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

Fifth Annual Fellows Retreat

April 19-20, 2007
Wyndham Gettysburg Hotel and Conference Center
Gettysburg, Pennsylvania

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Fifth Annual NHLBI DIR Fellows Retreat
Wyndham Gettysburg Hotel & Conference Center
Gettysburg, PA

April 19-20, 2007

Agenda

Thursday, April 19th, 2007

8:00 - 9:30 **Arrival, Registration, & Continental Breakfast**
Posters mounted at arrival in *Salon AB*
All attendees must sign-in at NHLBI registration table on arrival.

9:30 - 10:15 **Introduction & Welcome -** *Salon C*
Chairs: Drs. Steven Yee, LMI and Gabriela Viteri, LB
Fellows Advisory Committee Members
Dr. Herbert M. Geller, Director, Office of Education
Dr. Robert S. Balaban, Scientific Director, NHLBI

10:15 - 11:30 **Scientific Keynote Speaker**
Chair: Dr. Lee Ann Cohen, LCB
Dr. George Yancopoulos, Regeneron Pharmaceuticals

11:45 **Group Photo**

12:00 - 1:30 **Lunch** - *Salon D*

1:30 - 3:40 **Research Highlights by NHLBI Fellows -** *Salon C*
Chairs: Drs. Amar Sethi, VMB and Felipe Lisboa, LMI

1:30 Dr. Wenjun Zheng, Laboratory of Computational Biology
Towards the Mechanism of Dynamic Couplings and Translocation in Hepatitis C virus NS3 Helicase Using Elastic Network Model.

1:50 Ms. Natalie Porat-Shliom, Laboratory of Cell Biology and Tel-Aviv University
The Clathrin-Independent Endocytic Pathway and H-Ras: Interplay of Trafficking and Signaling.

2:10 Dr. Mikiei Tanaka, Laboratory of Biochemistry
Potential Biological Consequences of mRNA Oxidation-Induced Translation Errors.

2:30 - 2:40 **Break**

2:40 Dr. Jean-Yves Michael Metais, Hematology Branch.
MDS1-EVI1 and EVI1 Overexpression Results in Changes in the Behavior of Murine Hematopoietic Cells.

3:00 Dr. Lee Woodcock, Laboratory of Computational Biology
Exploring SCC-DFTB Paths for Mapping QM/MM Reaction Mechanisms.

3:20 Mr. Joshua A. Regal, Hematology Branch
A Large Mennonite Family with a Novel K570N TERT Gene Mutation: Association with a Clinical Spectrum of Bone Marrow Failure, Acute Myeloid Leukemia, and Acute Liver Failure.

3:40 **Room Check-in**

4:00 - 7:00 **Fellows Poster Session** - *Salon AB*
NHLBI Cores and Offices

7:00 - 8:00 **Dinner** - *Salon D*

8:00 - 8:30 **Awards Presentation & Remarks-** *Salon C*
Chairs: Drs. Steven Yee, LMI and Siddhartha Jana, LMC
Dr. Susan Shurin, Deputy Director, NHLBI
Fellows Award for Research Mentoring
Fellows Retreat Research Awards

8:45 - 11:00 **Karaoke Social Event -** *Salon D*

Friday, April 20th, 2007

8:00 - 9:15 **Breakfast** - *AB Foyer*

9:15 - 10:45 **Keynote Speaker -** *Salon AB*
Chair: Dr. Rodrigo Calado, HB
Dr. Alan Leshner, Chief Executive Officer, AAAS

10:45 - 11:00 **Coffee Break -** *AB Foyer*

11:00 - 1:00 **Career Development Workshop -** *Salon AB*
Chairs: Drs. Hongjun Liu, CB and Jing Zhang, PCCMB
Academic Industry: Dr. Jackob Moskovitz, University of Kansas
Pharmaceutical Industry: Dr. Carolyn Foster, Boehringer Ingelheim
Science Policy: Dr. Sheila Newton, NIEHS

1:00 - 2:30 **Lunch with Panelists -** *AB Foyer*

2:30 **Departure**
Please remember to hand in your evaluation forms to Jessica Llewellyn.
Buses meet at main entrance at 2 p.m.

Speaker Biographies

Alan I. Leshner, Ph.D., serves as the Chief Executive Officer for the American Association for the Advancement of Science (AAAS). Prior to joining AAAS, Dr. Leshner was Director of the National Institute on Drug Abuse (NIDA) from 1994-2001, the Deputy Director and Acting Director of the National Institute of Mental Health (NIMH), and the National Science Foundation (NSF), where he held a variety of senior positions, focusing on basic research in the biological, behavioral and social sciences, science policy and science education. He also served as a Professor of Psychology at Bucknell University and was a Fulbright Scholar at the Weizmann Institute of Science in Israel. Dr. Leshner is the author of a major textbook on the relationship between hormones and behavior, and has published over 150 papers for both the scientific and lay communities on the biology of behavior, science and technology policy, science education, and public engagement with science. He received his BS in psychology from Franklin and Marshall College, and M.S. and Ph.D. degrees in physiological psychology from Rutgers University. In addition, he holds honorary Doctor of Science degrees from Franklin and Marshall College and the Pavlov Medical University in St. Petersburg, Russia. Dr. Leshner is an elected fellow of AAAS, the National Academy of Public Administration, the American Academy of Arts and Sciences, and many other professional societies. He serves on the governing Council of the Institute of Medicine of the National Academies of Science and is a member of the Advisory Committee to the Director of National Institutes of Health. In 2004, Dr. Leshner was appointed to the National Science Board by the U.S. President, and represents AAAS on the U.S. Commission for UNESCO.

George D. Yancopoulos, M.D., Ph.D., graduated as the Valedictorian of both the Bronx High School of Science in 1976 and of Columbia College in 1980. He then received an M.D., Ph.D. degree in 1987 from Columbia University's College of Physicians & Surgeons. Following widely-recognized work in the field of molecular immunology at Columbia University with Dr. Fred Alt, for which he received the Lucille P. Markey Scholar Award, Dr. Yancopoulos left academia in 1989 as a founding scientist of Regeneron Pharmaceuticals, where he is now the Chief Scientific Officer and President of Regeneron Laboratories. Recent breakthroughs in Dr. Yancopoulos' group at Regeneron yielded the discovery of a new family of angiogenic growth factors (the "Angiopoietins") that work together with the VEGFs to mediate normal and pathologic blood vessel growth. Many of the discoveries of Dr. Yancopoulos and his group are in clinical studies to address major unmet medical needs - such as Axokine for obesity which is in Phase 3 clinical studies, cytokine antagonists known as Traps being studied in humans suffering from immunologic disorders such as asthma and rheumatoid arthritis, or angiogenic regulators in cancer and vascular disease. He is widely regarded as a world leader in many fields of biology and was elected to both the National Academy of Sciences and the American Academy of Sciences in 2004. According to a study by the Institute for Scientific Information, Dr. Yancopoulos was the eleventh most highly cited scientist in the world during the 1990's and the only scientist from the biotechnology industry on the list.

Carolyn Foster, Ph.D., is the head of the Competitive Technical Intelligence group for cardiovascular and autoimmune diseases at Boehringer Ingelheim Pharmaceuticals. Prior to this, she spent 20 years as a biochemical pharmacologist in Cardiovascular Drug Discovery at Schering Plough working on drugs to treat hypertension, atherosclerosis, thrombosis, and Parkinson's Disease. Her published research focuses on G-protein coupled receptors and platelet function. Dr. Foster studied chemistry at Swarthmore College, received an M.S. in biochemistry from Brown University and a Ph.D. in pharmacology from the University of Medicine and Dentistry of New Jersey. She did her post-doctoral research at the University of Pennsylvania in human genetics and is a Fellow of the New York Academy of Sciences, where she has served as a member the conference planning committee, Chair of the Biochemistry Section, and as head of the Biochemical Pharmacology Discussion Group, an academia-industry consortium. She is a member of the Society for Competitive Intelligence Professionals, the American Heart Association, and the American Society of Hypertension.

Jackob Moskovitz, D.Sc., received his doctorate degree in Medical Sciences from the Technion-Israel Institute of Technology in Haifa, Israel (in the Department of Biochemistry, School of Medicine, Mentors: Dr. Avram Hershko and Dr. Aaron Ciechanover, 2004 Nobel prize laureates in Chemistry). Dr. Moskovitz performed his post-doctoral training at Roche Institute of Molecular Biology (Laboratory of Dr. Nathan Brot and Dr. Herbert Weissbach) and NHLBI (Laboratory of Biochemistry with Dr. Earl Stadtman). His research focused on the methionine sulfoxide reductase system. In 2004, Dr. Moskovitz joined the faculty of the Department of Pharmacology and Toxicology, School of Pharmacy, at the University of Kansas as an Assistant Professor. His laboratory/research continues the research on methionine sulfoxide reductase system while focusing on its effect on aging and neurodegenerative diseases. Dr. Moskovitz also served as a consultant to biotechnology and pharmaceutical companies such as Upstate Biotechnology and Amgen.

Sheila Newton, Ph.D., presently serves as the Director of Policy, Planning and Evaluation for the National Institute of Environmental Health Services in Durham, NC, where she performs strategic and yearly research planning and intramural annual reporting on investments. Prior to this, Dr. Newton served as Senior Advisor for Environmental and Occupational Health for the Deputy Assistant Secretary for Health and as the Executive Secretary for Environmental Health Policy Committee. She also served as an assistant professor at the Howard University Cancer Center at Howard's College of Medicine. Dr. Newton received her MS and Ph.D. in biochemistry from the University of Massachusetts at Amherst.

NHLBI Core Facilities and Services

Animal MRI Core Facility (AMRI)

Stasia A. Anderson, Ph.D., Director
Building 10, Room 2N240, E-mail: andersos1@nhlbi.nih.gov
Phone: (301) 402-0908; Web: http://dir-intranet.nhlbi.nih.gov/amri

The Animal MRI/Imaging Core is a resource for biomedical imaging of small animal models in the NHLBI. The MRI Core develops and optimizes MRI methods for cardiovascular imaging of mice and rats. We provide imaging expertise, data interpretation and experimental design 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 in the Core are:

- Cine cardiac imaging for ejection fraction, ventricle size and wall thickness
- High resolution imaging of myocardium for identification of infarct
- Imaging aorta and vessels in live mice and rats
- Imaging atherosclerotic plaque
- Perfusion of skeletal muscle
- Cellular imaging: magnetic labeling and tracking cell transplants
- Targeted MRI contrast agent research
- 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.

Electron Microscopy Core Facility

Mathew P. Daniels, Ph.D., Director
Building 14E, Room 111B, E-mail: danielsm2@mail.nih.gov
Phone: (301) 496-2898, Fax: (301) 480-6560

The new NHLBI Electron Microscopy Core Facility has just moved into renovated space and has been taking on projects since last June. We provide contemporary transmission electron optical services to all investigators within the Institute. Services include transmission electron microscopy and scanning electron microscopy of cells and tissues, immunocytochemistry at the electron microscopic level including immuno-gold labeling, negative staining or rotary shadowing of macromolecule preparations (nucleic acids or proteins), computer-assisted morphometry and image analysis.

Flow Cytometry Core Facility

J. Philip McCoy, Jr., Ph.D., Director
Building 10, Rooms 4A07 and 4A11, E-mail: mccoyj@nhlbi.nih.gov
Phone: (301) 594-6950, Fax: (301) 480-4774

The mission of the NHLBI Flow Cytometry Core Facility is to provide investigators at the NHLBI access to state-of-the-art flow cytometry. This is done by having cytometers and software available in the core facility and by providing consultation to investigators who have cytometers available in their own laboratories or branches. 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. FCSExpress software (DeNovo Software) will be available for "offline" analysis of these files. For sorting experiments, each investigator is responsible for bringing appropriate media and test tubes.

Genomics Core Facility

Nalini Raghavachari, Ph.D.
Building 10, 8C103 B, 8C215
Phone: 301-435-2304; E-mail: nraghavachari@cc.nih.gov

The purpose of the genomics core facility is to provide NHLBI investigators high quality, state-of-the-art gene expression profiling using Expression and Exon arrays & genotyping services in a timely fashion using the Affymetrix platform. The core provides this high quality service by implementing rigorous standardization of protocols and multiple quality control checks at various points during sample processing and gene chip hybridization. We also provide integrated service to investigators in a collaborative manner for the design of microarray experiments, target preparation, streamlined data analysis applying complex statistical tools and validation of data by Taqman analysis on ABI 7900. Assistance will also be provided in the amplification of small amounts of RNA by applying our established linear amplification protocol.

Imaging Probe Development Center

Gary L. Griffiths, Ph.D., Director
9800 Medical Center Drive, Building 2B, Rockville MD
Phone: 301 217 5770 (Rockville); 301 496-3341 (Bethesda)
E-mail: griffithsgl@mail.nih.gov

The Imaging Probe Development Center (IPDC) is part of the NIH Roadmap for Medical Research (http://nihroadmap.nih.gov/), dedicated to Molecular Libraries and Imaging. Its mission is to produce known and novel imaging probes of a diverse nature, encompassing optical, radionuclide, MRI, and other imaging modalities. The IPDC is a trans-NIH initiative, and is operated and administered by NHLBI. It has recently relocated to its permanent laboratories at 9800 Medical Center Drive in Rockville, MD.

Currently, IPDC is collaborating with over a dozen NIH scientists, from throughout NIH, and is working on producing several dozen probes. IPDC has been set up with the capability to produce probes of almost any chemical composition, including peptides, carbohydrates, oligonucleotides, heterocyclic compounds, polymer-based materials, nanomaterials, protein conjugates, metal complexes, radiolabeled species, diverse organic targets, and conjugates incorporating two or more of the preceding classes of molecules.

IPDC solicits proposals from the entire NIH intramural community for the chemical synthesis of any type of imaging probe. At present, the cost of synthetic work undertaken on behalf of NIH scientists may be borne in whole, or in part, by the IPDC itself. NIH intramural scientists from any Institute or Center are invited to submit new proposals, or are invited to informally discuss ideas for probes with Dr. Griffiths, who will welcome your enquiries, and may be contacted as noted above.

Light Microscopy Core Facility

Christian A. Combs, Ph.D., Facility Director
Building 10, Room 5D19; E-mail: combsc@nhlbi.nih.gov
Phone: 301-496-3236; Pager: 301-768-2568

Daniela A. Malide, M.D., Ph.D., Facility Manager
Building 10, Room 5D19; E-mail: dmalide@nhlbi.nih.gov
Pager: 301-402-4719

The mission of the light microscopy core facility is to provide state of the art equipment, training, and image processing capabilities to assist researchers within the NHLBI-DIR in experiments involving light microscopy. Equipment within the facility includes several types of confocal microscopes, a two-photon microscope, and a standard epi-fluorescence widefield microscope. This range of instruments provides capabilities that include live cell imaging, deep tissue-level imaging, video-rate confocal imaging, spectral imaging, and simple widefield fluorescence and brightfield imaging of prepared slides Image processing capabilities include deconvolution, digital linear unmixing of spectrally overlapping fluorochromes, and 3D reconstruction as well as a custom in-house image processing programs for specific applications. It is intended that this webpage provide researchers with all of the

information necessary to plan their experiments based on the capabilities of the core instruments as well as to provide background information on the light microscopy techniques that are available.

Office of Biostatistics Research (OBR)

Nancy L. Geller, Ph.D., Director
Rockledge 2, Room 8210; 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 and collaborates in data management and analysis of some studies sponsored by the Division of Intramural Research. 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. 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.

Office of Technology Transfer and Development (OTTAD)

Vincent Kolesnitchenko, Ph.D., Technology Development Specialist
Rockledge 1, Room 6018; E-mail: kolesniv@nhlbi.nih.gov
Phone: (301) 594-4115; Fax: (301) 594-3080

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);
- Preclinical and 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, generates patentability reports and makes recommendations 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

Zu-Xi Yu, M.D., Ph.D., Facility Head
Building 10, Room 2N240; E-mail: yuz@helix.nih.gov
Phone: (301) 402-0908, Fax: (301) 402-4127

The Pathology Core is a Morphology Core Facility, which provides histopathological, immunocytochemical, and ultrastructural support for NHLBI intramural research. The Core Facility provides quality control for morphologic studies, experimental pathology (animal models) and optimizes use of supplies and equipment for all investigators at the NHLBI in which morphological studies and tissue-based molecular studies play a critical role. Services include standard light microscopy, transmission and scanning electron microscopy of cultured cells and tissues; histological and tissue preparation, sectioning and routine staining; frozen tissue section, immunohistochemistry and diagnostic pathology at both histological and ultrastructural level. Ongoing interaction of Pathology Core personnel with each investigator facilitates communication regarding 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

Rong-Fong Shen, Ph.D., Head
Building 10, Room 8C103C; E-mail: Shenr@nhlbi.nih.gov
Phone: (301) 594-1060, Fax: (301) 402-2113

The mission of the Proteomics Core is to assist NHLBI investigators on proteomic endeavors. The Core maintains state-of-the-art mass spectrometers, provides guidance for sample preparation, trains scientists for the use of instrument and data analysis, and develops analytical methodologies relevant to proteomics.

Transgenic Mouse Core Facility

Chengyu Liu, Ph.D., Chief
Building 50, Room 3305; E-mail: Liuc2@mail.nih.gov
Phone: (301) 435-5034, Fax: (301) 435-4819

The NHLBI Transgenic Core Facility is a state-of-the-art transgenic mouse laboratory established in 1999. Its main function is to assist NHLBI scientists making transgenic and knockout mice. Generally, the users of the facility are responsible for making the DNA constructs, and screening the potential positive ES cell clones and founder mice. The staff members at the facility are responsible for culturing, transfecting, and selecting ES cells, as well as microinjecting and implanting mouse embryos. After creation, the transgenic or knockout mice are transferred to each user's animal room for phenotypic analysis. We use the standard pronuclear microinjection method to make transgenic mice. Our standard strain of mice is B6CBAF1/J (C57BL/6J x CBA/J), but we can also make transgenic mice using FVB/NJ and C57BL/6J inbred strains if necessary. For creating knockout mice, we routinely use embryonic stem (ES) cells derived from 129 mouse strain to generate targeted ES clones. We have recently obtained two ES cell lines derived from C57Bl/6 mouse strain, and we will be happy to use these lines to generate C57Bl/6 knockout mice if any investigator is interested.

Poster Session Titles and Assignments

Posters are put up in the morning.

Authors of **odd** numbered posters present **4:00 to 5:30 p.m.**

Authors of **even** numbered posters present **5:30 to 7:00 p.m.**

Signal Transduction

1. Dexamethasone Suppresses Phospholipase D (PLD) Activation And Other Signaling Events Associated With Lipid Rafts In Mast Cells. F. A. Lisboa, M. A. Beaven; Laboratory of Molecular Immunology.

2. The Aging Regulator Klotho is a Secreted Wnt Antagonist and Essential for Organ Homeostasis. H. Liu, M. Fergusson, J. Liu, C. Liu, J. Chen, D. Malide, C. J. Kuo, P. M. Hwang and T. Finkel; Cardiology Branch.

3. The Clathrin-Independent Endocytic Pathway and H-Ras: Interplay of Trafficking and Signaling. N. Porat-Shliom, Y. Kloog, J. G. Donaldson; Laboratory of Cell Biology and Tel-Aviv University.

4. p53 Regulated SC02 Gene is Essential for Mitochondrial Respirattuin and Protection from Oxygen Toxicity. H. J. Sung, W. Ma, J. J. Hanisch, J. Y. Park, S. Matoba, J. Kang and P.M. Hwang; Cardiology Branch.

5. Mucolipin-1 Channel Activity is Regulated by Protein Kinase A Mediated Phosphorylantion. S. Vergarajauregui[1], R. Oberdick[2], K. Kiselyov[2], and R. Puertollano[1]; [1]Laboratory of Cell Biology; [2]Department of Biological Sciences, University of Pittsburgh.

6. The Molecular Basis of IL-21-mediated Proliferation. R. Zeng[1], R. Spolski[1], E. Casas[2], W. Zhu[2], D. E. Levy[2], and W. J. Leonard[1];[1]Laboratory of Molecular Immunology; [2]Departments of Pathology and Microbiology, New York University School of Medicine.

Biochemistry

7. NMR Solution Structure, Stability, and Interaction of the Recombinant Bovine Fibrinogen αC-Domain Fragment. R. A. Burton[1], G. Tsurupa[2], R. R. Hantgan[2], N. Tjandra[1], and L. Medved[2] ;[1]Laboratory of Molecular Biophysics; [2]Center for Vascular and Inflammatory Diseases and the Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine.

8. Small-Molecule Mimics of Superoxide Dismutase as Therapeutics. Y Che[1], B. R. Brooks[1], G. R. Marshall[2], D. P. Riley[2; 1]Laboratory of Computational Biology; [2]Metaphore, Inc.

9. Concerted Nitric Oxide Formation and Release from the Simultaneous Reactions of Nitrite with Deoxy- and Oxy-Hemoglobin. R. Grubina,[1] Z. Huang,[1] M. S. Joshi,[2] S. Basu,[2] D. B. Kim-Shapiro,[2] M. T. Gladwin[1]; [1]Vascular Medicine Branch [2]Department of Physics, Wake Forest University.

10. Function of the C1 Inserted Isoform of Nonmuscle Myosin II-C in Tumor Cell Lines and Mice. S. S. Jana, R. S. Adelstein; Laboratory of Molecular Cardiology.

11. PPARy Agonist Pioglitazone Restores the Mitochondrial Biogenesis Regulatory Program and Augments Respiratory Function in Insulin Resistant C2C12 Myotubes. I. Pagel-Lagenickel[1], B. S. Mantell[1], J. J. Joseph[1], J. Bao[1,] X. Xu[2], N. Raghavachari[2], M.

N. Sack[1; 1]Cardiology Branch, [2]Vascular Medicine Branch and the NHLBI Microarray Gene Expression Core Facility.

12. p53 Regulates Exercise Capacity and Skeletal Muscle Metabolism. J. Y. Park, J. W. Choi, H. J. Sung, Q. A. Ali, T. Matsumoto, W. Ma, P. Connelly, J. Fields, J. G. Kang, P. Hwang; Cardiovascular Branch.

13. Complexation of an ApoA-I Mimetic Peptide with Phospholipid Increases ABCA1-specific Cholesterol Efflux. A. A. Sethi, J. A. Stonik, S. J. Demosky, and A. T. Remaley; Vascular Medicine Branch.

14. Potential Biological Consequences of mRNA Oxidation-Induced Translation Errors. M. Tanaka[1], P. Jaruga[2], M. Dizdaroglu[2], P. B. Chock[1], E. R. Stadtman[1]; [1]Laboratory of Biochemistry, [2] Biochemical Science Division, NIST

15. Towards the Mechanism of Dynamic Couplings and Translocation in Hepatitis C Virus NS3 Helicase Using Elastic Network Model. W. Zheng[1], J. Liao[2], B. Brooks[1], S. Doniach[2]; [1]Laboratory of Cell Biology, [1]Stanford Univ.

Biophysics

16. Top-Down Approach in Protein RDC Data Analysis-*de novo* Estimation of the Alignment Tensor. K. Chen and N. Tjandra; Laboratory of Molecular Biophysics.

17. Theoretical Study of the Rhenium-Alkane Interaction in Transition Metal-Alkane S-Complexes. E. A. Cobar, R. Z. Khaliullin, R. G. Bergman, and M. Head-Gordon; Laboratory of Computational Biology.

18. Nanotube Confinement can Stabilize or Denature Protein Helices: Lessons for Helix Formation in the Ribosome Tunnel. E. P. O'Brien Jr., G. Stan, D. Thirumalai and B. Brooks; Lab of Computational Biology.

19. The Compliant Lever and Pivot (CLAP) Model for Myosin V's Mechanics. M. Riegelman, H. Bau, J. Sellers; Laboratory of Molecular Physiology.

20. Exploring SCC-DFTB Paths for Mapping QM/MM Reaction Mechanisms. H. L. Woodcock, M. Hodoscek, and B. R. Brooks; Laboratory of Computational Biology.

21. NMR Investigation of the Molecular Basis for Actin Filament Uncapping by CARMIL. A. Zwolak, N. Tjandra; Laboratory of Molecular Biophysics.

Cell Biology

22. Complexity of Arf Function at the Cell Periphery. L. A. Cohen, A. Honda, P. Varnai, T. Balla, J. G. Donaldson; Laboratory of Cell Biology.

23. MCOLN2 Localizes to Lysosomes and the Arf6-associated pathway. C. V. Karacsonyi, A. San Miguel, R. Puertollano; Laboratory of Cell Biology.

24. A Homogeneous ELISA Assay Detecting Chondroitin Sulfate Proteoglycan (CSPG) Production for the High-Throughput Screening of Small Molecules. T. L. Laabs[1], S.

Jeffries[3], HM Geller[1]; [1]Developmental Neurobiology Section, NHLBI, [2]Chemical Genomics Center, Oxford-Cambridge Biomedical Scholars Program.

25. A20 Attenuates Vascular Smooth Muscle Cell Proliferation and Migration Through Blocking PI3k/Akt Signaling In Vitro and In Vivo. A. B. Wang[1,2], H. L. Li[1], R. Zhang[1], Z.G. She[1], H.Z. Chen[1], Y. Huang[1], D.P. Liu[1] and C.C. Liang[1]; [1]National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College; [2]Laboratory of Molecular Cardiology.

Growth and Development

26. ENU Induced Mutation in the DNA Binding Domain of c-myb Causes Hematopoietic and Myocardial Defects. D. Alpert, R. Francis, X. Q. Zhao, B. Chatterjee, S. Sabol, Q. Yu, Y. Shen, L. Bracero, I. Kitabayashi and C. W. Lo; Laboratory of Developmental Biology; Molecular Oncology Division, National Cancer Center Research Institute, Tokyo, JAPAN.

27. Integrated analysis of muscle transcriptional regulatory networks in *Drosophila.* B. W. Busser[1], A. Singhania[1], A. A.Philippakis[2], S. Jaeger[2], M. F. Berger[2], S. Gisselbrecht[2], M. L. Bulyk[2], A. M. Michelson[1]; [1]Laboratory of Developmental Systems Biology; and [2]Division of Genetics, Brigham & Women's Hospital and Harvard Medical School, Boston, MA.

28. A Novel Mouse Model of Primary Ciliary Dyskinesia Exhibits Complex Structural Heart Defects and Ciliary Dyskinesia Due to a Mutation in Heavy Chain Dynein *mDNHC5.* S. Tan, R. Francis, S. Sabol, B. Chatterjee, Q. Yu, L. Bracero, J. Rosenthal, L. Leatherbury, and C. W. Lo; Laboratory of Developmental Biology.

29. Skeletal Anomalies in Novel Mutant Mouse Models with Congenital Cardiac Anomalies. B. Gibbs[1], D. Schimel[2], Q. Yu, and C. W. Lo[1 ;1]Laboratory of Developmental Biology, NHLBI and [2]Mouse Imaging Facility, NINDS.

30. Modulation of Connexin43 α1 on Directional Motility of Embryonic Fibroblast Cells. H. Park, X. Xu, C. Lo; Laboratory of Developmental Biology.

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32. Connexin43 in the Epicardium is Required for Normal Coronary Development. X. Q. Zhao, D. Y. Rhee, and C. W. Lo; Laboratory of Developmental Biology.

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35. Amplified Expression Profiling of Platelet Transcriptome Reveals Changes in Arginine Metabolic Pathways in Patients With Sickle Cell Disease. N. Raghavachari, X. Xu, A. Harris, J. Villagra, C. Logun, J. Barb, M. A. Solomon, A. F. Suffredini, R. L. Danner, G. Kato, P. J. Munson, S. M Morris, Jr., M. T. Gladwin; Vascular Medicine Branch.

36. Mitochondrial DNA Sequence Variation and Kinetics of Single $CD34^+$ Cells after Nonmyeloablative Allogeneic Stem Cell Transplantation. Y. G. Yao, R. W. Childs, S. Kajigaya, J. P. McCoy, Jr, N. S. Young; Hematology Branch and Flow Cytometry Core Facility.

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37. MDS1-EVI1 and EVI1 Overexpression Results in Changes in the Behavior of Murine Hematopoietic Cells. J. Y. Metais[1]*, R. Wieser[2], and C. E. Dunbar,[1]; [1]Hematology Branch; [2]Institute, Medizinische Universitaet Wien, KIMCL.

38. Mechanisms of Regulation of CXCR4/SDF1 Dependent Migration and Survival in Chronic Lymphocytic Leukemia. Y. Miura, Y. Herishanu, E. Lee, F. Gibellini, N. Njuguna, H. Mora-Jensen, A. Wiestner. Hematology Branch.

39. Real-Time MRI Guided Laser Transseptal Puncture. A. A. Elagha, O. Kocaturk, M. A. Guttman, C. Ozturk, P. V. Karmarkar; A. H. Kim, J. H. Kim, V. K. Raman, VJ Wright, W. H. Schenke, E. R. McVeigh, and R. J. Lederman; Cardiology Branch.

40. Ex Vivo Expansion of Retrovirally- Transduced Primate $CD34^+$ Cells Results in Preferential Engraftment and Persistence of Clones with MDS1/EVI1 Insertion Sites. T. J. Gomes, S. Sellers, R. E. Donahue, R. Adler, A. La Rochelle and C.E. Dunbar; Hematology Branch.

41. Fate Mapping of Neointima Formation in a Murine Vein-Arterial Grafts Model. J. Mellad, A. Wragg, M.C. Ma, S. Ding, L. Beltran, B. Cooley, M. Boehm; Cardiovascular Branch.

42. Resistance to Bortezomib in MCL Cells is Associated with Decreased Proliferation and Adaptive Changes of the Proteasome. H. Mora-Jensen, E. Rizzatti and A. Wiestner; Hematology Branch.

43. The Trendelenburg Position Avoids Ventilator Associated Pneumonia in Intubated and Mechanically Ventilated Pigs. A. Zanella, M. Cressoni, T. Kolobow; Pulmonary Critical Care Medicine Branch.

44. Breast Involvement in Patients with HTLV-1-associated Adult T cell Leukemia/Lymphoma. K. Sharma, J. Janik, C. Lee, D. O'Mahony, and J. Morris. Metabolism Branch, Center for Cancer Research, NCI.

45. Metabolic Stress Testing Predicts the Restorative Capacity of Mitochondrial Bioenergetics in Diabetes. I. Pagel-Langenickel[1], D.S. Schwartz[1], R. A. Arena[2], D.C. Minerbi[1], R. S. Balaban[1], D.J. Tripodi[1], M.N. Sack[1]. [1]Cardiology Branch; [2]Indiana University, IA.

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46. Rabbit ATG, but not Horse ATG and CsA Promotes Expansion of Functional $CD4^{+}CD25highFoxP3$ Regulatory T Cells of Healthy Controls. X. Feng, E. Solomou, N. Young; Hematology Branch.

47. Genetic Depletion of Sirt2 Augments Cellular Resistance to Hypoxia-Reoxygenation Injury. E. G. Lynn, C. J. McLeod, M. N. Sack; Cardiovascular Branch.

48. mESC-derived Vascular Progenitors Induced Rejective Responses in the Syngeneic Wound Repair Model as a Result of NK Attack. M. C. Ma, S. L. Ding, M. Konoplyannikov, J. Mellad, L. Beltran, C. Graham, and M. Boehm; Cardiovascular Branch.

49. The Pathologic Role of the Innate Immune System in Acetaminophen-Induced Liver Disease in Interleukin-13 Deficient Mice. S. B. Yee, M. Bourdi, M. J. Masson, L. R. Pohl; Laboratory of Molecular Immunology.

50. Sirt1 Regulates Autophagy. I. H. Lee[1], R. Mostoslavsky[2], D. B. Lombard[2], N. E. Bruns[1], M. Tsokos, F. W. Alt[2] and T. Finkel[1]; [1]Cardiology Branch, NHLBI;[2]Howard Hughes Medical Institute, The Children's Hospital, CBR Institute for Biomedical Research, Harvard University Medical School;[3] Laboratory of Pathology, NCI.

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51. Simultaneous Monitoring of MRgFUS Temperature and Tissue Stiffness Using 1D Magnetic Resonance Elastography. Y. Le, S. V. Primak, K. J. Glaser, A. Manduca, R. L. Ehman, J. P. Felmlee; Mayo Clinic College of Medicine, Rochester, MN.

52. Mapping 2D Strain in the Wall of the Carotid Artery Using Displacement-Encoded MRI. A. P. Lin[1], E. Bennett[2], L. Wisk[3], M. Gharib[1], S. Fraser[1], and H. Wen[2] ; [1]California Institute of Technology, Pasadena, CA, [2]Laboratory of Cardiac Energetics, NHLBI, [3]University of California Los Angeles, Westwood, CA..

53. High Resolution Three-Dimensional Modeling for Gene and Protein Expression Profiling. C. Rolfes, J. Rosenthal, V. Mangal, and C. W. Lo; Laboratory of Developmental Biology.

54. Hemodynamic Basis for Early Prenatal Death of Mouse Embryos with Endothelial Specific Deletion of *Klf2.* Q. Yu, L. Leatherbury, X Tian, J. Lee, M. Kahn, C. Lo; Laboratory of Developmental Biology.

55. The Imaging Probe Development Center - An NIH Roadmap Initiative to Provide Known and Novel Imaging Probes for the Advancement of Molecular Imaging. G. Kaur, H. Li, Z. Shi, A. Sulima, B.Teng, O. Vasalatiy, H. Wu, B. Xu, S. Cofiell, N. Neale, B. Ruddy, C. Wilson and G. L. Griffiths. Imaging Probe Development Center, NHLBI.

Abstracts in Order of First Author

Functional Genomic Analysis of Heart Development in *Drosophila*. S. M. Ahmad, S. E. Choe, L. H. Phun, A. M. Michelson; Laboratory of Developmental Systems Biology.

The *Drosophila* heart is a complex organ that exhibits a remarkable conservation of all major regulatory components when the molecular mechanisms governing its development are compared with those affecting the mammalian heart. In order to identify new genes involved in heart development, we used genome-wide transcriptional expression profiling of FACS-sorted mesodermal cells from *Drosophila* embryos that were either wild-type or genetically perturbed in ways that affect normal cardiac development, along with a "training set" of 51 genes previously known to be expressed in the heart, to arrive at a cardiac meta-analysis that could be used as a predictive tool. We then carried out a systematic validation of the meta-analysis predictions by large-scale histochemical in situ hybridizations, and found that the meta-analysis identified 73 novel genes expressed in the heart or its precursor, the cardiac mesoderm, with an accuracy of 55.7%. Combining the results of this meta-analysis, genes identified by other methods in our laboratory, and the published literature, the number of genes known to be expressed in the cardiac mesoderm and/or the heart is presently 185. We are currently evaluating the functional role of each of these genes in cardiac development by a large-scale RNA interference assay (an independent RNA interference screen by Dr. Y. Kim has already identified 54 of these genes as having a loss-of-function phenotype in the heart). We are also examining the *cis*-regulatory modules of these genes and their regulation by transcription factors involved in heart development in an attempt to determine transcriptional cardiac-specific regulatory codes.

ENU Induced Mutation in the DNA Binding Domain of c-myb Causes Hematopoietic and Myocardial Defects. D. Alpert, R. Francis, X. Q. Zhao, B. Chatterjee, S. Sabol, Q. Yu, Y. Shen, L. Bracero, I. Kitabayashi and C. W. Lo; Laboratory of Developmental Biology; Molecular Oncology Division, National Cancer Center Research Institute, Tokyo, JAPAN.

The c-myb oncogene encodes a transcription factor known to play an important role in regulating hematopoiesis during embryonic development. C-myb knockout (KO) mice die in utero from severe anemia around E14.5. We recovered a mutant mouse with an ENU induced point mutation in the DNA binding domain of c-myb. This mutant was recovered based on having high output cardiac failure as seen by fetal Doppler echocardiography. Similar to the c-myb KO mouse, homozygote mutant mice die around E14.5 due to a severe defect in the development of the definitive hematopoietic lineage. The fetal liver, the structure in which definitive hematopoiesis is first established, was hypoplastic and showed reduced cellularity. Analysis of the developmental potential of fetal liver cells using in vitro methylcellulose cultures showed a complete absence of multipotent hematopoietic colonies. This was likely due to the loss of hematopoietic stem cells, as FACS analysis showed a marked reduction in c-kit/Sca1 double positive cells. Analysis of E14.5 mutant embryos also revealed severe thinning of the compact myocardium accompanied by dense trabeculeation. Surprisingly, the myocardium of mutant embryos showed reduced TUNEL labeling, while phosphohistone-H3 immunostaining was increased. In contrast, in the fetal liver, phosphohistone-H3 and TUNEL labeling were both decreased. These results suggest an altered regulation of cell death vs. cell proliferation in the myocardium and fetal liver of the c-myb mutant embryos. Overall, the hematopoietic and myocardial defects seen in the ENU mutant were more severe than those in the c-myb KO mouse. These findings suggest the mutation in the DNA binding domain of c-myb may exert dominant negative effects that can disrupt protein-protein interactions required for both hematopoiesis and myocardial development. Gel shift experiments are underway to examine the effects of the point mutation on DNA binding affinity.

NMR Solution Structure, Stability, and Interaction of the Recombinant Bovine Fibrinogen αC-Domain Fragment. R. A. Burton[1], G. Tsurupa[2], R. R. Hantgan[2], N. Tjandra[1], and L. Medved[2] ;[1]Laboratory of Molecular Biophysics; [2]Center for Vascular and Inflammatory Diseases and the Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine.

According to the current hypothesis, the COOH-terminal portions of the two Aα chains of fibrinogen are folded into compact αC-domains that interact intramolecularly with each other and with the central region of the molecule; in fibrin, the αC-domains switch to an intermolecular interaction resulting in αC polymers. In agreement, our recent NMR study identified within the bovine fibrinogen Aα374-538 αC-domain fragment an ordered compact structure including a β-hairpin restricted at the base by a 423-453 disulfide linkage. To establish the complete structure of the αC-domain and to further test the hypothesis, we expressed a shorter αC-fragment, Aα406-483, and performed detailed analysis of its structure, stability, and interactions. NMR experiments on the Aα406-483 fragment identified a second loose β-hairpin formed by residues 459-476, yielding a structure consisting of an intrinsically unstable mixed parallel/anti-parallel β-sheet. Size-exclusion chromatography and sedimentation velocity experiments revealed that the Aα406-483 fragment forms soluble oligomers whose fraction increases with increasing concentration. This was confirmed by sedimentation equilibrium analysis, which also revealed that the addition of each monomer to an assembling αC oligomer substantially increases its stabilizing free energy. Additionally, unfolding experiments monitored by CD established that oligomerization of Aα406-483 results in increased thermal stability. Altogether, these experiments establish the complete NMR structure of the Aα406-483 αC-domain fragment and provide direct evidence for the above hypothesis. They also suggest that intra- and intermolecular interactions between the αC-domains, which increase their stability, are thermodynamically driven.

Integrated Analysis of Muscle Transcriptional Regulatory Networks in *Drosophila*. B. W. Busser[1], A. Singhania[1], A. A.Philippakis[2], S. Jaeger[2], M. F. Berger[2], S. Gisselbrecht[2], M. L. Bulyk[2], A. M. Michelson[1]; [1]Laboratory of Developmental Systems Biology; and [2]Division of Genetics, Brigham & Women's Hospital and Harvard Medical School, Boston, MA.

Embryonic muscles arise from an initially uniform field of uncommitted cells under the influence of multiple spatially-restricted signals, including the Wnt, Bmp, and Ras/MAPK pathways. We have proposed that cell-specific gene expression programs are controlled by cis-regulatory modules (CRMs) that integrate convergent inputs of multiple signal-activated and tissue- and cell-restricted transcription factors (TFs). Accordingly, a previously characterized muscle founder cell (FC) enhancer contains clusters of binding sites for Tcf, Mad and Pointed—TFs acting downstream of Wnt, Bmp and Ras, respectively—in combination with sites for the tissue-specific selectors, Twist and Tinman. We have examined the general relevance of this myogenic transcriptional regulatory model (TRM) using an integrated approach involving genome-wide expression profiling, cluster analysis of co-expressed genes, microarray-based determination of TF binding sites, computational prediction of CRMs, in silico evaluation of variant TRMs and empirical testing of candidate enhancers. Our findings suggest that a modified form of the previously characterized muscle TRM regulates only a subset of FC genes. These results led us to evaluate

the role of muscle identity TFs, which have been hypothesized to control the diversification of muscles, in guiding cell-specific gene expression programs. In support of this hypothesis, numerous FC genes were shown to be responsive to over-expression of muscle identity TFs. Interestingly, expression profiling experiments following over-expression of one identity TF, Slouch, supports its involvement in guiding two distinct temporal waves of myogenic gene transcription. These studies provide insights into the distinct spatial and temporal transcriptional codes that regulate gene expression during muscle development.

Small-Molecule Mimics of Superoxide Dismutase as Therapeutics. Y Che[1], B. R. Brooks[1], G. R. Marshall[2], D. P. Riley[2;] [1]Laboratory of Computational Biology; [2]Metaphore, Inc.

Many human diseases are associated with the overproduction of oxygen free radicals that inflict cell damage. Under normal circumstances, this radical burden is controlled by the superoxide dismutase (SOD). Protective and beneficial roles of SOD enzymes have been shown in many diseases, such as ischaemia-reperfusion injury, inflammation, Parkinson's disease, pulmonary disorder and cancer. Because of the limitations associated with enzyme therapies (cost, bioavailability, stability, immunogenicity), a series of manganese(II)-based macrocyclic complexes has been designed to be functional mimics of the SOD enzyme. These small molecules had both high catalytic SOD activity and selectivity, without reaction with nitric oxide, hydrogen peroxide, or peroxynitrite. Moreover, they are excreted intact with no detectable dissociation. Selective SOD mimics have successfully completed Phase I and II clinical trial in more than 600 human subjects. I am going to focus on discussing the role of molecular modeling in the design and development of these small-molecule mimics.

Top-Down Approach in Protein RDC Data Analysis-*de novo* Estimation of the Alignment Tensor. K. Chen and N. Tjandra; Laboratory of Molecular Biophysics.

In solution NMR spectroscopy the residual dipolar coupling (RDC), bearing on the two covalently bonded spin 1/2 nuclei, is usually used to refine the available structure. The value of RDC depends on the orientation of the bond vector with respect to the alignment tensor frame. The *de novo* protein structure determination with RDCs is challenging without knowing the alignment tensor. We present a top-down approach for RDC data analysis. Using only the RDCs measured from N-H bonds from residues in α-helix and CA-CO bonds from β-strand, we are able to extract the offset and amplitude from the RDC modulation-curve for each secondary structure element, which are used for global minimization. The alignment order parameters, axial component Azz and rhombicity R, and the orientation of the long principal axis of individual helix/strand with respect to the alignment frame can be determined in each of the eight quadrants of a sphere. For a helical rich protein Bax, the orientation parameters determined by this method are in average deviating by 11° away compared to the previously solved NMR structure. As expected the error in the direction estimation increases with any deviation from the canonical helix structure such as the dihedral angles and the curvature of the helix. For β-sheet protein Ubiquitin, the average deviation is 20°, which is determined from CA-CO RDCs. The larger discrepancy in β-strand orientation comes from both the diversity of β-sheet structure and the inferior quality of CA-CO RDCs. The top-down approach holds promise for providing a protein topological fold using only RDCs which is expected to speed up the structure determination using solution NMR spectroscopy.

Theoretical Study of the Rhenium-Alkane Interaction in Transition Metal-Alkane S-Complexes. E. A. Cobar, R. Z. Khaliullin, R. G. Bergman, and M. Head-Gordon; Laboratory of Computational Biology.

Metal-alkane binding energies have been calculated for $[CpRe(CO)_2]$(alkane) and $(CO)_2M(C_5H_4)C{\equiv}C(C_5H_4)M(CO)_2]$(alkane), where M = Re or Mn. Calculated binding energies were found to increase with the number of metal-alkane interaction sites. In all cases examined, the manganese-alkane binding energies were predicted to be significantly lower than those for the analogous rhenium-alkane complexes. The metal (Mn or Re)-alkane interaction was predicted to be primarily one of charge transfer, both from the alkane to the metal complex (70-80% of total charge transfer) and from the metal complex to the alkane (20-30% of the total charge transfer).

Complexity of Arf Function at the Cell Periphery. L. A. Cohen, A. Honda, P. Varnai, T. Balla, J. G. Donaldson; Laboratory of Cell Biology.

ARNO/cytohesin family proteins, are BFA insensitive exchange factors (GEFs) for ARF small GTPases that function in the cell periphery where they can promote cell ruffling at the PM, and promote cell migration. We show that ARNO/cytohesin GEFs are recruited by active ARF6 onto the plasma membrane through an interaction between their carboxy terminal PH domains, GTP Arf6 and inositol phospholipids. Once recruited to the plasma membrane ARNO/cytohesin family GEFs can recruit and activate ARF1 at the PM. This led us to examine a role for ARF1 in the organization of cytoskeletal proteins in the cell periphery. siRNA mediated knockdown of ARF1 leaves the Golgi intact, but alters the structure of focal adhesions in HeLa cells. Knockdown of ARF1, but not ARF6, severely retards cell spreading, and inhibits cell migration. These studies support an under appreciated requirement of ARF1 in cytoskeletal and focal adhesion organization in the cell periphery.

Real-Time MRI Guided Laser Transseptal Puncture. A. A. Elagha, O. Kocaturk, M. A. Guttman, C. Ozturk, P. V. Karmarkar; A. H. Kim, J. H. Kim, V. K. Raman, VJ Wright, W. H. Schenke, E. R. McVeigh, and R. J. Lederman; Cardiology Branch.

Objective. We report initial preclinical experience with real-time MRI-guided atrial septal puncture using a custom MRI-conspicuous blunt laser catheter that perforates only when energized. Background. Conventional needle septal puncture is guided by tactile and visual fluoroscopic feedback, often with adjunctive ultrasound guidance. Even in experienced hands the procedure risks non-target perforation and pericardial tamponade. Real-time MRI offers the potential advantage of superior device tracking and target imaging compared with ultrasound and X-ray, respectively, using a single imaging modality. Methods. We used a clinical excimer laser catheter (0.9mm Clirpath, Spectranetics) customized with receiver coils to impart visibility under MRI, and a 1.5T interventional MRI suite. Six swine underwent laser transseptal puncture under real-time MRI. Results. Embedded MRI-antennae accurately reflected the position of the laser catheter tip and profile *in vitro* and *in vivo*. Despite the increased profile from microcoils, the 0.9mm laser catheter traversed *in vitro* targets with reduced force compared with a conventional Brockenbrough needle. Laser puncture of the atrial septum was successful and accurate in all animals, corroborated by oximetry, pressure, angiography, and necropsy. The mean procedure time was 31 ± 0.4 minutes, with an average 3.8 ± 0.4 seconds of laser activation. There were no sequelae after prolonged observation. Necropsy revealed 0.9mm holes in the fossa ovalis in all animals. Intentional perforation of the aorta and of the atrial free wall was immediately evident, suggesting that real-time MRI guidance adds valuable safety information during these procedures. Conclusion. MRI-guided laser puncture of the interatrial septum is feasible in swine, and offers controlled delivery of perforation energy using an otherwise

blunt catheter. This technology may enable more advanced non-anatomic vascular connections.

Rabbit ATG, but not Horse ATG and CsA Promotes Expansion of Functional CD4+CD25highFoxP3 Regulatory T Cells of Healthy Controls. X. Feng, E. Solomou, N. Young; Hematology Branch.

$CD4^+CD25^+$ regulatory T cells (Treg) play important roles in suppressing immune responses and maintaining tolerance. Treg have the ability to prevent the development of autoimmune diseases, tumor progression, graft rejection and graft versus host disease (GVHD). Immunosuppressive drugs such as rabbit ATG (rATG), horse ATG (hATG) and Cyclosporine A (CsA) have been widely used in conditioning regimens for hematopoietic stem cell transplantation, for the treatment of autoimmune diseases, and in therapy of GVHD, but their effects on Treg remain to be elucidated. In the current study, we show that in vitro culture of human peripheral blood mononuclear cells (PBMC) with rATG, resulted in expansion of $CD4^+CD25$high cells and $CD4^+CD25$highFoxP3 cells. However, hATG and CsA decreased the percentage of $CD4^+CD25$high cells and $CD4^+CD25$highFoxP3 cells. The rATG treatment resulted in conversion of CD4+CD25- T cells into $CD4^+CD25^+$ T cells. In immunoblots, PBMC treated with rATG showed increased expression of FoxP3 and NFAT1 in $CD4^+CD25$- and $CD4^+CD25^+$ T cells. Moreover, these expanded Treg suppressed autologous T-cell proliferation on TCR stimulation by 64%, suggesting that expanded Treg were also functional. Culture supernatants of PBMC treated with rATG showed increased secretion of IL-10, compared to PBMC treated with hATG or CsA. No differences in INF-, IL-2 and IL-4 levels were observed. Our findings suggest that the therapeutic effects of rATG in the treatment of autoimmune diseases and GVHD might be related to its ability to foster expansion of functional Treg.

Skeletal Anomalies in Novel Mutant Mouse Models with Congenital Cardiac Anomalies. B. Gibbs[1], D. Schimel[2], Q. Yu, and C. W. Lo[1] ;[1]Laboratory of Developmental Biology, NHLBI and [2]Mouse Imaging Facility, NINDS.

We recovered many novel mouse mutants exhibiting structural heart defects from a prenatal fetal ultrasound screen of N-ethyl-N nitrourea (ENU) mutagenized mice. Interestingly, many of these mutants also showed a variety of skeletal anomalies. Analysis of the skeletal phenotypes in several of these mouse mutants indicated similarities with human syndromes in which specific skeletal defects are seen together with cardiac anomalies. One mutant referred to as Family 166 showed oligodactyly/syndactyly together with ventricular septal defects, syndromic presentations reminiscent of Holt-Oram syndrome. Another mutation identified as a point mutation in p53BP1 causes phenotypes similar to DiGeorge Syndrome. This included cardiac defects consisting of persistent truncus arterious and also craniofacial defects that include cleft palate and hypoplasia of the mandible. In addition, this mutant shows hypoplasia of the vertebral column with hemi and fused vertebrae and shortening of the forelimbs. In yet a third mutant family with a mutation in *DNH5C* encoding a dynein heavy chain protein, defects in the cilia were found causing Primary Ciliary Dyskinesia (PCD) and Kartagener's syndrome. These mutants exhibit hydrocephaly and other vertebral anomalies. Full-body computed tomography (CT) scanning showed a failure in fusion of the suture lines in the skull, dysmorphic vertebra, and abnormal bone structure. These findings suggest mouse models are invaluable for studying syndromic presentations involving cardiac and skeletal anomalies. Moreover, these results demonstrate that skeletal defects are a useful phenotyping tool for identifying mouse mutants with syndromic presentations.

Ex Vivo Expansion of Retrovirally- Transduced Primate $CD34^+$ Cells Results in Preferential Engraftment and Persistence of Clones with MDS1/EVI1 Insertion Sites. T. J. Gomes, S. Sellers, R. E. Donahue, R. Adler, A. La Rochelle and C.E. Dunbar; Hematology Branch.

There is increasing evidence that insertional activation of proto-oncogenes by retroviral vectors is a significant safety issue that must be addressed before clinical gene therapy can be further developed. The risk of insertional mutagenesis for replication-incompetent retroviral vectors has been assumed to be low until the occurence of T cell leukemias in children treated with HSC-directed gene therapy for X-SCID. We have previously reported a highly non-random occurence of 14 unique vector integrations in the first two introns of the MDS1/EVI1 proto-oncogene out of a total of 702 identified from myeloid cells of 9 rhesus macaques at least 6 months post-transplantion of retrovirally-transduced $CD34^+$ cells. To begin to investigate the factors contributing to the marked over-representation of MDS1/EVI1 insertions, we asked whether continued ex vivo expansion of transduced $CD34^+$ cells prior to transplantation would further select for clones with insertions in MDS1/EVI1 or other proto-oncogenes. Rhesus $CD34^+$ cells were transduced with the G1Na standard retroviral vector and were continued in culture for an additional 6 days under the same culture conditions, and then reinfused into the donor animal following 500x2 cGy TBI. At 6 months post-transplantation 5 out of 27 (19%) of the unique insertions identified in granulocytes were within the first two introns of MDS1/EVI1. This strongly suggests that the over-representation of this locus in engrafting cells is due to a potent immortalizing signal and that the need for extended ex vivo culture of target cells may select for insertion events activating this locus. It also suggests that strategies involving prolonged ex vivo expansion or selection of transduced cells could increase the risk of gene therapy utilizing integrating vectors targeting primitive hematopoietic cells.

Concerted Nitric Oxide Formation and Release from the Simultaneous Reactions of Nitrite with Deoxy- and Oxy-Hemoglobin. R. Grubina,[1] Z. Huang,[1] M. S. Joshi,[2] S. Basu,[2] D. B. Kim-Shapiro,[2] M. T. Gladwin[1]; [1]Vascular Medicine Branch [2]Department of Physics, Wake Forest University

Recent studies reveal a novel role for hemoglobin as an allosterically regulated nitrite reductase that may mediate nitric oxide (NO)-dependent signaling along the physiological oxygen gradient. Nitrite reacts with deoxyhemoglobin in an allosteric reaction that generates NO and oxidizes deoxyhemoglobin to methemoglobin. NO then reacts at a nearly diffusion-limited rate with deoxyhemoglobin to form iron-nitrosyl-hemoglobin, which to date has been considered a highly stable adduct and thus not a source of bioavailable NO. However, under physiological conditions of partial oxygen saturation nitrite will also react with oxyhemoglobin, and while this complex autocatalytic reaction has been studied for a century, the interaction of the oxy- and deoxy-reactions and the effects on NO disposition have never been explored. We have now characterized the kinetics of hemoglobin oxidation and NO generation at a range of oxygen partial pressures and found that the deoxy-reaction runs in parallel with and partially inhibits the oxy-reaction. In fact, intermediates in the oxy-reaction oxidize the heme iron of iron-nitrosyl-hemoglobin, a product of the deoxy-reaction, which releases NO from the iron-nitrosyl. This oxidative denitrosylation is particularly striking during cycles of hemoglobin deoxygenation and oxygenation in the presence of nitrite. These chemistries may contribute to the oxygen-dependent disposition of nitrite in red cells by limiting oxidative inactivation of nitrite by oxyhemoglobin, promoting nitrite reduction to NO by deoxyhemoglobin, and releasing free NO from iron-nitrosyl-hemoglobin.

Function of the C1 Inserted Isoform of Nonmuscle Myosin II-C in Tumor Cell Lines and Mice. S. S. Jana, R. S. Adelstein; Laboratory of Molecular Cardiology.

Nonmuscle myosin IIs play an important role during cytokinesis, cell migration and in establishing cell polarity. Three isoforms of nonmuscle myosin heavy chain II (NMHC) have been identified in vertebrates, NMHC II-A, II-B and II-C. Here we report on the role of an alternatively-spliced isoform, NMHC II-C1, which includes an 8 amino acid insertion in loop1 of NMHC II-C. We recently reported that expression of this isoform is markedly increased in a number of tumor cell lines and that inhibiting expression leads to a delay in cytokinesis, resulting in a decrease in cell proliferation in the A549 human lung tumor cell line (Jana et al. *J. Biol. Chem*. 2006 PMID: 16790446). We now report on a second tumor cell line showing increased expression of NMHC II-C1, the human breast cell line MCF-7. Unlike A549 cells which are adhesive to the underlying matrix, MCF-7 cells retain a rounded shape during cytokinesis and appear to be loosely attached to the surface. Decreasing NMHC II-C1 expression in MCF-7 cells leads to marked prolongation in a late stage of cytokinesis, from 3-3.5 h for control siRNA treated cells to 15-17 h for the NMHC II-C1 depleted cells, similar to our findings with A549 cells. This prolongation results in a 6-fold decrease in cell number at 120 h. Although A549 and MCF-7 cells differ morphologically during cytokinesis, in both cases inhibition of NMHC II-C1 leads to a delay in cell division. For *in vivo* studies of nonmuscle myosin II-C1 function we generated hypomorphic knockout mice using homologous recombination. These mice expressed decreased amounts of myosin II-C all of which lacked the C1-insert. Some of these mice show evidence for a cardiomyopathy as determined by echocardiography and histopathologic analysis. Of note is that expression of NMHC II-C1 in the heart is restricted to the embryonic stage.

MCOLN2 Localizes to Lysosomes and the Arf6-associated pathway. C. V. Karacsonyi, A. San Miguel, R. Puertollano; Laboratory of Cell Biology.

Mucolipins constitute a family of cation channels with homology to the transient receptor potential (TRP) superfamily. In mammals, the mucolipin family includes three members (MCOLN1-3). Homolog of mammalian mucolipins have been also described in *Drosophila* and *C. Elegans.* Mutations in MCOLN1 have been associated with mucolipidosis type IV, a lysosomal storage disease characterized by severe neurological and ophthalmologic abnormalities. MCOLN3 might also play a role in human pathologies, as mutations in this gene are responsible for the *varitint-waddler* mouse phenotype that is characterized by defects in pigmentation and hearing loss. However, little is known about the function and cellular distribution of MCOLN2. The aim of this project is to characterize the cellular localization and the possible roles of MCOLN2 in Hela cells.

The Imaging Probe Development Center - An NIH Roadmap Initiative to Provide Known and Novel Imaging Probes for the Advancement of Molecular Imaging. G. Kaur, H. Li, Z. Shi, A. Sulima, B.Teng, O. Vasalatiy, H. Wu, B. Xu, S. Cofiell, N. Neale, B. Ruddy, C. Wilson and G. L. Griffiths. Imaging Probe Development Center, NHLBI.

The Imaging Probe Development Center (IPDC) has been set up as part of the NIH Roadmap initiative (http://nihroadmap.nih.gov/) and is dedicated to the syntheses of known and novel imaging probes. IPDC services are currently being used by the NIH intramural community, and are also ultimately intended for use by the wider scientific community. The imaging probes under development are of a diverse nature encompassing optical, radionuclide, fluorescent and magnetic resonance agents. During its first year while located in temporary laboratory space IPDC successfully provided around one dozen probe compositions to collaborating scientists, including Coomassie dye analogs to enable extended protein analyses in native PAGE, a series of PAMAM dendrimer-chelate complexes labeled with gadolinium for MRI, fluorescent nucleotide analogs for DNA-RNA labeling, and a generally applicable multi-functional agent for the site-specific labeling of recombinant proteins near their C-terminal histidine tags. Now located in its newly opened and equipped permanent laboratories in Rockville, IPDC scientists have begun to work on the production of 50+ probes, as requested by scientists drawn from throughout the NIH Institutes. Examples of these ongoing projects, along with applications of the proposed probes, and their possible impacts on new findings in the imaging arena will be presented. The basic research aims of the IPDC will also be outlined, and suggestions and discussions concerning the preparation of new imaging probe proposals with interested NHLBI intramural scientists will be actively encouraged.

A Homogeneous ELISA Assay Detecting Chondroitin Sulfate Proteoglycan (CSPG) Production for the High-Throughput Screening of Small Molecules. T. L. Laabs[1], S. Jeffries[3], HM Geller[1]; [1]Developmental Neurobiology Section, NHLBI, [2]Chemical Genomics Center, Oxford-Cambridge Biomedical Scholars Program.

Chondroitin sulfate proteoglycans are the major inhibitory component of the glial scar that forms after a spinal cord injury. They are composed of a core protein and one or more linear glycosaminoglycan (GAG) chains. Removal of their GAG chains with the enzyme chondroitinase ABC leads to increased regeneration and functional recovery after spinal cord injury in an animal. We hypothesize that inhibiting the production and/or secretion of CSPGs shortly following spinal cord injury will greatly improve functional recovery. To this end, we are developing a homogeneous ELISA assay for detection of CSPGs in conditioned media and cell cultures. The assay utilizes two antibodies. One antibody recognizes the core protein component of the CSPG and the other recognizes the intact GAG chain, eliminating the need for the CSPG digestion step. The secondary antibody for each primary is bound separately to donor or acceptor beads. In the presence of CSPG core proteins bearing GAG chains these beads are brought together. When the donor bead is excited by a laser it releases singlet oxygen molecules which react with a chemiluminescer in the acceptor bead which then emits light at a detectable wavelength (AlphaScreen™ from PerkinElmer). Using cytokine treated primary mouse astrocytes as an in vitro model of the glial scar we aim to identify small molecules that inhibit CSPG production. We are miniaturizing this assay to a 1536-well mircotiter plate format to run a high throughput screen against approximately 100K compounds in dose response.

Simultaneous Monitoring of MRgFUS Temperature and Tissue Stiffness Using 1D Magnetic Resonance Elastography; Y. Le, S. V. Primak, K. J. Glaser, A. Manduca, R. L. Ehman, J. P. Felmlee**;** Mayo Clinic College of Medicine, Rochester, MN.

The purpose of this work is to study the feasibility 1D MRE monitoring of Focused Ultrasound Surgery (FUS). The key benefits of 1D MRE technique are: it can measure in real-time the temperature and stiffness change in the ablated tissue. First, PRF shift was measured by 1D MRE and regressed with the temperature measured by thermometer. Second, 1D MRE data were acquired along a cylindrical beam at the treatment focus where displacement and temperature were calculated. During short intervals, 2D MRE images were acquired using an external mechanical driver to induce motions. Temperature was calculated from these 2D images and compared with the 1D measurement. Finally, 1D MRE was used to measure the temperature and displacement at the same time during FUS treatment of *in vivo* tumor. Our results show that PRF shift calculated from 1D MRE data is in excellent agreement with the calibration curve, the

correlation coefficient is 0.996 and the slope is 0.008 ppm/°C. It is also shown that the 1D and 2D MRE measured temperature are consistent with each other. 1D MRE has an acquisition speed advantage to acquire tissue displacement at the focus and directly indicate protein denaturation and temperature simultaneously. This study shows feasibility of 1D MRE monitoring of the temperature and displacement during FUS treatment and supports further study in this area.

Sirt1 Regulates Autophagy. I. H. Lee[1], R. Mostoslavsky[2], D. B. Lombard[2], N. E. Bruns[1], M. Tsokos, F. W. Alt[2] and T. Finkel[1]; [1]Cardiology Branch, NHLBI;[2]Howard Hughes Medical Institute, The Children's Hospital, CBR Institute for Biomedical Research, Harvard University Medical School;[3] Laboratory of Pathology, NCI.

Autophagy is believed to be an important mechanism to maintain cellular viability during prolonged nutrient deprivation. Similarly, the sirtuin family of NAD-dependent deacetylases appears to be involved in the cellular adaptation to various stresses including limited nutrient availability. Here we demonstrate an essential role for the Sirt1 in the regulation of autophagy. In particular, we show that autophagy is not fully activated following starvation or the addition of rapamycin in Sirt1 knockdown cells, Sirt1 -/- mouse embryonic fibroblasts or in cells where Sirt1 activity is pharmacologically inhibited. Reconstitution with wild type but not a deacetylase inactive mutant of Sirt1 restores autophagy in Sirt1 -/- cells. In addition, increased expression of Sirt1 is sufficient to stimulate basal rates of autophagy. We further demonstrate that Sirt1 forms a molecular complex with several essential components of the autophagy machinery including Atg5, Atg6/beclin1 and Atg7. In vitro, Sirt1 can directly deacetylate some, but not all, autophagy related gene products. The absence of Sirt1 in both cells in culture and neonatal mouse tissues leads to markedly elevated acetylation of proteins known to be required for autophagy. Furthermore, the acetyltransferase p300 binds to and augments acetylation of the same autophagy-related proteins targeted by Sirt1 and knockdown of p300 stimulates autophagy. Finally, consistent with a protective effect of autophagy, we demonstrate that Sirt1-/- cells are more sensitive to the effects of acute nutrient withdrawal. These results suggest that the Sirt1 deacetylase is an important regulator of autophagy and provides a link between sirtuin function and the overall cellular response to limited nutrients.

Mapping 2D Strain in the Wall of the Carotid Artery Using Displacement-Encoded MRI. A. P. Lin[1], E. Bennett[2], L. Wisk[3], M. Gharib[1], S. Fraser[1], and H. Wen[2]; [1]California Institute of Technology, Pasadena, CA, [2]Laboratory of Cardiac Energetics, NHLBI, [3]University of California Los Angeles, Westwood, CA..

Atherosclerotic cardiovascular disease is the leading cause of death in the United States with over 19 million deaths worldwide where a large portion of these victims are asymptomatic. Therefore there is a considerable demand for the early diagnosis of atherosclerosis, otherwise known as hardening or stiffening of the arterial walls. A number of large epidemiologic studies have demonstrated that carotid artery wall stiffness contributes to systolic hypertension, increased cardiovascular risk, and risk of ischemic stroke. Furthermore, biomechanical models of the carotid artery have demonstrated increased atherogenesis in regions with high strain . We developed a displacement-encoded MRI (DENSE) sequence for imaging the motion of the carotid artery wall and mapping the 2D circumferential strain in wall. With increased resolution and regional accuracy, this technique could potentially give more efficacious risk indicators of atherosclerotic cardiovascular disease. Our first aim was to validate DENSE using both *in vitro* models and *in vivo* studies. Carotid stiffness has been shown to increase with age, therefore our second aim was to quantify strain using DENSE in two different age groups. Finally, in order to validate current biomechanical models of the carotid arteries, our third aim was to characterize strain at the bifurcation of the carotid artery.

Dexamethasone Suppresses Phospholipase D (PLD) Activation And Other Signaling Events Associated With Lipid Rafts In Mast Cells. F. A. Lisboa, M. A. Beaven; Laboratory of Molecular Immunology .

Antigen-induced aggregation of IgE receptors (FcεRI) and subsequent recruitment of activating kinases such as Lyn and Syk occurs in the vicinity of plasma membrane (PM) micro-domains known as lipid rafts. The ensuing phosphorylation of adaptor proteins such as LAT and LAB (NTAL), within lipid rafts, enables the assembly of additional effector molecules and further propagation of activating signals. Studies in our laboratory have shown that PLD2, but not PLD1, is localized in PM lipid rafts and its activation is blocked by glucocorticoid treatment. We have accordingly investigated whether the inhibition of PLD disrupts lipid raft function and signal propagation using membrane fractionation techniques, siRNA technology, and confocal microscopy in primary and transformed mast cell lines. We found consistent inhibition of antigen-induced migration of FcεRI to detergent-resistant membrane fractions in both dexamethasone- and anti-PLD siRNA-treated cells. Similar inhibition was noted with the co-localization of FcεRI and lipid raft markers in living cells when visualized by confocal microscopy. Both treatments also decreased LAT phosphorylation at Y171, although siRNA against PLD2 was much more effective in this regard than siRNA against PLD1, a finding consistent with the localization of PLD2 in PM lipid rafts. Collectively, these findings show dexamethasone inhibits assembly of lipid raft signaling complexes and may do so through inhibition of PLD2.

The Aging Regulator Klotho is a Secreted Wnt Antagonist and Essential for Organ Homeostasis. H. Liu, M. Fergusson, J. Liu, C. Liu, J. Chen, D. Malide, C. J. Kuo, P. M. Hwang and T. Finkel; Cardiology Branch.

Mice that fail to express the klotho gene product demonstrate evidence of accelerated ageing while overexpression of klotho can lead to life span extension. The mechanism by which klotho exerts these effects on mammalian lifespan are not well understood. Here we demonstrate that klotho can bind to multiple Wnt isoforms and inhibits their function. Consistent with these in vitro observations, analysis of tissues and organs from klotho deficient mice demonstrates that Wnt signaling is up-regulated. These results suggest that augmented Wnt signaling may contribute to the accelerated aging phenotype observed in klotho deficient mice. In support of this hypothesis, we further demonstrate that long term over activation of Wnt signaling in the klotho deficient mice leads to depletion of stem cells and accelerated senescence of progenitor cells in multiple tissues and organs. These further affect the normal processes of tissue maintenance and organ homeostasis, leading to animal aging. These results suggest that klotho is a novel secreted Wnt antagonist and that Wnt signaling play an unexpected but important role in mammalian aging.

Genetic Depletion of Sirt2 Augments Cellular Resistance to Hypoxia-Reoxygenation Injury. E. G. Lynn, C. J. McLeod, M. N. Sack; Cardiovascular Branch.

The family of class III histone deacetylases (sirtuins) is proposed to function as metabolic sensors and gene silencing proteins as well as function as mediators of stress tolerance and senescence. Sirt2 is one of the less well understood sirtuins. In the current study we have investigated the role of Sirt2 in mediating the effect of hypoxia-reoxygenation injury. Rat cardiac derived H9c2 cells exposed to 17-h hypoxia and 2-h reoxygenation 48-h post-transfection with Sirt2 siRNA exhibited increased cell survival, measured by LDH cell viability assay, relative to controls. Conversely, Sirt2 overexpression in H9c2 decreased cell viability in response to hypoxia-reoxygenation injury.

Affymetrix gene-chip analysis was performed to identify candidate cytoprotective genes modulated by Sirt2. The gene for the adapter protein 14-3-3-zeta was one of only four genes up-regulated in response to the genetic depletion of Sirt2 in H9c2. Real-time PCR and Western immunoblot analyses confirmed that levels of 14-3-3-zeta were significantly induced following Sirt2 depletion in H9c2. Sirt2-depleted H9c2 also exhibited increased association of 14-3-3-zeta with the pro-apoptotic protein Bad. Apoptosis in H9c2 induced by hypoxia-reoxygenation was significantly delayed by Sirt2-depletion. The current study indicates Sirt2 RNAi bestows resistance to hypoxia-reoxygenation injury in H9c2 by delaying apoptosis via a mechanism involving the increased sequestration of Bad by 14-3-3-zeta. This project was supported by the Division of Intramural Research.

mESC-derived Vascular Progenitors Induced Rejective Responses in the Syngeneic Wound Repair Model as a Result of NK Attack. M. C. Ma, S. L. Ding, M. Konoplyannikov, J. Mellad, L. Beltran, C. Graham, and M. Boehm; Cardiovascular Branch.

While mESCs are pluripotent and have great potential for *in vivo* application, little is known regarding their fate and effect after introducing different stages of mESC-derived cells into adult wound models. ESC are usually differentiated in serum-containing media, which contain many undefined factors that affect ESC differentiation into certain lineage, and makes it difficult to isolate a pure cell population. To address these problems, mESCs were differentiated into a mesodermal lineage in N2B27 media containing BMP4 and rmFGF. Brachyury positive and Flk-1 positive cells ($Bra^+/Flk\text{-}1^+$) were sorted out with FACS, and were cultured in medium supplemented with VEGF to induce endothelial differentiation. About 50% of the cells absorbed DiI-acLDL, and immunohistochemical analyses revealed that they were CD31, VE-Cadherin (VE-CAD), and Von-Willebrand Factor positive, and thus are believed to be endothelial progenitors. Surprisingly, FACS analysis showed that none of the above cells expressed $H\text{-}2D^b$ MHC class I. The tumorgenesis ability of the above cells in SCID immune-deficient mice revealed that teratomas formed only in the group injected with undifferentiated mESCs. 200,000 undifferentiated mESC and the above sorted cells were injected into right tibialis muscles 24 hours after femoral artery ligation of the ischemic model. Two weeks after surgery, tibialis muscles of both sides were harvested. This model is characterized by an inflammation reaction during the initial two weeks after femoral artery ligation. However, transplantation of mESC-derived vascular progenitor cells induced an increased degeneration response when compared to PBS. In addition, immuhistochemical study revealed that there are more T cell, Macrophage, infiltration among muscle fibers in the cell-injected groups than the control PBS-injected group. Y chromosome FISH and immunohistochemical double staining were performed in order to detect cell engraftment, and provide additional evidence for necrosis/apoptosis of the injected cells. These results suggest that early stage mESC-derived mesodermal lineage progenitor cells caused an inflammatory reaction and were rejected by the syngeneic host. This is possibly due to the lack of MHC class I expression on the ES derived progenitors, leading to the attack of local natural killer cells. Further studies will include inducing the expression of MHC class I on cells before injection and the injection of cells into NK depleted/deficient recipients.

Fate Mapping of Neointima Formation in a Murine Vein-Arterial Grafts Model. J. Mellad, A. Wragg, M.C. Ma, S. Ding, L. Beltran, B. Cooley, M. Boehm; Cardiovascular Branch.

A clinically relevant model of neointima formation and vascular remodeling is the vein-arterial graft, the clinical standard for vascular bypass surgery. However, a significant number of these grafts occlude due to neointima formation. When the jugular vein is grafted onto the femoral artery in our clinically-relevant mouse model, the vein undergoes both arterialization and stenosis, characterized by rapid proliferation of local vascular cells. The recently developed Cre-Lox system allows the *in vivo* tracing of vascular progenitors. Cre recombinase, under the control of a tissue-specific promoter, will excise a floxed stop codon and subsequently permit the expression of LacZ or a fluorescent protein cloned into the R26R locus. This genomic alteration irreversibly marks cells, and their progeny, which expressed the gene of interest at any point during development. We have characterized the offspring of Tie2-Cre x R26R-LacZ and Tie2-Cre x R26R-YFP mice, in which all endothelial and hematopoeitic stem cell–derived cells express LacZ or EYFP respectively. EYFP and LacZ staining of control vessels reveals endothelial cell labeling, with various adventitial cells also positive for the Tie2-lineage (TL) but negative for endothelial (CD31, VE-cadherin, VWF) and inflammatory (CD3, Mac2) markers. Staining of Tie2-R26R-LacZ veins grafted into wild-type animals shows extensive proliferation, as demonstrated by PCNA staining, of TL cells and their contribution to the neointima along with non-TL cells. These results confirm a diverse contribution of cells to the neointima and raise the possibilities of both endothelial-mesenchymal transition within the remodeling vessel wall as well as a role for TL adventitial progenitors. Future studies will characterize these TL cells by immunohistochemistry, FACS and *in vitro* differentiation assays. Additional lineage-specific Cre mice will also be used to examine the contribution of non-TL cells to the neointima.

MDS1-EVI1 and EVI1 Overexpression Results in Changes in the Behavior of Murine Hematopoietic Cells. J. Y. Metais[1], R. Wieser[2], and C. E. Dunbar,[1]; [1]Hematology Branch; [2]Institute, Medizinische Universitaet Wien, KIMCL.

We have found non-random patterns of retroviral integration in long-term hematopoietic repopulating cells in the rhesus macaque, with frequent integration events of MLV vectors into the MDS1-EVI1 gene complex. These findings, along with reports regarding frequent integration events in the same gene complex in patients with chronic granulomatous disease receiving MLV-transduced hematopoietic cells in a clinical trial and the ability of MLV vectors activating expression of this gene via integration to immortalize primary murine bone marrow cells, suggests these gene products could have important roles in normal and leukemic hematopoiesis. Expression from this gene complex can result in translation of at least three distinct proteins: MDS1, EVI1, and MDS1-EVI1. EVI1 has been the most studied protein of this locus. Its overexpression, as a consequence of chromosomal rearrangement or viral integration, is associated with leukemia. MDS1-EVI1 contains a PR domain that is lacking in EVI1 and is thought to possibly be antagonistic to EVI1, however the location of the integrations in our prior rhesus studies would indicate that overexpression of either gene product could be immortalizing. Both proteins share the same expression profile in normal tissues as well as most reports of myeloid leukemias. To investigate the impact of the three gene products on hematopoietic cells, we cloned murine mds1, evi1, and mds1-evi1 into the pMIEV-GFP retroviral vector and produced ecotropic vector particles. These were used to transduce the murine BaF3 hematopoietic cell line as a model to study the impact of expression of these various gene products. Gene expression analysis using Afflymetrix arrays demonstrated that both EVI1 and MDS1-EVI1 expression produced dramatic changes in gene expression profiles of these cells, compared to MDS1 and control vector. For instance, EVI1 transduced cells overexpressed oncogenes such as small G proteins belonging to the RAS family. There was modulation of genes implied in hematopoiesis, apoptosis, TGF beta signaling, and cell cycle. To assess changes in cell cycling of transduced BaF3 cells we used a flow cytometric assay, which unraveled an arrest in G1 phase only when EVI1 was overexpressed. These changes were concomitant to an increased metabolic activity as measured by an MTT assay. Further studies of these different pathways have to be performed in order to

confirm the results obtained by the DNA chips analysis. Primary murine bone marrow cells could be immortalized after transduction by both EVI1 and MDS1-EVI1 vectors, compared to MDS1 and control vectors. Mice have been transplanted with primary bone marrow cells transduced with all vectors, and are being followed for hematopoietic changes or leukemia. In conclusion, both MDS1-EVI1 and EVI1 overexpression appear to result in marked changes in the behavior of primitive hematopoietic cells.

Mechanisms of Regulation of CXCR4/SDF1 Dependent Migration and Survival in Chronic Lymphocytic Leukemia. Y. Miura, Y. Herishanu, E. Lee, F. Gibellini, N. Njuguna, H. Mora-Jensen, A. Wiestner. Hematology Branch.

Chronic lymphocytic leukemia (CLL) is characterized by the accumulation of mature B lymphocytes in the peripheral blood (PB) and bone marrow (BM). Leukemic cells from PB expressed CXCR4 more strongly than cells from BM. Expression of CD69, an activation marker, followed the inverse pattern. This data is consistent with a model in which CLL cells migrate along an SDF1 gradient to stroma cell niches in BM where they are activated. To determine whether the migration of CLL cells is induced in the BM milieu, we tested the migration of CLL cells in response to BM supernatant. Supernatant from the BM of patients with CLL was placed in the lower chamber of a transwell migration assay. CLL cells migrated in response to BM supernatant, to a degree comparable to SDF1 at 20 ng/mL. The BM milieu contains many chemokines and cytokines that induce the migration of CLL cells other than SDF1. To determine whether the inhibition of CXCR4 inhibited the migration of CLL cells in the BM milieu, we tested the effect of AMD3100, a CXCR4 inhibitor, on the migration of CLL cells in response to BM supernatant. AMD3100 resulted in inhibition of migration in the chambers containing BM supernatant as well as in the SDF1 chambers. In an *in vitro* culture system, S17 stroma cells attract CLL cells and extend their survival. AMD3100 inhibited the pro-survival effect of stroma. These results suggest that the interaction between leukemic cells and stroma represents a novel target for therapy of patients with CLL.

Resistance to Bortezomib in MCL Cells is Associated with Decreased Proliferation and Adaptive Changes of the Proteasome. H. Mora-Jensen**,** E. Rizzatti and A. Wiestner; Hematology Branch.

Mantle Cell Lymphoma (MCL) is a malignancy of B-cells. Bortezomib (BZM), an inhibitor of the proteasome, can induce responses in up to 50% of relapsed MCL patients. To investigate mechanisms of resistance to BZM, we generated BZM resistant MCL cell lines (BR). HBL-2-BR and Jeko-1-BR were up to 50-fold less sensitive to bortezomib than the parental cells. All BR subclones showed decreased sensitivity to three other proteasome inhibitors. No change in the sensitivity to BAY11-7082, an inhibitor of NF-kB signaling, was observed. Resistance to BZM was very slow to develop and required continuous exposure to increasing concentrations of BZM. The resistance was equally slowly lost over several months when cells were cultured without the drug, indicating adaptive changes that are potentially reversible. The resistant subclones grew significantly slower than the parental cells. BZM resistance in other systems has been associated with up-regulation of proteasome components and heat-shock proteins. Indeed, we confirmed an increased activity of the proteasome in all BR subclones compared to the parental cells. In addition, in the BR clones a relative resistance of the proteasome activity to BZM was evident. Surprisingly, BR cell lines were also able to survive with considerably lower proteasome activity than the parental cells. In summary, adaptive changes can lead to increased proteasome activity, relative resistance of the proteasome to the inhibitory effect of BZM, and the ability of BR cells to survive with lower proteasome function.

Nanotube Confinement can Stabilize or Denature Protein Helices: Lessons for Helix Formation in the Ribosome Tunnel. E. P. O'Brien Jr., G. Stan, D. Thirumalai and B. Brooks; Lab of Computational Biology.

As the ribosome translates mRNA to protein, the newly synthesized peptide is pushed through a tunnel inside the 50S subunit of the ribosome, and out into the cellular milieu. This `ribosome exit tunnel' is approximately cylindrical in shape with a length of 10 nm and an average diameter of 1.5 nm. Polymer theory predicts that confining a protein in an inert cylindrical tunnel of similar dimensions will stabilize helical conformations. However, experiments on different peptides inside the ribosome tunnel indicate that there is a sequence dependence as to whether or not helices are stabilized. This observation motivated us to ask whether a similar sequence dependence of helix formation can be observed in the simpler case of confinement by carbon nanotubes. To answer this question, we have examined the helical stability of different sequences under carbon nanotube confinement using Langevin dynamics simulations and a coarse-grained representation of the polypeptide. In agreement with polymer theory, entropic stabilization of the helix is observed for *all sequences* when the nanotube is weakly hydrophobic. However, we find a *strong* sequence dependence as the hydrophobic character of the nanotube wall increases. For an amphiphilic sequence, the helical stability *increases* as the hydrophobic character of the nanotube increases. In contrast, the helical stability of a polyalanine sequence decreases as the hydrophobic character of the nanotube increases. Decreasing the size of the `hydrophobic patch' lining the nanotube, which better mimics the chemical heterogeneity of the ribosome tunnel, increases the helical stability of the polyalanine sequence. Finally, we present simulation results of peptides inside a coarse-grained representation of the ribosome tunnel complex.

PPARγ Agonist Pioglitazone Restores the Mitochondrial Biogenesis Regulatory Program and Augments Respiratory Function in Insulin Resistant C2C12 Myotubes. I. Pagel-Lagenickel[1], B. S. Mantell[1], J. J. Joseph[1], J. Bao[1,] X. Xu[2], N. Raghavachari[2], M. N. Sack[1;] [1]Cardiology Branch, [2]Vascular Medicine Branch and the NHLBI Microarray Gene Expression Core Facility.

Insulin resistance in human skeletal muscle is associated with the dysregulation of mitochondrial function and downregulation of the mitochondrial biogenesis regulatory program. These skeletal muscle metabolic and regulatory perturbations presage the development of type 2 diabetes. As thiazolidinediones (peroxisome proliferator activated receptor gamma - PPARγ agonist mediated insulin sensitizers) delay the development of type 2 diabetes we propose that thiazolidinediones may modulate the insulin-resistance associated dysregulation of mitochondria. Insulin-resistance was induced in C2C12 myotubes following exposure to insulin and glucose. Insulin resistance in C2C12 myotubes exhibit downregulation of genes encoding mitochondrial biogenesis regulatory proteins and of the genes encoding uncoupling protein 3 and sirT1. This gene profile is paralleled by the reduction in mitochondrial and nuclear encoded electron transfer chain proteins. Similarly insulin resistant C2C12 myotubes have smaller mitochondria, consume less oxygen, exhibit decreased mitochondrial membrane potential and have diminished ATP content. Interestingly, pioglitazone does not restored insulin signaling. However, the levels of mitochondrial biogenesis regulatory genes, uncoupling protein 3 and sirT1 are normalized in concert with the restoration of electron transfer chain protein levels and the augmentation of oxygen consumption, inner mitochondrial membrane potential and cellular ATP levels. Pioglitazone mediates normalization of the mitochondrial biogenesis regulatory program and mitochondrial respiration in insulin-resistant C2C12 myotubes. This rescue of the mitochondrial respiratory phenotype in insulin resistant myotubes is independent of insulin signal

transduction. This study implicates pioglitazone-mediated activation of the mitochondrial biogenesis program as a putative mechanism whereby this PPARγ-activator may delay the onset of type 2 diabetes.

Metabolic Stress Testing Predicts the Restorative Capacity of Mitochondrial Bioenergetics in Diabetes. I. Pagel-Langenickel[1], D.S. Schwartz[1], R. A. Arena[2], D.C. Minerbi[1], R. S. Balaban[1], D.J. Tripodi[1], M.N. Sack[1]. [1]Cardiology Branch; [2]Indiana University, IA.

Skeletal muscle mitochondrial dysfunction is hypothesized to contribute to the pathophysiology of insulin resistance and type 2 diabetes. Whether restoration of mitochondrial function is required to reverse insulin resistance is unknown. To test this we evaluated skeletal muscle mitochondria in response to thiazolidinedione therapy in diabetes. All subjects (n=37) were treated with rosiglitazone for 12 weeks and underwent metabolic stress testing. Twenty-three individuals underwent pre- and post-therapy skeletal muscle biopsies. Muscle analyses were compared to individual's maximal exercise capacity (VO2max). Skeletal muscle culture studies were employed to investigate the interaction between insulin signaling and mitochondrial bioenergetics. Baseline VO2max correlated strongly with muscle mitochondrial copy number (r=0.56, p=0.018) and inversely with the duration of diabetes (r= -0.39, p=0.016). Employing citrate synthase as a marker of mitochondrial bioenergetics, increased enzyme activity in response to rosiglitazone was only evident in subjects with VO2max ≥28.1 ml/kg/min. In parallel, protein analyses showed that rosiglitazone coordinately upregulated oxidative phosphorylation proteins in subjects with preserved VO2max. In contrast, rosiglitazone improved insulin-sensitivity irrespective of aerobic capacity. This discordance between diabetic control and mitochondrial bioenergetics was replicated in insulin resistant skeletal muscle cultures, and here pioglitazone restored insulin signaling and mitochondrial bioenergetics through independent pathways. We demonstrate for the first time that in diabetic subjects VO2max reflects: 1) the duration of diabetes; 2) skeletal muscle mitochondrial content and 3) predicts the potential capacity of rosiglitazone to restore skeletal muscle mitochondrial bioenergetics. Conversely, skeletal muscle mitochondrial amelioration is not a prerequisite for thiazolidinedione mediated insulin sensitization.

Modulation of Connexin43 α1 on Directional Motility of Embryonic Fibroblast Cells. H. Park, X. Xu, C. Lo; Laboratory of Developmental Biology.

Connexin43 α1 (Cx43) is a member of gap junction family protein. Previously, it has been shown to play an important role in migratory cells such as NIH3T3 and cardiac neural crest cells. In the present study, we used mouse embryonic fibroblast (MEF) cells derived from wildtype (WT) and Cx43KO mouse embryos to further explore the mechanisms of the modulation of Cx43 in mediating directional cell movement. Using high resolution time-lapse imaging, we found MEF maintained active cell protrusion/retraction during wound closure, but this was accompanied by a marked loss in directional cell movement.

Cell migration behavior is dependent on the modulation of actin filaments, and the dynamic assembly and disassembly of focal adhesion complexes. β-actin is one of six different actin isoforms which have been identified that are involved in cell motility and cytoarchitecture. In migrating WT MEFs, aggregation of β-actin is observed at the leading edge of cell protrusions, known as lamellipodia, whereas Cx43KO fibroblasts failed to show β-actin aggregation in lamellipodial protrusions. Additionally, β-actin aligned parallel to the axis of cell motility in WT MEFs, while in Cx43KO, β-actin is distributed along the cell periphery with no directional alignment. These results suggest directional cell movement involves the dynamic regulation of the actin cytoskeleton by Cx43. Previous study in our lab has also indicated the loss of Cx43 may alter the dynamic turnover of focal adhesion contacts, sites of cell adhesion to the extracellular matrix and points of actin stress fiber insertion. In the present study, we examined vinculin, a major component of focal adhesion complex in WT and Cx43KO MEFs after nocodazole treatment, a reagent that induces focal contact disassembly. Immunostaining showed the expected dramatic loss of focal complexes in WT cells, but in KO cells, there was little or no change in vinculin distribution. Together these findings suggest Cx43 may play an important role in the dynamic turnover of the actin and tubulin cytoskeleton.

p53 Regulates Exercise Capacity and Skeletal Muscle Metabolism. J. Y. Park, J. W. Choi, H. J. Sung, Q. A. Ali, T. Matsumoto, W. Ma, P. Connelly, J. Fields, J. G. Kang, P. Hwang; Cardiovascular Branch.

Tumor suppressor p53 regulates mitochondrial respiration by transactivating the *Synthesis of Cytochrome c Oxidase 2* (*SCO2*) gene, an important component of the cytochrome c oxidase (COX) complex. In preliminary studies, we reported decreased exercise endurance in p53-deficient (p53-/-) mice though morphometric analyses revealed no significant differences with their wild-type counterparts. At this time, the influence of p53 on the bioenergetics of skeletal muscle, a major determinant of exercise capacity, remains unclear. To further characterize this cardiovascular phenotype, aerobic exercise capacity and ramping rotarod tests were performed. All animals were carefully examined to rule out detectable anomalies and their body mass compositions were measured. The exercise capacity of p53-/- mice were significantly decreased by ~40% compared to p53+/+ mice though no motor coordination abnormalities were detected. Biochemical studies revealed increased blood lactate levels in p53-/- mice after submaximal exercise, but interestingly, skeletal muscle mitochondrial oxygen consumption levels were minimally affected in the p53-/- mice. Upon detailed examination, we have obtained preliminary evidence revealing enlarged intermyofibrillar mitochondria by electron microscopy in p53-/- mice, indicative of abnormal mitochondrial biogenesis. Insights from our studies may allow the development of new strategies for modulating bioenergetic abnormalities through a tumor suppressor pathway with potential implications for improving global health.

The Clathrin-Independent Endocytic Pathway and H-Ras: Interplay of Trafficking and Signaling. N. Porat-Shliom, Y. Kloog, J. G. Donaldson; Laboratory of Cell Biology and Tel-Aviv University.

Ras is a small GTP binding protein that is involved in major cellular processes such as proliferation, membrane trafficking and actin re-organization. Here, we demonstrate that H-Ras traffics with the constitutive clathrin-independent endocytic pathway and that activation or expression of the constitutively active H-Ras mutant induces macropinocytosis, a stimulated form of the clathrin-independent pathway in HeLa cells. H-Ras co-localized with the clathrin-independent cargo molecule, MHCI, but not Transferrin and with the Arf6 Q67L vacuoles, indicating H-Ras internalization is through the clathrin-independent pathway. Furthermore, H-Ras was present on the characteristic recycling tubular endosomes. By contrast K-Ras, but not H-Ras, co-localized with Rab5 Q79L enlarged early endosomes. Using live cell imaging and biochemical assays we show that Arf6 Q67L alters H-Ras trafficking and hence reduces the levels of H-Ras-GTP by 50% following EGF stimulation. On the other hand, expression of the constitutively active mutant of H-Ras (G12V) stimulated the clathrin-independent pathway and caused the formation of macropinosomes. Our previous studies demonstrated that expression of EFA6, an Arf6 GEF, also induces macropinocytosis. In order to examine the potential interplay of Arf6 and H-Ras in macropinocytosis we depleted cells of Arf6 and found that Arf6 is required for the H-Ras (G12V) induced macropinocytosis. Altogether, our work demonstrates a new trafficking route for H-Ras and an alternative mechanism for H-Ras induced macropinocytosis involving Arf6.

Amplified Expression Profiling of Platelet Transcriptome Reveals Changes in Arginine Metabolic Pathways in Patients With Sickle Cell Disease. N. Raghavachari, X. Xu, A. Harris, J. Villagra, C. Logun, J. Barb, M. A. Solomon, A. F. Suffredini, R. L. Danner, G. Kato, P. J. Munson, S. M Morris, Jr., M. T. Gladwin; Vascular Medicine Branch.

In sickle cell disease, ischemia-reperfusion injury and intravascular hemolysis produce endothelial dysfunction and vasculopathy characterized by reduced nitric oxide and arginine bioavailability. Recent functional studies of platelets in patients with sickle cell disease reveal a basally activated state, which suggests that pathological platelet activation may contribute to sickle cell disease vasculopathy. Studies were therefore undertaken to examine transcriptional signaling pathways in platelets that may be dysregulated in sickle cell disease. We demonstrate and validate in the present study the feasibility of comparative platelet transcriptome studies on clinical samples from single donors by the application of RNA amplification followed by microarray-based analysis of 54 000 probe sets. Data mining an existing microarray database, we identified 220 highly abundant genes in platelets and a subset of 72 relatively platelet-specific genes, defined by 10-fold increased expression compared with the median of other cell types in the database with amplified transcripts. The highly abundant platelet transcripts found in the present study included 82% or 70% of platelet-abundant genes identified in 2 previous gene expression studies on nonamplified mRNA from pooled or apheresis samples, respectively. On comparing the platelet gene expression profiles in 18 patients with sickle cell disease in steady state to those of 12 black control subjects, at a 3-fold cutoff and 5% false-discovery rate, we identified _100 differentially expressed genes, including multiple genes involved in arginine metabolism and redox homeostasis. Further characterization of these pathways with real-time polymerase chain reaction and biochemical assays revealed increased arginase II expression and activity and decreased platelet polyamine levels. The present studies suggest a potential pathogenic role for platelet arginase and altered arginine and polyamine metabolism in sickle cell disease and provide a novel framework for the study of disease-specific platelet biology.

A Large Mennonite Family with a Novel K570N TERT Gene Mutation: Association with a Clinical Spectrum of Bone Marrow Failure, Acute Myeloid Leukemia, and Acute Liver Failure. J. A. Regal, R. T. Calado, S. Kajigaya, and N. S. Young; Hematology Branch.

Mutations in *TERT* (encoding telomerase reverse transcriptase) and *TERC* (telomerase RNA component) are associated with bone marrow failure syndromes, including acquired aplastic anemia. Low telomerase activity results in short leukocyte telomeres and predisposes the bone marrow to early senescence and exhaustion of the stem cell compartment. We have identified a six-generation pedigree carrying a novel, nonsynonymous *TERT* mutation (K570N). The index patient is a 26 year-old male with a ten-year history of severe aplastic anemia. The patient's paternal great-great-grandmother died of a severe blood disorder at age 65. The patient's father, an obligatory mutation carrier, had myelodysplastic syndrome when 33, evolving into acute myeloid leukemia and death. One of the proband's paternal aunts developed aplastic anemia as a young adult, and she tested positive for the mutation. Another heterozygous paternal aunt underwent a liver transplant at age 20 for submassive hepatic necrosis with fibrosis. Two additional paternal relatives died in their late 40s of liver disease, including a confirmed and an obligatory carrier. A third paternal aunt and two sisters carry the mutation but have only macrocytosis. Leukocyte telomere shortening tracked with the mutation in three generations analyzed. Following a 1:1 co-transfection of mutagenized and wild-type *TERT* vectors into telomerase-deficient VA13 cells, telomerase activity was approximately half of that following transfection with only wild-type *TERT*, implicating haploinsufficiency as the mechanism of telomere shortening. Our results confirm the association between aplastic anemia and *TERT* mutations and suggest that telomerase gene mutations may be related to hematological malignancy and severe liver disease.

The Compliant Lever and Pivot (CLAP) Model for Myosin V's Mechanics. M. Riegelman, H. Bau, J. Sellers; Laboratory of Molecular Physiology.

Myosin V is mechanistically fascinating molecular motor which serves *in vivo* as an actin based cargo transporter. A recent explosion of experimental data on myosin V has provided a clear picture of how it operates. Structurally, myosin V has two motor domains connected to long levers which are tethered via a coiled-coil. These long levers and the motors' high actin affinity allow the molecule to take multiple steps per diffusional encounter along actin in a hand over hand manner. Myosin V's most extraordinary feature is its ability to transmit intermolecular strain between its motors and coordinate their chemo-mechanical cycles. This synchronization has been shown to decelerate detachment of the leading motor and accelerate detachment of the trailing motor, effectively generating a strong forward bias on the molecular motion. The mechanistic details of this communication pathway remain largely unresolved. Most current models of molecular motors are based on biochemical kinetics and thermodynamics, and are not able to address mechanical questions. In this work, we demonstrate how recent, single molecule, biophysical experimental techniques can assist in formulating new quantitative models which provide a mechanistic view of how molecular motors function. A new model for myosin V, which we refer to as the Compliant Lever and Pivot (CLAP) model, is presented. The CLAP model is based on the analysis of single molecule experimental data for the step size of myosin V and mutants with truncated levers. This analysis provides new insights into previously unresolved features of the molecule's mechanics.

High Resolution Three-Dimensional Modeling for Gene and Protein Expression Profiling. C. Rolfes, J. Rosenthal, V. Mangal, and C. W. Lo; Laboratory of Developmental Biology.

High-resolution optical imaging is often necessary for accurate phenotypic assessment of anatomical structure in biological specimens. This is particularly important for tracking the dynamic structural changes that occur during normal and abnormal development. We have developed an automated high throughput imaging system that allows high resolution three-dimensional (3D) modeling. This automated approach, based on episcopic fluorescence image capture (EFIC), provides fine structural details in 2D image stacks that are aligned in perfect registration. As a result, high resolution 3D models can be rapidly rendered. We initially established the methodology for paraffin and polyethylene glycol embedded specimen. More recently, we have developed a parallel automated system for EFIC imaging of cryo-embedded specimen. With Cryo-EFIC imaging, specimen can be frozen immediately after sample collection without further processing, thereby eliminating the time-consuming sample preparation steps. More importantly, with cryoembedding, integrity of the nucleic acid and protein in the specimen are much better maintained. This grants us the opportunity to glean gene and protein expression data that can be integrated into 2D image stacks and 3D models of the specimen. We have successfully imaged mouse embryos and fetuses, neonatal mice, as well as adult mouse hearts by cryo-EFIC imaging. Experiments are underway to develop methods for profiling gene and protein expression using EFIC tissue sections, and integrating the expression data with the 3D models. The spatial mapping of gene and protein expression data will be invaluable for understanding the complex developmental mechanisms that modulate normal and abnormal development.

Complexation of an ApoA-I Mimetic Peptide with Phospholipid Increases ABCA1-specific Cholesterol Efflux. A. A. Sethi, J. A. Stonik, S. J. Demosky, and A. T. Remaley; Vascular Medicine Branch.

ABCA1-transporters promote cholesterol efflux to amphipathic helical containing peptides, but optimum state of lipidation for ABCA1-specific efflux is unknown. The synthetic peptide 37pA with class-A amphipathic helices was complexed with various amounts of phosphatidylcholine (DPPC) to produce small (peptide:lipid;1:9), medium (1:15), and large (1:30) discoidal complexes (10-20nm). The solubilizing capacity, as assessed by DMPC vesicle solubiliztion, of the small complex was reduced by 65% compared to the free peptide. The larger complexes were ineffective in solubilizing DMPC vesicles. Hemolysis was significantly reduced by the small complex when compared with the free peptide (7% RBC hemolysis/2h/80µg/ml vs. 27%, respectively). The small complex also increased ABCA1-specific cholesterol efflux by an almost doubling of Vmax when compared to the free peptide but with a reduced affinity (Vmax=9.7% cholesterol efflux/18 h; Km=8.1µg/mL vs. Vmax=5.0%/18h;Km=1.2µg/mL, respectively). The medium size complex increased Vmax to 13.0%/18h and Km to 21.9µg/mL. Addition of more phospholipid did not further increase ABCA1-specific cholesterol efflux but increased ABCA1-independent efflux by more than 5-fold from the control cell line. ABCA1-specific phospholipid efflux showed a similar pattern but ABCA1-nonspecific efflux from control HeLa cells was almost completely abolished with the addition of any phopsholipid to the peptide. Addition of phospholipd to 37pA enhances its ability to efflux cholesterol by the ABCA1 transporter and reduces its hemolytic potential, suggesting that partially lipidated apolipoproteins may be the endogenous lipid acceptors for ABCA1 and that partially lipidated apoA-I mimetic peptides may be the preferred therapeutic form of such peptides.

Breast Involvement in Patients with HTLV-1-associated Adult T cell Leukemia/Lymphoma. K. Sharma, J. Janik, C. Lee, D. O'Mahony, and J. Morris. Metabolism Branch, Center for Cancer Research, NCI.

Adult T cell leukemia/lymphoma (ATL) is an aggressive lymphoproliferative disorder with a poor prognosis caused by infection with human T cell lymphotrophic virus type 1 (HTLV-1). The acute and lymphomatous subtypes of ATL are associated with high circulating leukemic cell counts or rapidly progressing lymphadenopathy, hepatosplenomegaly, hypercalcemia, rising serum LDH and infiltration of the skin, bones, lungs, eyes, G.I. tract, heart and CNS with malignant T cells. We report three patients that presented with or developed clinical breast involvement with ATL. All three patients were women of Jamaican origin, ages 46, 55 and 56 years. Patient 1 presented to her local physician with a right breast mass initially presumed to be a primary breast cancer. Two other patients developed clinical breast disease at the time of progression of their ATL. At least one breast mass was palpable in each patient. In addition, one patient had clinical skin involvement overlying the breast mass. The breast lesions could be imaged on mammography and CT-scan. Biopsies in each patient revealed infiltration of the breast with T cell lymphoma consistent with ATL. Immunohistochemistry was CD2+, CD3+, CD4+, CD5+, CD7- and CD25+. A review of the literature found a single case of malignant gynecomastia occurring in a male with ATL. The breast masses in two of our patients resolved with systemic treatment of their ATL. ATL may involve the female breast and should be considered in the setting of breast mass in patients with ATL.

p53 Regulated SC02 Gene is Essential for Mitochondrial Respirattuin and Protection from Oxygen Toxicity. H. J. Sung, W. Ma, J. J. Hanisch, J. Y. Park, S. Matoba, J. Kang and P.M. Hwang; Cardiology Branch.

We have recently reported that p53 dose dependently modulates the balance between the utilization of oxidative and glycolytic pathways for energy generation in mice and in human cancer cells (Matoba et al, *Science* 312, 1650-3, 2006). We demonstrated that this p53 effect is directly mediated by the Synthesis of Cytochrome c Oxidase 2 (*SCO2*) gene that is vital for cytochrome c oxidase (COX) function as the center of eukaryotic oxygen consumption. However, why p53 regulates an energy generating pathway utilizing oxygen is currently unclear. To address this question, we have now created a homozygous SCO2 knockout (SCO2-/-) human cell line. Unexpectedly, SCO2 -/- cells display improved growth properties under hypoxic conditions indicating increased sensitivity to ambient oxygen tension. Consistent with this observation, preliminary data indicate SCO2 -/- cells have increased basal levels of reactive oxygen species (ROS) compared to wild-type cells. The source of ROS is unclear at this time, but other preliminary data characterizing this cell line will be discussed. Together, our studies indicate a direct antioxidant role for p53 as guardian of the eukaryotic genome by promoting the elimination of intracellular free oxygen, the precursor of toxic free radical generation. By creating a primordial anaerobic eukaryotic cell through the targeted disruption of a p53 regulated "metabolic" function, our findings support the oxygen toxicity hypothesis that forms the basis of the original mitochondrial endosymbiotic theory.

A Novel Mouse Model of Primary Ciliary Dyskinesia Exhibits Complex Structural Heart Defects and Ciliary Dyskinesia Due to a Mutation in Heavy Chain Dynein *mDNHC5*. S. Tan, R. Francis**,** S. Sabol, B. Chatterjee, Q. Yu, L. Bracero, J. Rosenthal, L. Leatherbury, and C. W. Lo; Laboratory of Developmental Biology.

Primary ciliary dyskinesia (PCD) encompasses a spectrum of diseases related to dysfunctional ciliary motion associated with chronic sinopulmonary disease, situs abnormalities and complex cardiac defects (CCDs). PCD is commonly associated with mutations in dynein, which affect ciliary motility. However, the relationship between ciliary function, SAs and CCDs remains controversial. Through ENU mutagenesis, a mutant mouse line with phenotypes consistent with PCD was recovered based on abnormal fetal echocardiography. Subsequent necropsies of harvested embryos revealed situs anomalies, with some embryos exhibiting CCDs like superior-inferior ventricular positioning. DNA sequencing showed an in frame deletion of sequences from the N-terminus of mouse dynein heavy chain DNHC5, in a region containing a putative dynein interacting domain. The frequency of anomalies were examined by necropsies of 16 harvested litters constituting 110 fetuses. Normal anatomy was seen in all heterozygous and wildtype (89) embryos. Of the 21 homozygous mutants, approximately one third each showed situs solitus (6), situs inversus (7) and heterotaxy (8). Four heterotaxic embryos exhibited CCDs. Scanning EM of respiratory epithelial in wildtype animals showed the presence of outer dynein arms (ODAs) in the cilia. Conversely, in homozygous mutants, ODAs were missing in most but not all cilia. In cilia of heterozygous animals, intermediate phenotypes were observed, ranging from normal ODAs to none. Functional analysis of ciliary motion by videomicroscopy of the tracheal epithelium showed immotile or dyskinetic cilia in homozygous mutants, while ciliary motion in heterozygotes ranged from rapid synchronous beating as seen in wild-type animals to dyskinetic or absent beating. This mutant mouse line is a valuable model for studying PCD, and may help to elucidate the role of ciliary motion in situs determination and cardiac development.

Potential Biological Consequences of mRNA Oxidation-Induced Translation Errors. M. Tanaka[1], P. Jaruga[2], M. Dizdaroglu[2], P. B. Chock[1], E. R. Stadtman[1]; [1]Laboratory of Biochemistry, [2] Biochemical Science Division, NIST

Reactive oxygen species (ROS) are generated in living mammalian cells. This leads to oxidation of intracellular biomacromolecules. Much attention has been focused on the effects of DNA, protein, and lipid oxidation. However, since RNAs are less protected against ROS than nuclear DNAs, they are more susceptible to oxidation. We report the detection of elevated levels of oxidized nucleic acid bases, including 8-hydroxyguanine, using GC/MS methods, in polyA mRNA in HEK293 cells after mRNA synthesis was inhibited by 5,6-Dichlorobenzimidazole 1-β-D-ribofuranoside for 8h, the average half life of mRNA. When cells were either transfected with radiolabeled mRNA or pulse-labeled mRNA, the labeled mRNA was found to be extensively oxidized. There results suggest that under physiological conditions, intracellular mRNAs are constantly subjected to oxidative modification. When HEK293 cells were transfected with a reporter mRNA followed by 12 h incubation, it was found that the reporter mRNA exhibited a time-dependent decrease in its translational efficiency. Concomitantly, there was an accumulation of full-length modified and short polypeptides derived from the transfected mRNA due to translation errors. The short polypeptides were derived, in part, from premature termination of the translation process. Both the modified and short polypeptides were highly susceptible to proteasome-catalyzed degradation. Thus, translation errors caused by mRNA oxidation may contribute, in part, to the reported high quantity of nascent polypeptides degraded and to the accumulation of protein aggregates observed in neurodegenerative diseases and in age-dependent changes in protein modification and thermostability, in conjunction with disease- and age-related reductions in proteolytic activity.

Mucolipin-1 Channel Activity is Regulated by Protein Kinase A Mediated Phosphorylantion. S. Vergarajauregui[1], R. Oberdick[2], K. Kiselyov[2], and R. Puertollano[1]; [1]Laboratory of Cell Biology; [2]Department of Biological Sciences, University of Pittsburgh.

Mucolipins constitute a family of cation channels with homology to the transient receptor potential family. Mutations in mucolipin-1 (MCOLN1) have been linked to mucolipidosis type IV, a recessive lysosomal storage disease characterized by severe neurological and ophthalmologic abnormalities. At present, little is known about the mechanisms that regulate MCOLN1 activity. Here, we addressed whether MCOLN1 activity is regulated by phosphorylation. We identified two protein kinase A (PKA) consensus motifs in the C-terminal tail of MCOLN1 containing serine 557 and serine 559. Serine 557 was the principal phosphorylation site, as mutation of this residue to alanine caused greater that a 75% reduction in the total levels of phosphorylated MCOLN1 C-terminal tail. Activation of PKA with forskolin promoted MCOLN1 phosphorylation both in vitro and in vivo. In contrast, addition of the PKA inhibitor H89 abolished MCOLN1 phosphorylation. We also found that PKA-mediated phosphorylation regulates MCOLN1 channel activity. Treatment with forskolin decreased MCOLN1 channel activity, while H89 treatment increased MCOLN1 channel activity. The stimulatory effect of H89 on MCOLN1 function was not observed when serine 577 and serine 559 were mutated to alanine, indicating that these two residues are essential for PKA-mediated negative regulation of MCOLN1. This study constitutes the first example ofregulation of a member of the mucolipin family by phosphorylation.

A20 Attenuates Vascular Smooth Muscle Cell Proliferation and Migration Through Blocking PI3k/Akt Signaling In Vitro and In Vivo. A. B. Wang[1,2], H. L. Li[1], R. Zhang[1], Z.G. She[1], H.Z. Chen[1], Y. Huang[1], D.P. Liu[1] and C.C. Liang[1]; [1]National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College; [2]Laboratory of Molecular Cardiology.

A20 was originally characterized as a TNF-inducible gene in human umbilical vein endothelial cells. It is also induced in many other cell types by a wide range of stimuli. Expression of A20 has been shown to protect from TNF-induced apoptosis and also functions via a negative-feedback loop to block NF-kappaB activation induced by TNF and other stimuli. However, there are no reports on whether A20 can inhibit vascular smooth muscle cell proliferation in vivo. Here, we examined the effects of A20 on neointimal formation after balloon injury and TNF-α-induced vascular smooth muscle cells (VSMCs) proliferation and migration, as well as related molecular mechanisms in vitro and in vivo. We introduced adenovirus expressing A20 or GFP into rat carotid arterial segments after balloon injury. The effects of A20 were evaluated 14 days after gene delivery with morphometry and immunohistochemical staining for proliferating and apoptotic cells. Ad-A20 infection resulted in a significantly lower intima to media ratio and a greater lumen area compared with Ad-GFP infected group. Proliferation index was significantly reduced 14 days in Ad-A20 infection group. However, apoptotic index and caspase-3 activity were not significantly different between any groups at 14 days. In vitro experiments were performed to show that A20 markedly inhibited TNF-α-induced proliferation and migration in VSMCs. Further studies showed that A20 expression blocked artery injury- and TNF-α-activated PI3K/Akt/GSK3β/CREB pathway in vivo and in vitro. In conclusion, A20 attenuates neointimal formation after arterial injury as well as cell proliferation and migration in response to TNF-α in VSMCs through blocking PI3K/Akt/GSKβ-dependent activation of CREB.

Exploring SCC-DFTB Paths for Mapping QM/MM Reaction Mechanisms. H. L. Woodcock, M. Hodoscek, and B. R. Brooks; Laboratory of Computational Biology.

A new first-order procedure for locating transition structures (TS) that employs hybrid quantum mechanical/ molecular mechanical (QM/MM) potentials has been developed. This new technique (RPATh+RESD) combines the Replica Path method (RPATh) and standard reaction coordinate driving (RCD) techniques in an approach that both efficiently determines reaction barriers and successfully eliminates two key weaknesses of RCD calculations (i.e. hysteresis/discontinuities in the path, the sequential nature of the RCD procedure). In addition, we have extended CHARMM's QM/MM reaction pathway methods, the Replica Path (RPATh) and Nudged Elastic Band (NEB) methods, to incorporate SCC-DFTB wavefunctions. This newly added functionality has been applied to the Chorismate Mutase catalyzed interconversion of chorismate to prephenate, which is a key step in the shikimate pathway of bacteria, fungi, and other higher plants. The RPATh+RESD barrier height (DE++ = 5.7 kcal/mol) is in good agreement with previous results from full energy surface mapping studies. Full reaction paths were independently mapped with RPATh and NEB methods and showed good agreement with the final transition state from the RPATh+RESD "gold standard" and previous high level QM/MM transition states. The SCC-DFTB TS geometry most closely approximates the MP2/6-31+G(d) QM/MM result. However, the barrier height is underestimated and possibly points to an area for improvement in SCC-DFTB parameterization. In addition, the Steepest Descents (SD) minimizer for the NEB method was modified to uncouple the in-path and off-path degrees of freedom during the minimization which significantly improved performance. The convergence behavior of the RPATh and NEB was examined for SCC-DFTB wavefunctions and it was determined that in general both methods converge at about the same rate, although the techniques used for convergence may be different. For instance, RPATh can effectively use the adopted basis Newton Raphson (ABNR) minimizer, where NEB seems to require a combination of SD and ABNR.

Human Embryo Atlas Constructed by Magnetic Resonance (MR) Imaging and Episcopic Fluorescence Image Capture (EFIC). S. Yamada, C. Rolfes, X. Q.

Zhao, S. Anderson, C.W. Lo; Laboratory of Developmental Biology,

During the period of major organogenesis, embryos undergo complicated morphogenetic changes. To understand and analyze such dynamic ontogenic movements, it is important to visualize embryonic structures in three-dimensions (3D). To construct a high resolution 3D atlas of the human embryo covering the dynamic period of organogenesis, human embryos from the Kyoto Collection at Carnegie stages 13 to 23 were imaged by MRI and also with a novel histological technique referred to as episcopic fluorescence image capturing (EFIC). EFIC imaging provides high resolution 2D image stacks in perfect registration, thus allowing for rapid high resolution 3D volume rendering. Embryos were first MRI scanned using a 7T super-conducting magnet, followed by EFIC imaging. Using data generated in this manner, we constructed a web accessible digital Human Embryo Atlas, with each Carnegie stage represented by serial 2D image stacks shown in three views: the native sagittal plane, and in digitally resectioned frontal and transverse planes. In addition, Quicktime movies of 3D rendered images of the embryos were incorporated into the Atlas together with QTVRs to allow for direct viewer interaction with the 3D volumes. Some abnormal human embryos were also imaged in a parallel manner by MRI and EFIC, and the 2D image stacks and 3D volumes of the abnormal embryos were incorporated into the Atlas. This digital Atlas of the Human Embryo should serve as a invaluable resource for morphologic information of normal human development, and for studying the etiology of human congenital anomalies.

Mitochondrial DNA Sequence Variation and Kinetics of Single CD34$^+$ Cells after Nonmyeloablative Allogeneic Stem Cell Transplantation. Y. G. Yao, R. W. Childs, S. Kajigaya, J. P. McCoy, Jr, N. S. Young; Hematology Branch and Flow Cytometry Core Facility.

Hematopoietic stem cell transplantation long has been used in clinics but the fate and potentiality of the individual transplanted stem cells after transplantation has not been fully resolved. We applied the single-cell analysis method for mtDNA mutation to assess the kinetics of hematopoietic stem cells and committed progenitor cells (HSC) in donors and recipients after transplantation. A total of 1958 single CD34$^+$ cells from six HLA-matched sibling sample pairs were determined for mtDNA sequence variation and donor- or recipient-specific individual CD34$^+$ clones were recognized based on the observed mutations. Consistent with the nonmyeloablative regimen, variable levels of mixed donor/recipient CD34$^+$ cell chimerism in recipients were observed early after transplantation, and this chimerism could be found in a patient who survived renal cell cancer for 8 years after transplantation. There was no overall reduction of mtDNA heterogeneity among CD34$^+$ cells from the recipient after transplantation. Serial assessment in samples collected from donors over multiple time periods showed the persistence of certain CD34$^+$ clones marked by specific mutations. Taken together, our analysis for individual CD34$^+$ cells supported the idea that HSCs remain quiescent *in vivo* and suggested that HSCs that are proliferating and populating the circulation in the donor continue a similar pattern in the host.

The Pathologic Role of the Innate Immune System in Acetaminophen-Induced Liver Disease in Interleukin-13 Deficient Mice. S. B. Yee, M. Bourdi, M. J. Masson, L. R. Pohl; Laboratory of Molecular Immunology.

Recently, we have shown that endogenous interleukin (IL)-13 protected mice from acetaminophen (APAP)-induced liver disease (AILD). Several proinflammatory cytokines and chemokines downstream of reactive metabolite formation appear to be regulated by IL-13, as their serum expression was found to be higher in APAP-treated IL-13 knockout (KO) than wild-type (WT) mice. In the present study, we evaluated the pathologic role of proinflammatory factors in IL-13 KO mice following challenge with 200 mg APAP/kg. When interferon (IFN)-gamma neutralizing antibody (NAb) was administered to APAP-treated IL-13 KO mice, IFN-gamma levels decreased significantly and AILD was attenuated. Since natural killer (NK) cells and NK cells with T-cell receptors (NKT cells) are major sources for IFN-gamma, the importance of these innate immune cells was investigated. Administration of NK 1.1 NAb to APAP-treated IL-13 KO mice depleted both NK and NKT cells, attenuated liver injury and abrogated IFN-gamma levels, suggesting these cells play a critical role by secreting injurious IFN-gamma. Additionally, based on observations of elevated neutrophil (polymorphonuclear leukocyte, PMN) chemoattractants, along with immunohistochemically-determined greater hepatic PMN accumulation in APAP-treated IL-13 KO mice compared to WT, the role of PMNs was also investigated. Administration of PMN NAb to APAP-treated IL-13 KO mice greatly reduced hepatic PMN accumulation and attenuated liver injury, suggesting PMNs contribute to AILD in this susceptibility model. Collectively, these results indicate IL-13 modulates the pathologic role of the innate immune system in AILD and suggests IL-13 is an important hepatoprotective cytokine in drug-induced liver disease.

Hemodynamic Basis for Early Prenatal Death of Mouse Embryos with Endothelial Specific Deletion of *Klf2.* Q. Yu, L. Leatherbury, X Tian, J. Lee, M. Kahn, C. Lo; Laboratory of Developmental Biology.

Klf2, a transcription factor expressed in endothelial cells, is suggested to be an important molecular transducer of fluid shear stress in endothelial cells. Mice with endothelial targeted deletion of *Klf2* using Tie2Cre and a floxed *Klf2* KO allele die between embryonic day 12.5 to 14.5 (E12.5-14.5). Noninvasive fetal ultrasound interrogations were used to investigate possible hemodynamic mechanism leading to the death of conditional Tie-Cre/*Klf2* KO mice. Using a Visualsonics 660 biomicroscope with a 30 Mhz transducer, two dimensional images of the hearts were acquired in 3 or 4 chamber views. Further Doppler derived blood flow parameters were obtained in the heart, descending aorta and umbilical cord. From E11.5 to E13.5, cardiovascular function in wildtype and heterozygous *Klf*2 KO embryos were found to be similar. At E11.5 homozygous KO mice showed normal heart rates and excellent myocardial function with increased ejection fraction (EF). However, cardiac output was elevated without valvular regurgitation. This high cardiac output state progressed to heart failure at E12.5 and E13.5. This is indicated by pericardial effusion, bradycardia, decreased EF, decreased peak umbilical venous velocities, with 60% of the mutants dying by E13.5, and the rest expiring at E14.5. These findings indicate prenatal lethality of the *Klf2* KO is due to heart failure elicited by high cardiac output brought on secondarily by decreased systemic vascular resistance.

The Trendelenburg Position Avoids Ventilator Associated Pneumonia in Intubated and Mechanically Ventilated Pigs. A. Zanella, M. Cressoni, T. Kolobow; Pulmonary Critical Care Medicine Branch.

Ventilator associated pneumonia (VAP) occurs in 9-27% of ICU intubated patients. Gastric reflux is believed to be a major risk factor for VAP. The semirecumbent (SR) position reduces gastric reflux, hence is recommended for intubated patients. This study was to assess, in intubated and mechanically ventilated pigs, the effect of body positioning on onset of VAP. Twenty-six pigs were intubated and randomized into 4 groups: 8 were placed in SR position (30° above horizontal), and 18 in the Trendelenburg position (10° below horizontal). Twelve pigs in the Trendelenburg position were mechanically ventilated for 72h: 6 of these received enteral feeding, and 6 did not; the other 6 in the Trendelenburg position were mechanically ventilated for 168h and received enteral feeding. No antibiotics were administered. Eight pigs in the SR position developed pneumonia; a median of 6 lobes of the lungs in each pig was heavily colonized. None of the 18 pigs kept in the Trendelenburg position

developed pneumonia. The microbiological analysis showed absence of lung bacterial colonization in 16 pigs in the Trendelenburg position; in 2 pigs ventilated for 168h, 1 and 2 lobes, respectively, had low level of bacterial colonization. In this pig model, following tracheal intubation, the Trendelenburg position prevented VAP. Enteral feeding, in the Trendenburg position, was not a risk factor for VAP. The SR position was invariably a risk factor for VAP.

The Molecular Basis of IL-21-mediated Proliferation. R. Zeng[1], R. Spolski[1], E. Casas[2], W. Zhu[2], D. E. Levy[2], and W. J. Leonard[1];[1]Laboratory of Molecular Immunology; [2]Departments of Pathology and Microbiology, New York University School of Medicine.

Interleukin-21 (IL-21) is a type I cytokine that modulates functions of T, B, NK, and myeloid cells. The IL-21 receptor (IL-21R) is closely related to the IL-2 receptor β chain and is capable of transducing signals through its dimerization with the common cytokine receptor γ chain (γ_c), the protein whose expression is defective in humans with X-linked severe combined immunodeficiency. To clarify the molecular basis of IL-21 actions, we investigated the role of tyrosine residues in the IL-21R cytoplasmic domain. Simultaneous mutation of all six tyrosines greatly diminished IL-21-mediated proliferation, whereas retention of tyrosine 510 (Y510) allowed full proliferation. Y510 efficiently mediated IL-21-induced phosphorylation of Stat1 and Stat3, but not of Stat5, and $CD8^+$ T cells from Stat1/Stat3 double knockout mice exhibited decreased proliferation in response to IL-21 + IL-15. In addition, IL-21 weakly induced phosphorylation of Shc and Akt, and consistent with this, specific inhibitors of the MAPK and PI3K pathways inhibited IL-21-mediated proliferation. Collectively, these data indicate the involvement of the Jak-STAT, MAPK, and PI3K pathways in IL-21 signaling.Towards the mechanism of dynamic couplings and translocation in Hepatitis C virus NS3 helicase using elastic network model.

Connexin43 in the Epicardium is Required for Normal Coronary Development. X. Q. Zhao, D. Y. Rhee, and C. W. Lo; Laboratory of Developmental Biology.

Connexin43 (Cx43) knockout mice exhibit anomalous coronary vessel development accompanied by the formation of infundibular pouches. These abnormal pouches result in the obstruction of the pulmonary outflow tract and ultimately, death of the mice at birth. These pouches contain ectopic vascular smooth muscle, endothelial cells and fibroblasts. All three of these cell lineages are epicardially derived and thus, strongly suggest a role for epicardial derivatives in the cardiac anomalies in the Cx43 KO mouse. In this study, we show significant Cx43 deficiency leads to a change in epicardial cell morphology with perturbation in the organization of the actin cytoskeleton and focal adhesion contacts. Motion analysis to examine epicardial cell motile behavior revealed altered cell protrusive activity accompanied by a reduction in directionality but an increase in the speed of cell locomotion. Using a 3-dimensional collagen gel assay, we showed an enhanced epithelial-mesenchymal cell transition (EMT) in the Cx43 KO epicardium, a process crucial in the production of coronary vascular progenitors. This change in EMT was accompanied by an increase in the expression of vascular endothelial growth factor (VEGF). Whole mount PECAM staining of the embryonic heart showed altered patterning of the coronary vascular plexuses and defects in the remodeling and maturation of the coronary vascular tree. The expression of a number of other genes associated with EMT and coronary vasculogenesis were also altered, including members of the Tgfβ and Fgf families. These findings demonstrate an important role for Cx43 in coronary vasculogenesis, and suggests this may involve a role in the regulation of epicardial EMT. Towards the mechanism of dynamic couplings and translocation in Hepatitis C virus NS3 helicase using elastic network model

Towards the Mechanism of Dynamic Couplings and Translocation in Hepatitis C Virus NS3 Helicase Using Elastic Network Model. W. Zheng[1], J. Liao[2], B. Brooks[1], S. Doniach[2]; [1]Laboratory of Cell Biology, [1]Stanford Univ.

Hepatitis C virus NS3 helicase is an enzyme that unwinds double-stranded DNA or RNA in an ATP-dependent reaction. Despite recent progress, the detailed mechanism of the coupling between ATPase activity and helicase activity remains unclear. Based on an elastic network model (ENM), we apply two computational analysis tools to probe the dynamic mechanism for the allosteric coupling between ATP binding and polynucleotide binding in this enzyme. Perturbation analysis identifies a network of hot-spot residues that dynamically couple the ATP-binding site and the polynucleotide-binding site. Several of these residues have been identified by mutational experiments that study the dynamic couplings between these two sites. The conformational changes among different crystal structures of NS3 helicase are found to be dominated by the lowest frequency mode. This dominant mode corresponds to the hinge motion of the highly flexible domain 2. The motion of this mode simultaneously modulates the opening/closing of the domain 1-2 cleft where ATP binds, and the domain 2-3 cleft where the polynucleotide binds. Furthermore, a small twisting motion of domain 1, observed in both mode #1 of the apo structure and the simulated ATP-binding induced conformational change, fine-tunes the binding affinity of the domain 1-3 interface for the polynucleotide, which facilitates the translocation of a single-stranded polynucleotide in an inchworm-like manner. By exploiting a structural homology of the ATP binding domain with a similar domain in bovine F1 ATPase we show that the ATP induced conformational change predicts a functional movement which is consistent with the ATP-dependent stepping observed in single molecule optical trap assays by the Bustamante Lab.

NMR Investigation of the Molecular Basis for Actin Filament Uncapping by CARMIL. A. Zwolak, N. Tjandra; Laboratory of Molecular Biophysics.

Proper regulation of the actin cytoskeleton is crucial to the cell. F-actin capping protein (CP) binds to the actin filament "barbed" end to prevent further monomer addition. CARMIL (capping protein, ARP2/3, myosin I linker) regulates CP by sequestering free CP, or by removing CP from a previously capped filament. Little is known about the molecular basis for actin filament uncapping by CARMIL, although CP binding activity has been localized to its CAH3 (CARMIL homology) domain. NMR studies are being carried out in order to determine the structural changes that CP undergoes in response to CARMIL-CAH3 binding. Since NMR has historically been limited to studying low molecular weight proteins, this study presents a technical as well as a biological challenge. The high molecular weight of this protein complex (74KD) necessitated deuteration of CP as well as incorporation of pulse sequence elements optimized for large proteins. Titrations were performed using isotopically labeled CP or CARMIL-CAH3 into which the non-isotopically labeled binding partner was added, and the NMR spectrum was measured at each molar ratio. Titration using labeled CP showed chemical shift changes in a large number of resonances, suggesting a broad conformational change upon CARMIL-CAH3 binding. To determine which regions of CP are most affected by binding, backbone resonance assignment of the NMR spectra is being carried out. This will be carried out using a suite of 3- and 4-dimensional NMR experiments optimized for large proteins. This will provide a detailed understanding of the interaction between CP and CARMIL-CAH3.

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The Battle of Gettysburg

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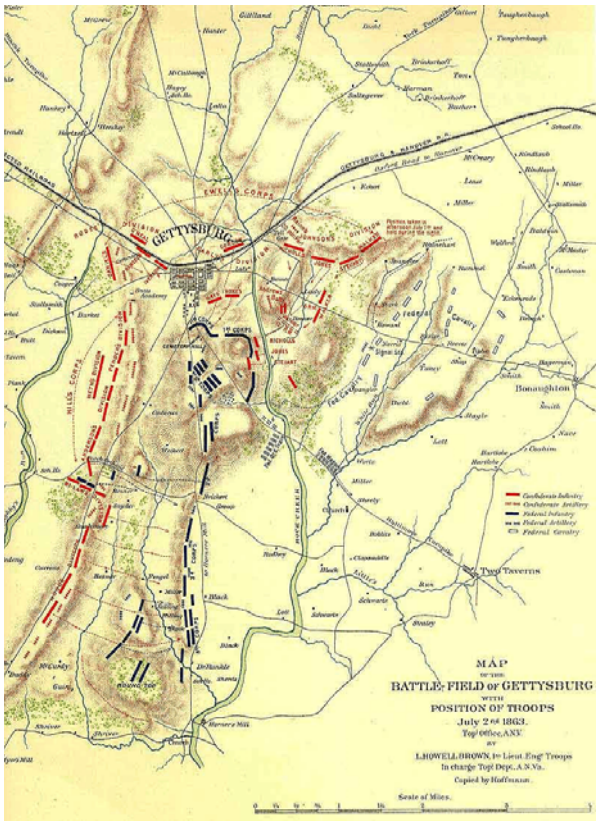

The Battle of Gettysburg (July 1 - July 3, 1863), fought in and around the town of Gettysburg, Pennsylvania, as part of the Gettysburg Campaign, was the bloodiest battle of the American Civil War and is frequently cited as the war's turning point. Union Major General George Gordon Meade's Army of the Potomac defeated attacks by Confederate General Robert E. Lee's Army of Northern Virginia, ending Lee's invasion of the North.

Following his brilliant success at Chancellorsville in May 1863, Lee led his army through the Shenandoah Valley for his second invasion of the North, hoping to reach as far as Harrisburg, Pennsylvania, or even Philadelphia, and to influence Northern politicians to give up their prosecution of the war. Prodded by President Abraham Lincoln, Maj. Gen. Joseph Hooker moved his army in pursuit but was relieved almost on the eve of battle and replaced by Meade.

The two armies began to collide at Gettysburg on July 1, 1863, as Lee urgently concentrated his forces there. Low ridges to the northwest of town were defended initially by a Union cavalry division, which was soon reinforced with two corps of Union infantry. However, two large Confederate corps assaulted them from the northwest and north, collapsing the hastily developed Union lines, sending the defenders retreating through the streets of town to the hills just to the south.

On the second day of battle, most of both armies had assembled. The Union line was laid out resembling a fishhook. Lee launched a heavy assault on the Union left flank, and fierce fighting raged at Little Round Top, the Wheatfield, Devil's Den, and the Peach Orchard. On the Union right, demonstrations escalated into full-scale assaults on Culp's Hill and Cemetery Hill. Across the battlefield, despite significant losses, the Union defenders held their lines.

On the third day of battle, July 3, fighting resumed on Culp's Hill, and cavalry battles raged to the east and south, but the main event was a dramatic infantry assault by 12,500 Confederates against the center of the Union line on Cemetery Ridge. Pickett's Charge was repulsed by Union rifle and artillery fire at great losses to the Confederate army. Lee led his army on a torturous retreat back to Virginia. Between 46,000 and 51,000 Americans were casualties in the three-day battle. That November, President Lincoln used the dedication ceremony for the Gettysburg National Cemetery to honor the Union dead and redefine the purpose of the war in his historic Gettysburg Address.