approach provides a promising and powerful tool for generating desired cardiac lineages from hPSCs that could be utilized in both basic research and clinical transition study.

In the Clutches of Maxwell's Demon: Below-Equilibrium DNA Topology Simplification by Type II Topoisomerases. T.R. Litwin, S.K. Sarkar, A.H. Hardin, I.J. Holt, K.C. Neuman. Laboratory of Molecular Biophysics.

Topoisomerases are divided into two classes: type I topoisomerases, which are ATP-independent enzymes that cut a single strand of DNA to relax supercoils, and type II topoisomerases, which are ATP-dependent enzymes that transiently cut both strands of one segment of DNA and pass another segment of DNA through the cut. Type I topoisomerases simplify topology to create a distribution of supercoils defined by thermal equilibrium. Type II topoisomerases use part of the energy from ATP hydrolysis to simplify DNA topology beyond thermal equilibrium such that the distribution of conformations is narrower, but the mechanism for this process is unresolved. Specifically, it is not known how type II topoisomerases acting locally on the nanometer scale can simplify the global topology of DNA, an extended substrate much larger than the enzyme. We propose that type II topoisomerases have a higher binding affinity for more highly supercoiled DNA and that this preferential binding can help explain below-equilibrium topology simplification. We developed a nitrocellulose filter binding assay to determine the topology-dependent binding of Topoisomerase IV, a bacterial type II topoisomerase. Computer simulations were also run to determine how empirical levels of differential binding can lead to non-equilibrium topology simplification.

The Relationship Between Sleep and Evolutionary Fitness in *Drosophila melanogaster*. A.S. Lobell, S.T. Harbison; Laboratory of Systems Genetics.

Nearly all animals sleep. While the evolutionary conservation of sleep suggests that it provides significant fitness benefits, the relationship between sleep and evolutionary fitness is not known. Here we investigate this relationship in *Drosophila melanogaster* by quantifying the phenotypic and genetic covariation between sleep and ovariole number, the egg development pipeline within the fly ovary. Ovariole number strongly affects reproductive fitness in female flies by limiting maximum fecundity. We measured ovariole number in both ovaries of thirty female flies from 200 lines of the *Drosophila* Genetic Reference Panel (DGRP) and quantified the asymmetry between ovaries. Ovariole number shows significant phenotypic variation among DGRP lines ($P<0.0001$) and exhibits high broad-sense heritability ($H^2 = 0.59$) while ovariole asymmetry also shows significant differences among lines ($P<0.0001$) but with lower heritability ($H^2 = 0.05$). Further, we calculated the coefficient of environmental variation (CV_E) for these measures and found that the environmental sensitivity in ovariole number is influenced by underlying genotype ($H^2 = 0.23$). We identified Single Nucleotide Polymorphisms (SNPs) significantly associated with each of these traits and compared them to 8,197 SNPs previously associated with fourteen sleep parameters. This approach allowed us to identify variants, genes, and genetic networks common to sleep and ovariole phenotypes.

Determining the extent to which sleep covaries with traits critical to an organism's fitness will provide insight into why sleep has been so strongly conserved over evolutionary time. Genetic correlations between fitness-enhancing genes and sleep also may help elucidate sleep's biological function.

Cancer cell migration, blebbing and the actin cortex. J.S. Logue, R.S. Chadwick[2], C.M. Waterman[1], . [1]Laboratory of Cell and Tissue Morphodynamics. [2]Section on Auditory Mechanics, Laboratory of Cellular Biology.

Metastasis is a frightening and often deadly scenario to those suffering from cancer. Despite all that we have learned about this process, treatments that specifically block metastasis still remain beyond our reach. Work described here, examines a mode of cell migration that is used specifically by cancer cells within a physiologically relevant context. Recent studies have shown that cancer cells will often bleb. Simply put, a bleb is an intracellular pressure driven circular protrusion of the plasma membrane. When cells are confined, blebs are able to push themselves between tight spaces and during the retraction phase pull a cell forward. In addition to intracellular pressure, whether or not a bleb forms is determined by how well the plasma membrane is connected to the underlying actin cortex. Throughout this study, we have confined human melanoma A375 cells under agarose. By combining spinning disk confocal imaging, Total Internal Reflection Fluorescence (TIRF) microscopy and Atomic Force Microscopy (AFM) we are studying bleb dynamics, mechanics and identifying proteins important to the actin cortex and that impact blebbing. One of these proteins, called Ezrin, has been demonstrated to link the plasma membrane to the underlying actin cortex. In addition to this fundamental function of Ezrin, it has also been shown to interact with specific modifiers of the actin cytoskeleton. A protein called EGF receptor pathway substrate 8 (Eps8) was recently shown to bind a Pro-X-X-Pro related motif in Ezrin via its SH3 domain. Therefore, we are testing whether Ezrin, besides linking the plasma membrane to the actin cortex, reorganizes the actin cortex in concert with proteins, such as Eps8, to influence blebbing. Eps8 is the prototype of a family of proteins that are capable of actin capping and bundling. Recently, our studies have uncovered a role for the Ezrin-Eps8 complex in cancer cell migration. More specifically, augmenting the formation of the Ezrin-Eps8 complex affects the actin cortex in such a way that melanoma cells produce fewer but larger blebs. We are currently examining the role of this complex in the overall ability of melanoma cells to migrate by blebbing when confined. Interestingly, Ezrin and Eps8 are highly expressed in cells derived from metastatic sites; therefore, these studies may provide a deeper understanding of this correlation and potentially reveal new targets for the prevention of metastasis.

Exploring myosin-5 elasticity under strain. A. Lopata[1,2], A. Nagy[2], N. Billington[2], M. Peckham[1], P. Knight[1], J. Sellers[2]; [1]University of Leeds, UK; [2]Laboratory of Molecular Physiology.

The structure and enzymatic properties of myosin-5 moving processively along actin under unloaded conditions have already been described previously. While pulling a cargo through the viscoelastic cytoplasm, the molecule will experience variable forces. This work aims to study how these forces alter the structure and enzymology of myosin-5 using single particle electron microscopy and single molecule super resolution fluorescence microscopy.

Thus, a myosin 5 chimera has been engineered, so that the C-terminus has an actin binding sequence. When this recombinant myosin is allowed to interact with actin filaments, the molecule will walk along the actin until the tail regions forms a bond with the actin filament. This system is expected to result in the motor "stalling" while interacting with actin. This would simulate a case where inside the cell the myosin 5 stalls while trying to move its cargo through a narrow pore in the cytoskeleton.

Negative staining electron microscopy will be used to image the motor domains and levers of stalled myosin 5 and their structures will be compared to those of myosin 5 molecules moving under unloaded conditions. It is likely that the predicted coiled-coil tail of myosin 5 is elastic and might extend under these conditions, which will be measured. In addition, TIRF microscopy will be applied to determine how strain affects the kinetics of the two heads of myosin under stalled conditions.

Whole Exome Sequencing to Establish a Genetic Etiology for Pentalogy of Cantrell: In Rare Diseases the Code can be Difficult to Decipher. M. A. Markowitz[1], X. Ma[1], L. Leatherbury[2], Y. Yang[3], J. Zhu[3], J. Chong[4], M. J. Bamshad[4], R. S. Adelstein[1], E.K. Grant[1, 2]; [1]NHLBI Laboratory of Molecular Cardiology, [2]Children's National Medical Center, [3]NHLBI Genomics Core Facility and [4]University of Washington Centers for Mendelian Genomics.

Pentalogy of Cantrell (POC) is an extremely rare and often fatal syndrome including defects of the abdominal wall, diaphragm, pericardium, sternum and heart. Patients affected by POC frequently have an extrathoracic cardiac position known as *ectopia cordis*. The syndrome affects less than 1 in 200 000 live births. A murine phenotype similar to POC has been generated in our laboratory from a point mutation (R709C) in the nonmuscle myosin IIB heavy chain (MYH10). Generation of this mouse model has prompted us to conduct a clinical study in which we are performing whole-exome sequencing of human families affected by POC to determine whether a genetic etiology can be established. In order to interpret the sequencing results and better understand the environmental and genetic mechanisms of this disease, we are conducting a review of the phenotypic features of all previously reported POC cases including our eleven NIH enrolled subjects. Preliminary analysis of the available sequencing results thus far has identified a possible point mutation in nonmuscle myosin IIB in one family, however further testing is required to determine whether this mutation is causative. We hope to increase the likelihood of identifying a genetic etiology for POC through worldwide recruitment of families affected by this extremely rare disorder.

Emerging Evidence of Lysosomal Degradation of α-Synuclein by Cysteine Cathepsins. R. P. McGlinchey, J. C. Lee; Laboratory of Molecular Biophysics.

Cytosolic accumulation of α-synuclein (α-syn) is a feature of Parkinson's disease (PD). This accumulation may be counteracted by mechanisms of protein degradation that involve the proteasome and lysosome. Specifically, the aspartyl lysosomal protease, Cathepsin (Cat) D, has been suggested to be the main 'synuclease' involved in the clearance of α-syn *in vivo*. However, only C-terminal truncated species are generated by Cat D *in vitro*, arguing that other mechanisms are needed to explain complete α-syn degradation. Here, we show that intact purified lysosomes from mouse brain and liver harbor cysteine cathepsin activity, which results in degradation of α-syn. Using purified proteases, cysteine Cat B, L, K, and V are identified as candidate 'synucleases' that are fully capable of proteolyzing α-syn in contrast to Cat D. In addition, since α-syn associates with membranes and adopts a highly helical structure, we have investigated the effects of various lysosomal lipid compositions on cathepsin degradation of α-syn. Our data clearly show that lipids stimulate cathepsin activity, highlighting the importance of protein conformation during proteolysis. This work offers new mechanistic insights into α-syn clearance in the lysosome as well as the groundwork to explore cysteine cathepsins as new PD therapeutic targets.

ATP Binding Induced Conformational Change in RECQ Helicase. M. Mills, K. Neuman; Laboratory of Molecular Biophysics.

RecQ helicases are a family of DNA helicases critical for repair of recombination errors. These enzymes catalyze DNA strand separation via the binding and hydrolysis of ATP and are essential for resolving complex DNA structures such as double Holliday junctions and D-loops. Mutations in three human RecQ family proteins have been identified in several disorders linked to premature aging and a high predisposition to cancer. Studies of the structural dynamics of RecQ helicases are necessary to fully understand the mechanism of this important class of proteins. The RecQ family contains a highly conserved core domain which consists of two RecA-like helicase domains, a zinc binding domain, and a dsDNA-binding winged helix domain. Crystal structures of *E. coli* RecQ and human RecQ1 indicate that the enzyme binds ATP in the cleft between the two RecA-like domains. This structural detail is consistent with the nucleotide binding motif of SF1 family helicases, in which ATP hydrolysis drives translocation along ssDNA. However, it is not currently known how the binding and hydrolysis of ATP is coupled to ssDNA translocation in RecQ. Using FRET and molecular dynamics simulations, we have investigated the ATP-dependent dynamics of the core of *E. coli* RecQ. We show that binding of ATP induces a conformational change in the protein that has implications for its DNA-binding state. These studies provide insight into the mechanism of RecQ's ATP-dependent activity.

An Alternative Mechanism for Actin Arc Formation at the Immunological Synapse of Jurkat T cells. S. Murugesan, J. Yi, D. Li, L. Shao, E. Betzig, X. Wu, J.A. Hammer; Laboratory of Cell Biology.

The T cell's actin cytoskeleton undergoes striking rearrangements at the immunological synapse (IS) upon contact with an antigen presenting cell to form segregated concentric domains named the distal, peripheral and central supramolecular activation clusters (dSMAC, pSMAC, and cSMAC). The dSMAC corresponds to a lamellipodial actin network of branched actin arrays that undergo polymerization-driven retrograde flow, while the pSMAC corresponds to a lamellar actin network of concentric actin arcs that undergo myosin II-dependent contraction. Together, these forces drive the centripetal transport of signaling molecules to create a mature IS. Here we addressed how the concentric actomyosin arcs in the pSMAC are assembled. Currently, it is thought that the branched actin network, which is created by Arp2/3-dependent nucleation, is converted by debranching and crosslinking into concentric arcs. Using structured-illumination microscopy, we identified for the first time linear actin filaments arranged perpendicular to the plasma membrane that are embedded in the branched actin network. As these filaments flow inward, they splay out and reorient into concentric arcs with an inherent antiparallel organization required for myosin II-dependent contraction. These perpendicular filaments are accentuated by inhibiting Arp2/3, which collapses the branched network. Importantly, inhibiting myosin II results in disorganized filaments in the pSMAC that fail to reorient into concentric arcs. Thus, arc assembly can occur independently of Arp2/3-dependent nucleation, and myosin II contractility is required for reorienting the perpendicular filaments emanating from the dSMAC into concentric arcs in the pSMAC. Finally, we are addressing the nucleation of perpendicular filaments, possibly by the formin INF2.

The Macrophage Scavenger Receptor CD163 Suppresses Airway Hyperreactivity in a Murine Model of Experimental Cockroach-induced Asthma. E.M. Mushaben[1], C. Dai[1], X. Yao[1], G.Z. Nugent[2], K.M. Keeran[2], Z. Yu[3], A. Aponte[4], M. Gucek[4], S.J. Levine[1]. Laboratory of Asthma and Lung Inflammation[1], Laboratory of Animal Surgery and Resources Core Facility[2]; Pathology Core Facility[3]; and Proteomics Core Facility[4].

The macrophage scavenger receptor, CD163, which is expressed exclusively by cells of the monocyte-macrophage lineage, binds and internalizes hemoglobin-haptoglobin complexes during hemolysis. CD163 also induces both anti-inflammatory and pro-inflammatory responses, however, the role of CD163 in asthma, an airway inflammatory disease, has not been defined. Since CD163 is expressed by alveolar macrophages and can influence inflammatory responses, we hypothesized that CD163 might modulate the severity of cockroach-induced allergic asthma, which is an important cause of asthma in inner cities. Wild-type (WT) C57BL/6 and *Cd163*$^{-/-}$ mice received intranasal challenges of German cockroach (*Blattella germanica*) extract (GCE) to induce allergen-mediated inflammation. GCE-challenged *Cd163*$^{-/-}$ mice had significant increases in airway hyperreactivity (AHR) to methacholine as compared to WT mice, whereas there were no differences in airway inflammation and mucous

cell metaplasia. In addition, GCE-specific levels of IgE and IgG1 were elevated in serum from *Cd163*$^{-/-}$ mice as compared to WT mice, indicating an increase in allergic sensitization. We also found that bronchoalveolar lavage fluid levels of the C-C chemokine, CCL24, which mediates eosinophil chemotaxis were elevated as compared to WT mice. Since CD163 is a scavenger receptor, we hypothesized that CD163 might suppress AHR by binding to GCE proteins. Pull-down experiments of GCE proteins were performed utilizing human recombinant CD163 protein, followed by 1D gel electrophoresis and mass spectroscopy, which identified binding of the *B. germanica* egg yolk protein, vitellogenin, to CD163. We conclude that cockroach-induced AHR is attenuated via the binding of the cockroach protein, vitellogenin, to CD163. These data identify a novel, protective function for macrophage-associated CD163 as a negative regulator of GCE-mediated AHR in an experimental murine model of asthma.

Directly Measured Inflammation in Psoriasis Skin Associates with Vascular Inflammation Beyond Traditional Risk Factors. H.B. Naik, P Krishnamoorthy, J. Dave, S. Rose, N.N. Mehta; Section of Inflammation and Cardiometabolic Diseases.

Inflammation is critical to development and progression of atherosclerosis. Psoriasis, a chronic inflammatory disease characterized by skin and systemic inflammation, is associated with increased cardiovascular diseases (CVD). We have previously demonstrated increased vascular inflammation by FDG PET CT (18F-fluorodeoxyglucose positron emission tomography/computed tomography) in psoriasis. It is currently unknown whether directly-quantified skin inflammation is related to CVD. We hypothesized that increased skin inflammation is associated with increased vascular inflammation persisting beyond adjustment for CVD risk factors. Clinical and laboratory measures of inflammation were prospectively evaluated in 41 consecutive adult psoriasis patients (NCT01778569). Psoriasis severity was assessed using Psoriasis Area Severity Index (PASI). Aortic vascular inflammation was assessed using a validated measurement from clinical trials, the target-to-background ratio (TBR), assessed by FDG PET/CT. Multivariate linear regression was used to assess for association between TBR and PASI, adjusting for age, sex, hypertension, hyperlipidemia, diabetes, tobacco use, alcohol and body mass index. Analyses were conducted using STATA 8.0 software. No statistically significant difference was seen in demographic or metabolic risk factors between psoriasis severity groups. Framingham 10-year risk scores were low in our cohort (males mean 6.9, SD 5.4; females mean 4.5, SD 4.3; $p=0.11$). In unadjusted analyses, hsCRP ($\rho=0.34$, $p=0.03$) and psoriasis body surface area ($\rho=0.79$, $p<0.001$) were significantly associated with PASI. As expected, CVD risk factors were each associated with TBR ($p<0.001$ for all). Furthermore, as PASI increased, we observed increased TBR (unadjusted $\beta=0.007$, $p<0.01$) beyond CVD risk factors (adjusted $\beta=0.002$, $p=0.012$). Directly assessed skin inflammation independently associated with vascular inflammation beyond CVD risk factors. These findings suggest that inflammation *in vivo* in humans heightens the risk for CVD. Whether treatment of this inflammation modulates CVD risk is unknown, and the topic of ongoing study.

Regulation of UPF1 binding to 3'UTRs. T. Nguyen, S.L. Baker, J.R. Hogg; Laboratory of Ribonucleoprotein Biochemistry

The nonsense-mediated mRNA decay (NMD) pathway is a surveillance mechanism utilized by cells to discard mRNAs containing premature termination codons. The selective decay of aberrant mRNAs is carried out by the RNA helicase UPF1 and several additional NMD factors.

We have previously demonstrated that selective UPF1-mediated RNA decay occurs in a two-step process: recognition of long 3'UTRs by UPF1, followed by commitment to decay. We have found that the length-sensing role and commitment steps can be decoupled through inefficient translational readthrough that allows UPF1 binding to mRNPs but inhibits decay. Together these observations imply that the kinetics of UPF1 displacement might play a role in determining the decay fate of UPF1-bound mRNAs.

We are currently investigating the process of UPF1 displacement from mRNPs. Preliminary evidence suggests that UPF1 is capable of regulating its own binding to mRNAs. Additionally, a second RNA helicase, MOV10, which has been recently shown to bind to 3'UTRs in close proximity to UPF1, might also play a role in active UPF1 displacement. Here we perform biochemical assays to interrogate the role of UPF1 and MOV10 helicases in determining mRNP protein composition. Furthermore, we perform dual color immunofluorescence microscopy to observe how localization of these two factors might relate to their biochemical functions in the NMD pathway.

Cytokine and T Cell Changes Upon *in vivo* Ibrutinib Therapy for CLL –Targeting CLL and Microenvironment. C.U. Niemann, A. Biancotto, B. Chang, J. Buggy, M. Farooqui, A Wiestner. Laboratory of Lymphoid Malignancies.

Chronic lymphocytic leukemia (CLL) cells are highly dependent on the microenvironment. In clinical trials, targeted therapy for CLL by the BTK inhibitor ibrutinib causes redistribution of CLL cells from lymph nodes to peripheral blood that could be due to direct or indirect effects on microenvironmental cells. In addition to blocking B cell antigen receptor signaling; cell signaling, adhesion, and migration are inhibited by ibrutinib. BTK is not expressed in T cells; however ITK is known to be directly inhibited by ibrutinib,

The effect of ibrutinib on T cell subsets, T cell activation, and cytokine profiles in serum was assessed in CLL patients in an ongoing phase II trial.

Inflammatory cytokines in patient serum decreased during ibrutinib treatment in accordance with *in vitro* data showing decreased secretion from T cells. This may correlate with an overall inhibition of inflammatory signaling. Activation of CD4+ T cells was also inhibited. Proliferation of both activated (HLA-DR+) and non-activated CD4+ T cells in peripheral blood decreased upon 2-3 months of ibrutinib treatment. The Th17 T cell subset was also diminished in patients upon ibrutinib treat-

ment. However, the Th1 and Th2 subsets were not significantly changed upon treatment.
This inhibition of inflammatory drive and T cell activation during ibrutinib treatment may take part in the very significant clinical results achieved for CLL patients on ibrutinib treatment. These changes may be due to direct effect of ibrutinib on T cells and macrophages of the microenvironment and indirect effects through inhibition of B cell interaction with microenvironment cells.

Antimicrobial Peptides Piscidins King at a Central Glycine to Maximize their Hydrophobic Moments. B. S. Perrin Jr[1], R. Fu[2], R. M. Venable[1], E. Mihailescu[3], C. Grant[4], Y. Tian[4], S. Opella[4], R. W. Pastor[1], M. Cotten[5]. [1]Laboratory of Computational Biology, [2]National High Magnetic Field Laboratory, Tallahassee, FL, [3]IBBR, University of Maryland, Gaithersburg, MD, [4] Dept. of Chemistry and Biochemistry, UCSD, La Jolla, CA, [5]Hamilton College, Clinton, NY.

Piscidin 1 (p1) and the less active piscidin 3 (p3) are α-helical, amphipathic, and cationic antimicrobial peptides. They adsorb onto the outer membrane of pathogenic species and induce leakage beyond a threshold peptide concentration. While different mechanisms of membrane disruption have been proposed, an atomic-level description of the events leading to cell death is lacking and elucidating these mechanisms will aid in the antibiotic design. Here, the dominant structures, orientations, and positions of p1 and p3 in two different lipid model bilayers (3:1 DMPC/DMPG and 1:1 POPE/POPG) are studied by solid-state NMR spectroscopy, all-atom molecular dynamics (MD) simulations, and neutron diffraction. The tilts of the helical axis, τ, are between 83 and 93° with respect to the bilayer normal for all systems and analysis methods. The average azimuthal rotation, ρ, is 235°, which results in burial of hydrophobic residues in the bilayer. The refined NMR and MD structures reveal a slight kink at Gly13 of the α-helix; this kink is characterized by a small difference in τ (<10°) and significant difference in ρ (~20°). This kink in ρ maximizes the hydrophobic moment for the α-helix. The depths of insertion are similar in DMPC/DMPG. In contrast, p1 and p3 are 2.2 and 1.2 Å less inserted in POPE/POPG.

KMT2D histone methyltransferase is a positive regulator of T cell development. K. Placek, K. Cui, G. Hu, W. Jin, K. Zhao, Systems Biology Center.

Gene regulatory elements are associated with histone H3 lysine 4 (H3K4) methylation and H3K27 acetylation and DNase I hypersensitivity. Whilst much is known about changes in histone modification patterns and chromatin accessibility during T cell differentiation, little is known about the role of histone modifying enzymes in this process. KMT2D is a histone methyltransferase that has been reported to facilitate establishment of enhancer landscape by regulating H3K4me1 and H3K27ac. To determine whether KMT2D plays a role in T cell development, we deleted KMT2D in T cells. Our data show that KMT2D deficiency results in reduced number of periphery T cells. Moreover in the absence of KMT2D, differentiation of naïve CD4+ T cells into regulatory T (Treg) cells is compromised. ChIP-seq revealed a co-localization of KMT2D with active chromatin marks in CD4+ T cells. We showed that DNaseI hypersensitive sites (HS) bound by KMT2D tend to decrease in accessibility upon KMT2D deletion that leads to reduced expression of nearby genes. The decreased HS are linked to genes involved in Treg differentiation and are enriched in Ets motif important for CD4+ T cells development. We showed that KMT2D co-localizes with Ets1 and Elf1 in naïve CD4+ T cell and in Tregs. Our data suggest that KMT2D regulates CD4+ T cell development and differentiation of Tregs by maintaining chromatin accessibility at genes involved in CD4+ T cell differentiation.

High-resolution analysis of genome-wide long-distance chromatin interactions using sureHi-C. G. Ren, W. Jin, K. Cui, Q. Tang, K. Zhao, Systems Biology Center.

Transcription is often regulated by promoter-enhancer (P-E) interactions. In order to achieve unbiased and high-resolution mapping of P-E interactions, we have developed a new technique termed super-resolution Hi-C (sureHi-C) by using a combination of different restriction enzymes for digesting chromatin, followed by proximity-mediated re-ligation and next generation sequencing. Using this technique, we analyzed the genome-wide long-distance chromatin interactions in mouse ES cells and CD4 T cells. With limited sequencing depth, we achieved a resolution of about 1kb. We identified 2000 and 2015 topological domains in ES and CD4 T cells, respectively. Among the 66,941 interaction clusters identified in CD4 T cells, 98% are intra-chromosome interactions, and only 2% interactions are inter-chromosome interactions. Comparison with H3K27ac and p300 ChIP-Seq data revealed that these interactions are highly enriched in potential enhancer regions, suggesting that these interactions may be critically involved in regulating transcription in T cell. Indeed, by analyzing gata3- and stat6-deletion-induced gene expression changes, we found that the more interactive a factor binding site is, the more likely it regulates gene expression. Our high-resolution interaction data predict numerous potential enhancers. To experimentally test the enhancer activity, we employed CRISPR to delete the genomic regions. One example is a potential enhancer located 120kb away from the Bcl2 promoter. We found that deletion of this region in EL4 cells significantly decreased the Bcl2 expression and increased apoptosis, indicating a critical role of this region as an enhancer. In summary, sureHi-C is an unbiased high-resolution technique for mapping chromatin interactions.

Effects of Heterologous Expression of Human Cyclic Nucleotide Phosphodiesterase 3A (hPDE3A) on Redox Regulation in Yeast. D.K. Rhee, J.C. Lim, S. Hockman, F. Ahmad, Y.W. Chung, S. Liu, A. Hockman, V. Manganiello; Cardiovascular Pulmonary Branch.

In yeast, cyclic nucleotide phosphodiesterases (PDEs) are components of RAS/cAMP signaling pathways involved in regulation of H_2O_2-induced oxidative stress. We have used *S. cerevisiae* as a model system to study effects of exogenous WT human (h)PDE3A and K13R hPDE3A (a putative ubiquitinylation site mutant) on H_2O_2-induced oxidative stress. H_2O_2-stimulated ROS production

was markedly increased in yeast expressing K13R hPDE3A, named OxiS1, compared to that in mock (control) or yeast expressing WT hPDE3A, named OxiR1. H_2O_2 increased ubiquitinylation, phosphorylation, and activity of WT hPDE3A, but not K13R hPDE3A. In OxiR1, H_2O_2-induced ROS production was reduced, and YAP1 and YAP1-dependent anti-oxidant genes involved in recovery from oxidative stress were upregulated and accompanied by reduction of Tsa1p. However, in OxiS1, accumulation of YAP1 and YAP1-dependent genes was greatly reduced, resulting in failure to recover from oxidative stress. These changes in anti-oxidant gene expression did not directly correlate with differences in cAMP/PKA signaling, since despite differences in PDE activities, PKA activity was increased in OxiR1 and mock, but was lower in OxiS1. During exposure to H_2O_2, however, the activity of Sch9p, a TORC1-regulated rpS6 kinase and negative regulator of PKA in *S. cervesiae*, was rapidly reduced in OxiR1, but maintained in OxiS1, consistent with reduced activation of PKA and reduced phosphorylation of PKA substrates in OxiS1. These results suggest that, during oxidative stress, PKA activity may be regulated by TOR-Sch9 signaling, and that post-translational modifications of hPDE3A are critical in its ability to regulate the onset of cellular recovery from oxidative stress.

Vascular Inflammation by FDG PET-CT is Associated with Aortic Wall Properties by MRI. A. Sadek, J. Dave, J. Hasan, P. Krishnamoorthy, S. Rose, H Naik, E Weiner, M.A. Ahlman, D.A. Bluemke, N.N. Mehta; Section of Inflammation and Cardiometabolic Diseases.

Psoriasis is an inflammatory skin disease associated with cardiovascular (CV) events. Aortic vascular inflammation (VI) by [18F] Fluorodeoxyglucose Positron Emission Tomography-Computed Tomography (PET-CT) predicts future CV events and is increased in psoriasis. Furthermore, increased aortic wall thickness (WT) and decreased aortic distensibility (AD) by Magnetic Resonance Imaging (MRI) predict future CV disease. We utilized a sample with increased VI to characterize WT and AD to further our understanding of these imaging outcomes.

PET-CT and MRI exams were obtained in psoriasis (n=53). To measure VI, regions of interest were placed on PET images to measure the maximal aortic standard uptake value (SUVmax). To measure WT, wall boundaries of the aorta were traced on each MRI slice ,and to measure AD, aorta contours were traced throughout the cardiac cycle .The relationship between VI, AD and WT were analyzed for unadjusted (Spearman's rho) and adjusted (multivariable linear regression) models.

CV risk factors were associated with SUVmax, WT and AD ($p<0.01$ for all).After adjusting for CV risk factors, both WT ($\beta= 8.75$, $p <0.001$), and AD ($\beta= -247$, $p< .001$) were independently associated with VI.

In psoriasis, increased VI may be associated with structural and functional vessel abnormalities. Larger ongoing outcome studies will inform biological significance of these findings.

Imaging Flow Cytometry in the NHLBI Flow Cytometry Core Facility: Combining Statistical Power with High Resolution Single Cell Imaging. L. Samsel, J. P. McCoy; NHLBI Flow Cytometry Core Facility.

Imaging flow cytometry (IFC) is a powerful technology which combines the speed, sensitivity, and statistical strength of flow cytometry with the morphological and spatial characterizations possible with microscopy. Whereas typical microscopy-based analysis may involve manual scoring of a small number of cells, IFC automates image analysis, utilizes tens of thousands of cells per sample, and provides an immense capacity for single cell analysis, reducing operator-dependence, user bias, and low sensitivities/high variabilities. Further, IFC provides classification and quantification of a spectrum of morphologies. Powerful features of IFC include acquisition of hundreds of cells per second, 20-, 40-, or 60X magnification, extended depth of focus (from 4u in standard mode to 16u with extended depth of field), and up to 10 fluorescence channels along with brightfield and scatter imagery. Image analysis possibilities include location of accumulating proteins, co-localization of proteins to each other or in sub-cellular compartments such as lysosomes or mitochondria, shape changes, nuclear translocation and membrane trafficking, characterization of rare event morphology, cell death and apoptosis, "spot" counting and average spot size/intensity of DNA damage and repair proteins and mitochondria, etc., autophagy, nuclear imagery classifications such as lobe counting in neutrophils, among others. Sophisticated analysis software permits training of software to find similar images and identify features best suited to quantify changes among samples. These capabilities greatly enhance the investigation of multiple biological processes, concomitantly, at the single cell level.

Developing Assays of Angiogenesis in Cyanotic Congenital Heart Disease Patients with Aortopulmonary Collateral Vessels. N. Sandeep, Y. Mukoyama; Laboratory of Stem Cell and Neuro-Vascular Biology.

Cyanotic congenital heart disease patients often experience abnormal angiogenesis manifesting as abnormal aortopulmonary collateral (APC) blood vessel connections. Traditional methods of identifying APCs, cardiac catheterization and MRI, each have limitations and risks. We wish to develop highly sensitive in vitro and in vivo angiogenesis assays using only patient plasma to detail the presence and severity of APCs. In pilot experiments, we tested the ability of the in vitro human umbilical vein endothelial cell (HUVEC) sprouting and in ovo chick chorioallantoic membrane (CAM) assays to detect differences in plasma angiogenic activity with extensive quantification measurements. As proof of principle experiments, we first tested the HUVEC and CAM assays with serum from mice with and without oral neoplasm. While serum from both mice induced strong HUVEC sprouting, only serum from the neoplastic mouse induced new capillary formation in the CAM. Next, HUVECs were tested with plasma from patients either with (n=6) or without (n=7) APCs. Our initial trial with quantification measurements demonstrated minimal differences in HUVEC sprouting between both patient groups. When plasma from both patient groups was applied to the CAMs, vessel hemorrhage and overall decreased vessel density was observed in the area of ap-

plication, suggesting that all human plasma is toxic to CAMs. We are currently optimizing both assays to improve their differentiating capabilities. We are also attempting to profile angiogenic factors (e.g. VEGF-A, VEGF-C, VEGF-D, sFlt-1, SDF1-a, etc.) in patient plasma using commercially available ELISA multiplex panels. This information will provide insights in APCs pathogenesis.

Calmodulin Regulates the Behavior of Abnormal Spindle to Coordinate Mitotic Spindle Dynamics. T. Schoborg, B. J. Galletta, N. M. Rusan; Laboratory of Molecular Machines & Tissue Architecture.

Autosomal Recessive Primary Microcephaly (MCPH) is a neurodevelopmental disorder characterized by reduced brain volume and head size, leading to mild mental retardation. Mutations in at least seven different genes have been linked to this disorder, most of which are involved in mitotic spindle function. The most frequent lesions map to the *Abnormal Spindle-Like, Microcephaly Associated* (*ASPM*) locus, the vertebrate ortholog of the *Abnormal Spindle (Asp)* gene from *Drosophila melanogaster.* Although *Asp* appears to be required to focus microtubule (MT) minus ends at spindle poles during mitosis, its molecular function and etiology remain poorly understood. Here we provide evidence that Asp directly interacts with Calmodulin (CaM) and both associate with kinetochore microtubules during mitosis. The interaction between CaM and Asp is mediated through at least five separate IQ domains present in the C-terminal region of Asp. In support of their functional dependence, we show they exhibit nearly identical and dynamic streaming behavior towards MT minus ends. This occurs during metaphase and early anaphase and is lost upon CaM inhibition. During telophase/cytokinesis, both proteins colocalize with the minus ends of the central spindle MTs, a behavior that appears to be necessary for correct assembly of the contractile ring machinery. Our results suggest that CaM plays a key role in Asp function, providing significant insight into the molecular mechanisms underlying MCPH.

Generation of HERV-E specific CD8+ T-cells for immunotherapy of renal cell carcinoma. C. Scrivani, E. Cherkasova, and R.W. Childs. Hematology Branch.

A CD8+ T-cell clone was isolated and expanded in our lab from a patient who showed prolonged renal cell carcinoma (RCC) tumor regression following an allogeneic hematopoietic stem cell transplant. This clone was shown to kill kidney cancer cells in vitro.

An HLA-A11 restricted 10mer peptide (CT-RCC-1) was identified as the target of these cytotoxic T-cells. This peptide is derived from a newly discovered human endogenous retrovirus type E (CT-RCC HERV-E) located on chr. 6. The HERV-E-derived transcripts encoding this peptide are spliced variants sharing a common region in the retroviral 5'LTR. Expression of CT-RCC HERV-E is restricted to clear cell RCC (ccRCC) cell lines and fresh tumors, with no expression observed in normal tissues or any non-clear cell renal carcinomatumors.

The HLA-A11 phenotype is present in only ~16% of the human population whereas HLA-A2 is the most common HLA subtype. Here we attempt to isolate and expand HLA-A2+ restricted T-cells targeting peptides derived from the common region of CT-RCC HERV-E transcripts. We have generated a CD8+T-cell population that is only been shown to respond to a HLA-A2+ restricted peptide derived from this common region and to a HERV-E^+/HLA-$A2^+$ccRCC cell line while not responsive to ccRCC lines that have been shown to be HERV-E negative and/or HLA-A2 negative.

Our long-term goal would be to sequence the T-cell receptor from a clonal population of HERV-E peptide specific HLA-A2 + restricted T-cells. Retroviral vectors carrying TCR sequence might be used further for immunotherapy for kidney cancer.

Programmed Death-1 Receptor and Myeloid-Derived Suppressor Cells Have Protective Roles in Drug-Induced Allergic Hepatitis in Mice. K. M. Semple[1], M. Chakraborty[1], A. Fullerton[1], D. E. Kleiner[2], L. R. Pohl[1]. [1]Molecular and Cellular Toxicology Section, [2]Laboratory of Pathology, NCI.

Although clinical evidence suggests that many cases of drug-induced liver injury (DILI) are caused by an allergic hepatitis, detailed experimental proof for this mechanism of toxicity has remained elusive due to the lack of animal models. We have hypothesized that the relatively low frequency of these adverse events is due in part to the tolerogenic nature of the liver. To test this idea, immune inhibitory activities of programmed death-1 and myeloid-derived suppressor cells were blocked in female Balb/cJ mice prior to treatment with halothane, an inhalation anesthetic that appears to cause allergic hepatitis in humans. Nine days after female Balb/cJ mice were treated for the second time with halothane, histological examination of the liver revealed zones of necrotic and apoptotic hepatocytes that were associated with clusters of lymphocytes, macrophages, plasma cells, and eosinophils. Liver proteins covalently modified by a reactive metabolite of halothane *in vivo* were shown to activate hepatic $CD4^+$ T cells and react with isotype specific IgG1 and IgE serum antibodies. In addition, the severity of liver injury was significantly reduced when $CD4^+$ T cells were depleted from the liver. These findings are important because they indicate experimentally for the first time that DILI can be mediated by an allergic reaction against the liver and deficiencies in hepatic tolerance may predispose patients to this disease.

Cardiometabolic risk factors predict vascular disease beyond Framingham risk score in the systemic inflammatory disorder psoriasis. S. Rose[1,2], P. Krishnamoorthy[3], J. Dave[1], H. Naik[4], M. P. Playford[1], N. N. Mehta[1], [1]Section of Inflammation and Cardiometabolic Diseases, [2] NIAMS, [3] Englewood Hospital, Englewood, NJ. [4]NCI.

Objective: To understand how cardiometabolic disease (CMD) assessed by body mass index (BMI), insulin resistance (IR) and HDL function relate to cardiovascular disease (CVD) beyond traditional risk factors in psoriasis.

Background: Systemic inflammatory disorders, including psoriasis, increase CVD susceptibility beyond traditional Framingham risk factors. CMD is enriched in

psoriasis, yet it is unclear how obesity, insulin resistance, and HDL dysfunction affect CVD risk in psoriasis. FDG PET/CT detects vascular inflammation, and when present in the aorta, is associated with future CVD events. Therefore, we determined whether CMD measures predicted greater vascular disease assessed by FDG PET CT in psoriasis beyond Framingham Risk Score (FRS).

Methods: FDG-PET/CT was utilized to evaluate aortic vascular inflammation (defined as maximum standardized uptake value [SUVmax]). A CMD risk score (CMS) was assigned for each psoriasis patient based on BMI, HOMA-IR, and HDL efflux assessments, whereby a point was given for a value that was more adverse than the published cutoffs for these values (range -1 to 3, with -1 being the best and awarded to normal BMI, IR and high HDL function; 3 being the most adverse). The relationships between aortic SUVmax and CMD measures were established using linear regression, and the added contribution of this CMS to vascular disease estimation beyond FRS was determined using likelihood ratio testing.

Results: Our population (n=65) had low FRS (mean 6.7 +/- 1.5), however, there was still a significant association between individual components of the CMS and FRS ($p<0.001$ for each BMI, IR and HDL efflux). Significant associations were observed for CMS and FRS (β 0.134, $p<0.001$), FRS and aortic SUVmax (β 0.119, $p<0.001$), and CMS and aortic SUVmax (β 0.409, $p<0.001$). After adjusting for traditional CVD risk factors, CMS score >0 was associated with greater vascular inflammation (RR 1.5-fold, $p<0.001$), and this CMS also predicted increased vascular inflammation beyond FRS (χ^2 82, $p<0.001$).

Conclusions: Measures of obesity, insulin resistance and HDL dysfunction independently predict the presence of vascular inflammation in psoriasis beyond FRS. Thus, this CMS may serve as a powerful indicator of CVD risk in this population and should be considered important an determinant of CVD risk in this population.

TGF-ß signaling positively regulates lymphatic endothelial cell sprouting in vitro. B. Shepherd, J. James, Y. Mukouyama. Laboratory of Stem Cell and Neuro-Vascular Biology. Genetics and Developmental Biology Center.

The lymphatic system is a specialized endothelial cell network responsible for maintaining interstitial fluid homeostasis and serving as a conduit for immune cell transport throughout the body. During lymphatic development, a subset of venous endothelial cells differentiate into lymphatic endothelial cells (LECs) and sprout from the cardinal and intersomitic veins to form lymphatic network. Molecular signals regulating initial lymphatic network patterning and migration are unclear. We previously showed that Transforming Growth Factor ß (TGFß) signaling, a critical molecular pathway required for cardiovascular development, is also required for lymphatic network patterning. Genetic disruption of the TGFß pathway in embryonic mouse skin reduced LEC sprouting and lymphatic network branching complexity in vivo; however, the precise mechanism of TGFß action during developmental lymphangiogenesis remains to be elucidated. Therefore, we generated an in vitro assay to measure the direct effects of TGFßs on LEC sprouting. Using human dermal lymphatic microvascular endothelial cell (HMVEC-dLy) or FACS-isolated primary mouse LECs cultured in 3-D type I Collagen gels, we found that addition of TGFßs significantly enhanced LEC sprouting compared to non-treated controls. Live imaging of LEC sprouting in culture provided further insights into the behavior of individual LECs and lymphatic sprouts in cultures exposed to TGFß treatment. Our results show that TGFß positively regulates LEC sprouting and targeted migration in vitro.

Novel IgM-derived antibody-drug conjugate targeting Fcμ-receptor selectively kills chronic lymphocytic leukemia through intracellular cytotoxic payload delivery. M. Skarzynski[1], B. Vire[1], J. D Thomas[2], C. G Nelson[2], A. David[3], GeorgAue[1], T. R Burke[2], C. Rader[4,5], A. Wiestner[1]. [1]Hematology Branch, [2]Chemical Biology Laboratory, Molecular Discovery Program, Frederick National Laboratory for Cancer Research, CCR, [3]Laboratory of Viral Diseases, NIAID, [4]Experimental Transplantation and Immunology Branch, CCR, NCI, [5]Department of Cancer Biology and Department of Molecular Therapeutics, The Scripps Research Institute, Scripps Florida, Jupiter, FL.

FcμR, originally named TOSO after a spiced sake traditionally consumed during New Year celebrations in Japan, was identified by Kubagawa et al. as the long sought after IgM Fc receptor. FcμR binds IgM Fc very strongly [mean(SD)=10(9.2)nM] and IgM-FcμR complexes are rapidly internalized (>80% internalized within 5 minutes) upon binding. These findings combined with the discovery of overexpression in B-cell chronic lymphocytic leukemia (CLL) provided the rationale for the design of an ADC targeting FcμR.

In our study, 29 out of 30 CLL patient samples (97%) evaluated by flow cytometry had high FcμR expression by CLL cells [mean(SD)=89.1(7.1)%] and the remaining patient had moderate CLL FcμR expression (57.9%). In contrast, FcμR expression by T cells [mean(SD)=8.9(5.2)%] was significantly lower ($p<0.0001$).

We went on to show that an ADC consisting of the CH2-CH3-CH4 IgM regions conjugated to monomethyl auristatin F can kill CLL cells [mean(SD)=78.8(10.1)%, $p<0.0001$ versus scaffold treated control] but not T cells [mean(SD)=8.4(3.8)%, $p=0.32$ versus scaffold treated control] in peripheral blood mononuclear cell (PBMC) samples obtained from CLL patients.

This ADC also greatly reduced tumor burden in NOD/SCID/IL-2Rγnull (NSG) mice xenografted with 4 different CLL PBMC patient samples [mean reduction(SD)=74(20)% in blood and 65(19)% in spleen, n=30] without harming human T cells or murine cells.

In summary, FcμR is a promising new target for therapy of CLL and possibly other malignancies. Our preclinical results provide translational evidence for the clinical utility of IgM-derived protein scaffolds to target FcμR expressing cells.

A Novel Actin-Adhesion Structure Involved in Nuclear Positioning Requires the Formin FMN2. C.T. Skau, C. M. Waterman. Laboratory of Cell and Tissue Morphodynamics.

Active positioning of the nucleus is critical to a variety of cell functions, including migration, particularly in

3D environments. Although previous studies establish that integrins and actin-binding proteins at the nuclear envelope are critical for nuclear positioning, it is not known if this is mediated by linking the nucleus to the adhesion/actin system used for migration, or if nucleus-ECM coupling is accomplished via a specialized cytoskeletal system. We sought to test the hypothesis that a dedicated adhesion-actin system is responsible for maintenance of nuclear position in migrating cells. To test this, we examined the interplay between actin, adhesions, and the nucleus in fibroblasts using fluorescence microscopy. We identified novel adhesive structures underneath the nucleus termed subnuclear adhesions that are distinct from the canonical focal adhesions found near the leading edge of migrating cells. Unlike focal adhesions at the leading edge, the long-lived subnuclear adhesions assemble in bursts and slide toward the front of the cell. Additionally, we find that subnuclear adhesions have reduced levels of several actin-binding proteins and elevated levels of myosin IIB. We also show that a specific set of actin fibers connecting two subnuclear adhesions can help control nuclear shape. Furthermore, we find that the formin FMN2 localizes underneath the nucleus and is essential for organization of these subnuclear actin fibers. In fact, cells lacking FMN2 migrate poorly in 3D environments and exhibit uncoordinated changes in nuclear position. Together, our data reveal a previously unidentified mechanism for control of nuclear position via a novel adhesive structure.

Molecular modeling of membrane curvature induction by a peptide. A. Sodt, R. Pastor. Laboratory of Computational Biology.

Simulations offer not only a detailed molecular view of biological processes but also the opportunity to apply conditions that are ideal for extracting parameters and properties necessary to model complex disease-related phenomenon. The properties of the lipid membrane are examined here. The ideal conditions, those of a single amphipathic helical peptide embedded in a lipid bilayer, are applied to extract the curvature energetics of the system (how much force the embedded peptide exerts to bend the membrane). The resulting model, which relates the fraction of peptide surface coverage necessary to stabilize highly curved structures such as tubes and liposomes, can be used to predict the energetics of intermediates in, for example, endocytosis and vesicle fusion. The simulations contradict textbook descriptions of lipids and embedded proteins – that they have a particular shape and prefer similarly shaped membrane surfaces.

Development of in vitro and in vivo human disease models to study mechanisms regulating vascular calcification. C. St. Hilaire, H. Jin, J-M. Davaine, G. Chen, M. Boehm. Laboratory of Cardiovascular Regeneration.

We identified a novel disease causing calcifications in the lower-extremity arteries. This disease – Arterial Calcification due to Deficiency of CD73 (ACDC) – results from biallelic inactivating mutations in the gene encoding CD73. CD73 converts extracellular AMP to adenosine; adenosine elicits a signaling cascade via the four adenosine receptors (ARs). We hypothesize that AR signaling modulates calcification processes. Alkaline phosphatase (ALPL) regulates calcification and ACDC cells have an increase in ALPL mRNA under osteogenic conditions. Genetic rescue or exogenous adenosine reduced ALPL mRNA in ACDC cells compared to controls. We found that activation of the A1 or A3 receptors inhibited ALPL activity in ACDC cells, whereas a pan/A2b agonist did not. This suggests that CD73-mediated A1/A3 signaling modulates calcification. To identify pathways downstream of AR signaling we used our in vitro disease model along with pharmacological inhibitors of specific proteins and found that inhibition of MEK/ERK, PI3 Kinase, or mammalian target of rapamycin (mTOR) prevented the upregulation of ALPL under osteogenic conditions. Further, application of rapamycin prevented in vitro calcification. To further assess the mechanisms underlying this disease phenotype we generated induced-pluripotent stem cells (iPSCs) from ACDC and control patient fibroblasts (iACDC and iControl). IPSCs injected into immunocompromised mice form teratomas and we used this characteristic to test the calcification potential of iACDC cells. CT analysis discovered that iACDC-derived teratomas form distinct nodules of calcification that are not present in iControl teratomas. This novel in vivo disease model can be used to study CD73- and A1/A3AR-mediated calcification and screen pharmacological inhibitors of the calcification process.

Monocyte Subsets Predict Vascular Inflammation Beyond Traditional Risk Factors of Cardiovascular Disease in Psoriasis. E. Stansky, S. Rose, P. Krishnamoorthy, E. Weiner, J. Dave, N.N. Mehta, Section of Inflammation and Cardiometabolic Diseases, FACS Core.

Psoriasis (PSO), a systemic inflammatory disorder, is associated with increased susceptibility to cardiovascular disease (CVD), and circulating blood monocytes are thought to play a role. Monocytes are considered to be a heterogeneous population, and while the CD16- classical monocytic subset has been associated with early atherosclerosis, the role of CD16+ subsets is unclear. The current study utilized 18-Flurodeoxyglucose (FDG)-positron emission tomography (PET) computed tomography (CT) imaging, a reliable indicator of vascular inflammation, atherosclerotic plaque activity and future CV events, along with flow cytometry to evaluate the relationship between vascular inflammation and monocytic subsets in PSO. In vivo vascular inflammation, defined by maximum standardized uptake value (SUV) in the aorta, was measured by FDG-PET/CT in PSO patients (n=26). Monocytic subsets were evaluated based on differential expression of CD14 and CD16 in the peripheral whole blood: classical (CD14++CD16-), intermediate (CD14++CD16+), and nonclassical (CD14+CD16++). After adjusting for traditional CVD risk factors, demographics, HDL efflux and BMI, the relationship between monocytic subsets and aortic vascular inflammation was determined using linear regression analysis. FDG-PET/CT imaging demonstrated vascular inflammation in the aorta that significantly correlated with total monocyte count ($\beta=0.26$, $p<0.01$), classical ($\beta=0.02$, $p<0.01$), intermediate ($\beta=-0.30$, $p<0.01$), and nonclassical subsets ($\beta=0.22$, $p<0.01$). In opposition to the other subpopulations, an increase in the intermediate subset was associated with

decreased aortic vascular inflammation. Total monocyte count as well as all three monocytic subsets independently predict aortic vascular inflammation beyond traditional risk factors in PSO. For the first time, we demonstrate that CD16+ cell populations may have relevance in CVD, and that intermediate monocytes may protect from CVD.

Cardiac prolyl hydroxylation influences protein degradation and stability. A. Stoehr, A.M. Evangelista, S. Menazza, E. Murphy. Laboratory of Cardiac Physiology.

The posttranslational protein modification prolyl hydroxylation plays an important role in regulation of HIF-1α or β_2-adrenergic receptor protein levels. In the presence of oxygen and α-ketoglutarate, prolyl hydroxylases (PHDs) add OH groups to proline residues. So far, only a few proteins have been shown to undergo prolyl hydroxylation. We undertook a non-biased proteomic approach to define prolyl hydroxylated proteins in the heart. We immunoprecipitated tryptic digests of rat heart homogenates with an antibody that recognized prolyl hydroxylation and the samples were run on an orbitrap LC MS/MS. Surprisingly, given the cytosolic and nuclear localization of PHDs, we found many prolyl hydroxylated mitochondrial proteins (7 of 23). In the homogenate 25 prolyl hydroxylated peptides from 23 proteins were identified, including 4 glycolytic proteins. We hypothesized that prolyl hydroxylation might alter protein levels. To test this we treated iPS cell-derived human cardiomyocytes with dimethyloxalylglycine (DMOG**)**, an inhibitor of PHDs, and MG132, a proteosome inhibitor and investigated the protein levels of several proteins by Western blot. Fructose-bisphosphate aldolase A protein level increased in presence of DMOG and MG132 in comparison to control (1.6- and 1.5-fold, $p<0.05$). Pyruvate kinase enzymes M1/M2 PKM protein levels remained unchanged with DMOG or MG132. Histidine triad nucleotide-binding protein 1 protein levels decreased in presence of DMOG (-~50% vs. ctr, $p<0.05$). These data suggest that prolyl hydroxylation is an important modification which influences protein degradation or stability of so far unknown targets.

Ischemic preconditioning preferentially increases protein S-nitrosylation in subsarcolemmal but not interfibrillar mitochondria. J. Sun[1], T. Nguyen[1], A. Aponte[2], E. Murphy[1]; [1]Laborotary of Cardiac Physiology, Systems Biology Center, and [2]Proteomic Core Facility.

Nitric oxide (NO) and protein S-nitrosylation (SNO) have been shown to play important roles in ischemic preconditioning (IPC)-induced acute cardioprotection. The majority of proteins that show increased SNO following IPC are localized to the mitochondria, and our recent studies suggest that caveolae transduces acute NO/SNO cardioprotective signaling in IPC hearts. Due to the close association between subsarcolemmal mitochondria (SSM) and the sarcolemma/caveolae, we tested the hypothesis that SSM, rather than the interfibrillar mitochondrial (IFM), are major targets for NO/SNO signaling derived from caveolae-associated eNOS. Following either control perfusion or IPC, SSM and IFM were isolated from Langendorff perfused C57BL/6J mouse hearts in the presence of EDTA and neocuproine in the dark to prevent SNO decomposition. SNO was analyzed in these two populations of mitochondria using a modified biotin switch method with fluorescence maleimide fluors. The SNO content was higher in SSM compared to IFM, and IPC significantly increased SNO content only in SSM, but not in IFM. Consistent with these findings, eNOS and caveolin-3 were detected in SSM, but not in IFM, and IPC resulted in a further significant increase in eNOS/caveolin-3 levels in SSM. In conclusion, these results suggest that SSM are the major target for protein SNO in the IPC mouse heart, suggesting that the SSM may be the preferential target of sarcolemmal signaling-derived post-translational protein modification (caveolae-derived eNOS/NO/SNO), thus providing an important role in cardioprotection.

High Density Lipoprotein suppresses inflammasome activation in response cholesterol crystals and other particulates. S. G. Thacker, D. O. Sviridov, S. Demosky, A. T. Remaley. Liboprotein Metabolism Section.

Background: Cardiovascular disease is a major cause of mortality in western cultures. Inflammation plays a major role in the initiation and progression of atherosclerosis. Activation of the inflammasome has been shown to an important component of the inflammation seen in atherosclerosis. While the initial source of the inflammation in atherosclerosis is not known, it has recently been shown that cholesterol crystals can be detected early in disease development and are potent activators of the inflammasome. While HDL is known to have anti-inflammatory effects and elevated levels of HDL appear to have a protective effect on the development of atherosclerosis, its effects on inflammasome activation are unknown. We hypothesize that HDL will be able to ameliorate activation of the inflammasome.

Methods and Results: A human macrophage cell line was used in all experiments. Treatment of macrophage cells with cholesterol crystals and other particulates resulted in a robust activation of the inflammasome, and treatment of these cells with HDL blunted this response. Interestingly HDL's anti-inflammatory effects could be detected both on cells and on the crystals. The suppressive effects appear to be mediated by the protein portion of HDL, and are related to the ability of HDL to mediate cholesterol efflux. HDL was shown to stabilize lysomes and inhibit phagocytosis.

Conclusions: Our results demonstrate a novel way in which HDL is anti-inflammatory. HDL's ability to antagonize the activation of the inflammasome provides another possible benefit from treatments to increase HDL levels.

Sirt3 modulates nutrient-dependent NLRP3 inflammasome regulation. J. Traba[1], T. Okoli[1], M. Pelletier[2], K. Han[1], R.M. Siegel[2], M.N. Sack[1]. [1]Laboratory of Mitochondrial Biology in Cardiometabolic Syndromes, Cardiovascular and Pulmonary Branch, [2]NIAMS.

Caloric restriction is the only intervention known to increase lifespan in several organisms, including mammals. The molecular mechanism by which caloric restriction mediates its effects is not completely understood, but the Sirtuin family of protein deacetylases is thought to

be involved at least partially. Many degenerative pathophysiologic situations associated with aging, such as obesity, diabetes, Alzheimer's disease or ischemia-reperfusion are characterized by a certain degree of inflammation and hyperactivation of the NLRP3 inflammasome, an interleukin-1 β releasing process. Here we have studied the release of IL-1β in peritoneal mouse macrophages after fasting and we have found that it is significantly decreased compared to fed conditions. Interestingly, the presence of the mitochondrial sirtuin, Sirt3, provides an additional inhibition of IL-1β in release. Consistently, the knock-down of Sirt3 in human THP-1 macrophages exacerbates IL-1β in release with no change in TNF-α, whereas the knock-down of other sirtuins, such as Sirt1, Sirt2 or Sirt5 have no effect. Conversely, the genetic depletion of Gcn5L1, a component of the mitochondrial acetyltransferase machinery, known to counter the deacetylation effects of Sirt3, decreased IL-1βrelease in THP-1 and J774A.1 cells. We began to explore the mechanisms underlying the effects of Sirt3 in inflammation and found that Sirt3 regulates mitochondrial biogenesis, AMP-activated protein kinase activation and autophagy in macrophages. These results support that the modulation of mitochondrial protein acetylation regulates IL-1βproduction and may influence the development of inflammasome-associated diseases in vivo.

Semaphorin3G-Neuropilinp2 signaling influences arterio-venous alignment in the skin. Y. Uchida, Y. Mukouyama; Laboratory of Stem Cell and Neuro-Vascular Biology.

In developing limb skin, the pattern of sensory nerves provides an anatomical template that controls the arterial patterning, resulting in a congruence of sensory nerve and arterial vessel branching. However, what controls venous vessel branching remains unclear. Our time-course analysis of venous development in the limb skin revealed that venous remodeling and subsequent vascular smooth muscle coverage begin adjacent to remodeled arteries at E15.5 when arterial development has completed. At E17.5, remodeled veins become aligned with remodeled arteries but there is a space (~10μm) between arteries and veins to prevent their direct attachment. We recently found that the repulsive axon guidance molecule Semaphorin3G (Sema3G) is preferentially expressed by arteries, while its receptor Neuropilin2 (Nrp2) expression is restricted to veins and lymphatic vessels. This result suggests that Sema3G-Nrp2 signaling may regulate arterio-venous alignment. Indeed, both *Sema3G* and *Nrp2* mutants exhibit a close alignment and association between arteries and veins in the E17.5 limb skin. In adult ear skin vasculature, large-sized arteries and veins are aligned with each other, but no alignment between small-sized arteries and veins is evident. Interestingly, *Sema3G* mutants exhibit arterio-venous alignment in the small-sized arteries and veins. We are currently investigating whether such close association between arteries and veins influences the circuratory system in the skin such that the mutants may exhibit defective blood flow dynamics and body temperature control.

Experimental Pain Phenotyping in Adults with Sickle Cell Anemia and Normal Volunteers. K. Vaughan, D. Darbari, [1]M. Quinn, [2]C.A. Seamon, [2]G. Wallen, [2]I. Belfer, [1]J.G. Taylor VI. [1]Genomic Medicine Section, Hematology Branch, [2]University of Pittsburgh and Nursing Department, Clinical Center, Division of Blood Diseases, Children's National Medical Center.

Sickle cell anemia's (SCA) hallmark is an acute vaso-occlusive pain crisis (VOC) which occurs with hypoxia, intra-vascular erythrocyte sickling and ischemia reperfusion injury. Because frequent pain alters sensory perception, we hypothesized that adults with SCA experience hypersensitivity to experimental pain as observed in other chronic painful conditions.

Eighteen subjects with SCA and 19 normal volunteers (NV) underwent quantitative thermal testing (cold and heat) for temperature at first detection, threshold for sensing pain, and pain tolerance as endpoints. Pressure pain threshold and tolerance were measured bilaterally with an algometer. Ischemic pain testing was performed on the non-dominant arm. Testing was at baseline at least 2 weeks since a painful event requiring hospitalization.

The only significant difference for thermal testing was temperature at first cold detection (SCA mean 24.5±6.3 ˚C vs. NV mean of 26.6±4.7 ˚C, P=0.05). There were no other significant differences between SCA subjects and NV for cold pain threshold (P=0.84), cold pain tolerance (P=0.82), first heat detection (P=0.21), heat pain threshold (P=0.68) or heat pain tolerance (P=0.38). Furthermore, there were no differences in pressure for pain thresholds at the thumb (P=0.31), forearm (P=0.44) or trapezius (P=0.10), nor were there pain tolerance differences. Surprisingly, ischemic pain QST showed no differences for initial pain threshold (468.3±263.1 seconds for SCA vs. 569.6±395.8 seconds for NV, P=0.69) or pain tolerance (642.5±245.4 seconds for SCA vs. 774.4±453.5 seconds for NV).

Detection of cold temperature change was the only observed sensory difference. Of particular interest were the ischemic tests, where SCA subjects tolerate ischemia as well as NV despite the expectation this promotes forearm hypoxia, erythrocyte sickling and potentially vaso-occlusion. The absence of a difference in onset of ischemic pain contradicts the SCA VOC paradigm. Further studies are necessary to further elucidate the pathophysiology of pain in SCA.

A mitochondrial protein GCN5L1 regulates hepatic glucose production and insulin signaling pathway. L. Wang, I. Scott, L. Zhu, K. Han, M. Sack; Cardiovascular and Pulmonary Branch.

Type 2 diabetes (T2D) is one of the major threats to human health. Typically, increased hepatic glucose production and impaired insulin sensitivity are major contributors to hyperglycemia characteristic for T2D. GCN5L1 is a cytosolic and mitochondrial localized protein with sequence homology to the nuclear acetyltransferase GCN5. GCN5L1 depletion blunts mitochondrial acetylation and induces mitophagy. As acetylation is a central nutrient-sensing post-translational modification and GCN5L1 is regulated by nutrient levels in the liver, we used Cre-loxp

technology and generated GCN5L1 knockout mice that specifically deleted GCN5L1 in liver (GCN5L1 LKO). We found that primary hepatocytes from GCN5L1 LKO mice showed impaired insulin signaling pathway. Moreover, glucose productions under the basal level and in response to glucagon stimulation were dramatically decreased in GCN5L1 LKO primary hepatocytes. In parallel, hepatic expression levels of the key gluconeogenic enzymes, PEPCK and *G6Pase,* were significantly down regulated in GCN5L1 LKO hepatocytes. Our findings provide novel insights into an in-vivo role of GCN5L1 in regulating hepatic gluconeogenesis and insulin signaling pathway in a physiological range.

Metabolomic Profiling in Psoriasis Patients May Provide a Link between Inflammation and Cardiometabolic Diseases. E. Weiner[1], S. Rose[1], J. Doveikis[1], P. Krishnamoorthy[2], M. P. Playford[1], J. M. Gelfand[3], N. N. Mehta[1]. [1]Section of Inflammation and Cardiometabolic Diseases, [2]Englewood Hospital, Englewood, NJ. [3]University of Pennsylvania, Philadelphia, PA.

Psoriasis is an inflammatory skin disease associated with cardiovascular disease and metabolic disorders. The inflammatory milieu of psoriasis provides an *in vivo* model to understand the biochemical cascades that may lead to these derangements. This study aimed to interrogate these mechanisms in plasma from psoriatic patients and healthy controls. We enrolled patients with psoriasis (n=60) and compared plasma from these patients to controls matched for age, sex, and body mass index (n=30). The samples were split for analysis on the GC/MS and Orbitrap Elite accurate mass LC/MS/MS platforms (Metabolon, North Carolina, USA). Results were adjusted for age, sex, and body mass index. One of the most striking changes involved increased lipolysis and β-oxidation of fatty acids in the psoriasis group, as evidenced by the accumulation of various essential, medium-chain, and long-chain fatty acids ($p<0.001$ correcting for false discovery rate). We also observed a reduction in plasma bile acids in patients with psoriasis, which may have important implications for other aspects of lipid metabolism and homeostasis. Trending ($0.05<p<0.10$) or significant ($p\leq0.05$) reductions in the conjugated primary bile acids glycocholate and glycochenodeoxycholate and secondary bile acids taurodeoxycholate, glycodeoxycholate, glycolithocholate sulfate, and glycoursodeoxycholate were noted in patients with psoriasis. In conclusion, profound metabolic alterations were observed in plasma samples from psoriasis patients compared to healthy controls. Targeted pathway analyses will further elucidate potential mechanisms underlying the links between inflammation and cardiometabolic diseases.

Neuro-vascular wiring in obese mice. T. Yamazaki, W. Li, Y. Mukouyama. Laboratory of Stem Cell and Neuro-Vascular Biology.

Diabetic neuropathy is a common diabetic complication that leads to a deficient sensory system. How metabolic factors such as high blood glucose in the circulatory system lead to damage to neighboring nerves remains elusive. We have recently established a high-resolution whole-mount imaging protocol for adult ear skins to visualize branching morphogenesis of nerves and blood vessels. Using the whole-mount imaging system, we have examined what happens to neuro-vascular wiring in diet-induced obesity (DIO) mice, which model a severe pathology found in human diabetic neuropathy. We have found a collapse of sensory nerve bundles and depletion of αSMA $^{+}$ vascular smooth muscle cells (VSMCs) in the DIO mice. This surprising result suggests that neuronal damage occurs early in diabetes. We are currently investigating the possibility that chronic inflammation-induced recruitment of macrophages and dendritic cells may trigger the disruption of neuro-vascular wiring. We have found a significant increase and accumulation of CD11b^{+} inflammatory macrophages in the DIO skin, suggesting that inflammatory macrophages may play a major role in obesity-induced axon degeneration. We attempt to carry out an anti-inflammation treatment and a systemic macrophage/monocyte depletion. These studies will provide insight to understanding pathogenesis of diabetic neuropathy.

Disabled-1 Attenuates House Dust Mite-induced Airway Inflammationby Suppressing the Recruitment of Intraepithelial Mast Cells. X. Yao, M. Gao, K. S. Meyer, K. J. Keeran, G. Z. Nugent, K. R. Jeffries, X. Qu, Z. X. Yu, Y. Yang, N. Raghavachari, and S. J. Levine; Laboratory of Asthma and Lung Inflammation, Cardiovascular and Pulmonary Branch.

Apolipoprotein E in the lung binding to LDLR on airway epithelial cells attenuates airway hyperresponsiveness (AHR) and mucous cell metaplasia in house dust mite (HDM)-induced asthma. Disabled-1 (Dab1), a cytosolic adaptor protein interacts with multiple members of the LDLR family. The *scrambler* (*scm*) mutant mouse (*Dab1^{scm}*), with a spontaneous autosomal recessive mutation in the *Dab1* gene and wild type mice received intranasal HDM challenges for 5 weeks. We found that Dab1 is expressed in the cytoplasm of ciliated airway epithelial cells and *Dab1* mRNA levels are increased in the lung following HDM challenges. HDM-challenged *Dab1^{scm}* mice had increased numbers of airway inflammatory cells with associated increases in Th1, Th2, and Th17 cytokines and the C-C chemokines, CCL22 and CCL24 as well as enhanced mucous cell metaplasia and AHR. Gene expression analysis identified increased expression of *mouse mast cell protease-1* (*Mcpt1*) in the lungs of HDM-challenged *Dab1^{scm}* mice, correlated with an increased number of mouse mast cell protease-1 (mMCP-1) protein positive intraepithelial mast cells. Mast cell mediators: IL-3, IL-25, TGF-**β** and TSLP, were also increased in the lungs of HDM-challenged *Dab1^{scm}* mice. Mast cell chymase inhibitor-Chymostatin inhibited the increases in airway inflammation, mucous cell metaplasia and AHR. We identified a novel function for airway epithelial Dab1 in regulating the pathogenesis of HDM-induced asthma by attenuating the recruitment of mMCP-1-positive intraepithelial mast cells to the airway. These findings suggest that therapeutic strategies aimed at enhancing the activation of the Dab1 signaling pathways in the airway might reduce the severity of HDM-induced asthma.

Proteome-Wide Identification of PRG-3-Interacting Proteins Reveals a Functional Interaction among PRG Family Members. P. Yu[1], C. Agbaegbu[1, 2], D.A. Malide[3], Y. Katagiri[1], H.M. Geller[1]. [1]Developmental Neurobiology Section; [3]Light Microscopy Core Facility, [2]Department of Physics, Georgetown University, Washington, DC.

The integral membrane protein plasticity-related gene-3 (PRG-3), alternatively named lipid phosphate phosphatase-related protein-1 (LPPR-1), belongs to a novel subclass of the lipid phosphate phosphatase (LPP) superfamily, which shares homology with LPPs but with no detectable lipid phosphatase activity. PRG-3 has been shown to induce membrane protrusions or neurite protrusions in several cell lines and primary neurons. However, the mechanism of action for this effect remains unknown. In this study, we aimed to identify the binding partners of PRG-3 through affinity purification and proteomics as an initial step towards identifying its mechanism. By coupling mass spectrometry-based proteomics analysis with co-immunoprecipitation, we identified putative PRG-3 binding proteins involved in several different biological processes such as protein localization and transport, cellular homeostasis, and lipid biosynthetic process. In particular, we have identified mammalian target of rapamycin (mTOR), phosphatase and tensin homolog (PTEN) and most interestingly, another three PRG family members PRG-1, -2 and -5, associated with PRG-3. Their interactions were further confirmed by co-immunoprecipitation and co-localization analysis using confocal microscopy. More importantly, we revealed a novel role of PRG-5 in facilitating the localization of PRG-3 and other PRG family members from endomembranes to the plasma membrane which resulted in an increased induction of protrusions.

Regulators of MT Dynamics in the Spindle. A.L. Zajac, C.J. Fagerstrom, N.M. Rusan; Laboratory of Molecular Machines and Tissue Architecture.

Centrosomes are major microtubule (MT)-organizing centers that help build the mitotic spindle and position it within the cell to establish polarity. Many MT-associated proteins and MT-nucleating proteins are concentrated in the centrosome, but the mechanism by which centrosomes coordinate MT nucleation, stabilization, and anchoring are not fully understood. Several whole genome screens for centrosome and spindle mutants have identified new genes involved in these processes. We have begun to investigate two genes, patronin and ssp3, that have a short spindle phenotype (Goshima et al., 2007) for potential roles in MT regulation at the centrosome. Patronin was previously shown to be a MT minus-end binding and stabilization protein that localizes to spindle MTs and centrosomes (Goodwin and Vale, 2010; Wang et al., 2013). We performed a targeted yeast-2-hybrid screen that identified potential interactions between several centrosomal proteins and the NT of patronin. Since this region is not involved in MT binding, it may regulate patronin's activity and localization. We have generated a lethal allele of patronin using CRISPR for future studies of its function in live drosophila. Ssp3 does not have a known effect on MT dynamics. It streams on astral and spindle MTs during mitosis towards the centrosomes. Ssp3 accumulates along MTs and around centrosomes only on the astral side of the spindle, a unique localization pattern to our knowledge. Future studies examining the effect of loss of these two proteins in drosophila will provide insight into their role in spindle formation as well tissue-specific developmental functions.

Identification of H2A.Z-interacting Proteins Using a High-throughput in Vivo Protein-protein Interaction Assay. Y. Zhang[1,2], W. Jin[1], Q. Tang[1], B. Ni[2], K. Zhao[1], [1]Systems Biology Center, [2]Third Military Medical University, China.

Epigenetic mechanisms play critical roles in regulating cellular differentiation. Incorporation of histone variant H2A.Z into chromatin is an important epigenetic process that facilitates chromatin opening and targeting of key transcription factors to their sites. Although chaperones mediating H2A.Z deposition into chromatin have been identified, little is known about how H2A.Z is targeted to specific loci in the genome and how H2A.Z interacts with downstream effector proteins. In order to systematically identify H2A.Z-interacting proteins, we developed an unbiased in vivo screening assay by combining the Retrovirus-based Molecular Two Hybrid system (ReMTH) with the next generation sequencing (ReMTH-Seq). Using ReMTH-Seq, we identified more than 300 potential H2A.Z-interacting proteins. We further validated Osr1 as an H2A.Z-interacting protein by co-transfecting H2A.Z-VNN and Osr1-VNC expression constructs and by co-immunoprecipitation assays. Using ChIP-Seq, we show that Osr1 is co-localized with H2A.Z to chromatin regulatory regions in the genome. We are currently testing the functional relationship between H2A.Z and Osr1. Our data show that ReMTH-Seq is a sensitive genome-wide technique to screen for protein interactions.

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