NATIONAL GRADUATE STUDENT RESEARCH CONFERENCE 2011

OCTOBER 17-18, 2011

NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND

EXPERIENCE THE NATIONAL INSTITUTES OF HEALTH

6TH NIH NATIONAL GRADUATE STUDENT RESEARCH CONFERENCE

OCTOBER 16–18, 2011

NATCHER CONFERENCE CENTER | BETHESDA, MARYLAND

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AGENDA

Sunday, October 16, 2011: Hyatt Regency Bethesda

3:00 – 9:00 PM **Check In and Registration**

5:15 – 5:30 PM **Welcome and Opening Remarks**

Jackie A. Lavigne, PhD, MPH, Co-Chair, NGSRC Organizing Committee

5:30 – 6:15 PM **NIH 101**

What is the NIH, how is it organized, and how does it work?
Sharon L. Milgram, PhD, Director, NIH Office of Intramural Training & Education

6:15 – 9:00 PM **DineArounds*** (organized by the NIH Fellows Committee)

Monday, October 17, 2011: Natcher Conference Center, Bethesda

7:30 – 8:00 AM **Registration**

Outer Lobby

8:00 – 9:00 AM **Light Refreshments with the NIH Institute/Center Training Directors**

Lower Level Lobby

9:00 – 9:45 AM **Concurrent Scientific Sessions** (presenters are senior postdoctoral fellows)

Session I
Balcony A

- Transitions in Chromatin Structure Require Multiple Chromatin Remodeling Systems
  Stephanie A. Morris, PhD
  National Cancer Institute
- Study of the Gut Microbiome: From Translational Research to Evaluation of Cancer Risk
  Roberto Flores, PhD, MPH
  National Cancer Institute

Session II
Balcony B

- Interactions between SPATE Family Proteins and Mammalian Cells
  Matt Wenham, DPhil
  National Institute of Diabetes and Digestive and Kidney Diseases
- RecQ Helicases in DNA Repair
  Marie Rossi, PhD
  National Institute on Aging

9:45 – 10:00 AM **Morning Break**

10:00 – 11:30 AM **Poster Session I – Upper Level Atrium;** details to be provided

11:30 – 12:30 PM **Lunch with NIH Postdoctoral Fellows and NIH Investigators**

Lower Level Lobby
Participants will have an opportunity to interact with current NIH trainees and investigators.

12:30 – 2:00 PM **Poster Session II – Upper Level Atrium;** details to be provided

2:00 – 3:00 PM **Career Workshop I: Advancing Your Career at the NIH**

Lower Level, Room E1/E2
Lori M. Conlan, PhD
Director, Office of Postdoctoral Services, OITE

3:00- 3:15 PM **Afternoon Break**

3:15 – 4:00 PM** **Keynote Scientific Presentation**

Lower Level, Room E1/E2
How to Build a Dog: Genetics of Complex Traits in the Canine
Elaine A. Ostrander, PhD
National Human Genome Research Institute

4:00 – 5:00 PM **Career Workshop II**

Lower Level, Room E1/E2
NIH Postdoctoral Fellows who recently transitioned to a variety of scientific jobs, including tenure-track academic positions and positions as science policy analysts, will share advice on critical job search skills, their own job searches, and their current positions.

- Bobbie Ann Austin, PhD
  Assistant Director, Association for Research in Vision and Ophthalmology
- Sydella Blatch, PhD
  Assistant Professor, Department of Biology, Stevenson University
- Sandeep Dayal, PhD
  Science Policy Analyst, Office of Science Policy Analysis
  National Institute of Diabetes and Digestive and Kidney Diseases, NIH
- Britta Hahn, PhD
  Assistant Professor, Department of Psychiatry
  University of Maryland School of Medicine
- Orlando Lopez, PhD
  Commissioner's Fellow, Food and Drug Administration
- Marci Scidmore, PhD
  Associate Professor, Department of Microbiology and Immunology
  Cornell University

5:00 – 5:30 PM** **Closing Remarks, Festival Evaluation, Raffle**

Lower Level, E1/E2
Michael M. Gottesman, MD
Deputy Director for Intramural Research, NIH

6:00 PM **FelCom Networking Event; Dinner on Your Own**

Tuesday: Bethesda and Other Campuses

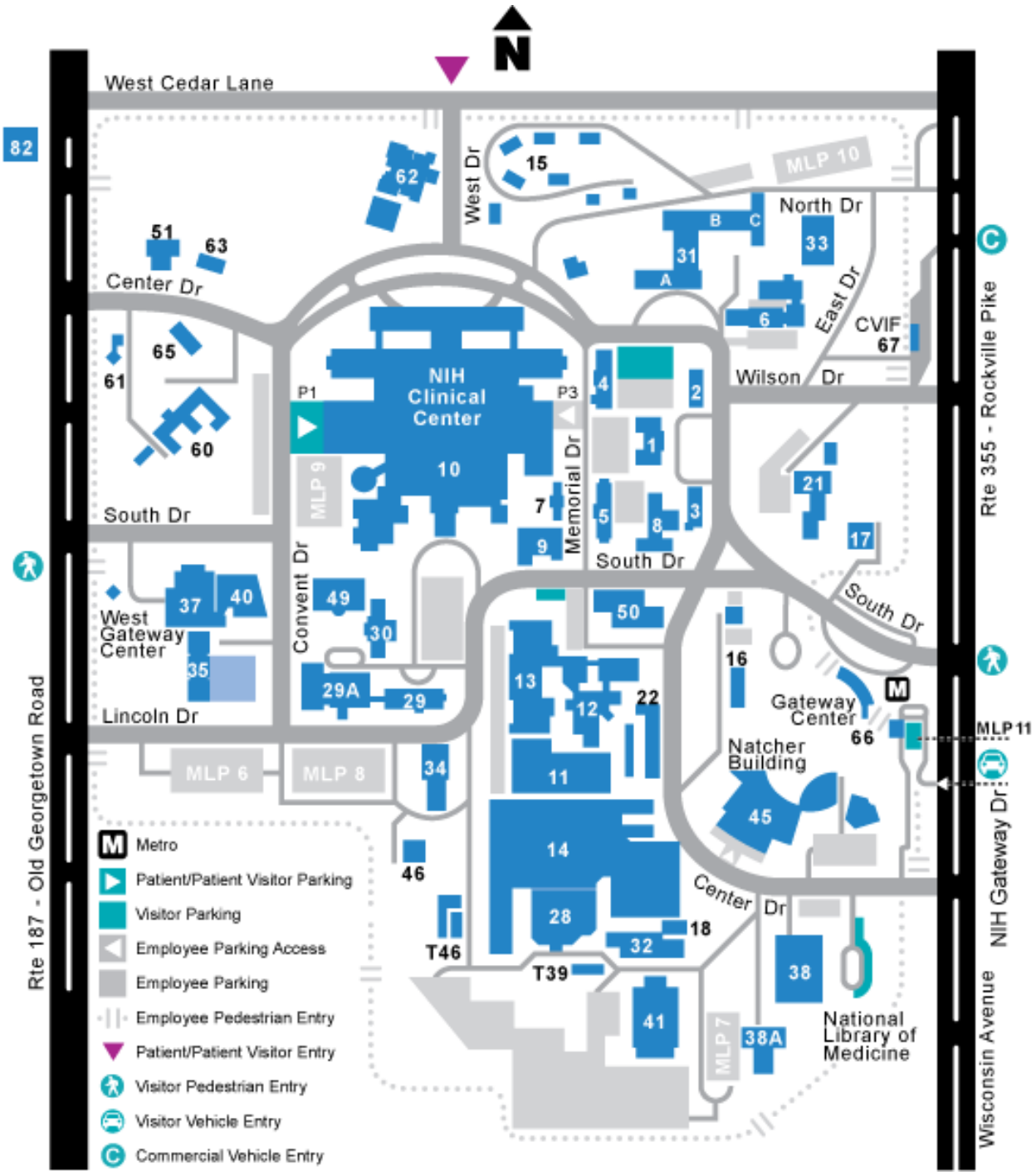
Breakfast on Your Own

Meetings with NIH scientists

* *Reservations will be made for groups of 10 at a selection of Bethesda restaurants. Conference participants can sign up to "dine" at the restaurant that interests them most. Each group will be "hosted" by a current NIH postdoc. Diners will cover the cost of their own dinners.*

** *Event will be held jointly with Resident Research Career Day at the NIH.*

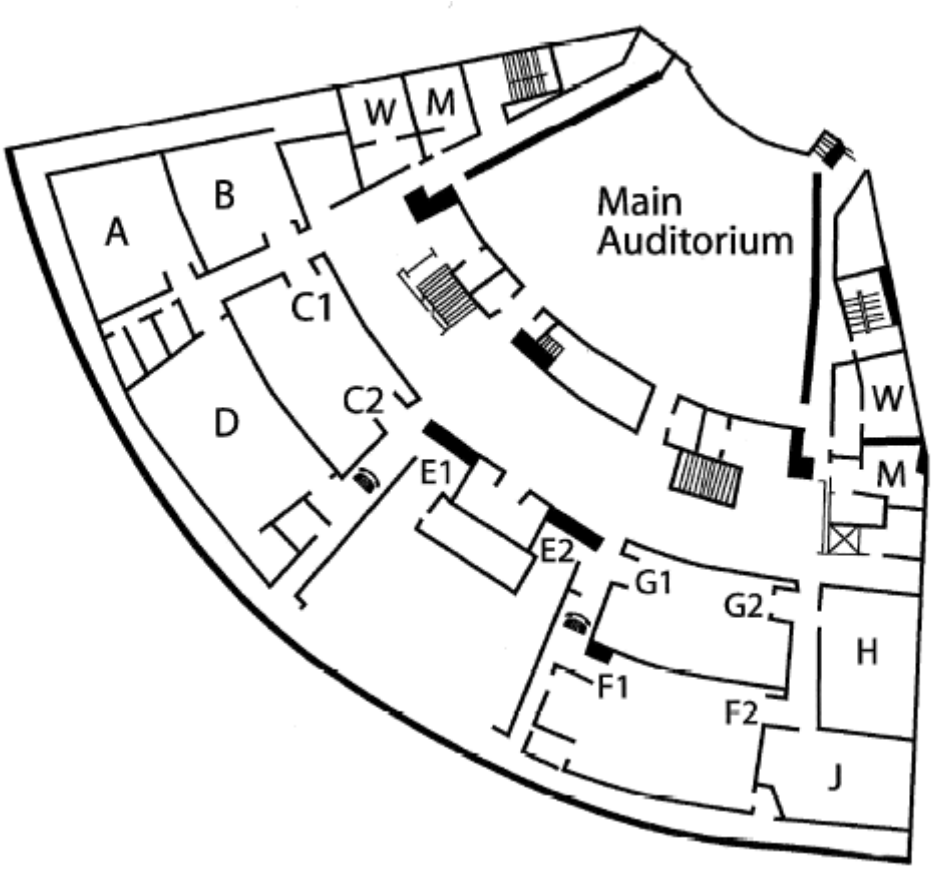
THE NATIONAL INSTITUTES OF HEALTH CAMPUS MAP



THE NATCHER CENTER CONFERENCE FLOOR PLAN- P2 LEVEL

This diagram depicts the P2 Level of the Natcher Conference Center.

Upstairs on the Atrium (Lobby) Level of the Natcher Conference Center you will find the entrance to Balconies A, B and C, and poster presentations.



NIH INSTITUTES & CENTERS

The Office of the Director (OD)
The Office of the Director is the central office at NIH for its 27 Institutes and Centers. The OD is responsible for setting policy for NIH and for planning, managing, and coordinating the programs and activities of all the NIH components. OD's program offices include the Office of AIDS Research and the Office of Research on Women's Health, among others.

National Cancer Institute (NCI) - Established in 1937
NCI leads a national effort to eliminate the suffering and death due to cancer. Through basic and clinical biomedical research and training, NCI conducts and supports research that will lead to a future in which we can prevent cancer before it starts, identify cancers that do develop at the earliest stage, eliminate cancers through innovative treatment interventions, and biologically control those cancers that we cannot eliminate so they become manageable, chronic diseases.

National Eye Institute (NEI) - Established in 1968
NEI conducts and supports research that helps prevent and treat eye diseases and other disorders of vision. This research leads to sight-saving treatments, reduces visual impairment and blindness, and improves the quality of life for people of all ages. NEI-supported research has advanced our knowledge of how the eye functions in health and disease.

National Heart, Lung, and Blood Institute (NHLBI) - Established in 1948
NHLBI provides leadership for a national program in diseases of the heart, blood vessels, lung, and blood; blood resources; and sleep disorders. Since October 1997, the NHLBI has also had administrative responsibility for the NIH Women's Health Initiative. The Institute plans, conducts, fosters, and supports an integrated and coordinated program of basic research, clinical investigations and trials, observational studies, and demonstration and education projects.

National Human Genome Research Institute (NHGRI) - Established in 1989
NHGRI supports the NIH component of the Human Genome Project, a worldwide research effort designed to analyze the structure of human DNA and determine the location of the estimated 30,000 to 40,000 human genes. The NHGRI Intramural Research Program develops and implements technology for understanding, diagnosing, and treating genetic diseases.

National Institute on Aging (NIA) - Established in 1974
NIA leads a national program of research on the biomedical, social, and behavioral aspects of the aging process; the prevention of age-related diseases and disabilities; and the promotion of a better quality of life for all older Americans.

National Institute on Alcohol Abuse and Alcoholism (NIAAA) - Established in 1970
NIAAA conducts research focused on improving the treatment and prevention of alcoholism and alcohol-related problems to reduce the enormous health, social, and economic consequences of this disease.

National Institute of Allergy and Infectious Diseases (NIAID) - Established in 1948
NIAID research strives to understand, treat, and ultimately prevent the myriad infectious, immunologic, and allergic diseases that threaten millions of human lives.

National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) - Established in 1986
NIAMS supports research into the causes, treatment, and prevention of arthritis and musculoskeletal and skin diseases, the training of basic and clinical scientists to carry out this research, and the dissemination of information on research progress in these diseases.

National Institute of Biomedical Imaging and Bioengineering (NIBIB) - Established in 2000
NIBIB improves health by promoting fundamental discoveries, design and development, and translation and assessment of technological capabilities in biomedical imaging and bioengineering, enabled by relevant areas of information science, physics, chemistry, mathematics, materials science, and computer sciences.

Eunice Kennedy Shriver **National Institute of Child Health and Human Development (NICHD)** - Established in 1962
NICHD research on fertility, pregnancy, growth, development, and medical rehabilitation strives to ensure that every child is born healthy and wanted and grows up free from disease and disability.

National Institute on Deafness and Other Communication Disorders (NIDCD) - Established in 1988
NIDCD conducts and supports biomedical research and research training on normal mechanisms as well as diseases and disorders of hearing, balance, smell, taste, voice, speech, and language that affect 46 million Americans.

National Institute of Dental and Craniofacial Research (NIDCR) - Established in 1948
NIDCR provides leadership for a national research program designed to understand, treat, and ultimately prevent the infectious and inherited craniofacial-oral-dental diseases and disorders that compromise millions of human lives.

National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) - Established in 1948
NIDDK conducts and supports basic and applied research and provides leadership for a national program in diabetes, endocrinology, and metabolic diseases; digestive diseases and nutrition; and kidney, urologic, and hematologic diseases. Several of these diseases are among the leading causes of disability and death; all seriously affect the quality of life of those who have them.

National Institute on Drug Abuse (NIDA) - Established in 1973
NIDA leads the nation in bringing the power of science to bear on drug abuse and addiction through support and conduct of research across a broad range of disciplines and rapid and effective dissemination of results of that research to improve drug abuse and addiction prevention, treatment, and policy.

National Institute of Environmental Health Sciences (NIEHS) - Established in 1969
NIEHS reduces the burden of human illness and dysfunction from environmental causes by, defining how environmental exposures, genetic susceptibility, and age interact to affect an individual's health.

National Institute of General Medical Sciences (NIGMS) - Established in 1962
NIGMS supports basic biomedical research that is not targeted to specific diseases. NIGMS funds studies on genes, proteins, and cells, as well as on fundamental processes like communication within and between cells, how our bodies use energy, and how we respond to medicines. The results of this research increase our understanding of life and lay the foundation for advances in disease diagnosis, treatment, and prevention. NIGMS also supports research training programs that produce the next generation of biomedical scientists, and it has special programs to encourage underrepresented minorities to pursue biomedical research careers.

National Institute of Mental Health (NIMH) - Established in 1949
NIMH provides national leadership dedicated to understanding, treating, and preventing mental illnesses through basic research on the brain and behavior, and through clinical, epidemiological, and services research.

National Institute on Minority Health and Health Disparities (NIMHD) - Established in 1993
The mission of NIMHD is to promote minority health and to lead, coordinate, support, and assess the NIH effort to reduce and ultimately eliminate health disparities. In this effort NIMHD will conduct and support basic, clinical, social, and behavioral research, promote research infrastructure and training, foster emerging programs, disseminate information, and reach out to minority and other health disparity communities.

National Institute of Neurological Disorders and Stroke (NINDS) - Established in 1950
The mission of the NINDS is to reduce the burden of neurological diseases—a burden borne by every age group, every segment of society, and people all over the world. To accomplish this goal the NINDS supports and conducts research, both basic and clinical, on the normal and diseased nervous system, fosters the training of investigators in the basic and clinical neurosciences, and seeks better understanding, diagnosis, treatment, and prevention of neurological disorders.

National Institute of Nursing Research (NINR) - Established in 1986
NINR supports basic and clinical research that develops the knowledge to build the scientific foundation for clinical practice, prevent disease and disability, manage and eliminate symptoms caused by illness, and enhance end-of-life and palliative care.

National Library of Medicine (NLM) - Established in 1956
NLM collects, organizes, and makes available biomedical science information to scientists, health professionals, and the public. The Library's Web-based databases, including PubMed/Medline and MedlinePlus, are used extensively around the world. NLM conducts and supports research in biomedical communications; creates information resources for molecular biology, biotechnology, toxicology, and environmental health; and provides grant and contract support for training, medical library resources, and biomedical informatics and communications research.

Center for Information Technology (CIT formerly DCRT, OIRM, TCB) - Established in 1964
CIT incorporates the power of modern computers into the biomedical programs and administrative procedures of the NIH by focusing on three primary activities: conducting-computational biosciences research, developing computer systems, and providing computer facilities.

Center for Scientific Review (CSR) - Established in 1946
CSR is the focal point at NIH for the conduct of initial peer review, the foundation of the NIH grant and award process. The Center carries out peer review of the majority of research and research training applications submitted to the NIH. In addition, the Center serves as the central receipt point for all such Public Health Service (PHS) applications and makes referrals to scientific review groups for scientific and technical merit review of applications and to funding components for potential award. To this end, the Center develops and implements innovative, flexible ways to conduct referral and review for all aspects of science.

John E. Fogarty International Center for Advanced Study in the Health Sciences (FIC) - Established in 1968
FIC promotes and supports scientific research and training internationally to reduce disparities in global health.

National Center for Complementary and Alternative Medicine (NCCAM) - Established in 1999
NCCAM is dedicated to exploring complementary and alternative medical (CAM) practices in the context of rigorous science; training CAM researchers and disseminating authoritative information.

National Center for Research Resources (NCRR) - Established in 1962
NCRR provides laboratory scientists and clinical researchers with the environments and tools they need to understand, detect, treat, and prevent a wide range of diseases. With this support, scientists make biomedical discoveries, translate these findings to animal-based studies, and then apply them to patient-orientated research.

NIH Clinical Center (CC) - Established in 1953
CC is the clinical research facility of the National Institutes of Health. As a national resource, it provides the patient care, services, and environment needed to initiate and support the highest quality conduct of and training in clinical research.

BIOGRAPHICAL SKETCHES

Bobbie Ann Austin, PhD

Dr. Austin is Assistant Director, Science Policy & Programs for the Association for Research in Vision & Ophthalmology (ARVO). Since 2008 she has worked as a science liaison to the staff, committees, outside organizations, and the administration. She helps develop and evaluate scientific programs and prepares grant proposals for new initiatives. Dr. Austin also prepares and reviews the scientific accuracy of publications, newsletters, press releases, Web content, and social media. She works on advocacy by monitoring policies and regulations that affect members and assembles/supervises scientific working groups that respond to requests for feedback or policy statements. She received her PhD from the University of North Dakota School of Medicine & Health Services in Grand Forks, ND.

Sydella Blatch, PhD

Dr. Blatch is an Assistant Professor of Biology at Stevenson University, in Baltimore County, Maryland. At Stevenson, Dr. Blatch teaches general biology and animal physiology courses. She also does research with students on functions of the B-vitamin folic acid in relation to unknown microorganisms in the fruit fly Drosophila melanogaster. Prior to joining Stevenson, Dr. Blatch studied the molecular biology of mouse epigenetics as a postdoctoral fellow at the Eunice Kennedy Shriver National Institute of Child Health and Human Development, at the National Institutes of Health. During this time she also worked as an adjunct faculty member at Howard University in Washington, DC and Prince George's County Community College in Maryland. Dr. Blatch received her PhD in biology from Arizona State University, focused on insect nutritional physiology. During graduate school, Dr. Blatch was the co-president of the Black Graduate Student Association of ASU, worked with the Minority Access to Research Careers Program, and created The Shades Mentoring Program, a campus-wide multicultural mentoring program for undergrad and graduate students in the sciences. In graduate school, she was awarded a National Science Foundation Graduate Research Fellowship and several different campus awards for her efforts in diversity and in the community. Dr. Blatch received her BS degree in biology, with a concentration in zoology, from the University of Maryland – College Park.

Lori M. Conlan, PhD

Dr. Conlan trained as a biochemist, receiving her BS in biochemistry from Michigan State University and her PhD in biochemistry and biophysics from Texas A&M University. She worked for several years as a postdoc at the Wadsworth Center, New York State Department of Health, before transitioning from the lab to focus on career issues for the next generation of scientists. Dr. Conlan started her life outside the lab as the Director of the Science Alliance, an international career development program for graduate students and postdocs sponsored by the New York Academy of Sciences. She then joined the Office of Intramural Training & Education at the NIH in 2008 as Director of the Office of Postdoctoral Services where she assists the 4000 NIH postdocs with their personal career choices. She currently is serving as a board member for the National Postdoctoral Association (NPA). Dr. Conlan speaks throughout the nation on career options for scientists, including participating in an AAAS Webinar on non-traditional careers.

Sandeep Dayal, PhD

Dr. Dayal is Health Science Policy Analyst in the Office of Scientific Program and Policy Analysis at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). From 2004 to 2010 he was an Intramural Research Training Award Postdoctoral Fellow in the Laboratory of Molecular Biology at the NIDDK. There, as a joint postdoc in the laboratories of Drs. Gary Felsenfeld and Marty Gellert, he investigated the role of chromatin remodeling in class switch recombination – a DNA recombination event in human B lymphocytes that leads to the production of different antibody isotypes. During his fellowship, Dr. Dayal served on various NIH committees supporting the postdoctoral community, including FelCom, the NIH Fellows Committee and the NIH Career Symposium Planning Committee. Dr. Dayal earned his BS degree in Cell and Molecular Biology from California State University Northridge, MS degree in Cell and Molecular Biology from Eastern Michigan University, and PhD from the University of Texas MD Anderson Cancer Center in Houston.

Roberto Flores, PhD, MS, MPH

Dr. Flores is a Cancer Prevention Fellow at the National Cancer Institute. He earned his MS and PhD in Nutritional Sciences with emphasis on nutritional biochemistry and cancer biology from the University of Arizona, Tucson. Dr. Flores also holds a Master's in Public Health with a focus on Vaccine Science and Policy from the Johns Hopkins School of Public Health. During his Public Health training, he evaluated national HPV vaccine implementation and cervical cancer screening programs in India. His previous work as a Lab scientist has been related to natural history studies of human papillomavirus (HPV) infection in men as well as transitional and quality control studies for molecular screening of HPV. Dr. Flores is working with James Goedert, MD in the Infectious and Immunoepidemiology Branch, Division of Cancer Epidemiology and Genetics. His research interest is the association of the gut microbiome and cancer risk. While at NCI, Dr. Flores directed a pilot study to assess the reproducibility of sample collection and functional assays for gut microbiota studies. He is also involved in a project assessing the role of estrogen recirculation and microbial metabolism in relation to breast cancer risk.

Michael M. Gottesman, MD

Dr. Gottesman is Deputy Director for Intramural Research at NIH, and Chief of the Laboratory of Cell Biology, National Cancer Institute (NCI), National Institutes of Health (NIH). He was born on October 7, 1946 in Jersey City, New Jersey and attended Harvard College where he graduated summa cum laude in biochemical sciences in 1966. He was married the same year to Susan Kemelhor. He graduated from Harvard Medical School with an MD magna cum laude in 1970 and completed a medical internship and residency at the Peter Bent Brigham Hospital in Boston. His research training began at Harvard in the laboratories of William Beck and Bert Vallee, and continued in the laboratory of Martin Gellert at the National Institutes of Health as a Research Associate from 1971 to 1974. Dr. Gottesman spent a year as an Assistant Professor at Harvard Medical School and then, together with his wife who is a bacterial geneticist, joined the permanent staff of the National Cancer Institute in 1976. He became Chief of the Molecular Cell Genetics Section of the Laboratory of Molecular Biology in 1980, Chief of the Laboratory of Cell Biology in 1990, Acting Director of the National Center for Human Genome Research (NCHGR) from 1992 to 1993, and also Acting Scientific Director of the NCHGR (April 1993 to October 1993). He has been Deputy Director for Intramural Research, NIH, since November 1993, and was Assistant Surgeon General (Rear Admiral), Public Health Service from 1997 to 2000.

At the NIH his research interests have ranged from how DNA is replicated in bacteria to how cancer cells elude chemotherapy. He has published over 400 peer-reviewed papers. Using chloramphenicol resistance as a model, he was one of the first to show that drug resistance

genes could move from one replicon to another in bacteria. Applying the tools of molecular and somatic cell genetics to the study of cAMP-resistance and anti-microtubule drug resistance in mammalian cells, he isolated and characterized cAMP-dependent protein kinase mutants and conditional a- and b-tubulin mutants. He was among the first to exploit novel techniques of DNA transfer in somatic cells, while using them as tools to demonstrate the role of cAMP-dependent kinase in growth regulation and to study the effect of microtubule defects on mitosis. The work on anti-microtubule drug resistance led to studies on multidrug resistance in human cancer cells. During the past 20 years he has identified and characterized the human MDR1 gene, the first of the mammalian ABC transporter genes to be described as that responsible for resistance of cancer cells to many of the most common anticancer drugs. This research has shown that this gene encodes a protein that acts to pump anticancer drugs out of drug-resistant human cancers. In addition to the development of strategies to circumvent multidrug resistance in cancer, these studies have led to a new generation of selectable vectors for gene therapy, and the discovery of other members of the ABC transporter family as drug-resistance genes. At least 20 human ABC transporters contribute to anticancer drug resistance, and identification of their substrates has enabled the development of new lead compounds that specifically kill MDR cells. Most recently, his laboratory has used gene expression analysis to correlate patient response to chemotherapy with expression of MDR genes.

Dr. Gottesman's professional activities include many active memberships in professional societies and editorial boards. He was elected a Fellow of the American Association for the Advancement of Science (AAAS), 1988; elected to the Institute of Medicine of the National Academies, November 2003; and the Association of American Physicians (AAP), March 2007. His awards and honors are numerous and, to name a few, include the Milken Family Foundation Cancer Research Award, 1988; Rosenthal Foundation Award, 1992; American Society of Pharmacology and Experimental Therapeutics (ASPET) Award, 1997; Public Health Service Commissioned Corps Meritorious Service Medal, 1999; NIH Director's Award, 2002; Department of Health and Human Services (DHHS) Secretary's Award for Distinguished Service, May 2005; NIH Office of Technology Transfer Hottest Technology Award, "MDR-1 Selective Compounds," September 2009 on-site competition at the Mid-Atlantic Region of the Federal Laboratory Consortium (FLC); and the 6th Alan S. Rabson, M.D. Award for NCI Intramural Cancer Research, January 2010.

Dr. Gottesman has been actively involved in initiating several training and mentoring programs for high school students and teachers, college students, graduate students, and postdoctoral students. As Deputy Director for Intramural Research at the NIH, he has initiated an NIH-wide lecture series, reformulated tenure and review processes in the intramural program, initiated multiple trans-NIH research initiatives, and instituted training programs for disadvantaged students. He has also overseen the creation of loan repayment programs for laboratory and clinical researchers, a clinical research training program for medical students, and formalized institutional training and mentoring for postdoctoral fellows at the NIH.

Britta Hahn, PhD

Dr. Hahn is an Assistant Professor at the University of Maryland School of Medicine, Dept. of Psychiatry, Maryland Psychiatric Research Center. She is conducting research on cognitive dysfunction in schizophrenia and possible remediation by nicotinic acetylcholine receptor agonists, such as nicotine. From 2003 to 2008, Dr. Hahn conducted postdoctoral research in the NIH, National Institute on Drug Abuse Intramural Research Program, using functional Magnetic Resonance Imaging techniques to study cognition and the mechanisms of nicotine-induced attentional enhancement. Dr. Hahn earned her MSc in Neuroscience and her PhD in Behavioral Pharmacology from King's College London, UK, and her first degree in Psychology from Hamburg University, Germany.

Jackie A. Lavigne, PhD, MPH

Dr. Lavigne is the Chief of the Office of Education in the National Cancer Institute (NCI)'s Division of Cancer Epidemiology and Genetics (DCEG). She received her bachelor's degree in French from Colby College in Waterville, ME. She then earned a PhD in molecular toxicology and an MPH with a concentration in epidemiology and biostatistics from the Johns Hopkins Bloomberg School of Public Health in Baltimore, MD. She completed her postdoctoral training in the NCI's Cancer Prevention Fellowship Program (CPFP), conducting research on the role of insulin-like growth factors in diet-related cancer. Subsequently, she joined NCI's Center for Cancer Research as a Scientific Program Specialist, became Associate Director of the CPFP in 2006, and has served as the Chief of the Office of Education in DCEG since 2008. In this role, she oversees the division's fellowship training program, which was recently awarded the inaugural Alexander D. Langmuir Award for Training Program Excellence and Innovation by the North American Congress of Epidemiology. She is experienced in mentoring and training junior scientists and received the NCI's Outstanding Mentor Award in 2008.

Orlando Lopez, PhD, GWCPM

Dr. Lopez is a Biomedical Engineer and Scientific Reviewer with the Division of Cardiovascular Devices in the Center for Devices and Radiological Health at FDA. He participates in the regulatory review of premarket approval applications of emerging medical device technologies, contributes to the development and modification of existing guidelines and protocols for testing specific types of devices, and serves as scientific expert in technical workgroups and as reviewer to various scientific journals. From 2009 to 2011, Dr. Lopez was a Commissioner's Fellow with the Office of the Commissioner and the Center for Devices and Radiological Health at FDA. During his Commissioner's Fellowship, Dr. Lopez contributed his broad scientific expertise to a variety of projects with public health impact surrounding the evaluation and regulation of medical devices associated with cardiovascular, interventional, and medical imaging technologies. Dr. Lopez continues to play a significant role in regulatory science efforts at the Division of Biology in the Office of Science and Engineering Laboratories of CDRH on the development and integration of a wide range of biomedical engineering, informatics, imaging and computational principles aimed to improve the predictive power of preclinical studies, establish preclinical-to-human correlations and provide recommendations for science-led regulations that ultimately improve the clinical outcomes of interventional therapies. Prior to the FDA, Dr. Lopez was a biomedical engineer and postdoctoral research associate at the Magnetic Resonance Imaging and Spectroscopy Laboratory of the National Institute on Aging at the National Institutes of Health, where he conducted applied preclinical research on imaging-based evaluations of cardiovascular function and muscle bioenergetics on animal models. Dr. Lopez earned an undergraduate degree in biomedical engineering from Boston University and a doctorate in biomedical engineering from the Mayo Clinic College of Medicine with emphasis in biomedical imaging and biomechanics. Most recently, Dr. Lopez earned a graduate certificate in project management from The George Washington University.

Sharon L. Milgram, PhD

Dr. Milgram received a BS degree in physical therapy from Temple University in 1984 and a PhD in cell biology from Emory University in 1991. She completed a postdoctoral fellowship at the Johns Hopkins University before joining the faculty at the University of North Carolina at Chapel Hill in 1994. At UNC Dr. Milgram rose to the rank of Full Professor with Tenure in the Department of Cell and Developmental Biology. Her research focuses on cell signaling and protein trafficking in polarized cells; it has been published in journals including Journal of Cell Biology, Journal of Clinical Investigation, the Proceedings of the National Academy of Sciences, and the Journal of Biological Chemistry. Her research was supported by grants from the NIH, the Cystic Fibrosis Foundation, and the American Heart Association. Dr. Milgram held a number

of administrative positions at UNC including Associate Director of the Medical Scientist Training Program (MSTP), Director of the Interdisciplinary Biomedical Sciences Graduate Program, and Director of the Summer Undergraduate Research Experience. She founded and advised the UNC Office of Postdoctoral Services and served on the advisory committee of the Signal Xi National Postdoctoral Survey. Dr. Milgram served as principle investigator on a number of nationally-funded training grants including an NSF-funded program for undergraduate students, and NIH grants to support predoctoral students in Cell and Molecular Biology as well as an Initiative to Maximize Student Diversity grant spanning the Schools of Medicine and Public Health at UNC. In 2007 Dr. Milgram joined the NIH Office of the Director as the Director of the Office of Intramural Training & Education. She is also a Senior Scientist in the National Heart, Lung, and Blood Institute.

Stephanie Morris, PhD

Dr. Morris is currently a postdoctoral fellow in the laboratory of Dr. Gordon Hager at the National Cancer Institute (NCI). She graduated in 2000 from Wesleyan University in Middletown, Connecticut, with a BA in biology, and neuroscience and behavior. Prior to starting graduate school, Dr. Morris worked for two years as a research technician at the Albert Einstein College of Medicine in the Bronx, New York, where she designed and conducted analytical ultracentrifugation experiments in the Laboratory for Macromolecular Analysis & Proteomics. She received a Ford Foundation Predoctoral Fellowship during her graduate studies at the University of North Carolina at Chapel Hill where her research focused on histone modifications during transcription elongation. Dr. Morris completed her PhD in biochemistry and biophysics in 2007 and accepted a postdoctoral position at the NCI in the Laboratory of Receptor Biology and Gene Expression where she studies chromatin remodeling during nuclear receptor-mediated gene regulation. In 2009, Dr. Morris was awarded one of 10 UNCF-Merck Postdoctoral Fellowships to fund her research at the NCI, and in 2010 received a National Institutes of Health Fellows Award for Research Excellence. She currently has nine peer-reviewed publications, three of which are first author, and is preparing another submission for review. In addition to her postdoctoral research responsibilities at the NIH, she led the development of a new chromatin postdoctoral seminar series, and is a volunteer editor for the NIH Fellows Editorial Board. In 2011, she was appointed Senior Editor of this Board, which assists NIH fellows in the preparation of manuscripts and other scientific documents.

Elaine A. Ostrander, PhD

Dr. Ostrander is Chief of the Cancer Genetics Branch at the National Human Genome Research Institute of NIH where she also heads the Section of Comparative Genetics. Dr. Ostrander received her PhD from the Oregon Health Sciences University in 1987 and did her primary postdoctoral training at Harvard. She then went to University of California, Berkeley and the Lawrence Berkeley National Labs where she began the canine genome project, initiating work on the canine meoitic linkage maps. She moved to the Fred Hutchinson Cancer Research Center and University of Washington in 1993, rising to the rank of Member in the Human Biology and Clinical Research Divisions, and Head of the Genetics Program. She moved to NHGRI in 2004. Her laboratory at NHGRI works in both human and canine genetics, focusing on the genetics of complex traits such as cancer and autoimmune disease and genetics of morphology. She is the winner of several awards, including the 1995 American Cancer Society Junior Faculty Award, 2000 Burroughs Wellcome Innovation Award, and the Asa Mays Award. She has published over 200 papers and articles, and leads a team of postdocs and students on projects aimed at finding genes contributing to human breast and prostate cancer, regulating variation in canine morphology, understanding the population dynamics of modern domestic dog breeds, and finding genes that contribute to behavior.

Marie L. Rossi, PhD

Dr. Rossi received her BS in Biology from Nazareth College of Rochester in 2002. She then entered a graduate program in the Department of Biochemistry and Biophysics at the University of Rochester and in 2003 joined the laboratory of Dr. Robert A. Bambara researching mechanisms of lagging strand DNA replication. In 2007, she completed her PhD in Biochemistry at Rochester and joined the laboratory of Dr. Vilhelm A. Bohr at the National Institute on Aging. As a postdoctoral fellow in the Bohr laboratory, she has been investigating the biochemical characteristics of the RecQL4 helicase and its functions in the complex network of DNA repair processes. During her tenure as a scientist, she has been selected to present her research to a variety of local, national, and international audiences and has several scientific publications to her credit. Upon completion of her postdoctoral fellowship, she will be seeking employment as a researcher or writer in the biotechnology/pharmaceutical industry.

Marci Scidmore, PhD

Dr. Scidmore is an Associate Professor in the Department of Microbiology and Immunology at the Cornell College of Veterinary Medicine and has been associated with the department since 2001. She currently studies the host-pathogen interactions of the obligate intracellular bacteria Chlamydia. From 1997 to 2001, she was a senior staff fellow, and from 1993 to 1997, she was a postdoctoral research fellow at Rocky Mountain Laboratories, National Institutes of Allergy and Infectious Disease, National Institutes of Health. Dr. Scidmore earned her BA degree in Molecular Biology from University of California at San Diego in 1987, and her PhD degree in Molecular Biology from Princeton University in 1993.

Matt Wenham, DPhil

Dr. Wenham joined NIH in January 2010, as a postdoctoral fellow in the research group of Dr. Harris Bernstein at the National Institute of Diabetes and Digestive and Kidney Diseases. Matt grew up in Adelaide, South Australia, and completed his Bachelor of Science and Honours degrees in biochemistry at the University of Adelaide, before moving to Melbourne to work at the University of Melbourne. In 2005, Matt was awarded a Rhodes Scholarship and moved to the UK to complete his PhD at the University of Oxford. He undertook his doctoral research with Professor Gillian Griffiths at the Sir William Dunn School of Pathology and the Cambridge Institute for Medical Research, working on T cell immunodeficiencies and the cell biology and protein trafficking of cytotoxic T lymphocytes. Matt previously chaired a ministerial advisory council on youth affairs for the South Australian Government and was awarded an Australian Centenary Medal in 2001 for services to youth affairs. He has served in the Australian Army Reserve and is a graduate of the reserve officer course at the Royal Military Academy Sandhurst, UK. He is currently a member of the NIH Fellows Committee and Visiting Fellows Committee, serves as NIH liaison to the National Postdoctoral Association, and edits the NIDDK fellows' newsletter. Matt has previously undertaken a science policy fellowship with Scientists and Engineers for America and is current a Fellow with the Institute on Science for Global Policy.

POSTER SESSION OVERVIEW

POSTER SESSION 1:

Biomedical Engineering/Imaging
Biophysics
Chemistry
Membranes
MMP/ECM/Cytoskeleton
Modeling/Computational Biology/Informatics
Neurobiology – Cognition and Behavior
Neurobiology – Electrophysiology
Neurobiology – Receptors, Channels, Transporters
Neurological Diseases
Physiology
Protein Complexes
Protein Structure/Function
Public Health/Global Health
Regenerative Medicine/Tissue Engineering
Social/Behavioral Science
Stem Cells
Structural Biology
Toxicology/Environmental Health

POSTER SESSION 2:

Cancer
Epidemiology/Biostatistics
Genetics/Genomics
HIV/AIDS
Immunology
Microbiology and Viruses
Mitosis/Chromosomes/Cell Cycle
Regulation of Gene Expression
Signaling

NOTE: Shaded text indicates Conference invitees who were, at the last minute, unable to attend.

POSTER SESSION 1

MONDAY, OCTOBER 17

10:00 – 11:30 AM

BIOMEDICAL ENGINEERING/IMAGING

1.1 **Kvar Black,** Light-Activated Cancer Therapy with Biomimetic Multifunctional Anti-EGFR Bioplasmonic Nanoparticles

1.2 **Cynthia Davies-Venn,** Albumin Binding Peptide Conjugate for Near-Infrared Fluorescence Lymphatic Imaging

1.3 **Jonathan Kuo,** Bioengineering Approach to Understanding Temporomandibular Joint Pathobiology

1.4 **Elyssa Monzack,** Effects of Simvastatin Treatment on 2-D and 3-D in vitro Models of Heart Valve Disease

1.5 **Johnny Tam,** Human Retinal Capillaries Are Disrupted before the Onset of Diabetic Retinopathy

1.6 **Stefano Young,** Image Quality Tradeoffs in a Model Breast Tomosynthesis System: Angular Span and Angular Sampling Scheme

BIOPHYSICS

1.7 **Kimberly Stroka,** Leukocyte Transmigration Is Mediated by Endothelial Cell Contractile Forces and Substrate Stiffness

CHEMISTRY

1.8 **Angie Garcia,** Synthetic, Theoretical, and Clinical Studies of Cannabinoid Analogues for CNS Disorders

1.9 **Thomas Umile,** Biomimetic Manganese Porphyrins Catalyze the Rapid Generation of Chlorine Dioxide from Chlorite

MEMBRANES

1.10 **Sajjan Koirala,** Reconstitution of the Minimal Mitochondrial Fission Machinery in Yeast and Humans

1.11 **Sunandini Sridhar,** PI-4-KINASES at the Lysosome: PI4KIIIß Is a Novel Regulator of Lysosomal Membrane Proteins

MMP/ECM/CYTOSKELETON

1.12 **Doug Brownfield,** Orientation of Mammary Epithelium Is Determined by Stromal Collagen during Branching Morphogenesis

1.13 **Corinne Fairchild,** Tetraspanin18 Restricts Neural Crest Migration by Modulating Cadherin6B mRNA and Protein Levels

1.14 **Ernest Heimsath,** Biochemical Analysis of the Mammalian Formin FMNL3

1.15 **Ziba Razinia,** ASB2a Mediates Filamin Degradation via Interaction with the Actin Binding Domain of Filamin

MODELING/COMPUTATIONAL BIOLOGY/INFORMATICS

1.16 **Matthew Banegas,** Evaluating and Comparing Breast Cancer Risk Projections for Hispanic and non-Hispanic White Women

1.17 **Pradeep Kota,** Dynamic Coupling within NBD1 Influences Thermostability and Function of CFTR

1.18 **Jessica Larson,** Chromatin States Accurately Predict Cell Differentiation Status

1.19 **Chao Ma,** LiCABEDS, a Novel Ligand Classification Algorithm for in-silico Drug Design

NEUROBIOLOGY – COGNITION AND BEHAVIOR

1.20 **Meaghan Bychowski,** AVP Infusion into the Brains of Male Rats Rescues Progesterone Induced Impairment in Social Behavior

1.21 **Lauren Dobbs,** The Role of Mesopontine Acetylcholine in Methamphetamine Behavioral and Neurochemical Responses

1.22 **Nao Gamo,** Role of HCN Channels in D1 Receptor Stimulation and Stress-Induced Prefrontal Cortical Dysfunction

1.23 **Sung-joo Lim,** Investigating the Learning Mechanism of a Video Game Task

1.24 **Sarah Schipul,** Distinctive Neural Processes during Learning in Autism

1.25 **Adam Snyder,** The Countervailing Forces of Selection and Binding in Vision: a Human EEG Study

1.26 **Mathieu Wimmer,** Aging Reduces the Ability to Sustain Sleep/Wake States in Mice

NEUROBIOLOGY – ELECTROPHYSIOLOGY

1.27 **Paul Aparicio,** Does the Middle Face Patch Contain Cells that Detect Faces?

1.28 **Devon Crawford,** Astrocytes Support Depolarization-induced Presynaptic Plasticity in Hippocampal Neurons

1.29 **Shannon Farris,** Activity- and Translation-dependent Degradation of Arc mRNA in Neuronal Dendrites

1.30 **Michael Gertner,** gamma-Secretase Activity Is Critical for mGluR-dependent Long-term Depression at Hippocampal CA3-CA1 Synapses

1.31 **Jessica Lucas,** Neural Mechanisms Underlying Spinal Cord Injury Pain

1.32 **Andrew Piekarz,** Increased Resurgent Sodium Current in DRG Neurons Following Contusive Spinal Cord Injury

1.33 **Letitia Weigand,** A Role for ATP and P2X Receptors in Mechanical Activation of Nodose C-fibers in Guinea Pig Lungs

NEUROBIOLOGY – RECEPTORS, CHANNELS, TRANSPORTERS

1.34 **Travis Hage,** Sodium-Activated Potassium Channels Are Functionally Coupled to Persistent Sodium Currents

1.35 **Amy Moritz,** Kinetic Regulation of DAT Transport Capacity by Reciprocal Phosphorylation and Palmitoylation

1.36 **Hideaki Yano,** Pair up to Pare down: Different Modes of Functional Selectivity in GPCR Heteromers

NEUROLOGICAL DISEASES

1.37 **Fatima Ali-Rahmani,** H63D-HFE Reduces Brain Cholesterol: A Potential Mechanism for Increased Risk of Alzheimer Disease

1.38 **Loai Alzghoul,** Dysregulation of Serotonin during Early Development Alters Interhemispheric Connectivity in Rodents

1.39 **Cristin Davidson,** Treatment of Niemann-Pick Type C Disease with Different Cyclodextrins

1.40 **Emily Foran,** Motor Neuron Impairment Mediated by a Sumoylated Fragment of the Astroglial Glutamate Transporter EAAT2

1.41 **Akina Hoshino,** Structure-Function Analysis of ProSAAS as an Aß1-42 Anti-Aggregant

1.42 **Kristen Maynard,** Role of DSCAM in Cortical Dendrite Development

1.43 **Karienn Montgomery,** Novel Age-dependent Learning Deficits in a Mouse Model of AD: Implications for Translational Research

1.44 **Roshini Prakash,** Enhanced Cerebral but Not Peripheral Angiogenesis in the Goto-Kakizaki Model of Type 2 Diabetes Involves VEGF

1.45 **Rachel Reith,** Purkinje Cell Degeneration: a Link Between Tuberous Sclerosis Complex and Autism

1.46 **Armando Salinas,** Reduced Ethanol Preference and Consumption in CART Knockout Mice

1.47 **Kathleen Schoch,** Spectrin and Sodium Channel Proteolysis Are Reduced in Brain-injured Mice Overexpressing Calpastatin

1.48 **Scott Webster,** An Oral Vaccine against a RAGE-Aß Complex Immunogen on AD-like Pathology and Cognitive Function in APP/PS1 Mice

PHYSIOLOGY

1.49 **Jason Baik,** Chemical Synthesis of New Organelles in vivo

1.50 **Joseph Faust,** Peroxisomes and Lipid Metabolism in Drosophila melanogaster

1.51 **Albert Gonzales,** STIM1 Is Essential for the Coupling of Sarcoplasmic Reticulum Calcium Stores to TRPM4 and BKCa Channel Activit

1.52 **Abid Kazi,** Deptor Knockdown Enhances mTOR Activity and Protein Synthesis in C2C12 Myocytes

PROTEIN COMPLEXES

1.53 **Shireen Sarraf,** Proteomic Identification of Substrates of the E3 Ligase, Parkin, Essential in Mitophagy

PROTEIN STRUCTURE/FUNCTION

1.54 **Chad Brocker,** Characterization of ALDH7A1 and the Potential Mechanisms Underlying Pyridoxine-Dependent Epilepsy

1.55 **Zhiyun Ge,** Lon Protease: Mechanism of Substrate Recognition and Degradation

1.56 **Blake Hildreth III,** The Mid-Region, NLS, and C-terminus of PTHrP Influence Murine MSC Differentiation

1.57 **Katarzyna Walkiewicz,** Correlating Changes in Structure and Function of Bacteroides thetaiotaomicron TetX2 to Fitness during Adaptation

PUBLIC HEALTH/GLOBAL HEALTH

1.58 **Ashley Hazel,** Understanding the Ecology of Sexually Transmitted Disease (STD) Risk Among African Pastoralists

REGENERATIVE MEDICINE/TISSUE ENGINEERING

1.59 **Zhao Huang,** Functionalizing and Patterning Extensible Protein-based Materials for Use as Tissue Engineering Scaffolds

1.60 **Balendu Jha,** Employment of Structural and Biochemical Cues for Enhanced Peripheral Nerve Regeneration

1.61 **Eileen Pedraza,** Sustained, Controlled Delivery of Oxygen from Hydrolytically Activated Silicone Scaffolds

1.62 **Talar Tokatlian,** Engineering Cell-triggered Release from Hydrogel Scaffolds

SOCIAL/BEHAVIORAL SCIENCE

1.63 **Akiko Mizuno,** The Neural Basis of "Anchoring-and-Adjustment" during Mentalizing of Personality Traits in Autism

1.64 **Kelly Young-Wolff,** The Interactive Effects of Distal and Proximal Stressors on Alcohol Consumption among Adults

STEM CELLS

1.65 **Katie Russell,** High-capacity Assay to Quantify the Clonal Heterogeneity in Potency of Mesenchymal Stem Cells

1.66 **Robyn Sussman,** The Role of USP22 in Pluripotency and Differentiation

1.67 **Rebecca Thomason,** Investigating an Interchangeable Potential between Heart and Gut Mesothelium in the Embryo

STRUCTURAL BIOLOGY

1.68 **Gemma Comellas,** Structural Studies of Alpha-Synuclein Fibrils and the Lipid Catalyzed Fibrillation Pathway

1.69 **Lisa McMath,** Characterizing Iron Storage Proteins from Mycobacterium tuberculosis

1.70 **Alex Vecchio,** Structure-Function Elucidation of Selective Substrate Binding and Catalysis by Cyclooxygenase-2

TOXICOLOGY/ENVIRONMENTAL HEALTH

1.71 **Azita Cuevas,** Mechanisms of Microvascular Dysfunction in a Murine Model: The Role of Particulate Matter Composition

1.72 **Carmine Leggett,** Identification and Characterization of Novel Arylamine N-Acetyltransferase Small Molecule Inhibitors

1.73 **Mariya Liyasova,** Blood Test for Toxins in Jet Airplane: Mass Spectrometry to Identify Exposure to Tricresyl Phosphate

1.74 **Bingxuan Wang,** The Complex Regulation of MnSOD Expression by the AhR Agonist PCB126

ABSTRACTS - SESSION 1

BIOMEDICAL ENGINEERING/IMAGING

1.1 **Light-Activated Cancer Therapy with Biomimetic Multifunctional Anti-EGFR Bioplasmonic Nanoparticles**

Kvar C. Black, Phillip B. Messersmith, Biomedical Engineering, Northwestern University

Epidermal growth factor receptor (EGFR) upregulation is present in multiple types of solid tumors including those of the breast and oral cavity, and they are attractive cell-surface targets for intervention. Anti-EGFR antibody therapy is an important clinical approach to provide targeted therapeutics to tumors, however a significant percentage of cancers still become resistant to these treatments, leading to even more invasive and fatal disease. Therefore, more robust strategies that are able to monitor and modulate tumor progression and treatment response with multifunctional agents are needed. Due to their sub-cellular size, targeted metal nanoparticles offer both diagnostic and thermal therapeutic potential in bioimaging modalities at the cellular level. However to fully realize their capability against cancer, a versatile chemistry to form multifunctional nanoparticles is needed. Catechols are employed in nature for diverse functions including adhesion, photoprotection, and neuromodulation, and have a versatile chemical repertoire that includes hydrogen bonding, coordination to metals, p-stacking with aromatics, and redox activity, and therefore offer a robust biomimetic toolbox to assemble multifunctional nanomaterials. In this study, polydopamine-based metal nanocrystals of controlled sizes, shapes, and compositions were formed to tune the plasmons throughout the NIR and visible portions of the electromagnetic spectrum. Covalent conjugation chemistry was employed to react molecules including polyethylene glycol (PEG) polymers and anti-EGFR antibodies onto metal nanoparticle surfaces. Finally, EGFR-expressing cells targeted with biomimetic nanoparticles were imaged and photothermally ablated with light in optical systems.

1.2 **Albumin Binding Peptide Conjugate for Near-Infrared Fluorescence Lymphatic Imaging**

Cynthia A. Davies-Venn, Center For Molecular Imaging, University of Texas Health Science Center Houston, Baylor College of Medicine; Bonnie Angermiller, Nathaniel Wilganowski, Pradip Ghosh, Center For Molecular Imaging, University of Texas Health Science Center Houston; Barrett R. Harvey, Center for Immunology and Autoimmune Diseases, University of Texas Health Science Center Houston; Grace Wu, Eva M. Sevick-Muraca, Center For Molecular Imaging, University of Texas Health Science Center Houston

Objectives: The aim of this study was to develop and characterize a novel peptide imaging agent for non-invasive near-infrared fluorescence imaging of the lymphatic system. Our imaging agent consists of a cyclic albumin binding domain (cABD) peptide, RLIEDICLPRWGCLWEDDK, conjugated to a bright near-infrared fluorophore, IRDye800CW, allowing for enhanced vascular retention and signal. Methods: cABD-IRDye00 was synthesized using solution conjugation followed by HPLC purification. Characterization of the peptide conjugate was performed using mass spectrometry to confirm the molecular weight, fluorescence spectroscopy to assess optical properties, serum binding assays and SDS-Page to determine binding affinity and specificity, and fluorescence imaging of C57BL/6 mice to monitor lymphatic uptake and retention. Results: cABD-IRDye800 showed a greater fluorescent yield than commonly used ICG, a high affinity and specificity for albumin with IC50 and Kd in nanomolar range and superior retention characteristics within mouse lymphatics when compared to IRDye800. Based on its favorable characteristics for lymphatic imaging, cABD-IRDye800 was further used to show a lack of lymphatic vessels in the inguinal region of a VEGFR3 +/- mouse model of Milroy's lymphedema. These findings were subsequently confirmed by intravital imaging which showed a lack of dye uptake into the lymphatic vessels of VEGFR3 mice when compared to wild type C3H mice. Conclusion: cABD-IRDye800 has utility for assessing lymphatic function in mouse models of human lymphatic disease and the potential for use in clinical diagnostic imaging of lymphatic vasculature. [Funding Sources: Diagnostic Cancer Imaging using NIR Fluorescent Agents and FDPM, NCI-R01 CA112679 and the HHMI Med into Grad Initiative.]

1.3 **Bioengineering Approach to Understanding Temporomandibular Joint Pathobiology**

Jonathan Y. Kuo, Hai Yao, Clemson University

Temporomandibular joint (TMJ) disorders are a national health problem affecting more than 10 million Americans. It is believed that pathological mechanical loading of the TMJ disc may trigger a cascade of molecular events leading to degeneration, but this process is poorly understood. We hypothesize that sustained mechanical loading affects the nutrient transport and environment of the disc resulting in changes to metabolism, composition, and function associated with disc degeneration. Therefore, our goal was to characterize disc properties in an animal model to translate techniques for human diagnostic applications. Porcine disc mechanical and biochemical properties were characterized through a variety of novel techniques combining biological and engineering principles. The relationship of strain and nutrient transport (O2 and glucose) were examined via diffusion and conductivity to better understand nutrient pathways. These studies were combined with experiments measuring consumption rates to reveal the relationship between nutrient environment and metabolism. Our studies demonstrated that proliferation, ATP production, and matrix synthesis were affected by availability of nutrients. Transport experiments revealed that increases in compressive strain significantly impeded solute transport. Oxygen and glucose consumption rates were highly substrate dependent and significantly higher than in other cartilage. The results of these studies suggest that high nutrient consumption coupled with slow transport makes the disc susceptible to steep nutrient gradients caused by abnormal mechanical loading. By examining disc properties via predictive 3D models, this research provides translational applications in human diagnostics and potential tissue regeneration applications. [NIH T32DE017551 and F31DE020230]

1.4 **Effects of Simvastatin Treatment on 2-D and 3-D In Vitro Models of Heart Valve Disease**

Elyssa L. Monzack, Kristyn S. Masters, University of Wisconsin-Madison

Purpose: Statins (3-HMG-CoA reductase inhibitors), currently the nation's most prescribed drug, are used to lower cholesterol. However, pleiotropic effects show potential for ameliorating calcific aortic valvular disease, a prevalent clinical problem. Clinical studies have yielded conflicting results regarding the statin-valve relationship. This work examines statins' effects on in vitro valve disease models, providing mechanistic insight throughout disease progression. Methods: For 2-D studies, valvular interstitial cells (VICs) were cultured on multiple substrates over a variety of time courses in culture conditions that included +/- simvastatin, +/- TGF-b1, and +/- osteogenic stimuli. In 3-D work, mechanical stimuli were introduced via VIC encapsulation in tensioned collagen gels or via static and dynamic culture of whole leaflets in a bioreactor (15% strain, 60 Hz), in either untreated or statin-treated medium. Results: Our 2-D disease model showed that while simvastatin did not have a preventive effect, it did exhibit both inhibitory and disease-regressive actions, which were substrate-dependent. Additionally, the reduction in calcific nodules correlated with a reduction in Rho kinase. Simvastatin's effects in 2-D were mirrored in 3-D with inhibition of contraction of the VIC-encapsulated collagen gels. Gene expression results were more nuanced, with statin treatment resulting in the elevation of different disease markers depending on the culture environment. Conclusions: While initial work in 2-D indicated that simvastatin may have a net benefit to heart valve disease, leaflet cultures and gene expression analyses revealed a more complex picture involving a decrease in some disease markers, but an increase in others. [NIH/NHLBI, K. Masters, 1R01 HL093281]

1.5 **Human Retinal Capillaries Are Disrupted Before the Onset of Diabetic Retinopathy**

Johnny Tam[1], Kavita P. Dhamdhere [2], Pavan Tiruveedhula [2], Silvestre Manzanera [2], Shirin Barez [2], Marcus A. Bearse, Jr. [2], Anthony J. Adams [2], Austin Roorda [1,2] [1] Joint Graduate Group in Bioengineering, UC Berkeley and UCSF [2] School of Optometry, UC Berkeley

Purpose. To establish, using noninvasive imaging, that specific capillary channels in the human retina are affected in diabetes, even before the onset of clinical changes. Methods. Detailed images of retinal capillaries were generated noninvasively in human subjects with Type 2 diabetes and no diabetic retinopathy (DR). Videos of capillary flow were acquired using an adaptive optics scanning laser ophthalmoscope (AOSLO). By applying motion contrast enhancement algorithms to AOSLO videos, images of the parafoveal capillary network were generated without using injected contrast agents. Within the parafoveal capillary network, arterio-venous (AV) channels directly connect pre-capillary arterioles to post-capillary venules. We identified the location of these channels and quantified tortuosity. Measures of capillary dropout and leukocyte flow were also calculated in order to explore the relationship between early microvascular changes. Results. The tortuousity of AV channels was significantly increased in the patients with Type 2 diabetes and no DR ($p<0.05$). No signs of DR were present in these patients, as confirmed by grading of fundus photographs by a retina specialist. Although some evidence of capillary dropout and leukocyte flow retardation were observed, differences were not statistically significant. These results are consistent with prior studies, which have had difficulty establishing statistically significant changes in the microcirculation prior to the onset of diabetic retinopathy. Conclusion. Although it is often difficult to find consistent changes in the microcirculation due to large intersubject variability, AOSLO images show that one of the earliest signs of diabetes in the retina is disruption of AV channels, occurring even before the onset of DR.

1.6 **Image quality tradeoffs in a model breast tomosynthesis system: angular span and angular sampling scheme**

Stefano Young; University of Arizona Predrag R. Bakic; University of Pennsylvania Robert J. Jennings, Kyle J. Myers, and Subok Park; FDA Division of Imaging and Applied Mathematics

Digital breast tomosynthesis (DBT) promises to improve breast cancer screening by removing anatomical masking effects seen in mammography. It is difficult, however, to quantify the improvement offered by DBT and to objectively compare different DBT prototype systems. Because clinical DBT studies are costly and the patient population is often limited, it is useful to develop simulation tools to aid in quantitative DBT system evaluation and optimization. Effective optimization requires a rigorous, task-based approach to assessing image quality. In this paper, we present a new simulation framework for task-based image quality assessment in DBT. We also demonstrate the utility of this framework in determining the optimal x-ray source angular span and angular sampling scheme for detecting a small cancerous mass embedded in randomly-varying anthropomorphic phantoms. Our new framework has advantages over existing approaches to DBT assessment, particularly in the large anthropomorphic phantom ensemble, the handling of multiprojection correlation information, and the ability to exploit powerful photon-electron transport routines. In addition, we implemented covariance decomposition as a method for accelerating the assessment and optimization process.

BIOPHYSICS

1.7 Leukocyte Transmigration is Mediated by Endothelial Cell Contractile Forces and Substrate Stiffness

Kimberly M. Stroka, Helim Aranda-Espinoza, University of Maryland College Park

Leukocyte transmigration through the vascular endothelium is a crucial step in the immune response. However, in the cardiovascular disease of atherosclerosis, an excess of leukocytes adhere to and transmigrate through the endothelium, and progression of this disease is associated with arterial stiffening. Little is known about how these biomechanical changes in the vascular microenvironment affect leukocyte-endothelial cell (EC) interactions. In this study we investigated the biophysics of leukocyte transmigration in an in vitro model of the vascular endothelium. We modeled healthy versus diseased blood vessels through manipulation of substrate stiffness using polyacrylamide gels, coated with extracellular matrix protein and plated with human umbilical vein EC (HUVEC) monolayers. HUVECs were treated with tumor necrosis factor-alpha to induce an inflammatory response. We observed that leukocyte transmigration through HUVEC monolayers increased with matrix stiffness below the endothelium, and we hypothesized that matrix stiffness changed the biophysical properties of the endothelium to produce this effect. We found that (1) inhibition of myosin light chain kinase (MLCK)-dependent EC contraction reduced transmigration on stiff, but not soft, substrates, and (2) transmigration led to EC retraction and large hole formation in monolayers on stiff substrates, while the endothelium on soft substrates remained intact. Together, these results provide strong evidence that the mechanical properties of the sub-endothelial matrix regulate MLCK-mediated EC contractile forces during leukocyte transmigration. These results also suggest that the biomechanical changes that take place in vasculature during CVD are important regulators of the immune response.

CHEMISTRY

1.8 Synthetic, Theoretical, and Clinical Studies of Cannabinoid Analogues for CNS Disorders

Angie Garcia, Dan Borchardt, PhD, Chia-En Chang, PhD, Michael Marsella, PhD, University of California, Riverside; Milan Fiala, MD, Mathew Mizwicki, PhD, University of California, Los Angeles

With the discovery of cannabinoid receptors, interest in the endocannabinoid system for its therapeutic potential has grown. Focusing on CNS disorders, we have designed and synthesized compounds active against amyotrophic lateral sclerosis (ALS), a neurodegenerative disease, affecting the brain and spinal cord, with poor effective treatment. Our aim has included three integrated projects: i) to design novel cannabinoid ligands to test against SOD-1 stimulated macrophages of ALS patients (in collaboration with Dr. Milan Fiala, MD at UCLA's department of medicine; ii) to investigate the thermal isomerization of cannabinoid analogues via a tandem Knoevenagel – Diels-Alder cascade reaction. This work builds on previous contributions by Tietze, and was reported in our 2009 JACS paper. Our interest in thermal isomerization stems from a report that found a significantly higher observation of withdrawal symptoms in cannabis smokers versus those who were administered equivalent dosages of cannabinoids orally, after abruptly quitting. One hypothesis targets unnatural cannabinoid isomers as the origin of withdrawal symptoms. In collaboration with Dr. Chia-En Chang (UCR), computational docking analysis found little difference in CB1 receptor affinity among natural and unnatural THC isomers. iii) to develop a computational program that compiles many attributes of FDA approved drugs, and attempts to classify drugs by indication rather than affinity. Preliminary data has focused on a pool of ca. 300 drugs, and results to date are promising and non-intuitive with respect to which attributes are weighed most heavily. In summary, these projects have focused specifically on issues associated with ALS, but are intended to be more globally applicable. [Research funded by UC grant.]

1.9 **Biomimetic Manganese Porphyrins Catalyze the Rapid Generation of Chlorine Dioxide from Chlorite**

Thomas P. Umile, John T. Groves, Princeton University

Anthropogenic chlorite ion is consumed by a variety of heme-containing enzymes, affording a range of products including chlorate, chlorine dioxide (ClO2), and molecular oxygen (O2). The molecular mechanisms of these differing transformations are important to the understanding of hemoprotein reactivity as well as the development of catalysts for the synthesis of the disinfectant ClO2. We have used synthetic, water-soluble manganese porphyrins as biomimetic models and discovered a dramatic, catalytic generation of ClO2 gas from chlorite ion under ambient conditions. The mechanism of ClO2 formation was investigated by rapid-mixing, stopped-flow spectrometry. Products were quantified by UV-Vis spectroscopy, HPLC-ion chromatography, and iodometry. Chlorite reacts with the Mn(III) porphyrin in a rate-determining step to generate high-valent oxoMn(V) and hypochlorous acid (HOCl). HOCl can further, reversibly oxidize Mn(III) to oxoMn(V), and oxoMn(V) oxidizes two chlorite ions to two ClO2 molecules in sequential steps. More electron-deficient porphyrins are the fastest, most efficient catalysts (ca. 500 turnovers, 0.5 TO/s), presumably by facilitating formation of the oxoMn(V) intermediate. By a slower Mn-catalyzed pathway, ClO2 is converted to chlorate, although the ClO2 can be effectively isolated via helium sparging (ca. 60% yield). Computer simulation of the mechanism using experimentally-measured rate constants was in agreement with spectroscopically-measured ClO2 production and decay. We anticipate that an understanding of this mechanism will allow us to develop related catalysts as O2-evolving systems. In addition, the reported system represents an efficient, catalytic alternative for safely and cleanly generating the industrial oxidant and disinfectant ClO2. [NSF, DOE]

MEMBRANES

1.10 **Reconstitution of the Minimal Mitochondrial Fission Machinery in Yeast and Humans**

Sajjan Koirala, Qian Guo, Janet M. Shaw Department of Biochemistry, School of Medicine, University of Utah, Salt Lake City, UT 84112

Mitochondrial fission regulates organelle copy number and mitochondrial function in metabolism, development and apoptosis. The dynamin-related GTPases Dnm1 and Drp1 are required for mitochondrial fission in yeast and human, respectively. Dnm1/Drp1 assembles into spirals that encircle and divide mitochondria. In yeast, a tail-anchored protein called Fis1 and an adaptor protein called Mdv1 recruit Dnm1 to the membrane, where Mdv1 co-assembles with Dnm1 and stimulates Dnm1 GTPase activity. Whether Fis1 participates in Dnm1 assembly or post-assembly membrane scission events is unknown. Although Fis1 is conserved in humans (hFis1), an Mdv1 homolog is absent. Instead, humans have at least two different membrane anchored proteins (hFis1 and hMff) whose roles in fission are poorly defined. Using yeast cells expressing only Dnm1 and a mitochondrial-tethered form of Mdv1, we demonstrated that Fis1 is dispensable for Dnm1 recruitment, assembly, and mitochondrial membrane scission in vivo. Importantly, the absence of Fis1 did not affect distribution of fission complexes on the mitochondrial membrane. Thus, Fis1 acts to direct essential fission components to the correct cellular membrane but does not determine the site of complex assembly. Similar tethering studies reveal that hDrp1 and hMff (but not hFis1) are sufficient to reconstitute mitochondrial fission in yeast. We postulate that the hMff protein combines the hDrp1 GTPase recruitment and effector functions that are carried out separately by Fis1 and Mdv1 in yeast. To test this model, we are using biochemical methods to determine how the cytoplasmic domain of hMff affects the assembly and GTPase activities of hDrp1. Our findings suggest that the conserved role(s) of Fis1 in yeast and human is yet to be identified. [Funding: NIHR01GM53466 to Janet M. Shaw]

1.11 **PI-4-KINASES at the lysosome: PI4KIIIß is a novel regulator of lysosomal membrane proteins**

Sunandini Sridhar, Dennis Shields and Ana Maria Cuervo, Albert Einstein College of Medicine

PI-4-Kinase isoform PI4KIIIß plays a key role in maintaining the structure and function of the Golgi apparatus. Moreover, the PI4KIIIß yeast ortholog Pik1 has also been implicated in maintainence of vacuole morphology.The goal of our work is to determine whether a lysosomal function for PI4KIIIß is conserved in mammals and to characterize its contribution in the interplay of Golgi to lysosome trafficking. Immunoblot and immunoflourescence analysis of subcellular fractions revealed that PI4KIIIß associates to lysosomes but it does not undergo degradation in this compartment under normal nutritional conditions.Analysis of compartment-specific isoforms of PI4KIIIß by bidimensional electrophoresis demonstrated a clear distinction between the Golgi, cytosolic and lysosome-associated populations of this protein.The lysosomal variant of PI4KIIIß binds to the cytosolic side of the lysosomal membrane in a dynamic manner.Analysis of cell lines stably knocked-down for PI4KIIIß, revealed that lysosomal biogenesis is not affected globally as cathepsins and other lysosomal components delivered via the manose-6-phosphate pathway still reach this compartment normally.However, we found a marked reduction in levels of lysosome-associated membrane proteins (LAMPs) in lysosomes isolated from PI4KIIIß knock-down cells. Changes in LAMPs levels did not result from reduced stability of these proteins at the lysosomal membrane, but rather from their mis-stargeting to abnormal large vesicular cytosolic compartments.Our current efforts are directed towards characterizing this novel function of PI4KIIIß in Golgi to lysosomal traffic of LAMPs and the mechanisms that regulate this process. [Work supported by NIH/NIA grant AG-021904, Ellison Medical Foundation Award and Training Program in Cellular and Molecular Biology

MMP/ECM/CYTOSKELETON

1.12 **Orientation of Mammary Epithelium is Determined by Stromal Collagen during Branching Morphogenesis**

Doug G. Brownfield, University of California, Berkeley

The mammary epithelium has a unique, arboreal architecture resulting from the dynamic and reciprocal interaction between cellular and extracellular components. Whereas individual aspects of mammary gland structure have been studied in detail (invasion into the fat pad, side branching, lumen formation, and involution among others), few have focused on mechanisms governing epithelial architecture as a whole. Here we show that during branching morphogenesis (3-8 weeks after birth), the orientation of the mouse mammary epithelial branches is dramatically enhanced along a single axis. Furthermore, we observed that areas of aligned collagen fibers distal to the epithelium orient along that same axis in 3-week old fat pads, well before significant ductal elongation occurs. Using a novel three-dimensional branching model in a collagen matrix, we found that pre-alignment of the collagen fibrils had a dominant effect on branch orientation. Modulation of RhoA activity demonstrated that while necessary for contraction-mediated alignment, RhoA is dispensable for "sensing" collagen alignment. Current work is focused on identifying factors critical for alignment-sensing during branching morphogenesis and to further probe the relationship between collagen organization and epithelial orientation using mouse strains with mutations in either Col1a1 or Col1a2. Taken together, this work demonstrates that architecture of the mammary epithelium is at least partially pre-determined by the organization of stromal collagen within the fat pad and that the mechanism(s) for alignment-sensing act independently of those used for generation of alignment [Funding from the National Cancer Institute and National Science Foundation Graduate Research Fellowship Program].

1.13 **Tetraspanin18 Restricts Neural Crest Migration by Modulating Cadherin6B mRNA and Protein Levels**

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Unlike typical neuroepithelial cells in the developing central nervous system, neural crest cells undergo an epithelial to mesenchymal transition (EMT), detach from the neural tube, and migrate great distances to give rise to diverse structures, such as the peripheral nervous system, melanocytes and craniofacial skeleton. The mechanisms that regulate neural crest EMT remain incompletely understood. Tetraspanin 18 (Tspan18) is a member of the tetraspanin family of transmembrane proteins that have been implicated in cell signaling, motility and adhesion. Tspan18 is abundantly and specifically expressed in chick premigratory neural crest, but is down-regulated when neural crest cells begin to migrate, suggesting that Tspan18 may negatively regulate neural crest migration. Interestingly, mRNA and protein levels of the known neural crest EMT regulator Cadherin6B (Cad6B) decrease following Tspan18 knock-down, while neural crest migration is inconsistently enhanced. In contrast, Tspan18 overexpression impedes neural crest migration, which preliminary results suggest is due in part to Cad6B stabilization. Consistent with the fact that the neural crest transcription factor FoxD3 regulates neural crest cell-cell adhesion and promotes migration, FoxD3 represses Tspan18 expression. Taken together, these data suggest that Tspan18, as a readout of FoxD3, plays an important role in restricting neural crest migration by affecting levels of Cad6B. Experiments are in progress to identify proteins that interact with Tspan18 to gain insight into how dynamic interactions at the neural crest cell surface impact migration, and may provide clues to the mechanisms of EMT during metastasis. [Supported by NIH F31 GM087951 and a U of MN Grant in Aid.]

1.14 **Biochemical Analysis of the Mammalian Formin FMNL3**

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Actin filaments are integral to a large number of cellular structures. Due to this widespread use, cells must rigorously control when and where new actin filaments assemble. Nucleation of new filaments is a major regulatory step, and nucleation factors such as formin proteins act at this step. The Higgs laboratory has studied the 15 mammalian formin isoforms extensively, due to their potential to regulate assembly of many actin-based structures. FMNL3 (also called FRL2) is of interest because of its ability to assemble filopodia when transfected into multiple cell types. These finger-like protrusions are critical to specific types of cell motility. All formins contain a dimeric FH2 domain, which accelerates actin nucleation and also regulates barbed end elongation. In addition, a group of formins, including FMNL3, cause filaments to bundle into structures resembling those found in filopodia. While an FH2 construct is sufficient to bundle actin filaments, I demonstrate that FMNL3 is capable of severing actin filaments when the last 74 residues of its C-terminus are included. This construct (FH2-C) accelerates actin polymerization to a much greater extent than constructs containing only the FH1-FH2 domains or the FH2 alone, possibly due to barbed end production by filament severing. Additionally, the C-terminus interacts with actin monomers and inhibits polymerization and barbed end elongation, independent of FH2- barbed end binding. Lastly, we have identified two residues (L970 & I971) within a WH2-like motif of the FMNL3 C-terminus that are required for monomer interaction, as well as inhibition of polymerization and barbed end elongation.

1.15 **ASB2a Mediates Filamin Degradation via Interaction with the Actin Binding Domain of Filamin**

Ziba Razinia, Massimiliano Baldassarre, David A. Calderwood, Yale University School of Medicine

Filamins are an important family of actin-binding and crosslinking proteins that mediate remodeling of the actin cytoskeleton and maintain extracellular matrix connections by anchoring transmembrane proteins such as integrin adhesion receptors to actin filaments and intracellular signaling cascades. In this way, filamins mediate matrix-cytoskeletal signaling pathways via their actin-binding and integrin-binding functions and regulate re-organization of the actin cytoskeleton and cell migration. We recently found that filamins are targeted to proteasomal degradation by the E3 ubiquitin ligase specificity subunit ASBa and that acute degradation of filamins via this ubiquitin-proteasome pathway correlates with cell differentiation. Specifically, in myeloid leukemia cells retinoic acid-induced expression of ASB2a triggers filamin degradation and recapitulates early events critical for cell differentiation. ASB2a is thought to link substrates to the ubiquitin-transferase machinery, however, the mechanism by which ASB2a interacts with filamin to induce degradation remained unknown. Here, we use cell-based and biochemical assays to show that ASB2a sub-cellular localization to actin-rich structures is dependent on filamin and that the actin-binding domain (ABD) of filamin mediates interaction with ASB2a. Furthermore, we show that the ABD is necessary and sufficient for ASB2a-mediated filamin degradation. We propose that ASB2a exerts its effect by binding the ABD and mediating its polyubiquitination, so targeting filamins for degradation. These studies provide the molecular basis for ASB2a-mediated filamin degradation and unravel an important mechanism by which filamin levels can be acutely regulated. [This work was supported by funding from the NIH and AHA].

MODELING/COMPUTATIONAL BIOLOGY/INFORMATICS

1.16 **Evaluating and comparing breast cancer risk projections for Hispanic and non-Hispanic white women**

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Background: The National Cancer Institute Breast Cancer Risk Assessment Tool (BCRAT) has not been validated for Hispanic women. We evaluated BCRAT risk projections for Hispanic and non-Hispanic white (NHW) participants in the Women's Health Initiative (WHI). Methods: Data on 6,353 Hispanic and 128,976 NHW postmenopausal WHI participants aged 50 and older were used to calculate absolute risks of developing invasive breast cancer over the WHI Main and Extension Study periods, using the BCRAT and an Updated BCRAT with 1993-2007 SEER breast cancer incidence rates. We assessed model calibration by the observed-to-expected (O/E) ratio of number of breast cancers and discriminatory accuracy with the concordance statistic (AUC). Results: In the WHI Main Study, the BCRAT underestimated the number of invasive breast cancers in both Hispanics and NHWs by 18%. However, underestimation diminished in the Updated BCRAT. The BCRAT substantially overestimated breast cancer risk during the WHI Extension Study. The BCRAT had modest discriminatory power to predict breast cancer in Hispanic (AUC=0.63) and NHW women (AUC=0.58). For several risks factors, the magnitude of association significantly differed between WHI participants and that used by the BCRAT; this included age at first birth, number of breast biopsies, and family history. Conclusions: Although the BCRAT is reasonably well calibrated for prediction of invasive breast cancer in postmenopausal US Hispanics aged 50 and older, the Updated BCRAT performed better. Ultimately, a model unique to US Hispanic women is warranted to account for risk factors distinct to this population.

1.17 **Dynamic Coupling within NBD1 Influences Thermostability and Function of CFTR**

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Cystic fibrosis (CF) is caused by mutations in the gene encoding a chloride-ion channel called cystic fibrosis transmembrane conductance regulator (CFTR). Deletion of residue Phe508 in the first nucleotide-binding domain (NBD1) of CFTR (DelF508-CFTR) leads to misassembly and consequent degradation of DelF508-CFTR by cellular quality control systems in most CF patients. NBD1 contains a unique 32-residue segment termed the regulatory insertion (RI), which is absent in other ATP-binding cassette (ABC) transporters. We have recently reported that complete removal of RI enables cell surface expression of DelF508-CFTR, where it mediates regulated anion efflux and exhibits robust single chloride channel activity unattainable via partial removal of RI (Aleksandrov et al., J. Mol. Biol. 2010). Structural studies of the RI have been complicated due to its intrinsic flexibility. To elucidate the structural role of RI, we used discrete molecular dynamics (DMD) simulations and have identified allosteric coupling between RI and other regions of NBD1. Specifically, RI influences the dynamics of F508-loop and another critical loop (I539-loop) frequently modified to achieve DelF508-CFTR maturation. Our computational results also indicate that RI influences coupling of the F508-loop with the F1-like ATP-binding core subdomain of NBD1, so that RI removal overcomes the perturbations due to F508 deletion. Based on our results, we conclude that restriction of the conformational freedom of RI may ameliorate the thermostability of DelF508-CFTR, thereby decreasing the impact of the disease. We are currently conducting in vitro studies to investigate the consequences of perturbation of allosteric coupling on maturation and function of DelF508-CFTR. [Funding: NIH, CF Foundation]

1.18 **Chromatin States Accurately Predict Cell Differentiation Status**

Jessica L. Larson, Guocheng Yuan, Harvard University, and Dana-Farber Cancer Institute

Gene expression is controlled by the concerted interactions between transcription factors and chromatin regulators. While recent studies have identified global chromatin state changes across cell-types, it remains unclear to what extent these changes are regulated coordinately during cell-differentiation. Here we present a comprehensive computational analysis by assembling a large dataset containing genome-wide occupancy information of 5 histone modifications in 27 human cell lines (including 24 normal and 3 cancer cell lines) from the literature, followed by independent analysis at four different levels, corresponding to raw counts (bin), peak, gene, and multi-gene domain levels, respectively. We first analyzed the normal cell data, and found that chromatin states at any level can predict the differentiation status with nearly 100% accuracy. We identified 370 genes whose chromatin state diverges significantly across differentiation status. These "hotspot" genes are enriched with multiple developmental and cell signalling pathways and associated with multiple diseases. We found that the regulation of chromatin state changes is spatially coordinated. Chromatin domains are enriched with both "hotspot" genes and highly specialized gene clusters. For the latter, the domain pattern was persistent in nearly all cell types. We then applied our prediction model to analyze the cancer cell data. We found that the chromatin states in cancer cells were distinct from both embryonic and adult stem cells, and that the differences are in good agreement with a recently proposed three-module model. Taken together, our results have provided new insights into the role of chromatin state remodeling in development and diseases.

1.19 **LiCABEDS, a Novel Ligand Classification Algorithm for in-Silico Drug Design**

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Ligand classification, a technique that can be used for ligand screening and molecular property prediction, is an important and challenging field in virtual drug design and discovery. A successful predictive model may help researchers to understand the essential ligand-protein interaction and guide chemical modification in order to achieve better binding profile. However, reliable and robust algorithms that are widely applicable for ligand activity or property prediction are still under development. Thus, we have proposed a novel algorithm, Ligand Classifier of Adaptively Boosting Ensemble Decision Stumps (LiCABEDS), for virtual screening and quantitative structure-property relationship based on molecular fingerprints. In the recent publication, the performance of LiCABEDS was demonstrated through modeling ligand functionality (agonist or antagonist) for 5-HT subtype receptors. The algorithm was tested on four types of molecular fingerprint, and it achieved average 90.1% prediction accuracy on Molprint2D fingerprint. Software package with graphical user interface was developed to make the technology accessible to the scientific community. Furthermore, LiCABEDS was tested to model blood-brain-barrier passage on a dataset published by Zhao et al, with average prediction accuracy 96.4%. Currently I am applying LiCABEDS to investigate ligand selectivity for Cannabinoid receptor subtypes, aiming at separating selective ligands from unselective ones. Testing calculation showed that average 63% purity and 62% recovery rate were achieved for screening target-selective ligands from unselective ones. Further improvement was observed after feature selection and cross-validation.

NEUROBIOLOGY – COGNITION AND BEHAVIOR

1.20 **AVP Infusion into the Brains of Male Rats Rescues Progesterone Induced Impairment in Social Behavior**

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It is well established that social recognition is mediated by argenine vasopressin (AVP) produced by cells within the bed nucleus of the stria terminalis (BST) and medial amygdala (MeA); these cells send AVP projections to the lateral septum (LS). We have previously shown that progesterone (P) treatment impairs social discrimination, through its action on progestin receptors that are present in virtually all AVP cells in the BST and MeA. It has also been demonstrated that P treatment decreases AVP immunoreactivity in the BST and MeA; it could be this decrease in AVP that leads to impaired social recognition following P treatment. In the present experiment, we hypothesized that infusions of AVP into the LS would rescue the P induced impairment of social recognition in adult male rats. One week after adult male rats underwent cannulation surgery, they were given systemic injections of either a physiological dose of P or oil control. Social recognition testing was performed using the social discrimination paradigm. Immediately after the first exposure to a juvenile, each animal received bilateral infusions of either AVP or artificial CSF (aCSF) into the LS. Our results show that, as expected, control animals who received either oil and aCSF or oil and AVP exhibited normal social discrimination. In corroboration with previous experimental results from our lab, animals given P and aCSF showed impaired social discrimination. Interestingly, animals treated with P and AVP exhibited normal social discrimination, suggesting that AVP treatment rescued the impairment in social recognition caused by P. These data are exciting because they illustrate a mechanism by which physiological doses of P, acting on the AVP system, may have behaviorally relevant effects in male rats. [UW-Madison Psychology Department and the National Research Service Award (NRSA) T32 Gm007507]

1.21 **The Role of Mesopontine Acetylcholine in Methamphetamine Behavioral and Neurochemical Responses**

Lauren K. Dobbs, Gregory P. Mark, Oregon Health & Science University

Dopamine (DA) neuron activity and release within the nucleus accumbens (NAc) are thought to underlie the rewarding effects of methamphetamine (MA). Acetylcholine (ACh) in the ventral tegmental area (VTA) is also rewarding and capable of increasing terminal DA release. However, the effects of MA on the cholinergic system remain poorly characterized. We first tested the effect of MA on VTA ACh and NAc DA levels through a series of in vivo microdialysis studies in male C57BL/6J mice. We found that MA perfused directly through the VTA or administered systemically significantly increased DA levels within the VTA; however, only systemic MA induced a significant and prolonged increase in VTA ACh levels. The source of ACh in the VTA originates in the laterodorsal tegmental (LDT) and pedunculopontine tegmental (PPT) nuclei. Our next experiments sought to discriminate the contributions of the LDT and PPT to the prolonged MA-induced VTA ACh levels. To do this, we reversibly inhibited ACh neurons in the LDT or PPT with bilateral microinjections of the M2-preferring receptor agonist oxotremorine (OXO) and measured MA-induced changes in ACh and DA using in vivo microdialysis. Microinjection of OXO into the LDT, but not PPT, dose-dependently attenuated the MA-induced increase in VTA ACh levels. There was no effect of intra-LDT or PPT OXO on DA levels within the NAc. The last experiment investigated the role of LDT ACh in MA-induced locomotor activity, a behavior that may be related to MA's addictive potential. MA- and saline-induced locomotor activity was significantly inhibited by high-dose intra-LDT OXO. We conclude that MA acts upstream of the VTA, to specifically activate LDT, and not PPT, cholinergic neurons and that this may contribute to MA's rewarding or stimulatory effects.

1.22 **Role of HCN Channels in D1 Receptor Stimulation and Stress-Induced Prefrontal Cortical Dysfunction**

Nao J. Gamo, Min Wang, Brian P. Ramos, Amy F.T. Arnsten, Department of Neurobiology, Yale University School of Medicine

Schizophrenia is associated with impaired prefrontal cortical (PFC) function and dopamine D1 receptor signaling in PFC (Abi-Dargham et al 2002). The PFC uses working memory to guide behavior, but is incredibly sensitive to stress. Spatial working memory is maintained by PFC pyramidal cell networks that connect at dendritic spines, which show persistent firing while a cue is maintained. Strength of these connections is dynamically modulated by intracellular events in spines. During stress, excess catecholamines in PFC generate cAMP in part via D1 stimulation. cAMP increases the open state of hyperpolarization-activated cyclic nucleotide gated (HCN) channels and weakens PFC function (Wang et al 2007). Using rat behavior and monkey physiology, we examined whether stress or D1 stimulation impairs PFC function via opening of HCN channels. First, rats were trained in a spatial working memory task. Injection of the pharmacological stressor, FG7142, or local infusion of the D1 agonist, SKF81297, impaired performance, while co-infusion of the HCN blocker, ZD7288, prevented this impairment. Second, monkeys were trained in an oculomotor spatial working memory task, and PFC single-unit recording was combined with drug iontophoresis. The D1 agonist, SKF38393, suppressed persistent firing of neurons, while co-application of ZD7288 prevented this inhibition. These findings showed that stress exposure or D1 stimulation reduced PFC function via opening of HCN channels. This mechanism is relevant to psychiatric disorders associated with dysregulated cAMP signaling, e.g. mutation in the DISC1 (Disrupted in Schizophrenia 1) gene (Millar et al 2005), which may impair PFC function via disinhibited cAMP signaling and opening of HCN channels. [R37 AG06036, PO MH MH068789, RL1 AA017536 to AFTA]

1.23 **Investigating the Learning Mechanism of a Video Game Task**

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Adults have difficulty learning non-native speech categories even with extensive training or years of foreign-language exposure, presenting an opportunity to study adult learning and plasticity by measuring change in non-native speech categorization. Long-term training within response-feedback paradigms has produced modest non-native speech learning. Feedback-based training has been found to engage reinforcement learning mechanisms as caudate activity is modulated by reward. However, explicit trial-by-trial feedback is far removed from the natural learning environment, limiting the ability to generalize to speech category learning mechanisms. In contrast, we exploit a novel, video-game-based categorization training approach involving rich correlations among multimodal cues and more closely modeling natural learning environments (Wade & Holt, 2005). Even without explicit feedback, this training evokes efficient learning of novel non-speech sound and non-native speech categories (Wade & Holt, 2005; Lim & Holt, in press). The current study aims to discover the neural basis of learning this efficient auditory category learning using fMRI. This research provides an opportunity to collect online measurements of the experiences that engage the basal ganglia and change category representations throughout training. We predict that the videogame generates an internal reinforcement signal by increasing the level of engagement in the striatum, which ultimately increases perceptual sensitivity and the attention that may improve speech category learning. [This project is supported by a grant from the NSF (DGE0549352) and an award (T32GM081760) from the National Institute of General Medical Sciences.]

1.24 **Distinctive Neural Processes during Learning in Autism**

Sarah E. Schipul, Carnegie Mellon University; Diane L. Williams, Carnegie Mellon University and Duquesne University; Timothy A. Keller, Carnegie Mellon University; Nancy J. Minshew, University of Pittsburgh; and Marcel Adam Just, Carnegie Mellon University

Although numerous studies have examined learning-related changes in brain activation in neurotypical individuals, few have examined these effects in autism despite existing behavioral evidence of impaired learning in autism. This fMRI study compared the neural activation of 18 adult individuals with high-functioning autism and 18 IQ-matched neurotypical participants as they learned how to determine whether someone is lying or telling the truth, based on associated non-verbal cues. Participants watched pairs of computer-animated avatars uttering the same sentence but with different facial and vocal expressions. The findings (to appear in Cerebral Cortex) showed that the autism group did not show the same pattern as the control group of decreased activation in cortical association areas as they learned the task, despite exhibiting similar behavioral performance. The autism group also showed a significantly smaller increase in inter-region synchronization of activation (functional connectivity) with learning than did the control group. Finally, the autism group had decreased structural connectivity as measured by corpus callosum size, and this measure was reliably related to functional connectivity measures. Other studies in progress examine the disrupted neural basis of learning in autism in a spatial working memory task (again showing a smaller neural learning effect in autism) and in prototype learning. These findings constitute the first evidence that the neural processes during the learning of a complex task are impaired in autism and suggest that disrupted cortical connectivity in autism may constrain rapid learning in complex cognitive and social tasks. [Autism Centers of Excellence Grant (HD055748) from the National Institute of Child Health and Human Development]

1.25 **The Countervailing Forces of Selection and Binding in Vision: a Human EEG Study**

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Humans have limited cognitive resources to process the nearly limitless information available in the environment. Endogenous, or "top-down", selective attention to basic visual features such as color or motion is a common strategy for biasing resources in favor of the most relevant information sources in a given context. Opposing this top-down separation of features is a "bottom-up" tendency to integrate, or bind, the various features that constitute objects. We pitted these two processes against each other in a series of behavioral and electrophysiological experiments to test if top-down selective attention can overcome constitutive binding processes. Our results demonstrate that bottom-up binding processes can dominate top-down feature-based attention even when explicitly detrimental to task performance.

1.26 **Aging reduces the ability to sustain sleep/wake states in mice**

Mathieu E. Wimmer, Justin Rising, Ray Galante, Abraham Wyner, Allan Pack and Ted Abel, University of Pennsylvania

Life expectancy is on the rise worldwide. Within the US alone, it is estimated that 20% of the population will be over the age of 65 by 2030 (US Census estimate 2010). Importantly, normal aging produces sleep disturbances in humans and rodents, which might be linked to cognitive impairments and other deleterious effects of aging. We used EEG/EMG recordings from young (2-4 months-old) and aged (22-24 months-old) C57BL/6NIA mice to quantify wake, non-rapid eye movement (NREM) and rapid eye movement (REM) sleep. We found that aged animals show decreased wakefulness during the dark period, when mice are typically active. Conversely, aged mice showed decreased REM sleep during the light (rest) phase. We used a novel statistical approach to examine the architecture of wakefulness and sleep, which takes into account the uneven distribution of short and long bouts of wake, NREM and REM sleep in mice. The distribution of bouts in each state can be described as a spike (short bouts) and slab (long bouts) mixed distribution. Using this novel spike-and-slab analytical approach, we found that aged mice are unable to sustain wakefulness or NREM sleep. Lastly, we used fast Fourier transform of EEG signals to examine the spectral profile of each state in young and aged mice. Aged mice showed decreased slow wave activity, a marker of sleep intensity and sleep pressure. In addition, aging slowed theta peak activity, which is associated with arousal and exploratory behavior during wakefulness. In conclusion, aged mice showed difficulty maintaining wake and NREM sleep during the active period. These results are consistent with frequent awakenings during the night, lighter sleep and daytime sleepiness that have been reported with aging in humans.

NEUROBIOLOGY – ELECTROPHYSIOLOGY

1.27 **Does the middle face patch contain cells that detect faces?**

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Functional MRI in the monkey has revealed at least six patches of tissue in the ventral visual stream that prefer images of faces over objects. Neuronal recordings targeted to the largest patch ("middle face patch", MFP), hypothesized to be homologous to the human FFA, revealed that nearly all visually driven cells were at least 2x more responsive to faces over non-face images. These results support a view in which this region consists entirely of "face cells" exclusively involved in face processing. However, previous work is limited in two ways. First, it was not possible to distinguish between a single, large, face-selective cluster, several clusters, or a local, graded enrichment of "face cells". Secondly, previous studies restricted testing to image sets with limited shape variation. Using similar image sets and a luminance based model (e.g. "pixel neurons"), we found that 18-20% of the "pixels" would be face selective under the standard definition, suggesting that previous work may have overestimated the purity of face selective sites in the MFP. Using a custom built xray imaging system we spatially mapped spiking responses over multiple sessions in the MFP. Our computationally motivated image set contained variation in shape, size and position that reduced performance with pixel models (while remaining trivial for human observers), providing a more accurate test of face selective mechanisms. We found that the MFP contains a graded enrichment of faces selective sites that extends over a ~6mm region. Additionally, <50% of the sites remained face selective with our new image set. We conclude that while neural activity in the middle face patch is likely used in processing face like images, it does not contain an explicit representation of faces.

1.28 **Astrocytes Support Depolarization-induced Presynaptic Plasticity in Hippocampal Neurons**

Devon C. Crawford, and Steven Mennerick, Washington University in St. Louis

Glial cells are increasingly recognized as active participants in synaptic development and neural communication. The role of astrocytes in presynaptic plasticity remains unclear, however. We have been studying presynaptic muting of hippocampal glutamate terminals after depolarizing challenges. We hypothesized that factors released from astrocytes are necessary for induction of presynaptic muting. To test this hypothesis, we used defined networks of autaptic neurons in mixed astrocyte-neuron microisland cultures. To eliminate glial functioning, we lightly aldehyde-fixed astrocyte islands prior to neuronal plating. Calcein-AM verified that only 18% of fixed islands contained one or more viable astrocytes while 99% of islands were viable in non-fixed sibling cultures. Neurons on calcein-negative islands were viable based on calcein fluorescence and electrophysiological recording. Depolarization with a stimulus that reduced glutamatergic synaptic function by 70% in sibling control cultures failed to reduce EPSCs in neurons grown on fixed astrocytes, suggesting a permissive or instructive role for astrocytes in depolarization-induced presynaptic muting. Neuron-astrocyte co-culture-conditioned media added to fixed astrocyte cultures at DIV 7 rescued depolarization-induced silencing at DIV 10-11, even when the conditioned media was removed prior to the depolarization challenge. Conditioned media added during the depolarization challenge did not rescue silencing, however. These results suggest that soluble astrocytic factors play a permissive role in the development of homeostatic presynaptic silencing at glutamatergic hippocampal synapses. [This study was funded by NIH grants NS066611 (D.C.C.) and MH78823 (S.M.).]

1.29 **Activity- and Translation-Dependent Degradation of Arc mRNA in Neuronal Dendrites**

Shannon L. Farris, and Oswald Steward, University of California Irvine

The mRNA for the immediate early gene Arc is induced by synaptic activity or learning experiences. Arc is unique among neuronal mRNAs in that its newly synthesized transcripts are rapidly shipped out to dendrites where they accumulate near recently activated synapses. Studies have revealed that the localization of Arc mRNA may involve both targeting and degradation mechanisms. Here we assess whether there is activity-dependent degradation of Arc mRNA in vivo. We used a paradigm in which Arc transcription and delivery into dendrites is induced by electroconvulsive seizure and subsequent targeting to active synapses is achieved by high frequency stimulation of the medial perforant path. Simultaneous with targeting, Arc mRNA disappears from the non-activated portions of the dendrite. Local blockade of protein synthesis with cycloheximide prevented the activity-dependent decrease in mRNA in the inactive dendritic zones, indicating that Arc mRNA degradation is translation-dependent. Next we assessed whether the enzymes involved in mRNA degradation were present in the dendritic lamina and whether their levels were altered after stimulation paradigms that induced degradation. Immunostaining for Upf1 and Staufen, proteins necessary for nonsense mediated decay- a type of translation-dependent mRNA degradation, was diminished in the activated dendritic lamina in which Arc mRNA accumulates. These data indicate that Arc mRNAs are subject to degradation mechanisms, that this degradation is enhanced by synaptic activity and is dependent on active translation. Activity-dependent mRNA degradation occurring in dendrites is a novel finding that suggests a new mechanism to how neurons can selectively modify the spatial and temporal content of mRNA in response to activity. [NS12333 to OS]

1.30 **gamma-secretase activity is critical for mGluR-dependent long-term depression at hippocampal CA3-CA1 synapses**

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Autosomal dominant mutations in genes encoding presenilin 1 (PS1) and presenilin 2 (PS2) cause a familial form of Alzheimer's disease in humans. PS1 and PS2 are transmembrane aspartyl proteases and essential catalytic subunits of γ-secretase. Conditional ablation of PS1 and PS2 (cPS1/PS2 KO) in adult mouse forebrain causes aberrant neurotransmitter release at hippocampal CA3-CA1 synapses. However, the impact of γ -secretase-dependent proteolytic activity on synaptic plasticity is, as yet, unclear. I show that conditional knockdown of presenilin expression causes deficits in mGluR-LTD at CA3-CA1 synapses of cPS1/PS2KO mice. In contrast, NMDA-LTD at CA1 synapses is not altered, indicating specificity of γ -secretase action. To distinguish between γ -secretase-dependent and independent activity, I performed field recordings at CA1 synapses in the absence or presence of selective, small molecule γ -secretase inhibitors (L-685,458, DAPT), which block γ -secretase-dependent activity but leave expression intact. These findings demonstrate that γ -secretase inhibitors block mGluR-LTD, but not NMDA-LTD. In contrast, γ -secretase inhibitors do not alter basal synaptic transmission or paired-pulse facilitation, a measure of presynaptic release probability. I further show that γ -secretase inhibitors block mGluR-induced AMPAR internalization, as assessed by immunofluorescence and cell surface Westerns. These results demonstrate for the first time regulation of mGluR-dependent LTD by γ -secretase and reveal a novel, previously unappreciated role for γ -secretase in synaptic plasticity.

1.31 **Neural Mechanisms Underlying Spinal Cord Injury Pain**

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Chronic pain develops in most patients who suffer from spinal cord injury (SCI), and manifests as exaggerated responses to painful stimuli (hyperalgesia), pain in response to normally non-painful stimuli (allodynia), and, most commonly, constant spontaneous pain. The mechanisms driving SCI-pain have been difficult to identify, though it is hypothesized to develop as a result of thalamic disinhibition. Here we use an electrolytic lesion in the spinal thalamic tract of rats to model SCI-pain. We employ novel behavioral metrics to show that after spinal lesion rats develop both hyperalgesia and spontaneous pain. We tested the hypothesis that these behavioral abnormalities result from reduced inhibition from the zona incerta (ZI) to thalamic nuclei involved in the sensory aspects of pain (the posterior nucleus, PO) and the affective aspects of pain (mediodorsal nucleus, MD). With single unit extracellular electrophysiological recordings we demonstrate that SCI-pain results from decreased activity in the inhibitory nucleus ZI, which leads to a dramatic increase in spontaneous and evoked neuronal activity in both PO and MD thalamus. We show that SCI-pain can be reduced by stimulating the motor cortex, and demonstrate that this effect occurs through direct cortical inputs to ZI, thereby enhancing ZI inputs to PO and MD. These results support the hypothesis that SCI-pain results from reduced activity in ZI which corresponds with increased activity in thalamic nuclei involved with both sensory aspects of pain (PO) and affective aspects of pain (MD). Further, they provide evidence that MCS reverses sensory and affective components of pain by activating the incerto-thalamic pathway and highlight a novel pathway for clinical intervention. [F31 NS-070458 (JML), R01-NS-069568 (RM), DOD SC090123 (RM), R01-NS-31078 (AK)]

1.32 **Increased Resurgent Sodium Current in DRG Neurons Following Contusive Spinal Cord Injury**

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Spinal cord injury (SCI) results, not only in motor impairment below the site of the injury, but also chronic pain that can persist for years and significantly impact patient quality of life. Pain associated with SCI is neuropathic in origin and is hypothesized to result from trauma induced functional remodeling of central neurons, such that, post injury, these neurons become spontaneously hyperexcitable. A recent study, by Walters et al 2010 found that 3 days following a moderate thoracic (T10) spinal cord injury,75% of small diameter L4/L5 DRG neurons from rats exhibited elevated incidence of spontaneous activity (SA) that failed to decline over 8 months. Importantly, increased SA in DRG neurons correlated with increased mechanical and thermal hypersensitivity of sites below the injury level. Here we explored the hypothesis that altered expression or function of voltage gated sodium channels (VGSCs) contribute to increased SA and hyperexcitability of DRG neurons following SCI. To test this hypothesis we used the rat T10 contusive SCI model and harvested L1-L6 DRG neurons from sham and SCI rats at two weeks and assessed changes in function and expression of VGSCs using whole cell voltage-clamp electrophysiology and real time RT-PCR. No changes in VGSC current density was observed two weeks following SCI. Interestingly, we observed increased amplitude and distribution of resurgent sodium currents (INaR) in injured neurons. Our preliminary data indicates that increased INaR in injured neurons results from increased expression of the NaVB4 subunit, an important protein for INaR electrogenesis. Our data indicate that increased INaR might contribute to enhanced SA and hyperexcitability of below level neurons following SCI. This work was supported by NIH fellowship NS066663.

1.33 **A Role for ATP and P2X Receptors in Mechanical Activation of Nodose C-fibers in Guinea Pig Lungs**

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Activation of vagal afferent sensory C-fibers in the lungs leads to reflex responses that produce many of the symptoms associated with airway allergy. There are two subtypes of respiratory C-fibers whose cell bodies reside within two distinct ganglia, the nodose and jugular, and whose properties allow for differing responses to stimuli. We here used extracellular recording of action potentials in an ex vivo isolated, perfused lung-nerve preparation to study the electrical activity of nodose C-fibers in response to mechanical stimulati in guinea pig lung. We used smooth muscle agonists to induce bronchoconstriction, thereby producing a mechanical stimulus, and found that treatment with both histamine (HA; 30 μM; n=15) and methacholine (MCh; 10 μM; n=4) caused strong action potential discharge in nodose C-fibers (10 ± 2 Hz and 15 ± 8 Hz respectively), but not in jugular C-fibers (n=8). Responses to HA were significantly reduced ($P<0.05$) by blockade of smooth muscle contraction with isoproterenol (100 μM), suggesting that a component of the response is mechanical in nature. The HA-induced activation of the nodose C-fibers was blocked by pretreatment the H1 receptor antagonist pyrilamine (1 μM; n=5). As ATP activation of P2X3 receptors has been found to play a role in mechanosensory transduction, we examined the effect of P2X3 receptor inhibition on the mechanical activation of nodose C-fibers and found that pretreatment with the P2X3 receptor antagonist AF353 (100 μM; n=5) blocked the response to HA. These results suggest that ATP released within the tissues in response to rapid smooth muscle contraction may play a role in the mechanical activation of nodose C-fibers.

NEUROBIOLOGY – RECEPTORS, CHANNELS, TRANSPORTERS

1.34 **Sodium-Activated Potassium Channels are Functionally Coupled to Persistent Sodium Currents**

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We recently showed prominent sodium-dependent potassium currents IKNa were active under normal physiological conditions in many neuronal types. IKNa has the properties of a TTX-sensitive delayed outward current. Although the duration of the transient sodium current is only milliseconds, the activity of KNa channels persists well beyond 1 second, leading us to propose the involvement of the TTX-sensitive persistent sodium current (INaP). Using outside-out patches from the soma of tufted/mitral cells of the olfactory bulb, we recorded the activity of individual KNa and Na channels and demonstrated that the activity of KNa channels was dependent on INaP; treatments that blocked INaP, also removed KNa channel activity, while conversely, treatments that enhanced INaP also enhanced KNa channel activity. We further show that sodium influx through INaP is capable of activating KNa channels without increasing bulk intracellular sodium. While multiple labs have reported the activation of KNa by sodium influx, this is the first demonstration that has not relied solely upon whole cell recording, which may misrepresent ion channel activity distal to the recording site. In related studies, we find that inhibition of this functionally coupled current system dramatically increases spontaneous generation of action potentials in cultured neurons. KNa channels and INaP are found throughout the nervous system but we are only beginning to appreciate their role in neurophysiology. Many drugs used in the treatment of epilepsy and psychiatric disorders target INaP. Our results suggest IKNa may also be altered. [Supported by NS0661871-01]

1.35 **Kinetic regulation of DAT transport capacity by reciprocal phosphorylation and palmitoylation**

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The dopamine transporter (DAT) is a plasma membrane phosphoprotein that mediates the presynaptic reuptake of dopamine and is a site of action for psychostimulants. The level of DAT phosphorylation increases upon PKC activation, and this is associated with down regulation of transport activity. Our lab has identified Ser7 on the cytoplasmic N-terminus of DAT as a PKC-induced phosphorylation site. We have recently shown that DAT is also S-palmitoylated on Cys580 in the C-terminal domain, and found that inhibition of palmitoylation leads to reduced transport capacity. Here we identify a reciprocal relationship between these two post-translational modifications, as S7A DAT shows decreased phosphorylation and increased palmitoylation, while C580A DAT shows decreased palmitoylation and increased phosphorylation. In addition DAT palmitoylation is decreased by PKC activation and increased by PKC inhibition. To explore the functional relationship between these modifications, we examined regulation of transport by PKC in these two mutants. In the presence of concanavalin A, an endocytosis blocker, we found that S7A DAT does not down regulate with PKC activation, while C580A DAT, which has increased phosphorylation, down regulates to a greater extent than WT DAT. Although transport down regulation has been previously thought to occur solely through transporter endocytosis, our results indicate that DAT undergoes a kinetic down regulation of the transporter that is separate from endocytic mechanisms, and that transporter phosphorylation is essential for this process. Our findings also suggest a scenario in which phosphorylation and palmitoylation function synergistically to regulate DAT transport capacity. [Supported by NIDA grant DA13147 and ND EPSCoR (IIP-SG)]

1.36 **Pair up to Pare down: Different Modes of Functional Selectivity in GPCR Heteromers**

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G protein-coupled receptors (GPCRs) mediate neurotransmission of numerous molecules including dopamine (DA). Intriguing questions about the functional mechanisms of GPCRs center on the role of dimerization. A major obstacle to mechanistic understanding has been the inability to dissect heteromeric signaling from downstream signaling crosstalk. We have established a novel assay that distinguishes the two. It is based on luminescence complementation of two defined receptor fusion constructs, combined with bioluminescence resonance energy transfer between the complemented luciferase and fluorescent G proteins. DA receptors play a major role in cognitive, motivational, and motor functions. D1 and D2 DA receptors have been reported to colocalize in a small subset of striatal cells. Using our assay, we have investigated the G protein coupling of the D1R-D2R heteromer. Activation of Gs and Gi by the D1R and D2R protomers, respectively, was maintained in the heteromer. Depending on the dimerization partner (i.e. D2R-D2R vs. D2R-D1R), the potency of certain agonists such as NPA, but not others, was enhanced in the heteromer relative to the D2R homomer. We also uncovered a positive allosteric modulation across the dimer interface, whereby D1R G protein activation by certain agonists was enhanced by inverse agonist binding to the D2R in the heteromer. Taken together, such signaling regulation may account for differences in actions of related pharmacological agents and similar mechanisms likely extend to other GPCR heteromers. Exploitation of signaling differences between homomeric and heteromeric receptors may advance drug development. [Supported by NIH grants RR024158, DA022413, and MH54137, and Lieber Center for Schizophrenia Research]

NEUROLOGICAL DISEASES

1.37 **H63D-HFE reduces brain cholesterol: a potential mechanism for increased risk of Alzheimer Disease**

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The H63D variant of the hemochromatosis (HFE) gene, associated with deregulation of iron homeostasis, has been implicated as a risk factor for Alzheimer disease (AD). Recent studies showed that individuals with both Apolipoprotein E4 allele and H63D variant had a much greater risk and earlier onset of developing AD than individuals carrying either alone. Our global hypothesis is that expression of H63D-HFE establishes a permissive cellular milieu for pathogenic processes. Our specific hypothesis is that H63D expression alters cholesterol metabolism leading to disruption of lipid raft-mediated signaling pathways, specifically regulation of synaptic transmission. We investigated the effects of H63D on expression of cholesterol and sphingolipids, major lipid raft components using SHSY5Y human neuroblastoma cells transfected to stably express either wild type WT- or H63D-HFE. We found that cells expressing H63D had 50% less cholesterol and 6-fold increase in expression of a cholesterol efflux enzyme CYP46A1 than cells expressing WT-HFE. Depletion of cholesterol by exposing cells to simvastatin significantly decreased survival of H63D expressing cells compared to WT cells. Consistent with in vitro studies, H67D (human H63D) knock-in mice, showed a genotype and age dependent decline in brain cholesterol and changes in expression of proteins regulating cholesterol metabolism as well as a significant decrease in brain synaptophysin and an increase in caspase-3. Combined these data indicate that the H63D variant induces alterations in the expression of proteins involved in cholesterol efflux resulting in loss of brain cholesterol and proteins regulating synaptic transmission, eventually causing neuronal loss and development of AD. These data also have implications for use of statins to treat individuals with H63D-HFE variant.

1.38 **Dysregulation of Serotonin during Early Development Alters Interhemispheric Connectivity in Rodents**

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Previous work has shown that disruption of the rodent serotonin system during early development leads to numerous cellular, anatomical and behavioral abnormalities that resemble autism in humans. Imaging studies in autistic patients have highlighted a reduction in the size and shape of the corpus callosum (CC) as well as a decrease in functional connectivity among different cortical areas. The aim of this study was to analyze the integrity of CC and interhemispheric electrophysiological synchronization in rodents whose serotonin levels have been manipulated during critical periods of neural development. Both pharmacological manipulation and genetic mutations were used to alter serotonin levels. Specifically, rat pups were exposed postnataly (PN 8-21) to Citalopram or saline, furthermore, monoamine oxidase A knock out (MAO KO) and wild type mice were also tested. The integrity of the CC was examined immunohistochemically and quantified using stereology. Our data revealed: 1) reduced CC thickness and oligodendrocyte density especially in the rostral part of the CC were noted in the manipulated groups of both rats and mice, and 2) such changes were found more severely in males than females. Consequently, I am in the process of testing the effect of these anatomical changes on the functional connectivity between the primary somatosensory cortices by simultaneously recording local field potentials and single units. Our present findings suggest for the first time that serotonin plays an important role in the proper development of the corpus callosum. Furthermore, manipulation of serotonin levels leads to a long lasting alteration of interhemispheric connections. The current data further suggest a potential link between dysregulation of serotonin and autism.

1.39 **Treatment of Niemann-Pick Type C Disease with Different Cyclodextrins**

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Niemann-Pick type C (NPC) disease is a fatal neurodegenerative disorder characterized by widespread intraneuronal accumulation of unesterified cholesterol and glycosphingolipids (GSLs). While there is no cure for NPC disease, there have been recent advances in the treatment of this disorder utilizing the compound 2-hydroxypropyl-beta-cyclodextrin (HPBCD). We and others have shown significant disease amelioration by administration of HPBCD to NPC mice. However, it is possible that a different form or derivative of cyclodextrin (CD) will yield greater benefit. To test the efficacy of CDs, we administered different forms (alpha, beta, and gamma) and derivatives of CDs to Npc1 mice in vivo. Immunohistochemical and biochemical analyses were carried out to determine reduction of cholesterol and GSLs by each CD. We also performed in vitro binding assays to test the complexing ability of the CDs with cholesterol. In vivo results based on matched areas of neocortex indicate that the form of CD plays a key role in treatment efficacy. Alpha CDs, which show no cholesterol solubilization in vitro, provide no apparent benefit to treated Npc1 mice in vivo. However, in vivo data show that both beta and gamma CDs are capable of reducing intraneuronal storage. In vitro assays of beta and gamma CDs show differential cholesterol binding based on the derivative (hydroxypropyl vs. sulfobutylether), but do not directly correlate with efficacy shown in vivo. We are performing additional in vitro analyses to determine the binding capacity of CDs for other major storage metabolites in NPC disease. This should help determine whether direct binding of CD to a metabolite is critical for the mechanism of action of CD in NPC disease. [Funding generously provided by SOAR-NPC and NIH.]

1.40 **Motor neuron impairment mediated by a sumoylated fragment of the astroglial glutamate transporter EAAT2**

Emily L. Foran, Alex Bogush, and Davide Trotti, Thomas Jefferson University

Amyotrophic lateral sclerosis (ALS) is a progressive, paralytic, and ultimately fatal neurodegenerative disorder. During human ALS and in animal models of the disease, expression and activity levels of EAAT2, a high affinity glutamate transporter of glial origin, are dramatically reduced. Previously we showed that caspase-3 selectively cleaves EAAT2 at a unique site (-D502-T503-I504-D505-) located in the cytosolic C-terminus of the transporter (Boston-Howes et al. J Biol Chem. 2006). We showed that the C-terminus proteolytic fragment (CTE) derived from this caspase-3 cleavage of the EAAT2 is conjugated with the Small Ubiquitin-like Modifier (SUMO1). The SUMOylated C-terminus fragment (CTE-SUMO1) accumulates in the spinal cord of SOD1-G93A mice reaching 40% of EAAT2 immunoreactivity by disease end stage (Gibb et al. J Biol Chem. 2007). In a disease-dependent manner, CTE-SUMO1 partitions to the nucleus and co-localizes with PML nuclear bodies within spinal cord astrocytes, specifically. In addition astrocytic expression of CTE-SUMO causes, in vitro, activation of apoptotic cascade in co-cultured NSC-34 cells and axonal growth impairment in co-cultured primary motor neurons. Results from microarray experiments, performed in CTE-SUMO1 transduced spinal cord astrocytes, demonstrate alterations to proteins involved in vital nuclear, mitochondrial and repair processes. Also seen are alterations to several trophic factors, implicating a disruption to astrocytic-neuronal supportive interaction (Foran et al. Glia. 2011). Increasing evidence of alterations to SUMO and its substrates in neurodegeneration combined with the established role of astrocytes in the degeneration of motor neurons in ALS suggest that the generation of CTE-SUMO1 may be involved in ALS pathogenesis.

1.41 **Structure-Function Analysis of ProSAAS as an Aß1-42 Anti-Aggregant**

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Alzheimer's disease (AD) is a devastating neurodegenerative disease that affects 13% of Americans aged 65 and older. It is responsible for most cases of dementia and hallmarks of AD brains include neuronal loss in memory related areas, intracellular neurofibrillary tangles composed of hyperphosphorylated tau, and aberrant aggregation of Aß1-42 peptide into extracellular plaques. These tangles and plaques are hypothesized to contribute to the pathophysiology by disrupting normal cell function. Therefore, identifying molecular chaperones or drugs to prevent Aß1-42 from aggregating, represents a viable strategy for preventing or slowing down the progression of AD. We have recently identified such an anti-aggregant function for a 27 kDa neuronal secretory protein called proSAAS. ProSAAS was previously found to co-localize with hyperphosphorylated tau inclusions in human AD brain tissue. We therefore asked whether proSAAS would also be co-localized with amyloid plaques and whether proSAAS could prevent Aß1-42 aggregation. In the work presented here, we show that proSAAS is co-localized with amyloid plaques in the APP695/PS1-dE9 mouse by immunohistochemistry. Thioflavin T binding assay demonstrated that proSAAS can prevent the fibrillation of Aß1-42. Furthermore, Xenopus and Zebrafish proSAAS prevented Aß1-42 fibrillation despite limited sequence conservation. We then performed structure-function analysis using N-terminally truncated proSAAS proteins, differing by the number of (predicted) a-helical domains. We found that the anti-aggregation property resides in the central portion of proSAAS, residues 97-180. Additionally, our preliminary data suggest that proSAAS can block Aß1-42-mediated cytotoxicity in N2a cells. [This work was funded by 5R01DK049703-14].

1.42 **Role of DSCAM in Cortical Dendrite Development**

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Down Syndrome (DS) is one of the most common causes of mental retardation in humans, but its neurobiological basis is poorly understood. One candidate gene on chromosome 21 that may contribute to the developmental defects observed in DS is the Down Syndrome Cell Adhesion Molecule, or DSCAM. DSCAM has been implicated in a number of key developmental processes including axon guidance, dendritic patterning, and synapse formation. In order to better understand how increases in DSCAM dosage might contribute to the DS phenotype, we first sought to understand the normal function of DSCAM in the developing brain, particularly in the cortex. Here we report that DSCAM localizes to cortical pyramidal cell dendrites and peaks in expression at postnatal days 7-10, a period of robust dendrite outgrowth and differentiation. Mice with a spontaneous mutation in DSCAM show a number of striking morphological defects including an increase in brain size and alterations in cortical and hippocampal shape. Given that DS brains display abnormalities in cortical dendrite development, we used Golgi staining to examine dendrite arbors in mice lacking DSCAM. Interestingly, we found that pyramidal cell dendrites in DSCAM mutants are initially developmentally delayed, but after a period of compensatory overgrowth and subsequent pruning, adult mutants eventually establish normal dendritic arbors. Taken together, our results demonstrate that DSCAM plays a critical role in cortical development and early pyramidal cell dendrite differentiation. This suggests that increases in DSCAM dosage in DS may contribute to dendrite defects associated with mental retardation. [NSF Career Award 6396959; Kavli Initiative at Yale; NIH Training Grants 5T32 NS41228 HD007180]

1.43 **Novel Age-dependent Learning Deficits in a Mouse Model of AD: Implications for Translational Research**

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One hindrance to the development of interventions for Alzheimer's disease (AD) is the absence of assays that identify individuals at early preclinical stages ideal for intervention, and that translate well between rodent models and humans. Thus, there is significant interest in translational non-verbal tasks that are sensitive to mild decrements in vulnerable brain circuitry. The goal of the current study was to develop a mouse analog of human transfer learning and to determine if it was sensitive to pathological changes in mouse models of AD. The task involves a series of concurrent discriminations that contain 2 features (odor and digging medium) and then requires transferring the learned information into new configurations in which the irrelevant feature is altered (transfer learning). Initial lesion studies determined that transfer learning is hippocampal-dependent. Subsequent studies in 2 transgenic mouse models that have AD-like pathology (APPswePSEN1dE9)85Dbo/o and APPSwDI/mNOS2-/-) showed robust age-dependent deficits in transfer learning, and this impairment occurred earlier than did those assessed by the Morris water maze (in APP+PS1). An additional feature of the task is that it is resistant to previous experiences, making it ideal for within-subject design experiments. Current experiments are directed at determining which molecular changes, particularly at the synaptic level, may be related to onset and severity of transfer learning deficits. These data support that transfer learning is a sensitive, robust hippocampal-dependent assay for mouse models of AD, and indicate that this task should be a useful tool for assessment of therapeutic interventions. [NIA-R01-AG029421 & F31 AG037286]

1.44 **Enhanced Cerebral but Not Peripheral Angiogenesis in the Goto-Kakizaki Model of Type 2 Diabetes Involves VEGF**

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We previously reported enhanced cerebrovascular remodeling and arteriogenesis in a mild and lean model of type 2 diabetes. This study addresses how 1) cerebral neovascularization is regulated in a spatial manner 2) vascular endothelial growth factor (VEGF) and peroxynitrite (PN) signaling is the underlying mechanisms of increased angiogenesis. Vascular stereology was evaluated from 3-D images reconstructed from vessels in brain, eye and skeletal muscle of control Wistar and diabetic Goto-Kakizaki rats. Migration and tube formation properties of brain microvascular endothelial cells (BMEC) were analyzed as markers of angiogenesis. Vascular density, volume and surface area were progressively increased from rostral to caudal sections in both cerebral cortex and striatum in diabetic rats. Unperfused new vessels were prominent in the striatum and pericyte/endothelial cell ratio featuring vascular maturity was decreased in diabetes. Peripheral vascularization is impaired while increased in the retina. Cerebral microvessels and BMECs from diabetic rats expressed high levels of basal VEGF, nitrotyrosine and MT1-MMP and had greater migratory and tube formation properties. VEGF neutralizing antibody, PN inhibitor FeTPPs, c-src inhibitor PP2 and MMP inhibitor minocycline blocked the cell migration in diabetes. VEGF stimulation caused rapid and biphasic phosphorylation of VEGF receptor-2 and c-src, while FeTPPs prevented this. Thus diabetes increases and spatially regulates cerebral neovascularization. Increased VEGF-dependent angiogenic function in BMEC is mediated by PN and involves c-src and MT1-MMP activation. Future studies will identify how cerebral neovascularization in diabetes increases bleeding which impairs reparative angiogenesis and functional recovery after stroke.

1.45 **Purkinje Cell Degeneration: a Link Between Tuberous Sclerosis Complex and Autism**

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Tuberous sclerosis complex (TSC) is an autosomal dominant tumor-suppressor disorder caused by the loss of function of either TSC1 (hamartin) or TSC2 (tuberin). These proteins form a heterodimer to inhibit the mTORC1 pathway, which controls protein translation and cell growth. Interestingly, about half of children with TSC have autism. In patients with TSC-associated autism, studies have correlated the number and severity of cerebellar lesions with the severity of autistic behavior. Purkinje cells are the central output cell of the cerebellum. Loss of Purkinje cells has been demonstrated in the brains of a subset of autistic children as well as in some patients with TSC. Therefore, we hypothesize that the association between TSC and autism might be caused by Purkinje cell pathology. To understand the role of tuberin in Purkinje cells and the link between TSC and autism, we created a mouse model in which the Tsc2 gene was selectively deleted from Purkinje cells beginning at postnatal day 6. The deletion of Tsc2 caused Purkinje cell overgrowth leading to progressive cell death. Immunohistochemistry (IHC) confirmed activation of mTORC1. Additionally, IHC showed that both oxidative stress and endoplasmic reticulum (ER) stress were increased in Tsc2-null Purkinje cells. Upon injection of rapamycin, an mTORC1 inhibitor, we were able to rescue the cell death and ER stress phenotypes. Behavioral testing was performed on the mice toward the beginning of Purkinje cell death. The results of these behavioral tests indicate deficits in social interactions that are characteristic of autistic-like behavior. These data reveal a novel link between TSC and autism and provide a novel mouse model in which to explore the effects of rapamycin in the treatment of social behavioral deficits.

1.46 **Reduced Ethanol Preference and Consumption in CART Knockout mice**

Armando Salinas, Chinh T Q Nguyen, Dara Ahmadi-Tehrani, Richard A Morrisett, The University of Texas at Austin

Cocaine- and amphetamine-regulated transcript (CART) is a peptide neurotransmitter implicated in addiction to drugs of abuse. Several studies have characterized the role of CART in addiction to psychostimulants, but few have examined the role of CART in alcohol use disorders including alcoholism. The current study utilized a CART KO mouse model to investigate what role, if any, CART plays in ethanol appetitive behaviors. CART KO and wild type (WT) mice were produced by crossing heterozygote mice, weaned according to sex, and genotyped at 3 weeks of age. At 12-15 weeks, a 2-bottle choice, 23 hour access paradigm was used to compare ethanol preference and consumption between WT and KO mice. The mice were presented with escalating concentrations of an ethanol solution and water, each for four days with consumption levels measured daily. Preference for bitter and sweet solutions was also measured. Body weights were measured every other day and the position of the ethanol (or tastant) and water bottles was alternated daily. Ethanol metabolism rates following administration of a 2.5 g/kg i.p. were compared between genotypes and sexes. Sensitivity to ethanol was also examined using a loss-of-righting-reflex assay. CART KO mice consumed and preferred ethanol less than their WT counterparts in both sexes. This genotype effect could not be attributed to differences in bitter or sweet taste perception or ethanol metabolism rates. There was also no difference in ethanol sensitivity in male mice; however, CART KO female mice showed a greater ethanol sensitivity than the WT females. Taken together, these data demonstrate a role for CART in ethanol appetitive behaviors and as a possible therapeutic drug target for drug addiction and abstinence enhancement.

1.47 **Spectrin and Sodium Channel Proteolysis are Reduced in Brain-injured Mice Overexpressing Calpastatin**

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Calpains are activated early after traumatic brain injury (TBI), with subsequent proteolysis of substrates including cytoskeletal components and membrane receptors. Prolonged activity of calpains after trauma suggests that endogenous action or levels of its inhibitor, calpastatin, may be insufficient to fully inhibit calpain proteolytic activity. Therefore, we hypothesize that calpastatin overexpression will reduce calpain activity and calpain-mediated substrate proteolysis after TBI. Using calpastatin-overexpressing transgenic (TG) mice, we have demonstrated a reduction in protease activity, measured by fluorogenic assay on naïve cortical homogenates, in TG compared to wildtype (WT) mice, confirming functional activity of calpastatin. Immunoblots for alpha-spectrin revealed significantly less cortical and hippocampal calpain-mediated proteolysis in TG mice than WT littermates at 6 h and 24 h following controlled cortical impact (CCI) injury. Brain injury resulted in a reduction in cortical voltage-gated sodium channel (NaCh) protein, coupled with the appearance of fragments detected by NaCh-specific antibodies. TG mice exhibited a significant reduction in select NaCh breakdown products compared to WT at 6 h. At 24 h post-CCI, an 85 kDa I-II loop fragment was also attenuated in TG mice; however, other sodium channel fragments were equivalent to WT. Considering the structural and functional importance of spectrin and NaCh dynamics in neurons, preservation of these proteins by augmenting calpastatin levels after injury may contribute to a reduction in neuronal damage. [NIH grants F31 NS071804 (KMS), T32 DA0222738, P30 NS051220, P01 NS058484 (KES), and P01 NS056202 (DFM), and Kentucky Spinal Cord and Head Injury Research Trust grant 6-12]

1.48 **An oral-vaccine against a RAGE-Aß complex immunogen on AD like pathology and cognitive function in APP/PS1mice**

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder that gradually destroys a person's memory. Substantial evidence suggests that the amyloid beta (Aß) protein and the receptor for advanced glycation end products (RAGE) play an important and often deleterious role in the pathogenesis of AD. RAGE is a binding partner for Aß, facilitating the translocation of Aß from the periphery into the brain. RAGE is also involved in mediating Aß induced neurotoxicity as well as enhancing the inflammatory response induced by Aß. In addition, soluble forms of RAGE (sRAGE) and Aß bind together in the periphery forming high molecular weight complexes. These RAGE/Aß complexes are more highly immunogenic and less neurotoxic than Aß1-42 alone. We show here that there are elevated anti-RAGE and anti-Aß titers (occurring in a near 1:1 relationship) in samples analyzed from human AD patients, aged non-human primates, and AD transgenic mice (APPSWE-PS1). Also, we show that an in-vitro prepared RAGE/Aß complex induces a greater immunogenic response in both human peripheral blood mononuclear cells (PBMCs) and in immunized Balb-C mice than does either Aß1-42 or RAGE alone. Further, pretreatment with endogenous anti-RAGE antibodies isolated from our transgenic APPSWE-PS1 mice are able to prevent Aß1-42 induced neurotoxicity in cultured primary rat cortical neurons. Finally, we are currently examining the effectiveness of orally administered vaccine of either RAGE/Aß complex or Aß1-42 alone in decreasing the cerebral amyloid load and improving cognitive function in our AD TG mice. Our results to date support the hypothesis that a protein complex vaccine that targets both RAGE and Aß1-42 will provide a more effective treatment for AD than vaccination with Aß1-42 alone.

PHYSIOLOGY

1.49 Chemical Synthesis of New Organelles In Vivo

Jason Baik, University of Michigan; Gus R. Rosania, University of Michigan

We report a new cytoplasmic organelle that forms in mice treated with the antibacterial drug clofazimine. This drug has poor solubility and high lipophilicity, which leads to significant bioaccumulation in vivo. We found it can form membrane aggregates within cytoplasm in various cells. Most surprisingly, within macrophage-like cells, the drug formed membrane delimited polyhedral structures that superficially resembled crystals, but in fact correspond to a new organelle. Groups of male Balb/c mice were fed with clofazimine mixed chow for up to 2 months, and various organs were collected at different time points for biochemical and histological analyses. Light microscopy, confocal Raman imaging, and epifluorescence as well as TEM analysis showed clofazimine was sequestered in membrane complexes within the cells of liver, spleen and lymphatic organs. Clofazimine did not accumulate to a great extent in kidneys, muscle, brain and other organs. The difference in biodistribution was related to the formation of crystal-like organelles in phagocytes of spleen and liver. After detailed TEM analysis, the crystal-like organelles appeared filled with highly organized membrane lattice. By isolating these organelles from mouse spleen, we found that they were sensitive to osmotic stress, basic pH, and heat application, unlike pure clofazimine crystals. When freed from cells, they no longer maintained their regular needle-like morphology. The formation of a new organelle was accompanied with changes in organ size, cell numbers and population of inflammatory cells. The discovery of such "polyhedrosome" increases our understanding of a drug's pharmaceutical properties, while it also opens up many new therapeutic possibilities and opportunities for manipulating the structure and function of living organisms in a manner previously considered not possible.

1.50 Peroxisomes and Lipid Metabolism in Drosophila melanogaster

Joseph E. Faust, Michael Stern, James McNew, Rice University

Peroxisomes are organelles that carry out ß-oxidation of fatty acids and other lipid processing reactions. Defects in peroxisome biogenesis cause a spectrum of human diseases known as peroxisome biogenesis disorders (PBDs). The most severe PBD, Zellweger syndrome, is characterized by neuronal dysfunction, craniofacial malformation, and muscle weakness (hypotonia). There is currently no cure for PBDs and it is still unclear how the loss of peroxisomes leads to the disease state. We have begun to model PBDs in the fruit fly, Drosophila melanogaster, and hope to gain new insight into PDB etiology. Inhibition of peroxisome biogenesis was achieved by disrupting the pex3 gene, which is required for the early steps of peroxisome biogenesis as well as insertion of proteins into the peroxisomal membrane. Inhibition of peroxisome biogenesis in Drosophila is lethal during metamorphosis, a developmental stage that requires the catabolism of nutrients stored as triacylglycerol (TAG). Prior to metamorphosis, these larvae are hypersensitive to starvation, where metabolism of stored lipids is required. Flies with impaired peroxisome biogenesis in muscles have reduced locomotor function and accumulate TAG in muscles. Hypotonia is a characteristic of PBD patients, but is thought to be a secondary effect of nervous system dysfunction. We propose that peroxisome loss may directly affect muscle physiology by causing a buildup of potentially toxic lipids. The precise mechanism of lipotoxicty in not known, but it could result from increased mitochondrial ß-oxidation and elevated reactive oxygen species (ROS) production. Further experiment will determine the role of peroxisomes in muscle physiology and may lead to new therapeutic targets for the treatment of PBDs.

1.51 **STIM1 is Essential for the Coupling of Sarcoplasmic Reticulum Calcium Stores to TRPM4 and BKCa Channel Activit**

Albert L. Gonzales, and Scott Earley,Colorado State University

In cerebral arterial myocytes, pharmacologically distinct Ca2+ release events from the sarcoplasmic reticulum (SR) activate large-conductance Ca2+-activated K+ (BKCa) channels and melastatin transient receptor potential 4 (TRPM4) channels located on the plasma membrane. The stromal-interacting molecule, STIM1, is a single transmembrane protein consisting of a luminal Ca2+-sensing EF-hand and cytosolic interacting domains, and is localized in the SR membrane juxtaposed to the plasma membrane. To investigate the role of STIM1 in regulating Ca2+-activated ion channels, we used RNAi-mediated protein suppression and STIM1-interaction inhibitory peptides in rat cerebral artery smooth muscle cells. STIM1 siRNA treatment decreased STIM1 but not TRPM4 or BKCa protein expression. Using patch clamp electrophysiology, we found that Ca2+-dependent TRPM4 and BKCa channel activity was diminished following STIM1 siRNA treatment. Additionally, STIM1 inhibitory peptides targeting the STIM1-STIM1 or STIM1-ORAI1 interacting domains decreased TRPM4 channel activity. To investigate SR Ca2+ store load, we examined cyclopiazonic acid-induced Ca2+ release, and saw no difference between treatments. Using membrane specific fluorescent staining of the SR and plasma membranes, we found that SR membrane architecture and coupling with the plasma membrane was disrupted following our STIM1 siRNA or inhibitory peptide treatments. Thus, following down-regulation or inhibition of STIM1; SR Ca2+ stores were maintained; SR and plasma membrane coupling was disrupted; and Ca2+-dependent activation of plasma membrane ion channels were lost. This is the first evidence of a novel role for STIM1 in physically coupling SR Ca2+ stores with BKCa and TRPM4 channel activity in smooth muscle cells. RO1HL091905(SE); F31HL094145-01 (AG).

1.52 **Deptor Knockdown Enhances mTOR Activity and Protein Synthesis in C2C12 Myocytes**

Abid A. Kazi, and Charles Lang, The Pennsylvania State University, College of Medicine, Hershey, PA

Deptor is an mTOR binding protein that affects cell metabolism. We hypothesized that knock down (KD) of Deptor in C2C12 myocytes will increase protein synthesis via stimulating mTOR-S6K1 signaling. Deptor KD was achieved using lentiviral particles containing shRNA targeting the mouse Deptor mRNA sequence and control cells were transfected with a scrambled control shRNA. KD reduced Deptor mRNA and protein content by 90%, which increased phosphorylation of mTOR kinase substrates, 4E-BP1 and S6K1, and concomitantly increased protein synthesis. Deptor KD myoblasts were both larger in diameter and exhibited an increased mean cell volume. Deptor KD increased the percentage of cells in the S phase, coincident with an increased phosphorylation (S807/S811) of pRb protein which is critical for the G1—S phase transition. Deptor KD did not appear to alter basal apoptosis or autophagy as evidenced by the lack of change for cleaved caspase-3 and LC3B, respectively. Deptor KD increased proliferation rate and enhanced myotube formation. Finally, in vivo Deptor KD (~50% reduction) by electroporation into gastrocnemius of C57/BL6 mice did not alter weight or protein synthesis in control muscle. However, Deptor KD prevented atrophy produced by 3 days of hindlimb immobilization, at least in part by increasing protein synthesis. In conclusion, our data support the hypothesis that Deptor is an important regulator of protein metabolism in myocytes and demonstrate that decreasing Deptor expression in vivo is sufficient to ameliorate muscle atrophy. [Funding support: NIH (GM38032 and AA11290) to CH Lang and Pennsylvania Department of Health using Tobacco Settlement Funds (AA Kazi). The Department specifically disclaims responsibility for any analyses, interpretations or conclusions]

PROTEIN COMPLEXES

1.53 **Proteomic Identification of Substrates of the E3 Ligase, Parkin, Essential in Mitophagy**

Shireen A. Sarraf, Woong Kim, Steven P. Gygi, J. Wade Harper, Harvard Medical School

The E3 ubiquitin ligase, Parkin, is commonly mutated in recessive forms of Parkinson's disease, a neurodegenerative disorder linked with accumulation of mitochondrial dysfunction. In recent years, Parkin has been shown to translocate to the mitochondria after membrane depolarization in a PINK1-kinase dependent manner. Consequently, the outer mitochondrial membrane is extensively decorated with ubiquitin as a result of Parkin translocation and E3 ligase activity. Ultimately, the damaged organelles are eliminated via mitophagy, the selective autophagic removal of mitochondria. We are using a variety of proteomic approaches to identify interactors and substrates of Parkin and PINK1 which are important in the regulation and progression of mitophagy. We have confirmed the interactions of Parkin with known substrates, including Mfn1 and Mfn2, after CCCP-induced depolarization using mass spectrometry (LC-MS/MS) and the Comparative Proteomics Analysis Software Suite (CompPASS) to identify high-confidence candidate interaction proteins (HCIPs). Furthermore, we are using SILAC and mass spectrometry to identify ubiquitinated targets of Parkin by enriching for proteins containing a diglycine (diGly) signature after mitochondrial damage. We are currently validating potential substrates aiming to identify both substrates of Parkin whose proteasome-mediated degradation is essential for the initiation of mitophagy as well as those substrates which may regulate eventual engulfment of dysfunctional mitochondria by autophagosomes. Parkin and PINK1-mediated remodeling of the mitochondria signifies an important step in the progression of mitophagy and may ultimately provide information about the progression and possible treatment of neurodegenerative disorders.[Funding: NIH AG011085]

PROTEIN STRUCTURE/FUNCTION

1.54 Characterization of ALDH7A1 and the Potential Mechanisms Underlying Pyridoxine-Dependent Epilepsy

Chad N. Brocker, University of Colorado; Natalie Lassen, University of Colorado; Kathryn Kavanagh, University of Oxford; Udo Oppermann, University of Oxford; Vasilis Vasiliou, University of Colorado

Aldehyde dehydrogenases (ALDHs) catalyze the NAD(P)+ dependent conversion of aldehydes to their corresponding carboxylic acids. Mutations in human ALDH7A1 are responsible for pyridoxine-dependent epilepsy (PDE) and folinic acid-responsive seizures. The continuing aim of this study was to characterize the biochemical properties and functions of ALDH7A1 in an effort to better understand the broad phenotypic spectrum noted in PDE patients. ALDH7A1 expression in Chinese hamster ovary cells attenuated hyperosmotic stress-induced cell death caused by increased sucrose or sodium chloride (NaCl). Moreover, cells were protected from treatment with both 4-hydroxy-2-nonenal and hydrogen peroxide. siRNA knockdown in proximal tubular epithelial cells increased susceptibility to NaCl-induced hyperosmotic stress. Tissue distribution studies indicated highest expression in liver, kidney and brain, followed by pancreas and testes. Subcellular fractionation and immunochemistry revealed localization within the cytosol, nucleus and mitochondria, making it unique among ALDHs. Purified protein metabolized a number of aldehydic substrates including the osmolyte and methyl-group donor precursor, betaine aldehyde, lipid peroxidation-derived aldehydes and the lysine catabolyte, alpha-aminoadipic semialdehyde. Human ALDH7A1 was crystallized and structurally supports accommodation of the chemically diverse, experimentally verified substrate panel. Mapping and in silico mutagenesis of human PDE missense mutations suggest diverse and variable effects on protein function. In conclusion, ALDH7A1 is a novel ALDH expressed in multiple tissues and subcellular compartments that appears to play an important role in several biological processes. [NIH support: R01EY17963, R21AA017754 and F31AA018248]

1.55 Lon Protease: Mechanism of Substrate Recognition and Degradation

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The SmpB-tmRNA quality control system adds a peptide tag to the C-termini of the nascent polypeptides resulted from translation of mRNAs lacking an in-frame stop codon, marking them for proteolysis. We have shown that In Escherichia coli, in addition to the ClpXP system, Lon protease can also degrade tmRNA-tagged proteins, but with much lower efficiency. We then studied a unique case where in Mycoplasma pneumoniae (MP), Lon is the primary protease degrading the tmRNA tagged proteins due to the absence of Clp family proteases. We identified two discrete signaling motifs in the MP-tmRNA tag for Lon recognition. We also showed evidence that the MP-Lon and MP-tmRNA tag have co-evolved to allow enhanced Lon binding to the tag and more efficient degradation of the MP-tmRNA tagged proteins. The discrepancy in the substrate specificity between EC-Lon and MP-Lon promoted us to further explore the general substrate specificity of Lon in difference bacteria species. We made mutations in a predicted substrate recognition loop in Lon protease and tested the ATPase and protease activity of the mutants. We also identified preferred cleavage sites of Lon protease in multiple substrates. We are also interested in the role of Lon protease in the pathogenesis of Yersinia pestis. We performed a genome wide profiling of Lon substrates and identified novel substrates of Lon protease, many of which are master regulators of gene expression in Yersinia. We are now working on deciphering the biological significance of the degradation of these substrates by Lon protease and hopefully we can shed more light onto the role Lon is playing in Yersinia pathogenesis. This work was supported in part by National Institutes of Health Grants GM65319 and AI055621 to A. Wali Karzai.

1.56 **The Mid-Region, NLS, and C-terminus of PTHrP Influence Murine MSC Differentiation**

Blake E. Hildreth III, Krista M. Hernon, Brandlyn N. Marlow, John Leong, Thomas J. Rosol, Ramiro E. Toribio, Ohio State University

The N-terminus of parathyroid hormone-related protein (PTHrP) promotes bone and cartilage and inhibits fat formation from mesenchymal stem cells (MSC). However, scant information exists regarding its effect on myogenesis. Since the roles of the mid-region, nuclear localization sequence (NLS), and C-terminus is unknown, we investigated the effects of their deletion on MSC differentiation. MSC from mice lacking the mid-region, NLS, and C-terminus of PTHrP (KI) and wild-type (control) mice were grown in differentiation media (osteo-, chondro-, adipo-, and myogenesis). Secreted biomarkers were compared every 6 days with a 2-way RM-ANOVA. Lineage-specific mRNA at day 12 and 24, and histochemical/morphological indices at day 24 were compared with a t-test. KI MSC had greater alkaline phosphatase (ALP) activity ($P<0.0001$). However, KI MSC had reduced osteoblast maturation, demonstrated by less mineralization and osteocalcin at d24 ($P=0.02$ and 0.03). Similarly, KI MSC-derived cartilage pellets were smaller ($P<0.0001$) and expressed greater Ihh ($P=0.04$) but less Sox9 at day 24 ($P=0.04$). Although KI MSC produced more adipocytes ($P=0.02$) with increased Ppar? at day 24 ($P=0.09$), both genotypes had similar adipogenesis gene profiles. Interestingly, KI MSC produced a larger number of myocytes ($P<0.0001$) and expressed more desmin and myogenin at day 24 ($P=0.002$ and 0.03). Regions distinct from the N-terminus effect MSC differentiation. Increased ALP and Ihh by KI MSC, but less bone and cartilage formation, indicates that the mid-region, NLS, and C-terminus inhibit proliferation, but promote osteoblast and chondrocyte maturation. This is the first time these regions have been implicated in regulating adipo- and myogenesis, and they complement the inhibitory function of the N-terminus. [NIH NCRR-T32RR07073 and NIAMS-F32AR057597]

1.57 **Correlating changes in structure and function of Bacteroides thetaiotaomicron TetX2 to fitness during adaptation**

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The combination of experimental evolution to identify adaptive changes in proteins undergoing selection and their subsequent physicochemical characterization provides a physical basis for linking protein structure and function to organismal fitness. This is particularly relevant for the study of antibiotic resistance where the evolution of drug resistant bacteria is clinically important. Bacteroides thetaiotaomicron TetX2 was previously identified as a novel oxidoreductase with broad activity against tetracyclines. Experimental evolution was used to identify an adaptive mutation (TetX2T280A) within a bacterial population that confers higher resistance to minocycline and tigecycline. We have demonstrated that tetX2 promotes a rapid increase in resistance through adaptation, beyond that conferred by the T280A mutation alone. Strains carrying tetX2T280A have higher growth rates at minocycline concentrations corresponding to the conditions when the mutant was first observed. In vitro kinetic studies show that TetX2T280A has a 50% increase in kcat at 37°C but not at 25°C, which is in agreement with observed relative drug susceptibility tests and suggests that the mutation may alter TetX2 dynamics. We have determined the structure of the B. thetaiotaomicron TetX2 at 2.8 Å resolution and shown that it is a class A flavin dependent oxidoreductase. The structure reveals that the site of the mutation is ~18 Å away from the active site which supports an indirect mechanism for improved catalysis. We have gone on to identify other novel mutations to TetX2 using in vitro directed evolution and are now developing models that relate how adaptive changes in TetX2 kinetics quantitatively predict outcomes in the evolution of resistance (evolutionary dynamics).

PUBLIC HEALTH/GLOBAL HEALTH

1.58 Understanding the Ecology of Sexually Transmitted Disease (STD) Risk Among African Pastoralists

Ashley Hazel, University of Michigan

Our understanding of the ecological determinants of STD risk among remote, subsistence-level populations is extremely limited. Given their tangible dependence upon ecological resources (e.g. food sources, land), it is likely that individual subsistence strategies predict STD risk. We hypothesize that there is a relationship between ecological resource base and STD risk that is mediated by access to sexual partners. I tested for the presence of this hypothetical relationship among the Himba and Tjimba tribes of northwestern Namibia. These semi-nomadic, agro-pastoralists have high rates of STD, but ecological and cultural isolation have buffered them from the surrounding HIV/AIDS epidemic. However, imminent development projects and a rapidly changing way of life could lead to a dire epidemiologic transition. I integrated epidemiologic, ecologic and anthropologic methods to test the prediction that high status men and resource vulnerable women have the highest risk for STD. I asked reproductively aged individuals (n=446) living in 30 villages about their resource strategies and current sexual partners, including geographic location. Each participant submitted a genital swab; I use qPCR analyses to determine the presence of gonorrhea and chlamydia. By exploring the ecological underpinnings of STD risk, I expect to identify specific ways to predict and prevent the spread of HIV among the Himba and Tjimba, and offer novel insights into how ecological resources might drive infectious disease dynamics. [Funded by The Robert Wood Johnson Foundation, The Wenner-Gren Foundation, The American Philosophical Society, NIH training grant T32 AI049816, The University of Michigan's Rackham Graduate School and Center for Social Epidemiology]

REGENERATIVE MEDICINE/TISSUE ENGINEERING

1.59 Functionalizing and patterning extensible protein-based materials for use as tissue engineering scaffolds

Zhao Huang, Yang Lu, Taha Salim, Jun Lou, Kathleen S. Matthews, Rice University; Jan Patterson, Autumn Brawley, Sarah E. Bondos, Texas A&M University

Protein-based materials potentially combine excellent mechanical properties, functionality, and biocompatibility with the potential to engineer these properties using standard molecular biology techniques. However, existing procedures of producing and functionalizing these materials require harsh conditions, which severely compromised their applications. We overcame these challenges using a novel approach, producing materials using a Drosophila protein, Ubx, which does not self-assemble as part of its natural function but was found to form materials in vitro. In a gentle aqueous buffer (such as PBS), Ubx molecules self-assemble into a film within 2 hours and can be processed into fibers with tensile strength and extensibility comparable to natural elastin. Facile molecular biology tools in E. coli facilitate sequence engineering to functionalize Ubx materials. We are the first group to successfully integrate full-length, folded functional proteins into protein-based materials using gene fusion. Enhanced Green Fluorescent Protein (EGFP), mCherry, luciferase, and myoglobin were fused to Ubx to create fusion chimeras, which all retain the ability to self-assemble and the functions of the appended proteins. We have also established methods to create functional protein patterns in materials on the microscale to macroscale. Ubx materials are compatible with a variety of cells in vitro. Cells attach to Ubx materials and spread along nanoscale fibrils along the fibril axis. Fusion of integrin attachment sequences to Ubx enhances cell attachment. Overall, the mechanical properties, functionalization potential, and biocompatibility of Ubx materials have potential in a wide variety of applications such as biosensors and tissue engineering scaffolds.

1.60 **Employment of Structural & Biochemical Cues for Enhanced Peripheral Nerve Regeneration**

Balendu S. Jha, David G Simpson, Virginia Commonwealth University

Peripheral nerve injuries limit functional capacity and degrade quality of life. For long defect injuries, autologous grafts (AGs) remain the gold standard treatment. However, this material has well recognized limitations. Harvesting AG results in donor site morbidity. More importantly, the native axonal fragments in the graft must first degenerate before regenerating axons can penetrate it; thereby delaying regeneration. To address these limitations, we have developed a synthetic nerve guide (SG) that mimics the native tissue architecture. Air-gap electrospinning makes it possible to produce 3-D, macroscopic, cylindrical constructs composed of dense anisotropic arrays of fibres (nano-micron scale average diameter) oriented parallel to the long axis of the constructs. This unique design provides thousands of individual channels to direct axon growth. We also have developed a method to incorporate gradients of therapeutic reagents (NGF) into these grafts which can be tailored to provide sustained release at defined locations. To test the efficacy of our design, we reconstructed 15 mm lesions in the rodent sciatic nerve. Our SG promoted significantly enhanced functional recovery as measured by: (A) sciatic functional index- a 25 point improvement with respect to the AGs by day 60, (B) sensory withdrawal reflex- normal reflexes restored by day 45 with SG and not until day 60 with AG. After 7 weeks, SGs were populated with dense parallel arrays of myelinated & un-myelinated axons with an axon count 25% higher at the distal end than comparable AG. Our preliminary studies suggest that our novel SG's anisotropic architecture combined with the persistent NGF gradient act synergistically to increase the rate, extent, and fidelity of regeneration in the peripheral nerve injury.

1.61 **Sustained, Controlled Delivery of Oxygen from Hydrolytically Activated Silicone Scaffolds**

Eileen Pedraza, Chris Fraker, Maria Coronel, Cherie L. Stabler, University of Miami

It is critical to identify and develop methods to increase oxygen availability within scaffolds, particularly to serve as a bridge between implantation and the development of an adequate vascular network. In this study, we sought to design and develop an oxygen generating scaffold in the form of solid peroxide encapsulated in silicone. Macroporous silicone scaffolds containing the oxygen generating biomaterial of 25% w/w calcium peroxide (CaO_2, Sigma Aldrich), were fabricated with 85% ± 5% porosity using the solvent casting salt leaching technique. Scaffolds produced oxygen for over 2 weeks at a minimum rate of 100 mmHg per day within sealed oxygen chambers. Hydrogen peroxide and hydroxyl radical release were found to remain below negligible levels. Under hypoxic conditions, coculture of MIN6 cells or rat islets with the oxygen generating biomaterial resulted in a nearly a twofold increase in MTT viability over controls, with viability levels comparable to controls at 20% oxygen ($p<0.05$). Islets cultured in hypoxic conditions also demonstrated significant insulin dysfunction, while islets incubated with the scaffold illustrated insignificant changes in insulin function. We have illustrated a biomaterial capable of providing optimal, sustainable, and controlled delivery of oxygen thereby enhancing cell viability and function. Given the ubiquitous needs for optimal oxygen delivery within implants, we believe this material can provide benefit to numerous tissue engineered systems, particularly for highly vascularized and metabolic organs. [We acknowledge funding from the Juvenile Diabetes Research Foundation International and the Diabetes Research Institute Foundation. Pedraza is supported by a National Institutes of Health NIBIB predoctoral fellowship.]

1.62 **Engineering Cell-Triggered Release from Hydrogel Scaffolds**

Talar Tokatlian, Tatiana Segura, Chemical and Biomolecular Engineering Department, University of California, Los Angeles

Vascularization within tissue-engineered constructs remains the primary cause of construct failure following implantation. We are currently investigating two hypotheses to enhance hydrogel scaffold vascularization, both long-term mechanical support and cell-demanded release of non-viral DNA nanoparticles. Our preliminary in vivo studies show that after subcutaneous implantation for 3 weeks enzymatically degradable nano-pore hydrogels have cellular infiltration only at the periphery of the hydrogel, while hydrogels with micron sized interconnected pores (μ-pore) are extensively infiltrated. Significant positive staining for endothelial markers (PECAM) was also found in μ-pore implants but not in n-pore implants, even in the absence of pro-angiogenic factors. We believe that an open pore structure will increase the rate of vascularization through enhanced cellular infiltration and that the added delivery of DNA encoding for angiogenic growth factors will result in long lasting angiogenic signals. To deliver DNA, μ-porous hyaluronic acid-MMP hydrogels were then used to encapsulate DNA/poly(ethylene imine) polyplexes and transfect seeded mMSCs as they slowly degraded the matrix. Porous hydrogels also allow for cell spreading and migration without requiring extensive enzymatic degradation. Coupled with high loading efficiency of DNA polyplexes, μ-porous hydrogels then allow for sustained transfection and transgene expression of incorporated mMSCs for up to 10 days in vitro. These results suggest that DNA nanoparticle internalization and subsequent transgene expression could be controlled by both the protease expression of the seeded or infiltrating cells as well as the structural properties of the hydrogel. [NIH 1R21EB007730-01, NSF CAREER 0747539, CRCC, and NIH T32GM067555]

SOCIAL/BEHAVIORAL SCIENCE

1.63 **The Neural Basis of "Anchoring-and-Adjustment" during Mentalizing of Personality Traits in Autism**

Akiko Mizuno, and Marcel Adam Just, Carnegie Mellon University

In a process of making inferences about another person's mind, the recent study suggests that typically developed adults use the self as an anchor point and make an adjustment accordingly, based on the perceived difference relative to oneself. This process is called "anchoring-and-adjustment" and mediated by the medial prefrontal cortex (mPFC). Atypical understanding another person's thought, feelings, and intention, so called Theory of Mind (ToM), has been recognized as a pivotal characteristic of autism, and studies reported atypical mPFC activity not only for mentalizing about another person's mind, but also for introspecting one's own mind in autism. The present study hypothesized that disturbed mPFC recruitment, as a function of the perceived self-other discrepancy, may be the underlying basis of atypical social cognition in autism. By employing fMRI, the present study compared the mPFC activity and functional connectivity (synchronization of activation) with other cortical areas of ToM network between adults with autism and controls (n=7 in both groups) while they made judgments about how well a series of personality statements applied to themselves and to other people. The results indicate that only the autism group showed near absent mPFC activation during mentalizing about other individuals who are dissimilar from themselves, whereas similar mPFC activation was observed in both groups during menatlizing about other individuals who are similar to themselves. An interaction of extreme egocentrism and dysfunctional allocentrism is proposed as a theoretical account for an atypical understanding of social world in autism. [This research was supported by the Autism Centers of Excellence Grant HD055748 from the National Institute of Child Health and Human Development]

1.64 **The Interactive Effects of Distal and Proximal Stressors on Alcohol Consumption among Adults**

Kelly C. Young-Wolff, and Carol A. Prescott, University of Southern California Kenneth S. Kendler, Virginia Commonwealth University

Background: Childhood maltreatment (maltx) is a well-established risk factor for heavy alcohol consumption in adulthood. Less is known about the processes through which maltx contributes to adult drinking behaviors. One relatively unexplored mechanism is stress sensitization, whereby early adversity leads to psychobiological changes that increase risk for drinking following subsequent stressful life events (SLEs). In this study, we examine whether SLEs independent (iSLEs) or dependent (dSLEs) on a participant's actions interact with maltx to predict drinking in adulthood. Method. Past-year alcohol consumption and SLE exposure was assessed using structured clinical interviews in a sample of 4065 participants aged 20-61 from the Virginia Adult Twin Study of Psychiatric and Substance Use Disorders. Stress sensitization was examined using hierarchical multiple regression analyses to test for a maltx x SLE interaction. Results. 10% of males and 20% of females reported maltx. Among females not exposed to maltx, drinking quantity was roughly the same across iSLE exposure. In contrast, iSLEs were associated with heavier drinking among females exposed to maltx. Interaction effects were not attenuated after adjusting for early family risk factors. There was little evidence for maltx x iSLE interactions in males, or for maltx x dSLE interactions in either gender. Conclusions. Our results suggest that the association between SLEs independent of participant behavior and alcohol consumption is stronger among females with a history of maltx. Interaction effects were not attributable to early family environmental factors more broadly, suggesting that maltreatment may have direct effects on vulnerability to stress-related drinking. [MH/AA-49492, AA/DA-09095, AA-018611]

STEM CELLS

1.65 **High-Capacity Assay to Quantify the Clonal Heterogeneity in Potency of Mesenchymal Stem Cells**

Katie C. Russell, Michelle Lacey, Bonnie Barrilleaux, Kristin Meyertholen, Kim O'Connor, Tulane University; Donald Phinney, The Scripps Research Institute

The regenerative capacity of mesenchymal stem cells (MSCs) is contingent on their content of multipotent progenitors. Despite its importance to the efficacy of MSC therapies, the clonal heterogeneity of MSCs remains poorly defined. To address this deficiency, the current study presents a novel high-capacity assay to quantify the clonal heterogeneity in MSC potency and demonstrates its utility to resolve regenerative properties as a function of potency. The in vitro assay utilizes a 96-well format to (1) detect fluorescent MSC clones after limiting dilution, (2) generate matched clonal colonies, (3) differentiate 3 matched colonies per clone to quantify trilineage potential to exhibit adipo-, chondro- and osteogenesis as a measure of potency, and (4) cryopreserve the 4th matched colony of each clone in an undifferentiated state. All eight categories of trilineage potential were detected in human marrow MSCs. Multipotent MSCs had a higher proliferation potential than lineage-committed MSCs. The median growth rate was 0.85 day-1 for tripotent clones, 5-fold more than for unipotent clones. Colony-forming efficiency had a similar trend. At least 75% of unipotent MSCs were positive for senescence-associated beta-galactosidase vs. < 10% for tripotent MSCs. CD146 may be a potency marker, with a 2-fold difference in mean fluorescence intensity between tri- and unipotent clones. These data suggest a complex hierarchy of lineage commitment in which proliferation potential and CD146 expression diminish with loss of potency. The capacity of multipotent MSCs for ex vivo expansion and their differential expression of a potential potency marker will facilitate rapid production of efficacious MSC therapies with consistent progenitor content. [This work was funded by NIH and NSF.]

1.66 **The Role of USP22 in Pluripotency and Differentiation**

Robyn T. Sussman, and Steven B. McMahon, Thomas Jefferson University

USP22 is a ubiquitin hydrolase that we and others have recently shown cleaves mono-ubiquitin moieties from histones H2A and H2B. We have reported that USP22 is a stable component of the hSAGA transcription co-activator complex. We have also shown that USP22 is required for cell cycle progression in differentiated cells. We report that USP22 is important for mediating mammalian differentiation programs through transcriptional regulation. USP22 depleted mESCs are not as proficient as control cells in differentiating upon embryoid body formation as measured by alkaline phosphatase expression, which correlates with a more stem-like morphology. Furthermore, after embryoid-body induced differentiation, USP22-depleted mESCs show decreased expression of markers for all three germ layers compared to control cells. These cells show no change in their cell-cycle profile or BrdU incorporation. Before induction of differentiation, USP22-depleted mESCs display increased expression of the Sox2 transcription factor. This coincides with increased RNA Polymerase recruitment across the Sox2 locus and enhancer region, as well as a loss of histone H2B. Interestingly, ubiquitylated H2B levels increase across the sox2 locus and enhancer region upon USP22 depletion, which corresponds with USP22's enzymatic activity. These data suggest a model in which USP22 is important in maintaining steady-state Sox2 levels in ESCs and upon depletion of USP22, ESCs are somewhat defective in differentiation, potentially because of an overabundance of Sox2.

1.67 **Investigating an interchangeable potential between heart and gut mesothelium in the embryo**

Rebecca T. Thomason, Niki Winters, Emily Cross, David Bader, Vanderbilt University

The mesothelium is an epithelial sheet that covers organs in the coelomic cavity and is involved in development of the vascular system. In the developing heart, the proepicardial organ (PE), migrates to and over the heart to form the epicardium, and undergoes epithelial-mesenchymal transition (EMT) to give rise to the cells of the coronary blood vessels. The gut mesothelium (GM) serves as a major source of vascular smooth muscle cells for the gut tube in development. The role of mesothelial cells in both the heart and gut in the formation of blood vessels suggest that there may be similarities, possibly an interchangeable potential, in the development of coelomic organs. We have been characterizing the gut mesothelium in the avian embryo using histological and molecular techniques to determine how it develops and morphologically changes over time. To test the interchangeable potential of mesothelial cells, we used the chick-quail chimera system to transplant quail PEs into the peritoneal cavity of a chick embryo and quail GM cells into chick pericardial cavities. Our initial findings have revealed that both cell types have the potential to migrate into organs in the coelomic cavities, but PE cells do not incorporate into the endogenous GM, while GM cells will incorporate into the endogenous epicardium. However, in both systems, transplanted PE and GM cells are positive for smooth muscle. Taken together, our current data suggest that although the epicardium and GM appear similar in structure in the embryo and adult, and can potentially give rise to smooth muscle actin positive cells, we observe fundamental differences in how the mesothelium develops and its interchangeable properties in the heart and gut. [Serosal Mesothelium and Vascularization of the Gut 4043360241]

STRUCTURAL BIOLOGY

1.68 Structural Studies of Alpha-Synuclein Fibrils and the Lipid Catalyzed Fibrillation Pathway

Gemma Comellas, Luisel R. Lemkau, Andrew J. Nieuwkoop, Kathryn D. Kloepper, Julia M. George, Chad M. Rienstra, University of Illinois at Urbana-Champaign

Alpha-Synuclein (AS) fibrils are the major component of Lewy bodies, the pathological hallmark of Parkinson's disease (PD). AS fibrils are insoluble and do not form diffraction quality crystals; therefore, solution NMR or X-ray diffraction cannot be used to investigate the fibrils at an atomic level. Solid-state NMR offers unique access to insoluble protein systems. However, previous chemical shift assignments of AS fibrils (Heise et al. 2005; Kloepper et al. 2007) were lacking key stretches of amino acid residues. Here, we present the results from an extensive investigation employing solid-state NMR to present a detailed structural characterization and conformational dynamics quantification of the fibrils. Our results show that the core extends with a repeated structural motif, which disagrees with the previously proposed fold (Vilar et al. 2008). Additionally, our results demonstrate that the three single point mutations associated with early-onset PD are located in structured regions of the wild-type, and lead to major and minor structural perturbations by E46K and A53T mutation, respectively. These results, together with our investigations of the mass-per-length, provide the foundation for interpreting the structural restraints to obtain a 3D structure. The second part of this study focuses on the mechanism of fibril formation. AS is unstructured in solution and forms N-termini helices in the presence of anionic phospholipids. The tight interaction of AS with anionic phospholipids has shown to promote AS aggregation. Here, we present the results from capturing, at atomic-level, the transition from alpha-helical to beta-sheet fibrillar forms, as well as the changes in the phospholipid vesicles during fibrillation. [This work was supported by the NIH]

1.69 Characterizing iron storage proteins from Mycobacterium tuberculosis

Lisa M. McMath, Heidi Contreras, and Celia W. Goulding. Department of Molecular Biology and Biochemistry, University of California- Irvine, 2302 Natural Sciences I, Irvine, CA 92697, USA.

Like most pathogens, iron acquisition and regulation are critical for survival. Because of the poor solubility of iron in physiological conditions, both eukaryotes and prokaryotes have ferritin proteins, 24-subunit spherical assemblies that store iron and keep it bioavailable. The Mycobacterium tuberculosis (Mtb) genome encodes for two ferritin homologs: a heme-containing bacterioferritin (Mtb BfrA) and a non-heme ferritin (Mtb BfrB). Both are highly structurally conserved and share nearly identical roughly spherical architectures. The main structural difference is that bacterioferritins contain a non-covalently bound heme in a pocket created by the interface between two subunits of a dimer related by a non-crystallographic twofold axis; 12 heme groups per complex. Both BfrA and BfrB were crystallized and solved by X-ray crystallography to 2.9 Å and 2.5 Å, respectively. The typical ferritin fold was observed for each and density in the ferroxidase sites is observed, potentially indicating bound iron ions. Additionally, analysis of the Mtb BfrB crystal structure combined with sequence alignments with other structurally characterized bacterial ferritins revealed the presence of a 15-residue C-terminal extension. By protein pull-down experiments, we have shown that Mtb BfrB interacts with a newly described protein nanocompartment, Mtb encapsulin, and we postulate that this extension is the BfrB anchor peptide for the interaction. However, Mtb BfrA has neither a C-terminal extension nor an interaction with encapsulin. Hence, in an effort to gain a better understanding of Mtb iron metabolism, we report here the structural and functional characterization of the Mtb BfrA and BfrB assemblies, as well as a description of a novel association of Mtb BfrB with encapsulin. [This work has been supported by National Institutes of Health grant AI068135 (subcontract to CWG).]

1.70 **Structure-Function Elucidation of Selective Substrate Binding and Catalysis by Cyclooxygenase-2**

Alex J. Vecchio, Michael G. Malkowski, University at Buffalo, The State University of New York and The Hauptman-Woodward Medical Research Institute

The cyclooxygenases (COX-1 and COX-2) are membrane-associated heme-containing homodimers that generate prostaglandin H2 from arachidonic acid (AA, omega-6) in the committed step of prostanoid biogenesis and are the targets for nonsteroidal anti-inflammatory drugs. Both isoforms behave as heterodimers during catalysis, exhibiting half-of-sites reactivity. Although AA is the preferred substrate for both COX isoforms, only COX-2 can efficiently oxygenate omega-3 fatty acids and endocannabinoid substrates. The aim of these studies was to investigate the functional divergences between COX isoforms considering the structural convergences. To elucidate the atomic level interactions that are involved with catalytic binding and positioning of substrates, we determined the X-ray crystal structures of AA, the omega-3 fatty acids EPA and DHA, and the endocannabinoid 1-AG, bound to murine COX-2. Results indicate AA, EPA, DHA, and 1-AG bind in different conformations in each monomer constituting the homodimer in their respective structures, such that one monomer exhibits productive binding and the other unproductive binding of the substrate in the cyclooxygenase channel. Additionally, comparisons of these COX-2:substrate crystal structures revealed an alternate rotamer conformation of Leu-531 that allowed for these novel substrate conformations; but interestingly, mutagenesis of Leu-531 in COX-2 did not result in a significant decrease in Vmax or Km, in contrast to similar studies with COX-1. These findings suggest that the mobility of Leu-531 increases the accessible volume at the opening of the cyclooxygenase channel, and contributes to the observed ability of COX-2 to oxygenate a broader spectrum of substrates compared to COX-1. [NIH grant R01 GM077176 from the NIGMS]

TOXICOLOGY/ENVIRONMENTAL HEALTH

1.71 **Mechanisms of Microvascular Dysfunction in a Murine Model: The Role of Particulate Matter Composition**

Azita Cuevas, Eric N. Liberda, Qingshan Qu, Lung Chi Chen New York University School of Medicine, Department of Environmental Medicine

Size, chemical composition and emssion source of PM2.5 (<2.5μm) has been associated with changes in endothelial function in humans and animal models. This study collected PM2.5 from two cities in Gansu Province, China: Jinchang (JC), home to a large nickel refinery, and Zhangye (ZH), an upwind city, to identify the chemical components associated with vascular dysfunction. Although PM2.5 concentrations were similar, JC had levels of nickel, selenium, copper, and arsenic that were 76, 25, 17, and 7 fold higher than that measured in ZH. FVBN mice received a single oropharyngeal aspiration of aqueous suspension of PM2.5 (50μl@1mg/ml) from JC or ZH (N=6/grp). In addition, four groups of mice received a single aspirated dose of PM2.5 from ZH, spiked with one of the following elements at the same concentrations found in the JC PM (Ni=4.76; As=2.36; Se=0.24; Cu=2.43μg/mg) or water control (N=6/grp). The groups that demonstrated the most and least pulmonary inflammation were then evaluated for vascular response following repeated spiked PM2.5 aspirations (2x/wk for 3wks; N=6/grp). Secondary order mesenteric arteries were isolated, cut into 2-mm segments and mounted on a tension myograph. Contractile and dilatory responses were measured at 24hr post the last exposure. Lung lavage from the single exposure revealed significant pulmonary inflammation from the JC when compared to ZH ($p<0.001$). Significant differences were seen in vascular function among the groups spiked with Ni and Cu but not As and Se. These data suggest that pulmonary, as well as, vascular responses can be driven by specific components of PM2.5. Even short-term exposure to PM can induce an acute systemic inflammatory response and trigger endothelial damage and vascular dysfunction. [Support: NIH Fellowship F31 ES018236 (Cuevas, AK), R01ES015495 (Chen, LC), CIHR-DRA (Liberda, EN)].

1.72 **Identification and Characterization of Novel Arylamine N-Acetyltransferase Small Molecule Inhibitors**

Carmine S. Leggett, Mark A. Doll, John O.Trent, Ph.D., David W. Hein,Ph.D., University of Louisville School of Medicine

Many environmental arylamines play an important role in the etiology of cancer and require metabolic activation by acetylation. Arylamine N-acetyltransferase 1 (NAT1), a xenobiotic metabolizing enzyme, catalyzes the N-acetylation or O-acetylation of arylamine carcinogens. O-acetylation, which is preceded by cytochrome P450 N-hydroxylation, results in nitrenium ions that covalently bond with nucleophilic groups on DNA forming adducts. If unrepaired, these adducts can lead to mutagenesis and initiate cancer. Molecular epidemiology studies report that NAT1 expression and genetic polymorphisms play an important role in individual risk to many diseases such as breast, colorectal, lung, prostate, and pancreatic cancer. The purpose of this study is to use NAT1 as a molecular target for the development of small molecule inhibitors for cancer prevention. We utilized in silico screening of 20 million compounds to identify those that interact with NAT active sites. A total of 150 lead compounds were identified, scored and ranked based on their active site association. Sixty of these compounds were tested for their ability to inhibit recombinant human NAT1. The IC50 values of the most efficacious inhibitors (compounds 2, 10, 11 and 16) determined against human NAT1 in vitro were >2mM, 0.75, 191, 202 μM, respectively. The most potent inhibitor (compound 10) was further tested for inhibition of N-acetylation in situ in Chinese hamster ovary (CHO) cells. The IC50 values of compound 10 were 118 μM and 81.6 μM towards the N-acetylation of p-aminobenzoic acid and 4-aminobiphenyl. We report the in silico identification and verification of compound 10 as a potential new small molecule for cancer prevention.[NIH/NIEHS T32-ES011564]

1.73 **Blood Test for Toxins in Jet Airplane: Mass Spectrometry to Identify Exposure to Tricresyl Phosphate**

Mariya S. Liyasova, Bin Li, Lawrence M. Schopfer, Oksana Lockridge, Eppley Institute, University of Nebraska Medical Center; Florian Nachon, Patrick Masson, Département de Toxicologie, Institut de Recherche Biomédicale des Armées; Clement E. Furlong, Department of Medicine (Div. Medical Genetics) and Genome Sciences, University of Washington, Seattle, WA.

The aircraft cabin ventilation is supplied from unfiltered bleed air directly from the engine. Defective engine seals can result in the release of engine oil into the cabin air supply. Aircrew and passengers have complained of illness following such "fume events". Adverse health effects are hypothesized to result from exposure to tricresyl phosphate, mixed esters added to jet engine oil. Our goal was to develop a laboratory test for exposure to tricresyl phosphate. The assay was based on the fact that the active-site serine of butyrylcholinesterase reacts with the active metabolite of tri-o-cresyl phosphate, cresyl saligenin phosphate, to make a stable phosphorylated adduct with an added mass of 80 Da. No other organophosphorus agent makes this adduct in vivo on butyrylcholinesterase. Blood samples from jet airplane passengers were obtained 24-48 hours after completing a flight. Butyrylcholinesterase was partially purified from 25 ml serum or plasma, digested with pepsin, enriched for phosphorylated peptides by binding to titanium oxide, and analyzed by mass spectrometry. Of 12 jet airplane passengers tested, 6 were positive for exposure to tri-o-cresyl phosphate that is, they had detectable amounts of the phosphorylated peptide FGEpSAGAAS. No more than 0.05 to 3% of plasma butyrylcholinesterase was modified. None of the subjects had toxic symptoms. Four of the positive subjects were retested 3 to 7 months following their last airplane trip and were found to be negative for phosphorylated butyrylcholinesterase. In conclusion, this is the first report of an assay that detects exposure to tri-o-cresyl phosphate in jet airplane travelers. [U.S. Army Medical Research and Materiel Command (W81XWH-07-2-0034) and the NIH (U01 NS058056, P30CA36727, R01ES09883, and P42ES04696)]

1.74 **The Complex Regulation of MnSOD Expressions by the AhR Agonist PCB126**

Bingxuan Wang, Ian Lai, Kai Wang, Larry Robertson, Gabriele Ludewig, University of Iowa

PCB126 is the most potent Ah receptor agonist in the whole polychlorinated biphenyl (PCB) family and a major contributor of their toxicities. It is proved to produce oxidative stress which could ultimately lead to cancer. Although a number of mechanisms have been proposed for it, little is known on PCB126's influence on antioxidant enzymes, especially the manganese superoxide (MnSOD), one of the most efficient antioxidant enzymes. The aim of this project is to examine PCB126's effects on MnSOD expressions and to explore its mechanisms in vivo. PCB126's dose- and time- response studies were carried out in male Sprague Dawley rats and the mRNA, protein and activity levels of hepatic MnSOD were measured. The results indicated a dose-and time- dependent induction of MnSOD mRNA and a decrease of MnSOD activity while the protein levels remained unchanged. We attempted to study the mechanisms of such expression changes by gel shift assays on several redox-regulated transcription factors of MnSOD and immunoprecipitation-Western Blot on possible post-translational modifications of the protein. In addition, a manganese dietary study was done to study the effect of Mn on the activity of MnSOD in response to PCB126. Our results showed that Nrf-2 had significantly increased activity associated with PCB126 exposure. On the other hand, acetylation, nitration and phosphorylation of MnSOD protein could not account for the loss of its activity. Mn supplementation increased the inducibility of MnSOD mRNA but didn't reverse the drop of its activities by PCB126. In conclusion, we discovered a complex regulation of MnSOD expression by PCB126, the mechanisms of which calls for further elucidation. (Supported by NIEHS P42 ES013661 and DAMD17-02-1-0241)

POSTER SESSION 2

MONDAY, OCTOBER 17

12:30 – 2:00 PM

CANCER

2.1	**Kaushal Asrani,** Fn14 Receptor Signaling Promotes the Growth and Invasion of HER2-overexpressing Breast Cancer Cells
2.2	**Jessica Bell,** Novel Modulators of Rho Transcriptional Signaling in Prostate Cancer
2.3	**Gina Bernardo,** FOXA1 Actively Represses the Basal Breast Cancer Molecular Phenotype
2.4	**Cristel Camacho,** Loss of p15/Ink4b Accompanies Tumorigenesis Triggered by Complex DNA Double-Strand Breaks.
2.5	**Tracy Chow,** Understanding Mammalian Telomere End Processing to Improve Telomerase-based Cancer Therapies
2.6	**Aric Colunga,** The HSV-2 Mutant, DeltaPK Induces Multiple Death and Inflammatory Programs Associated with Tumor Elimination.
2.7	**Conrad Russell Cruz,** Combined Epigenetic and Immune-based Therapy for Relapsed Hodgkin's Lymphoma Post HSCT
2.8	**Sandra Indiviglio,** Defining the Ku Heterodimer's Role at Human Telomeres
2.9	**Michael Manning,** Prostate-Specific Antigen (PSA) Is an Immunomodulator
2.10	**Whei Moriarty,** Neuropilin-2 as a Mediator of Melanoma Metastasis
2.11	**Van Nguyen,** Targeting a Cancer-associated Biomarker with Diagnostic and Therapeutic Radioactivity
2.12	**Mariana Pavon-Eternod,** Transfer RNAs as Biomarkers and Regulators in Breast Cancer
2.13	**Elizabeth Toomey,** NR0B1 and BCL11B are Critical Up-regulated Targets of EWS/FLI
2.14	**Kathryn Tworkoski,** Analysis of the Melanoma Kinome: Focus on the TAM Family of Receptor Tyrosine Kinases
2.15	**Laura Wright,** Curcuminoids Inhibit PTHrP-mediated Breast Cancer Osteolysis Separate from Effects on Tumor Cell Growth

EPIDEMIOLOGY/BIOSTATISTICS

2.16	**Katherine Ahrens,** Prenatal Influenza Vaccination and the Risks of Preterm and Small for Gestational Age (SGA) Birth
2.17	**Elisabeth Beaber,** Breast Cancer Risk and Use of Oral Contraceptives by Type and Recency among Young Women
2.18	**Kristin Guertin,** Effect of Vitamin E and Selenium on F2-Isoprostanes, an Oxidative Stress Biomarker

2.19 **Lareina La Flair,** Intimate Partner Violence Victimization and Women's Alcohol Use: A Latent Class Analysis

GENETICS/GENOMICS

2.20 **Michael Guertin,** Accurate Predictions of Inducible Transcription Factor Binding Intensities in vivo

2.21 **Mee Kim,** Spatial and Temporal Characterization of Single Minded 1 (SIM1) Enhancers

HIV/AIDS

2.22 **Diana Chen,** Gag-Specific CD8+T Cells Have Moderate Susceptibility to Nef

2.23 **Renee Heffron,** Hormonal Contraceptive Use and Risk of HIV Transmission: A Prospective Cohort Analysis

2.24 **William Kowalczyk,** Decision Making and the Association of Missed Medication on Transmission Risk in a Sample of HIV+ MSM

2.25 **Tiffany Lucas,** Mechanistic Studies of an Unexpected HIV-1 Vpu Function on Retrovirus Assembly

2.26 **Eleftherios Michailidis,** Novel Mechanism of Inhibition of HIV-1Reverse Transcriptase by 4'substituted Nucleoside Analogs

2.27 **Nadeene Riddick,** CXCR6 and GPR15 are Efficient Entry Coreceptors used by SIVsmm from CCR5- and CCR5+ Sooty Mangabeys

2.28 **Tino Sanchez,** Discovery of Small Molecules Inhibiting HIV-1 Integrase and Cellular LEDGF/p75 Protein-Protein Interaction

2.29 **Ming Zeng,** Lymphoid Tissue Destruction during HIV Infection Depletes Naïve T Cells and Limits Immune Reconstitution

IMMUNOLOGY

2.30 **Michael Abt,** Resident Microbial Communities Regulate Anti-viral immunity

2.31 **Sandra Bonne-Année,** Macrophages and Neutrophils from Humans and Mice Kill Larval Strongyloides stercoralis

2.32 **Christopher Case,** Asc Modulates the Function of NLRC4 during Legionella pneumophila Infection in Macrophages.

2.33 **Wei-Jen Chua,** Unique Role of MR1-restricted MAIT Cells in Gut Homeostasis and Lung Bacterial Infection

2.34 **Jon Gabbard,** Passive Immunization with an Influenza M2e-specific Miniantibody Administered via Naked DNA Delivery

2.35 **Ritobrata Goswami,** PU.1 Regulates Chromatin Modifications of the Il9 Gene in Th9 Cells

2.36 **Matthew Gubin,** Coordinate Regulation of GATA3 and Th2 Cytokine Gene Expression by the RNA-binding Protein HuR

2.37 **Matthew Hufford,** Cytotoxic T Lymphocyte Effector Activity Is Regulated by Target Cell Type

2.38 **Brandon Kwong,** Liposome-coupled AntiCD40+CpG Induces Potent Anti-Tumor Responses with Minimal Systemic Side Effects

2.39 **Elliott Moseman,** Chemokine Receptor CX3CR1 Marks Effector and Effector Memory CD8+ T Cells after Viral Infection

2.40 **Simon Owino,** Placental CD44 Interacts with VAR2CSA-expressing, P. falciparum-infected Erythrocytes

2.41 **Deepali Sawant,** The Transcriptional Repressor BCL6 Controls the Th2 Inflammatory Activity of Regulatory T Cells

2.42 **Chao Shi,** Inflammatory Monocytes but not Neutrophils Are Essential for Defense against Listeria monocytogenes Infection

2.43 **Nicole Sullivan,** Relative Importance of B cells and T cells in Mucosal and Systemic Trypanosoma cruzi Protection

2.44 **Nekeithia Wade,** Understanding the Contributions of Lupus Susceptibility Loci in SLE-accelerated Atherosclerosis in Mice

MICROBIOLOGY AND VIRUSES

2.45 **Richard Adeyemi,** Parvovirus Minute Virus of Mice Induces an ATM-p53 Mediated DNA Damage Response That Enhances Viral Infection

2.46 **Jasdave Chahal,** Adenovirus E1B55K Protein Blocks Interferon-induced Inhibition of Viral Replication in Normal Cells

2.47 **Jenny An-Chun Chien,** A UDP-glucose Sensitive Rheostat for Cell Size

2.48 **Lindsey Crawford,** The Effect of the Cellular SUMOylation Pathway on KSHV Infection and Replication

2.49 **Marciela DeGrace,** Dissecting the Role of Trafficking Machinery in the Influenza A Life Cycle Reveals Novel Restriction Factors

2.50 **Shalon Ledbetter,** Probing the Starvation Stress Response in the Malaria Parasite, Plasmodium falciparum

2.51 **Emily Miller,** Inhibition of Ebola Virus Entry by a C-peptide Targeted to Endosomes

2.52 **Stacia Phillips,** Interaction Between the Human Cytomegalovirus Proteins UL94 and UL99 is Required for Viral Assembly

2.53 **Jessica Pierce,** Host Immune Status Affects the Regulation of Colonization by Candida albicans in the GI Tract

MITOSIS/CHROMOSOMES/CELL CYCLE

2.54 **Larry Bellot,** A Novel Role of Human APE1/Ref-1 and Its Acetylation in Structural Maintenance of the Genome

2.55 **Mengqiao Wang,** Asymmetrical Localization of a Lysine Deacetylase to the Bud Neck and the Daughter Spindle Pole Body

REGULATION OF GENE EXPRESSION

2.56 **Erin Clark,** Regulation of the Histone Demethylase LSD1 by the Glucocorticoid Receptor

2.57 **Leah Cushing,** miR-29 Regulation of ECM and Smooth Muscle Phenotype

2.58 **Heba Diab,** pH Homeostasis Is Critical in Managing Oxidative Stress and Iron Metabolism

2.59 **Tonibelle Gatbonton-Schwager,** Bryonolic Acid Transcriptional Control of Inflammatory and Antioxidant Genes in Macrophages

2.60 **Alison Heffer,** Evolution of the Hox Gene fushi tarazu in Arthropods

2.61 **Fu-Kai Hsieh,** Mechanism of FACT Action during Transcription through Chromatin by Pol II

2.62 **Maja Janas,** Regulation of MicroRNA-mediated Repression: Enter Ribosomal Proteins

2.63 **Mayumi Miller,** Cooperative Wnt Signaling Regulates Lung Progenitor Differentiation

2.64 **Sreejith Nair,** Critical Role of a Novel Transcription Co-regulator in Mammary Gland Development and Morphogenesis

2.65 **Bryan Nikolai,** HER-2 Signaling Affects SRC-3 Phosphorylation and Gene Regulatory Potential within the Cistrome

2.66 **Gabriel Rosenfeld,** Identifying Novel Gata4-regulated Molecular Networks during Cardiogenesis

2.67 **Anna Trofka,** The Her7 Node Asymmetrically Regulates the Hes6 Hub to Modulate the Segmentation Clock Network

SIGNALING

2.68 **Nicole Ashpole,** The Role of Calcium/Calmodulin-dependent Protein Kinase II Signaling in Neuronal Excitotoxicity

2.69 **Hashem Dbouk,** Gbetagamma-mediated Activation of p110beta Is Required for Transformation

2.79 **Amelia Hochreiter-Hufford,** A Beneficial Role of Apoptotic Cells in Muscle Development

2.71 **Courtney Netherland,** Win55, 212-2 Decreases Plasma Triglycerides in Cannabinoid Receptor Type 2 (CB2) Deficient Ldlr-null Mice

2.72 **Kelly O'Brien,** An Important Role for Akt3 in Platelet Activation and Thrombosis

2.73 **Mary Puckett,** Interception of Stress Signaling by the ASK1/IKK Signaling Node

2.74 **Amy Rines,** Snf1-related Kinase Enhances Cardiomyocyte Metabolism through Tribbles Homolog 3 Downregulation

2.75 **Jami Scheib,** The Phagocytic Receptor Jedi Signals Engulfment through the Tyrosine Kinase Syk

ABSTRACTS - SESSION 2

CANCER

2.1 **Fn14 Receptor Signaling Promotes the Growth and Invasion of HER2-overexpressing Breast Cancer Cells**

Kaushal V. Asrani and Jeffrey A. Winkles Departments of Surgery and Physiology, Center for Vascular and Inflammatory Diseases, and the Marlene and Stewart Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, MD, 21201

Human epidermal growth factor receptor (HER)-2 overexpression occurs in ~25% of all breast cancers and these cancers have a very poor prognosis. HER2 requires HER3 for cellular transformation and the HER2-HER3 heterodimer functions as a powerful oncogenic unit. Heregulin (HRG)-ß1 is a natural ligand for HER3 and elicits the formation of potent HER2-HER3 heterodimers. Fibroblast growth factor-inducible 14 (Fn14), a member of the TNF receptor superfamily, is overexpressed in breast tumors, where high expression levels strongly correlate with both the invasive HER2+/ER- intrinsic subtype and indicators of poor prognosis. Hence, we investigated whether HER2 activation in breast cancer cells could directly induce Fn14 gene expression. We found that transient or stable transfection of MCF-7 cells with a HER2 expression plasmid increased Fn14 protein levels. Breast tumor tissue from HER2-transgenic mice also showed increased expression of Fn14 when compared to normal mammary gland. Also, HRG-ß1 induced Fn14 protein expression in a dose- and time-dependent manner in MCF-7 cells. Both the HER2 and HRG-ß1-induced increase in Fn14 expression in MCF-7 cells could be blocked by treatment with the HER1/2 tyrosine kinase inhibitor lapatinib. Also, siRNA knockdown of HER2 and/or HER3 decreased Fn14 expression in HER2-overexpressing cells, as did treatment with the MEK inhibitor U0126 or the PI3K inhibitor Wortmannin. Stable knockdown of Fn14 in SKBR3 cells decreased anchorage-dependent/independent growth. Also, Fn14 knockdown in MCF-7/HER2 cells reduced invasiveness through Matrigel. Studies are in progress to determine the mechanisms underlying these effects and to investigate if Fn14 could be a new therapeutic target for HER2+ tumors which develop resistance to HER2-targeted drugs.

2.2 **Novel Modulators of Rho Transcriptional Signaling in Prostate Cancer**

Jessica L. Bell, Chris R. Evelyn, Jenny G. Ryu, Susan M. Wade, Richard R. Neubig, Scott D. Larsen, University of Michigan

RhoA/C play important roles in metastasis of multiple cancers, including prostate cancer. While Rho GTPases are known for their role in cytoskeletal reorganization, they have a less well understood downstream action on gene transcription. Current inhibitors of the Rho pathway are directed at downstream effectors; however, few target gene transcriptional modulation. We identified CCG-1423 using an SRE-Luciferase high throughput screen. It blocks gene transcription by acting on an unidentified target within the RhoA/C signaling pathways. It also inhibits invasion of PC-3 prostate cancer cells in a Matrigel model of metastasis. However, CCG-1423 exhibits modest acute cellular toxicity as shown by non-specific inhibition of gene expression (TK-Renilla) and a decrease in WST-1 cell viability. Therefore, our goals have been to: 1) improve potency and/or selectivity; 2) reduce cytotoxicity; and 3) identify the macromolecular target(s). SAR has focused on bioisosteric replacement of amide bonds and conformational restriction. Two of our best analogs (CCG-101425 and 100602) improve selectivity for inhibition of RhoA/C-mediated gene transcription and attenuate cytotoxicity. Both inhibit PC-3 prostate cancer cell invasion similarly to CCG-1423, but without associated cytotoxicity. We are currently focusing on development of probe molecules based on 100602 for use as affinity reagents to identify the macromolecular target(s) of the RhoA/C pathways. This work could provide a completely novel target for anti-metastatic therapies for prostate cancer. [NIH R01GM39561; University of Michigan Comprehensive Cancer Center Prostate SPORE; Fred W. Lyons, Jr. Fellowship]

2.3 **FOXA1 Actively Represses the Basal Breast Cancer Molecular Phenotype**

Gina M. Bernardo, Case Western Reserve University; Gurkan Bebek, Case Western Reserve University; Charles L. Ginther, University of California, Los Angeles; Steven T. Sizemore, Case Western Reserve University; Kristen L. Lozada, Case Western Reserve University; John D. Miedler, University Hospitals-Case Medical Center; Lee A. Anderson, University of California, Los Angeles; Andrew K. Godwin, Kansas University Medical Center; Fadi W. Abdul-Karim, University Hospitals-Case Medical Center; Dennis J. Slamon, University of California, Los Angeles; and Ruth A. Keri, Case Western Reserve University

Breast cancer is a heterogeneous disease comprised of at least five major subtypes. Luminal tumors confer a more favorable prognosis, which is attributed to Estrogen Receptor-a (ER) positivity. Expression of FOXA1 also correlates with the luminal subtype and survival, but can be expressed in a subset of ER-negative tumors. Similarly, FOXA1 is consistently expressed in luminal breast cancer cell lines even in the absence of ER. In contrast, basal breast cancers do not express FOXA1, and loss of FOXA1 in luminal cells increases migration and invasion, characteristics of the more aggressive basal subtype. To delineate an ER-independent role for FOXA1 in maintaining the luminal phenotype, we performed cDNA microarray analyses on FOXA1-positive, ER-positive (MCF7, T47D) and FOXA1-positive, ER-negative (MB-453, SKBR3) cells in the presence or absence of transient FOXA1 silencing. This resulted in three FOXA1 transcriptomes: luminal-dependent (consistent in all lines), ER-dependent (MCF7 and T47D only) and ER-independent (MB-453 and SKBR3 only). Gene set enrichment analyses, used as a phenotyping tool, revealed a transcriptional shift from luminal to basal gene expression. FOXA1 binds to both luminal and basal genes, suggesting it functions not only to transactivate luminal genes, but also to repress basal genes. From these results we conclude that FOXA1 controls plasticity between basal and luminal cells, playing a dominant role in repressing the basal phenotype, and thus tumor aggressiveness, in luminal breast cancers. Although it has been proposed that FOXA1-targeting agents may be useful for luminal tumors, these data suggest that this approach may give rise to more aggressive cancers. [DoD W81XWH-06-1-0712 and NIH T32-HD-07104-33 (G.M.B.); DoD W81XWH-08-1-0347 (R.A.K.)]

2.4 **Loss of p15/Ink4b Accompanies Tumorigenesis Triggered by Complex DNA Double-Strand Breaks.**

Cristel V. Camacho, Bipasha Mukherjee, Brian McEllin, Michael D Story, David A Boothman, Robert M Bachoo, Sandeep Burma, University of Texas- Southwestern Medical Center

DNA double-strand breaks (DSBs) are the most deleterious lesion inflicted by ionizing radiation. Although DSBs are potentially carcinogenic, it is unclear whether complex DSBs, such those induced by Fe ions, are more potently tumorigenic compared with simple breaks induced by gamma-irradiation. To determine whether Fe-induced DSBs are more potently tumorigenic than gamma ray-induced DSBs, we irradiated sensitized astrocytes deficient in Ink4a and Arf tumor suppressors and injected the surviving cells subcutaneously into nude mice. Using this model system, we find that Fe ions are potently tumorigenic, generating tumors with significantly higher frequency and shorter latency compared with tumors generated by gamma rays. Tumor formation by Fe-irradiated cells is accompanied by rampant genomic instability and multiple genomic changes, the most interesting of which is loss of the p15/Ink4b tumor suppressor due to deletion of a chromosomal region harboring the CDKN2A/CDKN2B loci. The additional loss of p15 in tumors derived from cells that are already deficient in Ink4a bolsters the hypothesis that p15 plays an important role in tumor suppression, especially in the absence of Ink4a. Indeed, we find that reexpression of p15 in tumor-derived cells significantly attenuates their tumorigenic potential, indicating that p15 loss may be a critical event in tumorigenesis triggered by complex DSBs. We are extending our studies using transgenic mice with glioma-relevant genetic alterations. Very strikingly, we observe rapid induction of high grade gliomas after intra-cranial exposure to Fe ions, which we have not observed with gamma-irradiation, underscoring the highly transforming nature of Fe particles. [National Aeronautics and Space Administration, National Cancer Institute]

2.5 **Understanding Mammalian Telomere End Processing to Improve Telomerase-based Cancer Therapies**

Tracy T. Chow, Yong Zhao, Jerry W. Shay and Woodring E.Wright, UT Southwestern Medical Center

Infinite proliferation is a hallmark signature of cancer cells that is tightly related to the maintenance of telomere lengths. Human telomere DNA contains hexameric repeats (5'-TTAGGG-3') and end in a single-stranded 3' telomeric G-rich overhang located at the end of a chromosome. The telomere overhang plays an essential role in telomere end protection and serves as a substrate for telomerase extension in most human cancer cells. However, little is known about how overhangs are generated and how overhang maturation is coupled with telomere replication. In particular, the differences in the biogenesis of leading versus lagging overhangs are unknown. We developed new differential-DNA-density-based biochemical techniques to separate leading versus lagging stands to study telomere overhang dynamics. Replicating cells were labeled with 5-bromodeoxyuridine (BrdU) during S-phase of the cell cycle. Differential BrdU incorporation into leading versus lagging telomeres allows for strand separation using cesium chloride gradients. It is thought that overhang maturation is tightly coupled with telomere replication. Surprisingly, we discovered that leading overhang processing was significantly delayed following telomere replication and involved several steps. In contrast, lagging overhangs immediately reached mature lengths post replication. These findings not only highlight the mechanistic differences underlying leading versus lagging overhang generation, but also predict multiple nucleases may be involved in the processing of leading overhangs. Understanding this multi-step overhang generation pathway will provide new targets for the development of anti-telomerase cancer therapies. [Department of Defense Predoctoral Cancer Research Program BC083357 awarded to Tracy Chow.]

2.6 **The HSV-2 Mutant, DeltaPK Induces Multiple Death and Inflammatory Programs Associated with Tumor Elimination.**

Aric G. Colunga, Jennifer Laing, Biquan Li, Laure Aurelian, University of Maryland-School of Medicine

Metastatic melanoma is a highly aggressive and resistant cancer. Oncolytic virotherapy is a strategy designed to circumvent resistance through virus replication-induced cancer cell lysis. Unfortunately, its modest efficacy is related to poor virus replication and failure to stimulate immunotherapeutic pathways. Unlike standard oncolytics, the growth compromised herpes simplex virus type 2 mutant, deltaPK (d-PK) causes melanoma cell death through the activation of multiple death pathways. Western blot analysis revealed activation of calpain and caspases-3 and -7. Ethidium homodimer staining demonstrated that these pathways provide the bulk of the death programs, as evidenced by the finding of 80% protection in infected cells treated with the combination of calpain (PD150606) and pan-caspase (zVAD-fmk) inhibitors. Additionally, d-PK upregulates the autophagy proteins Beclin-1 and LC-II, and the autophagy inhibitor 3-methyladenine (3-MA) reduces d-PK-induced cell death by 15-17%. Cell death is further decreased (to a total of 28-34%) by treatment with 3-MA together with the JNK inhibitor SP600125, indicating that these processes contribute independently to d-PK-induced cell death. d-PK treatment also induces inflammatory-related pathways, as illustrated by caspase-1 activation and interleukin (IL)-1beta release into the conditioned medium as seen by ELISA. Tumor burden is significantly reduced or eliminated by intratumoral injection of d-PK and is associated with protease activation and inflammatory processes. Collectively, the data indicate that d-PK provides a distinctly promising virotherapeutic approach through the synergistic activation of cell death programs and pro-inflammatory signaling. [Funding: AR053512, NIAMS; ES07263, NIEHS; 1F31CA154087-01, NCI]

2.7 **Combined Epigenetic and Immune-Based Therapy for Relapsed Hodgkin's Lymphoma Post HSCT**

Conrad Russell Y. Cruz, (1), Ulrike Gerdemann (1), Jessica A. Shafer (1), Ann M. Leen (1), Anas Younes (2), Terzah M. Horton (3), Cliona M. Rooney (1), Helen E. Heslop (1), and Catherine M. Bollard (1)

Hodgkin's lymphoma patients who relapse after standard therapy have limited options for long-term cure. The success of T cell therapy targeting Epstein Barr virus (EBV) proteins for relapsed Hodgkin's Lymphoma is tempered by the fact that ~70% of these patients present with EBV-negative disease. We thus chose a new target, MAGE A4, for use in immunotherapy. MAGE A4 is only expressed in tumors and immune-privileged germ cells, is expressed in 30% of EBV negative HL, and its expression is upregulated by modifying the epigenetic environment. Thus, we hypothesize that a combined immune-based and epigenetic therapy targeting MAGE A4 will be a feasible and effective approach for treating relapsed HL. We first verified whether we can generate MAGE-A4-specific T cells. Dendritic cells from HL patients & healthy donors were pulsed with MAGE A4 peptides and successfully expanded a polyclonal population of antigen specific CTLs. These cells recognized different epitopes within the protein, and evaluable CTL showed specific killing against autologous targets pulsed with MAGE and HLA-matched MAGE A4+ HL tumor lines. We then explored the effect of combining the MAGE A4 –specific CTL with the epigenetic-modifying drug decitabine. Increased expression of MAGE-A4 in tumors was previously shown, and culturing MAGE-A4 CTL with decitabine had no effect on CTL specificity and function. Analysis of decitabine treated HL patient peripheral blood samples showed increased numbers of MAGE-A4 specific T cells or an increase in the breadth of epitope specific activity of the T-cells in vivo. These results suggest that MAGE-A4-specific T cell immunotherapy, coupled with therapy with epigenetic-modifying drugs, is a novel and practical approach for the treatment of relapsed HL post-HSCT.

2.8 **Defining the Ku Heterodimer's Role at Human Telomeres**

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The Ku heterodimer, composed of Ku70 and Ku80, is a high affinity DNA end-binding (DEB) protein that is essential in humans for its role in preventing telomere loss, presumably by homologous recombination (HR). Ku is also central in the protection of telomeres from non-homologous end joining (NHEJ) and yet it is unclear how either function is achieved. Assumptions are that Ku functions directly by DEB, as in budding yeast, or by association with telomeric proteins. To target these properties, we mutated Ku. We found that a conditionally null Ku80flox/- cell line stably expressing a DEB defective Ku80 mutant (Ku80-DEB) was viable, signifying that Ku's DEB ability is not vital for its telomeric role. Co-immunoprecipitation (co-IP) assays showed separate mutations targeting a-helix 5 in Ku70 and Ku80 to be deficient in binding telomere specific factors TRF1 and TRF2, respectively. Ku80-DEB was proficient for binding TRF1 and TRF2 in a co-IP showing that Ku's ability to bind these factors is separate of its ability to bind DNA. Using protein fragment complementation assays (PFCA) to assess Ku70-TRF1 binding in vivo, punctate foci were observed, suggesting the interaction occurs at telomeres. During mitosis the foci disappear, suggesting Ku70-TRF1 comes off the telomere. Further analysis of these mutants is planned. In conclusion, Ku70-a5 is a conserved region crucial for NHEJ, which ironically telomeres are shielded from to avoid chromosome fusions. TRF2 is required to prevent telomere loss via HR, which presumably occurs upon the loss of Ku. We propose that TRF1 binding to Ku70-a5 blocks cancer-inducing NHEJ at telomeres while TRF2 binding to Ku80-a5 prevents telomere loss via HR, thereby providing two mechanisms for how telomeres are protected from aberrant DNA repair. [NIH NRSA 5F31AG034764-02, 3R01GM077509-03S1, 5R01GM077509]

2.9 **Prostate-Specific Antigen (PSA) is an Immunomodulator**

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Prostate specific antigen (PSA) is a 33kDa glycosylated serine protease that is associated with the progression of prostate cancer (PCa). Little is known about the biology of PSA despite its widespread use as a biomarker. We have determined the complement factors C3 and C5, both central to the complement system, are proteolytic substrates of PSA. In this study, we focused on PSA-mediated cleavage of C3. N-terminal sequencing was used to map the site of PSA hydrolysis and demonstrated that PSA cuts C3 at a canonical PSA cleavage sequence. Next, we determined that PSA can liberate activated C3b deposited on the surface of cells. We then confirmed the liberation of C3b results in a functionally downregulated complement response. Evidence of PSA-mediated C3 cleavage was observed in the expressed prostatic fluid of radical prostatectomy specimens as well as in the seminal plasma of healthy men. These findings suggest PSA may possess a previously uncharacterized immunomodulatory function. The ability of PSA to remove deposited C3b from the cell surface indicates PSA can potentially protect a malignant cell from complement-mediated lysis. PSA-mediated removal of C3b may suppress the immune system in the vicinity of prostate cancer cells, allowing the initiation and progression of disease. Evidence of PSA-mediated C3 cleavage in seminal plasma suggests PSA may have an uncharacterized role in fertility. Confirmation of PSA's immunosuppressive function would further validate PSA as a therapeutic target.

2.10 **Neuropilin-2 as a Mediator of Melanoma Metastasis**

Whei F. Moriarty, Stephanie Gerber, Hans Hammers, Johns Hopkins University School of Medicine; Rhoda M. Alani, Boston University School of Medicine

Tumor cell interactions with neighboring endothelial cells are critical for tumor cell survival and the metastatic process. Melanomas are notorious for their ability to metastasize at a relatively early stage of development. A prior study identified neuropilin-2 (NRP2), a cell surface receptor involved in angiogenesis and axonal guidance, as an important mediator of melanoma-endothelial cell crosstalk. To investigate the role NRP2 plays in melanoma progression, we chose a panel of melanoma cell lines representing different melanoma progression phases for NRP2 shRNA knockdown experiments. We found that NRP2 knockdown in a metastatic melanoma cell line, 1205Lu, showed a decrease in tumor growth in vitro and in vivo. In addition, 2 of the 6 scrambled control mice versus 0 of 7 in the NRP2 knockdown mice developed lung metastasis. We believe NRP2 plays a role in mediating melanoma metastasis. [Funding supported by the Joanna M. Nicolay Melanoma Foundation]

2.11 **Targeting a cancer-associated biomarker with diagnostic and therapeutic radioactivity**

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Our objective is to exploit a novel ligand-based delivery system for targeting radioactivity to cells expressing Interleukin 13 Receptor a2 (IL13Ra2), a tumor-restricted plasma membrane receptor overexpressed in majority of Glioblastoma Multiforme (GBM) and other peripheral tumors. Based on prior work, we designed a novel IL13Ra2-Targeted Quadruple Mutant of IL13 (TQM13) that would bind the tumor-restricted IL13Ra2 with high affinity, but not interact with the physiologically abundant IL13Ra1/IL4Ra heterodimer expressed in normal brain. We evaluated affinity of TQM13 towards the IL13 receptors using autoradiography, surface plasmon resonance (SPR) and functional assays. We explored in vivo targeting potential of radioiodinated TQM13 via biodistribution study and microPET imaging of mice bearing subcutaneous IL13Ra2-expressing G48a GBMs. To test in vivo efficacy, we treated groups of mice bearing subcutaneous G48a GBMs intratumorally with 131I-TQM13 or controls. TQM13 specifically bound IL13Ra2-expressing GBM cells and specimens, but not normal brain. SPR results demonstrated TQM13 bound strongly to recombinant IL13Ra2 (Kd˜5nM) but did not significantly bind the reconstituted physiologically abundant IL13Ra1/IL4Ra receptor nor functionally activate it in cells. Biodistribution and microPET images revealed a 7:1 tumor-to-background ratio at 24 hours post intravenous delivery. Importantly, only 131I-TQM13 demonstrated significant in vivo anti-tumor activity against IL13Ra2-expressing tumors. We successfully generated an optimized, biomarker-targeted scaffolding that demonstrated specific binding towards the tumor-restricted IL13Ra2 in vitro and potential to deliver diagnostic and therapeutic radioactivity in vivo. [NINDS 1R21NS067471-01, Dana Foundation Neuroimmunology Award]

2.12 **Transfer RNAs as Biomarkers and Regulators in Breast Cancer**

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Although many non-coding RNAs have been extensively studied in relation to cancer, transfer RNAs (tRNAs) are generally viewed as non-regulatory genes involved in protein synthesis. Recent literature, however, indicates tRNAs play a key role in cancer development and progression. In particular, a 2008 study demonstrated that modest over-expression of the initiator methionine tRNA leads to malignant transformation in mouse fibroblasts. Furthermore, the NIH reference human genome contains an unexpected diversity of tRNA genes: over 270 unique sequences are present among 49 isoacceptor families. Given the sequence and functional diversity of tRNAs, we hypothesized that altered tRNA expression in cancer cells could define distinct breast tumor signatures and contribute to tumorigenesis. Using a microarray method to profile tRNA abundance, we compare tRNA levels in cancer versus non-cancer cells. Our results show that tRNA levels are significantly elevated in cancer versus non-cancer cells, in both cell lines and patient tissue samples. This tRNA over-expression is selective and coordinates with amino acid properties. Nuclear- and mitochondrial-encoded tRNAs also exhibit distinct expression patterns, suggesting tRNAs may be useful as biomarkers for breast cancer. We are now focusing on how these elevated tRNA levels contribute to tumorigenesis, presumably by altering translational activity and regulation in the cell. To adress this question, we are using a ribosome profiling approach to globally determine how translation is altered upon over-expression of specific tRNAs. We are also examining whether changes in tRNA levels affect the expression of cancer-related genes preceded by upstream open reading frames. [NIH NRSA, NSF EAPSI, NIH Training Grant].

2.13 **NR0B1 and BCL11B are Critical Up-regulated Targets of EWS/FLI**

Elizabeth Toomey, Bianca Lui, and Stephen Lessnick, Huntsman Cancer Institute, University of Utah

Ewing's sarcoma is a pediatric bone and soft tissue associated tumor that results from the t(11;22) translocation. This translocation fuses the N-terminal portion of the EWSR1 gene to the C-terminal portion of the ETS family member, FLI1, creating an aberrant transcription factor, EWS/FLI. EWS/FLI acts as an oncogene and contributes to tumor formation by up- and down-regulating a number of target genes. Work from our lab has identified NR0B1 as a gene that is directly up-regulated by EWS/FLI and necessary for transformation of patient derived Ewing's sarcoma cell lines in soft agar assays and mouse xenograft models. NR0B1 normally functions in the development and function of the hypothalamic, pituitary, adrenal and gonadal axis. The goal of the current project is to understand the mechanism by which NR0B1 contributes to transformation in Ewing's sarcoma. To answer this question, we performed a protein purification to identify NR0B1 interacting partners. BCL11B, a protein that is involved in T-cell differentiation, was identified as an interacting partner. This interaction was verified using co-immunoprecipitation. EWS/FLI knock-down by shRNA followed by rescue with a cDNA construct revealed that like NR0B1, BCL11B is up-regulated by EWS/FLI. Chromatin immunoprecipitation revealed that EWS/FLI binds to the BCL11B promoter. BCL11B is also critical to the growth and survival of Ewing's sarcoma cell lines as demonstrated by cell cycle analysis, apoptosis assays, and soft agar assays. This work has identified two genes that are directly up-regulated by EWS/FLI and are necessary for the transformed phenotype in Ewing's sarcoma. Ongoing work aims to identify the significance of this protein-protein interaction. [National Cancer Institute R01CA140394]

2.14 **Analysis of the Melanoma Kinome: Focus on the TAM Family of Receptor Tyrosine Kinases**

Kathryn A. Tworkoski, Christina Zito, Garima Sighal, Sebastian Szpakowski, Antonella Bacchiocchi, Marcus Bosenberg, Michael Krauthammer, Ruth Halaban, and David F. Stern, Yale University

Current therapies for patients with metastatic melanoma afford patients a survival time of nine months. Recent data suggests that treatments directed against receptor tyrosine kinases (RTKs) may improve patient care. It is not, however, understood which RTKs are activated in melanoma and might therefore serve as therapeutic targets. We performed a phospho-array screen to identify active RTKs in a panel of 25 melanoma cell lines and melanocytes. The majority of melanoma cell lines were derived from tumors biopsied or resected at the Yale/New Haven Hospital and cell lines were used below passage 20. Immunoblotting was used to verify a subset of the results from the screen. Active RTKs included TYRO3, AXL, MERTK, EPHB2, MET, IGF1R, EGFR, KIT, HER3, and HER4. qRT-PCR verified that the presence of AXL or HER3 in a cell line generally correlated with the presence of the RTK in the parental tumor. Comparison of whole genome gene expression analysis with the phospho-screen revealed that some RTKs, such as FGFR3, may be activated via an autocrine loop. shRNA against AXL, HER3, and IGF1R reduced melanoma cell proliferation. Targeting AXL and IGF1R also reduced cell migration in a STAT3- and AKT-dependent manner, respectively. Knock-down of RTKs generated a cellular phenotype regardless of the RAS or RAF mutational status within a cell line. Our survey has identified which RTKs are most frequently and strongly activated in melanoma. Targeting these RTKs may have therapeutic benefit. [NSF Graduate Research Fellowship Program (KT), the Harry J. Lloyd Charitable Trust (DFS), National Cancer Institute grant number 1 P50 CA121974 (RH)]

2.15 **Curcuminoids Inhibit PTHrP-mediated Breast Cancer Osteolysis Separate From Effects on Tumor Cell Growth**

Laura E. Wright, The University of Arizona; Theresa A Guise, Khalid S Mohammad, Indiana University; Janet L Funk, The University of Arizona.

Curcuminoids are polyphenolic compounds isolated from the rhizome of turmeric (Curcuma longa L.), a botanical commonly used in Ayurvedic medicine. We previously found that curcuminoid-containing turmeric extracts inhibited osteoclast-mediated bone resorption in a translational model of rheumatoid arthritis and prevented rheumatoid synoviocyte expression of parathyroid hormone-related protein (PTHrP), a bone-resorptive peptide in rheumatic joints. Because PTHrP is also a critical breast cancer-derived mediator of lytic bone metastases, curcuminoid effects on breast cancer cell expression of PTHrP was tested. In vitro, the PTHrP-producing human breast cancer cell line MDA-MB-231 was pre-treated with curcuminoids (0.3-10 µg/ml) and stimulated with TGF-ß (5 ng/ml) for 24 hours. TGF-ß-stimulated PTHrP secretion was significantly inhibited by curcuminoids (IC50 = 1.8 µg/ml) independent of effects on cell proliferation (IC50 = 20 µg/ml). In vivo, female nude mice (n = 11/group) were inoculated with MDA-MB-231 cells (1 x 10^5) into the left cardiac ventricle and treated ip every other day with a curcuminoid-enriched extract (25 mg and 50 mg/kg/curcuminoids) or vehicle. Curcuminoid-treated mice had significantly reduced osteolytic bone lesion area (by 49% and 61% for low and high dose, respectively) compared to vehicle-treated mice ($p < 0.001$). In a separate study, using an identical dosing regime, orthotopic tumor volume in the mammary fat pad was not significantly reduced by curcuminoid treatment (n = 7/group). These initial data suggest that curcuminoids may decrease lytic bone metastases in breast cancer through inhibition of breast cancer cell expression of PTHrP separate from effects on tumor cell growth.

EPIDEMIOLOGY/BIOSTATISTICS

2.16 **Prenatal Influenza Vaccination and the Risks of Preterm and Small for Gestational Age (SGA) Birth**

Katherine A. Ahrens, Martha Werler, Carol Louik, Allen Mitchell/Slone Epidemiology Center at Boston University

Background: Influenza vaccination is routinely recommended for pregnant women, yet information on perinatal outcomes is lacking. Methods: Data were collected as part of the Boston University Pregnancy Health Interview Study (2006-2010), in which mothers of live born, non-malformed infants were interviewed within 6 months of delivery about various prenatal exposures, including vaccinations. Associations between any influenza vaccination two months before and during pregnancy and the outcomes of preterm birth (<37 weeks) and SGA (<10th percentile using both sex-specific reference values [r] and a customized standard [c]) were assessed using time-varying exposure survival analysis. To examine influenza vaccination timing, analyses were stratified by trimester of vaccination. The effect of exposure misclassification was modeled using probabilistic bias analysis with internal and external study validation data. Results: Prenatal influenza vaccination showed no association with the risk of preterm birth (adjusted hazard ratio 1.1, 95% confidence interval 0.76-1.5) and a slight protective association with SGA ([r] 0.87, 0.63-1.2; [c] 0.73, 0.54-1.0). Analysis by trimester of influenza vaccination yielded similar results to those overall. Maternal race was a strong downward confounder in SGA models. Taking misclassification of exposure status into account did not explain the observed protective effect of vaccination on SGA risk. Conclusion: We found no association between influenza vaccination and preterm birth and a possible protective association between influenza vaccination and SGA, which varied by SGA definition. Though limited by study size, these findings support no increased risk of preterm birth and SGA as a consequence of prenatal influenza vaccination.

2.17 **Breast Cancer Risk and Use of Oral Contraceptives by Type and Recency among Young Women**

Elisabeth F. Beaber, and Christopher I. Li, University of Washington, Fred Hutchinson Cancer Research Center; Diana S.M. Buist, Group Health Research Institute

Prior studies suggest current and recent oral contraceptive (OC) use may be associated with a modest increased breast cancer risk among young and/or premenopausal women, though many of these reports relied on self-reported use and have not characterized risks associated with newer hormonal preparations and use patterns. This case-control study at Group Health Cooperative, a large health maintenance organization, identified 1,107 health plan members ages 20-49 diagnosed with invasive breast cancer from 1990-2009 in the greater Seattle-Puget Sound region. We randomly selected 22,140 matched controls on age, year, and enrollment length. Multivariate-adjusted conditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI). We used electronic pharmacy records to calculate OC formulation, dose, and duration of use. Recent OC use (within 1 year of diagnosis) was associated with a 50% (95% CI=1.3-1.8) increased risk of breast cancer. Risk varied somewhat by OC type, with recent use of OCs containing the progestin ethynodiol diacetate (OR=2.6, 95% CI=1.4-4.7) or high dose estrogen (OR=2.7, 95% CI=1.1-6.2) associated with particularly high risks. Analyses further evaluating risks by breast cancer subtype and the impacts of parity, breast cancer family history, and screening mammography are in progress. Preliminary analyses suggest recent use of current OC formulations is associated with an elevated relative risk of breast cancer among women ages 20-49 that varies somewhat by OC formulation. Although breast cancer is rare among young women, the potential risk of breast cancer associated with certain formulations could impact OC recommendations by providers if these findings are confirmed. [NCI grants: R03CA141485 and U01CA063731]

2.18 **Effect of Vitamin E and Selenium on F2-Isoprostanes, an Oxidative Stress Biomarker**

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Oxidative stress plays a key role in the pathophysiology and progression of chronic obstructive pulmonary disease (COPD). The Selenium and Vitamin E Cancer Prevention Trial (SELECT) tested single or joint effects of vitamin E (400 IU/day all rac-alpha-tocopherol acetate) and selenium (200 µg/day selenomethionine) on prostate cancer in a randomized controlled trial in 35,533 men in North America. The Respiratory Ancillary Study (RAS) to SELECT investigated the effect of treatment on risk of COPD in the full sample, and on lung function outcomes in 2,920 men. RAS also measured urine F2-isoprostanes (F2s), an oxidative stress biomarker known to increase with smoking and COPD, in 517 men (mean age 64) after 36 months on supplement. Cigarette smokers (71%) and men with COPD (23%) were over-sampled. The median F2 value was 285 pg/mg creatinine (range 27-2218). In the placebo arm F2s were higher by 185 in smokers (vs. nonsmokers) and by 45 pg/mg creatinine in men with COPD (vs. no COPD). Compared to placebo, treatment with vitamin E attenuated oxidative stress by 56 pg/mg creatinine (p=0.01) and by 26 in the combination arm (p=0.29), while there was no effect in the selenium arm (p=0.87); effect sizes were in the same direction and of similar magnitude in never/former smokers without COPD. In the subgroup of men with COPD (n=116), treatment effects were similar in both vitamin E arms, although statistical power was limited. This study demonstrates that treatment with vitamin E attenuates F2s, a biomarker of oxidative stress, in men with and without COPD. Antioxidant intervention may offer a means to alter the oxidant/antioxidant imbalance in COPD and mitigate disease progression. [Funded by NIH RO1 HL071022, NCI/DCP grant CA37429, and NCCAM]

2.19 **Intimate Partner Violence Victimization and Women's Alcohol Use: A Latent Class Analysis**

Lareina N. La Flair, Catherine Bradshaw, Johns Hopkins University Bloomberg School of Public Health; Carla Storr, University of Maryland School of Nursing; Kerry Green, University of Maryland School of Public Health; Anika Alvanzo, Johns Hopkins School of Medicine; Rosa Crum, Johns Hopkins University Bloomberg School of Public Health

Intimate partner violence (IPV) is a major public health issue, yet little is known about the association between IPV victimization and problem drinking among women. Study objectives were to (1) identify subtypes of problem drinking among women, (2) examine the association between recent IPV and problem drinking classes, and (3) evaluate major depressive disorder (MDD) as potential mediator of the IPV-alcohol relationship. Data are from a cohort of 11,782 women identified as current drinkers in Wave 2 (2003-2004) of the National Epidemiological Survey on Alcohol and Related Conditions. Latent class analysis was used to categorize participants using 11 DSM-IV abuse/dependence criteria. The IPV measure was derived from questions regarding six abusive behaviors perpetrated by a spouse or romantic partner in the past-year. Latent class regression was used to evaluate the association between IPV and drinking class membership, accounting for MDD, prior alcohol use disorder, and sociodemographic variables. Three classes of problem drinkers were identified: Severe (Class 1: 1.9%; n=224), moderate (Class 2: 14.2%; n=1676), and non-symptomatic (Class 3: 83.9%; n=9882). Past-year IPV was associated with both severe and moderate classes (Severe AOR: 5.41, 95% CI 3.54-8.28; Moderate AOR: 1.82, 95% CI 1.35-2.45). Results show a strong association between IPV and problem drinking class membership. This study offers preliminary evidence that programs aimed at preventing problem drinking among women should consider IPV and MDD. This research was supported by National Institute of Alcoholism and Alcohol Abuse Grant F31AA 018935-01A1 awarded to Lareina N. La Flair and Grant AA016346 awarded to Rosa M. Crum.

GENETICS/GENOMICS

2.20 **Accurate Predictions of Inducible Transcription Factor Binding Intensities In Vivo**

Michael J. Guertin, Andre Martins, Adam Siepel, John Lis, Cornell University

Transcription factor (TF) binding intensity is influenced by both the DNA element's sequence and the surrounding chromatin landscape. To study the mechanisms influencing TF binding intensity, we focused on the Drosophila Heat Shock Factor (HSF), which inducibly binds a target DNA sequence element (HSE) following heat shock stress. We developed a novel experimental framework to quantify the genome-wide binding energy landscape for HSF in the absence of chromatin effects. Genomic binding energy constants and in vivo chromatin landscape data, generated during non-stressed conditions, were used to accurately predict stress-induced HSF binding profiles using a rules-based ensemble model. DNase I hypersensitivity and tetra-acetylation of H4 were the most influential covariates in predicting HSF binding affinity. We achieve a correlation coefficient of 0.7 between in vivo intensity and the predicted test set values. This analysis is unique because we used the chromatin state prior to TF binding to predict inducible binding. Furthermore, as a test of our model, we demonstrate that directly changing the chromatin landscape by increasing histone acetylation permits inducible HSF binding at HSEs. TFs bind to many sites in a genome and only a subset of sites are transcriptionally activated. We set out to determine the basis of context-dependent HSF activation. Comparing genes in which HSF was promoter bound, we found that pre-bound GAF correlated with gene activation, and in contrast, BEAF insulated unregulated genes from HSF activation. [This work was funded by an NIH grant GM25232 and a predoctoral training grant T32-GM007617]

2.21 **Spatial and Temporal Characterization of Single Minded 1 (SIM1) Enhancers**

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Obesity leads to an increased risk for a variety of health issues, most notably cardiovascular disease, type 2 diabetes, cancer, sleep apnea and liver disease. The Single Minded 1 (SIM1) gene is involved in the development the hypothalamus, which has essential roles in regulating food intake. In addition, SIM1 is important for the long-term regulation of energy homeostasis in adult mice. Rare coding mutations in SIM1 have been found to be more prevalent in severely obese individuals than in lean controls. Haploinsufficiency of SIM1 in humans and mice leads to severe obesity, suggesting that altered expression of SIM1 by way of regulatory elements, such as enhancers, can also predispose individuals to obesity. To further explore the role of SIM1 regulatory elements in obesity susceptibility, we have used comparative genomics and an in vivo transgenic enhancer assay in zebrafish to identify and characterize noncoding enhancers in the SIM1 genomic locus in a spatial and temporal manner. Of the eighteen tested sequences, three were found to have hypothalamus-specific enhancer activity. Due to the dual role of SIM1 in development and in adult energy homeostasis, the activity of these three enhancers was annotated from embryonic to adult age. We observed that the tissue-specific enhancer activity of these enhancers changed over time, suggesting that the developmental and physiological expression of SIM1 may be regulated by different enhancers. Further biochemical studies will be done to validate these enhancers' ability to regulate SIM1 expression. These results provide candidate enhancer sequences in which nucleotide variation in humans could lead to obesity susceptibility and demonstrate that enhancers can alter their spatial activity over time. [This research was supported by NIH NHGRI grant R01HG005058 and Amgen.]

HIV/AIDS

2.22 Gag-Specific CD8+T Cells Have Moderate Susceptibility to Nef

Diana Y. Chen, Arumugam Balamurugan, Hwee L. Hg, and Otto O. Yang, University of California, Los Angeles

Nef-mediated HLA class I down-regulation on infected cells can reduce the antiviral efficacy of cytotoxic T lymphocytes (CTL). It is unclear whether HIV-1 specific CTL vary in their susceptibility to this effect, because they differ in specificity and other functional properties that could affect the impact of Nef. Here we examined the factors that affect the ability of Nef to interfere with CTL antiviral activity. The susceptibility of CTL to Nef-mediated HLA downregulation was evaluated using a viral suppression assay and a chromium release assay using target cells infected with NL4-3 containing either wild type (WT) Nef or M20A mutated Nef that lacks HLA down-regulation function. Comparing CTL suppression of HIV-1 with WT versus M20A Nef, Gag -specific CTL generally showed similar efficiency in suppressing both viruses, whereas non-Gag restricted CTL had reduced antiviral activities against WT Nef virus. Specifically, CTL targeting the B*57 restricted Gag TW10 and QW9, and the B*15 restricted Gag RY10 epitopes, were minimally affected by Nef. Assessing the effect of Nef on the kinetics of infected cell killing by CTL, we observed that Gag-TW10 B*57- and Gag-RY10 B*15-restricted CTL showed little difference in the killing of infected cells with WT versus M20A Nef and were able to recognize infected cells very early, typically within seven hour of infection. For other CTL, recognition of infected cells was not until >11 hours of infection and was associated with increased susceptibility to Nef. Our data suggest that there may be epitope-specific determinants of Nef impact on the antiviral activity of HIV-1-specific CTL, and we are in the process of studying them.

2.23 Hormonal Contraceptive Use and Risk of HIV Transmission: A Prospective Cohort Analysis

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Background: Hormonal contraception, including daily oral pills and long-acting injectable methods, are used widely but their effects on HIV-1 risk are unclear. Methods: We followed 3790 heterosexual HIV-1 serodiscordant couples from 7 African countries for =24 months. Among hormonal contraceptive users and nonusers, we compared rates of HIV-1 acquisition in women and HIV-1 transmission from women to men using multivariate Cox proportional hazards regression and marginal structural modeling. Results: Among 1314 couples in which the HIV-1 seronegative partner was female, HIV-1 acquisition rates were 6.61 and 3.78 per 100 person-years in women currently using and not using hormonal contraception (adjusted hazard ratio [HR] 1.98, p=0.03). Among 2476 couples in which the HIV-1 seronegative partner was male, HIV-1 transmission rates from women to men were 2.61 and 1.51 per 100 person-years in those whose partners currently used versus did not use hormonal contraception (adjusted HR 1.97, p=0.02). Both injectable and oral hormonal contraceptive methods were associated with increased HIV-1 risk, although subgroup analyses were statistically significant only for injectable contraception. Results from marginal structural model analyses were consistent with those from the Cox proportional hazards regression. Conclusion: Women should be counseled about potential increased risk of HIV-1 acquisition and transmission with hormonal contraception, particularly injectable methods, and about the importance of dual protection with condoms to decrease HIV-1 risk. Strategies to encourage use of long-acting contraceptive methods with lower doses of exogenous hormones or non-hormonal methods should be promoted. [Funding provided by The Bill and Melinda Gates Foundation and the NIH.]

2.24 **Decision Making and the Association of Missed Medication on Transmission Risk in a Sample of HIV+ MSM**

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Recent reports suggest that antiretroviral therapy (ART) may be effective in preventing the spread of HIV (PREP, HPTN-052). As the effectiveness of ART is sensitive to adherence, it is important to examine the association between ART adherence and sexual transmission risk (TR) among HIV+ persons, especially in the context of known risk factors such as methamphetamine (MA) use and decision making deficits. HIV+ men who have sex with men (n=148) were recruited from an ongoing experimental RCT targeting MA use and ART adherence. Decision making deficits were assessed using the Iowa Gambling Task and its variant, measuring reward and punishment hypersensitivity, respectively. MA use, missed meds and TR were collected on the day-level. In a hierarchical model including all predictors, both MA use (OR: 4.2, p=.001) and missed meds (OR: 1.4, p=.001) on a given day independently predicted an increase in the odds of TR on that day. Across all participants, punishment sensitivity was associated with an increase in odds of TR (OR: 1.1, p=.001). However, the strength of association between missed meds and TR was decreased by punishment sensitivity (OR: .76, p=.001) and increased by reward sensitivity (OR: 1.54, p=.001). As dissemination occurs of the findings that ART may be protective against the spread of HIV, these data suggest that: (1) the troubling association between missed meds and TR should be monitored; (2) motivation for the linking of adherence and TR may be driven by prefrontal cortex dysfunction as hypersensitivity to reward and punishment modified this relationship; and (3) it will be important to examine how changes in the rewarding or punishing nature of this relationship could lead to increases or decreases in TR in the light of this brain-behavior relationship.

2.25 **Mechanistic studies of an unexpected HIV-1 Vpu function on retrovirus assembly**

Tiffany M. Lucas, University of Missouri; Edward Stephens, University of Kansas; Marc Johnson, University of Missouri

HIV-1 encodes six accessory genes (Vif, Vpr, Vpu, Tat, Rev and Nef), many of which counteract host proteins that are detrimental to the virus. For instance, Vpu restricts host proteins CD4 and tetherin, enhancing viral infectivity. However, we recently reported that under certain circumstances the opposite occurs, and Vpu prohibits the formation of infectious viral particles. This restriction occurs when HIV-1 particles are generated with the surface glycoprotein (Env) from the gammaretrovirus gibbon ape leukemia virus (GaLV). Interestingly, the closely related murine leukemia virus (MLV) Env is insensitive to Vpu-mediated restriction. Our initial studies showed Vpu excludes GaLV Env from viral particles, but fails to reduce Env abundance on the plasma membrane or release of virus-like particles. We sought to determine whether the mechanism of Vpu's restriction on GaLV Env is distinct from known functions of Vpu against CD4 or tetherin, an anti-viral protein. We investigated key features in Vpu's transmembrane domain and cytoplasmic tail. Regions required for Env restriction were similar to those previously reported for CD4 modulation. To determine whether Env restriction was specific to HIV-1 cores, we pseudotyped GaLV Env with other retrovirus and found only non-lentiviral cores Vpu-insensitive. Additionally, we found that restriction required neither proteasomal nor lysosomal pathways. Our studies find restriction of Env and CD4 require similar functional domains in Vpu. However, unlike CD4-targeting, Vpu fails to markedly reduce GaLV Env plasma membrane expression, require degradation and the restriction is viral core-type dependent. Our current model suggests some mechanistic overlap in Vpu restriction of Env and CD4. [NIH: R01 AI 073098, R21 AI087448]

2.26 **Novel Mechanism of Inhibition of HIV-1Reverse Transcriptase by 4'substituted nucleoside analogs**

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Nucleoside Reverse Transcriptase Inhibitors (NRTIs) are among the most important therapeutics against HIV-1 infections. They mimic the natural nucleosides in order to be incorporated at the viral nascent DNA by the viral reverse transcriptase (RT). Since they lack a 3'OH they inhibit RT by acting as chain terminators. EFdA and ENdA are deoxyadenosine analogs that suppress viral replication of wild-type and drug resistant HIV with very high potency. These compounds have a 4'-ethynyl substitution and retain a 3'OH. The purpose of this study was to characterize the mechanism of action of these compounds in a molecular level by using biochemical assays including primer extension, gel shift and footprinting assays. Using the triphosphate forms (TP) of EFdA and ENdA we found that they both inhibit HIV RT very efficiently by acting as chain terminators despite the presence of a 3'OH group. This inhibition arises mainly from a difficultly of the primer 3'-terminus to translocate following incorporation of the compounds. Therefore, we termed EFdA and ENdA as Translocation-Defective RT Inhibitors (TDRTIs). Our biochemical studies showed ENdA-TP inhibits HIV RT more potently than EFdA-TP. However, in cells it appears that ENdA is less stable and more toxic than EFdA. Here we show that the higher toxicity of ENdA is due to inhibition of human DNA polymerase ?. In addition, we have investigated the contribution of the 4'ethynyl group by comparing analogs with a methyl or ethyl groups at the 4'position. These results provide valuable insights into understanding the mechanism of RT inhibition by TDRTIs and could lead to the development of more effective therapeutics. [NIH Funding: AI076119, AI074389, AI076119-S1, and AI076119-02S1]

2.27 **CXCR6 and GPR15 are Efficient Entry Coreceptors used by SIVsmm from CCR5- and CCR5+ Sooty Mangabeys**

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Background: SIV infection of sooty mangabey (SM) natural hosts does not cause disease, despite robust virus replication. Understanding the virus/host relationship in natural hosts will enable better understanding of pathogenic HIV infection of humans. SIV uses CCR5 for entry into target cells, but SM and other natural hosts express very low CCR5 levels on CD4+ T cells. We recently identified a novel SM CCR5?2 deletion allele encoding a non-functional protein that is common in captive North American SM and present in wild West African SM. Methods: Using PCR screening and direct sequence confirmation, we CCR5-genotyped SM from Yerkes National Primate Research Center (n=202). We then used pseudotypes and infectious virus to assess entry coreceptor use by SIVsmm. Results: We found a frequency of 25% for the ?2 allele, and combined with a previously identified ?24 allele, 8% of SM at Yerkes are homozygous for CCR5-null mutations. SIVsmm infection was not significantly less prevalent in SM lacking functional CCR5 compared to CCR5+ animals, and CCR5-null animals displayed high-level viremia. SM PBMC were permissive for SIVsmm replication in the absence or presence of CCR5 in vitro, and Envs from both CCR5-null and wild-type infected animals used CXCR6 and GPR15 efficiently for entry in transfected cells, and GPR1 somewhat less efficiently. Conclusions: SIVsmm utilize CXCR6 and GPR15 for entry in vitro and likely in vivo. Thus, SIVsmm cell targeting in vivo may be defined by the distribution and use of these alternative coreceptors in addition to CCR5. This finding may further elucidate SIVsmm tropism and host cell targeting in vivo and raises the question of whether alternative coreceptor pathways are utilized in other natural hosts that also show restricted CCR5 expression.

2.28 **Discovery of Small Molecules Inhibiting HIV-1 Integrase and Cellular LEDGF/p75 Protein-Protein Interaction**

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Cellular co-transcription activator p75, also known as lens epithelial-derived growth factor (LEDGF/p75), plays an essential role in HIV-1 IN-led integration of viral DNA into the human genome. Designing compounds to disrupt LEDGF/p75-IN complexes serves as a novel mechanistic approach different from current antiviral therapies. In 2007, Raltegravir, a diketo-enol based IN inhibitor, was approved by the FDA for HIV infection. The evolution of HIV has afforded the viral species to adapt to external therapeutic pressure and Raltegravir-resistant viral strains have been well documented, increasing the need for new IN inhibitors with a different mechanism of action. Mechanistic studies show the majority of reported IN inhibitors effectively chelate with a magnesium ion in the catalytic active site, a region topologically different from the LEDGF/p75 binding site. Key LEDGF/p75 K364 I365 D366 amino acid residues from the IN binding domain laid a foundation for a ligand-based pharmacophore model. Herein we report funding from the National Institute of Allergies and Infectious Diseases led to the discovery of novel small molecules that successfully inhibited LEDGF/p75-IN complex formation. A small molecule database of 365,000 was searched and compounds that had fitness values above 2.0 were selected for prescreening in an in vitro luminescent proximity assay. Lead inhibitors expanded into training sets using substructure similarity tools led to the discovery of four novel classes of LEDGF/p75-IN inhibitors.

2.29 **Lymphoid Tissue Destruction during HIV Infection Depletes Naïve T Cells and Limits Immune Reconstitution**

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Highly active antiretroviral therapy (HAART) can suppress HIV-1 replication and normalize the chronic immune activation associated with infection, but restoration of naïve CD4+ T cell populations is slow and usually incomplete for reasons that have yet to be determined. By examining HIV-1- infected patients' lymphoid tissues (LTs) and utilizing an ex vivo culture system, We first found that fibroblastic reticular cells (FRCs) within human LTs are the major source of interleukin-7 (IL-7), the primary survival factor for naïve T cells, and that naïve T cells need to physically interact with FRCs to get access to the IL-7 produced by and presented on the surface of FRCs. We next showed that collagen deposition and progressive loss of the FRCs in LTs prior to treatment restrict both access to and the major source of IL-7 on which survival particularly of naïve T cells depends. Consequently, apoptosis within naïve T cell population increases significantly and thereby resulting in progressive depletion of both naïve CD4+ and CD8+ T cell populations. We further demonstrated that the extent of loss of FRCs and collagen deposition predicts the extent of restoration of the naïve T cell population after 6 month of HAART. The optimal restoration of the FRCs is highly dependent on initiating therapy in the early/acute stage of infection, which correlates with optimal reconstitution of naïve T cell population. Thus our findings strongly suggest that early initiation of HAART and the potential use of adjunctive anti-fibrotic therapies may avert or moderate the pathological consequences of LT fibrosis, thereby improving immune reconstitution. [We acknowledge the sources of funding for the project: NIH research grants AI028246, AI048484 and AI056997 to A.T.H. and AI074340 to T.W.S.]

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2.30 Resident microbial communities regulate anti-viral immunity

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Alterations in resident microbial communities are associated with enhanced susceptibility to multiple inflammatory diseases suggesting that commensal bacteria influence immune homeostasis and function. However, whether the microbiota can influence protective immunity to systemic infection remains poorly understood. Here, we demonstrate a previously unrecognized role for resident microbial communities in regulating anti-viral immunity following systemic (LCMV) or local (Influenza) viral infection. Depletion of bacterial communities via antibiotics (ABX) resulted in impaired innate and adaptive anti-viral immunity and delayed viral clearance. In addition, ABX-treated mice challenged with influenza virus exhibited severe bronchiole epithelial degeneration and increased mortality. As early as day 3 p.i., ABX-treated mice exhibited decreased levels of macrophage-associated proinflammatory cytokines and reduced expression of interferon-inducible anti-viral genes in the lung. Genome-wide transcriptional profiling of macrophages from naive ABX-treated mice revealed an inherent diminished expression of anti-viral defense genes. Further, these macrophages demonstrated impaired activation to viral infection or IFN stimulation ex vivo. These data indicate that resident microbial communities provide tonic immune stimulation critical to establish a gene expression profile predisposed to efficiently respond to viral pathogens.

2.31 Macrophages and Neutrophils from Humans and Mice Kill Larval Strongyloides stercoralis

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Strongyloides stercoralis (Ss) infects 30-100 million people worldwide, yet little is known about the immune response in humans. Previous studies on Ss in mice have demonstrated a role for eosinophils, neutrophils (PMN) and complement activation in the protective immune response. The goal of this study was to determine the role of macrophages (MF) in innate immunity to Ss in humans and mice. Human MF and PMN were isolated from the blood of healthy donors. When cultured independently, MF and PMN did not kill the larvae; however, larval killing did occur when both human MF and PMN were combined in vitro in the presence of complement. To examine the role of mouse MF in the immune response against Ss, bone marrow-derived MF were cocultured with PMN and larvae in vitro or placed in diffusion chambers with Ss into naïve mice. Larval killing only occurred in vitro if both MF and PMN were present. In addition, MF implanted in naïve mice killed the larvae within 7 days. To determine the phenotype of MF during the immune response to Ss, mice were infected subcutaneously with larvae and peritoneal exudates cells (PEC) were analyzed by flow cytometry to quantify classically activated MF (CAMF) and alternatively activated MF (AAMF). Analysis of PEC from mice with primary infections revealed both CAMF and AAMF levels higher than in control mice. To determine if CAMF and/or AAMF functioned in killing the larvae, MF were stimulated with IL-4 to induce AAMF or IFN-?/LPS to induce CAMF. AAMF, but not CAMF, killed larvae both in vitro and after 3 days in vivo. We conclude from these studies that both human and mouse MF, in conjunction with PMN, kill Ss. Furthermore, infection of mice with Ss results in the induction of AAMF which kill the parasite. [NIH grant 1R56AI076345-01A2]

2.32 **Asc Modulates the Function of NLRC4 During Legionella pneumophila Infection in Macrophages.**

Christopher L. Case and Craig Roy, Yale University

NLRs activate caspase-1 in response to multiple bacterial-derived signals in macrophages. Activation of caspase-1 by Legionella pneumophila occurs through a NLRC4-dependent pathway and a separate mechanism dependent on the adapter protein Asc. Activation through both pathways is required for maximal caspase-1 cleavage. However, caspase-1-mediated pore formation and cell death require signaling through NLRC4, while Asc is dispensable for these processes. We hypothesize that caspase-1 is regulated spatially in order to elicit these distinct downstream consequences. This possibility was investigated by monitoring the localization of various components involved in caspase-1 activation during L. pneumophila infection by immunofluorescence microscopy. Caspase-1 was found to localize to a punctate structure in infected cells containing Asc, but not in cells lacking this adapter. Endogenous Asc and GFP-NLRC4 also localized to caspase-1 puncta following infection. Formation of puncta correlated with cleavage of caspase-1 in immunoblot analyses, suggesting a role for Asc/NLRC4/caspase-1 complexes in caspase-1 cleavage. In live cell imaging studies, GFP-NLRC4 failed to relocalize to puncta in the absence of Asc, yet cells underwent pore formation and cell death. Furthermore, cells deficient for Asc underwent pore formation and cell death at a greater rate than wild-type cells. These data indicate that Asc mediates integration of NLRC4 into caspase-1 processing platforms and that recruitment of NLRC4 into an Asc complex can dampen cell death responses. This negative feedback role of Asc complexes may be important for regulating caspase-1-mediated responses during microbial infection. [Funded by an NSF predoctoral award to CLC and by NIH grant R01-AI048770 to CRR.]

2.33 **Unique Role of MR1-restricted MAIT Cells in Gut Homeostasis and Lung Bacterial Infection**

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Mucosal associated invariant T (MAIT) cells express an invariant TCR alfa chain, reside mainly in the mucosal tissues, and rapidly release cytokines upon TCR ligation. MAIT cells are restricted by the class Ib molecule MR1 and they require commensal microbes and B cells for peripheral expansion. Recent studies have implicated MR1 presentation to MAIT cells in bacterial infection. However, the question of the specific function and uniqueness of MAIT cells in mucosal system remains to be elucidated. We employed two murine models to investigate the role of MR1-restricted MAIT cells. To determine if MAIT cells play a key role in intestinal homeostasis in the presence of commensal microbes, we used the colitis model induced by dextran sodium sulfate. MR1 knockout (KO) mice failed to undergo epithelial regeneration in the descending colon compared with wild type (WT) mice in response to colitis. MR1 KO mice also showed higher levels of IL-17, IL-1b and IL-6 and decreased levels of IFNg and IL-12. The result implied that MR1 and MAIT cells play an important role in sustaining mucosal homeostasis in the presence of commensal microbes. To examine the role of MAIT cells in bacterial infection, we employed the aerosol infection model of Mycobacterium bovis BCG. After infection, MR1 KO mice showed significantly higher bacterial burden in the lung than WT mice. Bronchoalveolar lavage cells from BCG-infected MR-KO mice showed lower IFNg response when stimulated ex vivo with BCG and other mycobacterial antigens. The result demonstrated MAIT cells play a non-redundant role in providing immunity against bacterial infection. In conclusion, MR1 and MAIT cells play a unique role in both homeostasis and infection in the mucosal system. [Funding sources: NIH, MRCE, and McDonnell Academy]

2.34 **Passive Immunization with an Influenza M2e-specific Miniantibody Administered via Naked DNA Delivery**

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Influenza remains an important respiratory disease in humans with the recent emergence of the pandemic H1N1 highlighting the need for therapeutic tools able to provide rapid and broad protection. One appealing target for the development of therapeutic monoclonal antibodies (mAb) is the matrix 2 (M2) protein. The ectodomain of M2 (M2e) is highly conserved among human influenza viruses and, to a lesser extent, avian influenza viruses. Moreover, M2e has been shown to be both immunogenic, inducing specific antibody responses, and protective in murine models of influenza virus infection. Finally, M2e-specific mAbs have been shown to be protective in vivo. Passive immunization through the delivery of influenza-specific antibodies is appealing because of the potential for providing protection to certain high-risk groups which are often poor responders against standard vaccination approaches. This approach is not without drawbacks however, as exogenous antibodies have a limited serum half-life along with significant cost associated with production of therapeutic antibodies for human use. A novel and potentially cost-effective approach for the delivery of therapeutic antibodies resulting in prolonged expression is through antibody gene transfer using naked DNA. Here, we demonstrate that delivery of plasmid encoding a single-chain M2e-specific miniantibody results in significant serum titers over a period of weeks, which have potential for providing protection against influenza virus challenge. [NIH R01AI070847]

2.35 **PU.1 regulates chromatin modifications of the Il9 gene in Th9 cells**

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IL-9-secreting Th9 cells are generated when naïve CD4+ T cells are cultured with IL-4 and TGF-ß. The transcription factor, PU.1 is required for the development of Th9 cells. The expression of PU.1, which is abundantly expressed in Th9 cells, is dependent on TGF-ß dose. Dose dependent increase in TGF-ß leads to a concomitant increase in PU.1 expression and IL-9 production. Ectopic expression of PU.1 can convert Th2 cells to a Th9 phenotype and enhance IL-9 production from Th9 cells. The DNA binding domain and the transactivation domains of PU.1 are required for PU.1 to activate Il9, suggesting that as it binds Il9 directly, it recruits other factors to mediate gene activation. In the absence of PU.1 there is reduced recruitment of specific histone acetyl transferases (HATs) PCAF and Gcn5 at Il9 gene locus. We hypothesized that altered HAT recruitment would result in alteration in the acetylation of specific histone residues at the Il9 locus. H3 acetylation is decreased in PU.1-deficient Th9 cells at both the Il9 promoter and a second conserved non-coding sequence immediately 3' of the Il9 gene. Acetylation at H3 lysine 9, 14, and 18 and H4K5 is also diminished in PU.1-deficient Th9 cells demonstrating specific PU.1-mediated histone modifications that correlate with Il9 transcription. Overall these results indicate that PU.1 promotes expression of IL-9 by recruiting specific HATs and mediating the modification of specific histones at the Il9 locus.

2.36 **Coordinate Regulation of GATA3 and Th2 Cytokine Gene Expression by the RNA-binding Protein HuR**

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The posttranscriptional mechanisms whereby RNA-binding proteins (RBPs) regulate T-cell differentiation remain unclear. RBPs can coordinately regulate the expression of functionally related genes via binding to shared regulatory sequences, such as the Adenylate-Urydylate-Rich Elements (ARE) present in mRNA's 3' untranslated region (UTR). The RBP HuR posttranscriptionally regulates IL-4, IL-13 and other Th2-cell-restricted transcripts. We hypothesized that the ARE-bearing GATA-3 gene, a critical regulator of Th2 polarization, is under HuR control as part of a coordinate posttranscriptional regulation of the Th2 program. We report that GATA-3 mRNA half-life is increased after restimulation in Jurkat and murine Th2-skewed cells, as well as in human memory and Th2 cells. We demonstrate that HuR associates with the GATA-3 transcript 3'UTR and using a combination of in vitro and animal models of both loss- and gain-of-function, we show that HuR is a critical mediator of stimulus-induced increase in GATA-3 mRNA through the increase of its stability. These results suggest that posttranscriptional control plays a significant, highly combinatorial and yet underappreciated role in T-cell development and polarized effector function, in both murine and human systems. These data suggest that better understanding of HuR-mediated control of Th2 polarization may have utility in altering allergic airway inflammation in human asthmatic patients. [National Institutes of Health Grants R01AI080870-01and R21AI079341-01]

2.37 **Cytotoxic T Lymphocyte Effector Activity Is Regulated by Target Cell Type**

Matthew T. Hufford, Taeg S. Kim, Jie Sun, and Thomas J. Braciale, University of Virginia

Cytotoxic T lymphocytes (CTLs) are critical in a number of respiratory viral infections via their ability to release pro-inflammatory cytokines and to initiate targeted cell death. The stimulation of these anti-viral activities must not only be robust enough to achieve viral clearance but also regulated to minimize tissue damage. The factors controlling these effector activities at infection sites in vivo, though, are poorly understood. Utilizing a murine model of influenza infection, we observed that the expression of CTL anti-viral activity in the infected respiratory tract is dictated by the target cell encountered. Hematopoietic cells, notably infiltrating inflammatory mononuclear cells and neutrophils, trigger both CTL cytotoxicity and the release of pro-inflammatory cytokines (i.e. IFN-gamma). In contrast, non-hematopoietic cells, including the influenza-infected respiratory epithelium, stimulate only CTL cytotoxicity, which is sufficient in itself to achieve CTL mediated influenza viral clearance within the respiratory tract. Furthermore, CTL mediated cytotoxicity is dependent on MHC Class I expression while CTL cytokine production requires additional signaling, such as co-stimulation (i.e. CD80/86). The expression of co-stimulatory molecules is restricted to cells of hematopoietic origin in vivo and may, in part, reflect the ability of these cells to trigger CTL cytokine production. These findings suggest that the quality of CTL effector activity is dependent on a signal hierarchy in vivo, which is potentially important in controlling excess pulmonary inflammation while preserving optimal viral clearance during respiratory virus infections. [Supported by USPHS Grants RO1 AI-15608, RO1 HL-33391, R01 AI-37293 and U19 AI-083024 (TJB) and USPHS Training Grant T32 AI-055432-7 (MH)]

2.38 **Liposome-Coupled AntiCD40+CpG Induces Potent Anti-Tumor Responses with Minimal Systemic Side Effects**

Brandon Kwong, Haipeng Liu, Darrell Irvine, Massachusetts Institute of Technology

Immunomodulatory agents such as anti-CD40 antibodies and CpG oligonucleotides have previously been shown to stimulate potent anti-tumor immune responses. Despite therapeutic success in mice, the clinical usage of these compounds has been limited by the severe inflammatory toxicity observed in non-target organs following systemic exposure. This motivates the need for a strategy to localize immunostimulatory therapy and minimize the systemic dissemination of such potent immuno-agonists. In this study, anti-CD40 and CpG were coupled to the surface of liposome nanoparticles, which were injected intra-tumorally into mice bearing subcutaneously implanted tumors. Biodistribution analyses demonstrated that the nanoparticle carriers successfully sequestered anti-CD40 and CpG following local injection, limiting their draining into circulation while maintaining potent immunostimulatory activity in the tumor tissue and tumor-draining lymph node. Anti-CD40/CpG liposomes strongly inhibited tumor growth in mice, with an efficacy similar to equivalent doses of locally injected soluble immunotherapy. However, the systemic inflammatory effects induced by soluble intratumoral therapy, including serum markers of liver damage, inflammatory cytokine release, and overall weight loss, were eliminated by the use of liposome-anchored delivery. Furthermore, ongoing experiments have demonstrated the priming of systemic anti-tumor immunity following local treatment with anti-CD40/CpG liposomes, enabling mice to resist secondary tumor challenge at a distal site. Current studies are aimed at elucidating the immune effectors and mechanisms responsible for mediating the anti-tumor response induced by anti-CD40/CpG liposomes. [Funding: HHMI; Dana-Farber/HCC–MIT Bridge Project Fund]

2.39 **Chemokine Receptor CX3CR1 marks Effector and Effector Memory CD8+ T Cells after Viral Infection**

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A number of changes in surface marker expression characterize the differentiation of naïve to effector and memory CD8+ T cells. However, no single surface marker has been reported that durably characterizes effector memory (CD62L- and CCR7-) CD8+ T cells and allows distinction from other T cell subsets, in particular central memory CD8+ T cells (CD62L+ CCR7+). We report here through the use of CX3CR1-GFP reporter mice and adoptive transfer experiments, robust surface expression of chemokine receptor CX3CR1 by effector and memory CD8+ T cells following viral challenge. CX3CR1 expression by CD8+ T cells occurred relatively late after activation, appearing on virus specific T cells 4-5 days after LCMV infection. Expression of CD127 increased on CX3CR1hi cells over time and was markedly enriched during the memory phase. Memory CX3CR1hi CD8+ cells did not express central memory associated LN homing molecules CCR7 or CD62L, and failed to enter LNs, while preferentially localizing to peripheral tissues. Memory CX3CR1hi possessed potent cytolytic function while maintaining a modest proliferative capacity following viral rechallenge. In addition, heterogeneous CX3CR1 expression levels allowed distinction of subpopulations of memory CD8+ T cells. These findings characterize CX3CR1 as a robust and distinctly expressed marker to identify long-lived effector memory CD8+ T cell, which to date could only be approximated as cells that express memory markers, but lack lymph node homing receptors. Expression of CX3CR1 by effector memory CD8+ T cells may provide a potent tool for differentially visualizing their in vivo behavior alongside central memory T cells after rechallenge. [NIH Hematology T32 (5T32-HL07623-20), Harvard Medical School to EAM, NIH to UVA]

2.40 **Placental CD44 Interacts with VAR2CSA-expressing, P. falciparum-infected Erythrocytes**

Simon O. Owino, Demba Sarr, Samantha Burton, Briana Flaherty, David S. Peterson, and Julie M. Moore, University of Georgia

Plasmodium falciparum infected red blood cells (iRBCS) accumulate in the maternal blood space of the placenta during malaria infection, leading to pathologic consequences deleterious to pregnancy success. Fetal cell in contact with maternal placental blood is a syncytialized epithelium called syncytiotrophoblast (ST). The ST is rich in low sulfated chondroitin sulfate A (CSA), a principle ligand for VAR2CSA parasite protein, present on the surface of placenta-adherent iRBCs and confers binding to CSA. Importantly, critical proteoglycans bearing CSA on ST acting as anchors for iRBCs in the placenta have not been identified. Because our previous studies have shown that interaction of iRBCs with primary ST in vitro activates these cells to perform immunologic functions, examination of role for CSA-bearing proteoglycans with trans-membrane cytoplasmic domains and signaling functions, such as CD44, in placental iRBCs sequestration and resultant ST activation is warranted. Method. Mononuclear cytotrophoblasts were obtained by a series of enzyme digests of fresh placental tissue followed by density gradient separation and antibody purification. Cells were plated and syncytium formation monitored. ST membrane proteins extracted and incubated with magnet immobilized iRBCs. Fluorochrome-labeled monoclonal antibody against CD44 was used to target proteins bound onto iRBCs. As controls, ST membrane proteins were pre-treated with chondroitinase ABC enzyme and P. falciparum line null mutants for VAR2CSA were included. Results. By specifically incubating ST-derived CD44 with iRBCs we provide evidence that this proteoglycan can act as an in vivo receptor for VAR2CSA. Conclusion. Expression of VAR2CSA is required for epican interaction with iRBCs, and VAR2CSA/epican interaction is at least partially dependent on CSA. Acknowledgment. Funding from NIH grant [1R21AI090439-02]

2.41 **The transcriptional repressor BCL6 controls the Th2 inflammatory activity of Regulatory T cells**

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The transcriptional repressor Bcl6 is a critical arbiter of T helper cell fate, promoting the follicular helper (Tfh) lineage while repressing other T helper cell lineages. Bcl6-deficient (Bcl6-/-) mice develop a spontaneous and severe Th2-type inflammatory disease early in life, thus warranting assessment of the role of Bcl6 in Treg cell function. Bcl6-/- Tregs displayed enhanced suppressor function in vitro and were competent at suppressing Th1-mediated colitogenic inflammation in vivo. In contrast, Bcl6-/- Treg cells were uniquely defective in their ability to control Th2-type airway inflammation, and in fact, actively exacerbated the disease. Bcl6 deficiency promoted the expression of Th2 cytokines by Tregs, in part due to increased transcriptional activity of Gata3, and also due to increased expression of microRNA-21 (miR-21). Ectopic expression of miR-21 in naïve T cells in vitro induced polarization towards Th2-type, while antagomir-mediated miR-21 repression was able to specifically suppress Th2 differentiation. Our results define novel roles for Bcl6 in regulating Treg gene expression and in dictating the ability of Treg cells to control Th2 inflammation. Thus, Bcl6 represents a crucial regulatory layer in the Treg functional program. (This work was supported by NIAID grants 1R21AI079349-01A1 and 1R21AI090150-01 to A.L.D., AHA pre-doctoral fellowship 10PRE4620001 to D.V.S., and NIAID grant U19 AI070448 to M.H.K)

2.42 **Inflammatory monocytes but not neutrophils are essential for defense against Listeria monocytogenes infection**

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Listeria monocytogenes is a facultative intracellular bacterium that causes systemic infections in immunocompromised hosts. Early recruitment of myeloid cells, including inflammatory monocytes and neutrophils, to sites of L. monocytogenes infection is essential for the control of infection and host survival. Because previous experimental studies used depleting or blocking antibodies that affected both inflammatory monocytes and neutrophils, the relative contributions of these cell populations to defense against L. monocytogenes infection remain incompletely defined. Herein, we used highly selective depletion strategies to either deplete inflammatory monocytes or neutrophils from L. monocytogenes infected mice and demonstrate that neutrophils are dispensable for early and late control of infection. In contrast, inflammatory monocytes are essential for bacterial clearance during the innate and adaptive phases of the immune response to L. monocytogenes infection.

2.43 **Relative Importance of B cells and T cells in Mucosal and Systemic Trypanosoma cruzi Protection**

Nicole L. Sullivan, Christopher S. Eickhoff, Daniel F. Hoft, Saint Louis University

Trypanosoma cruzi is an intracellular parasite and the causative agent of Chagas disease. Previous work has shown that T cells are critical for systemic T. cruzi protection. However, the importance of T cells in mucosal protection and B cells in both mucosal and systemic T. cruzi protection have not been clearly defined. First, we examined the importance of T cells and B cells in the transfer of mucosal T. cruzi protection. Total spleen cells from T. cruzi infection-immune mice were able to transfer mucosal protection to naïve SCID mice as measured by reduced T. cruzi DNA present in the gastric mucosa after oral T. cruzi challenge. Additional adoptive transfer studies determined that CD8+ T cells, but not B cells, were important for the transfer of mucosal protection. Next, we wanted to investigate the importance of B cells in providing systemic T. cruzi protection. First, naïve B cell knockout mice infected systemically with a low dose of viable T. cruzi developed high levels of parasitemia and died as compared with WT mice, demonstrating that B cells are important in the initial control of T. cruzi. Second, using B cell KO mice, we determined that B cells are important for prolonged systemic protection as T. cruzi infection-immune B cell KO mice survive past naïve controls but over time succumb to challenge. B cells appear to be important in controlling antigen load and thus preventing chronic T cell exhaustion. In conclusion, we have determined that B cells are important for systemic, but not mucosal, T. cruzi protection, most likely due to antigen-specific antibodies reducing T. cruzi antigen load and preventing chronic T cell exhaustion. [This work was funded in part by the NIH-5R01AI040196 grant]

2.44 **Understanding the Contributions of Lupus Susceptibility Loci in SLE-accelerated Atherosclerosis in Mice**

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Persons with the autoimmune disease systemic lupus erythematosus (SLE) have an increased risk for cardiovascular disease (CVD). Studies in our laboratory are focused on determining the mechanisms by which SLE accelerates CVD development. We have previously shown that chronic autoimmunity is sufficient to increase atherosclerosis in the SLE-susceptible LDLr.Sle1.2.3 bone marrow chimera model. To determine the individual contribution of the 3 lupus-susceptibility loci, we hypothesized that Sle1 and Sle3, which lead to a break in tolerance and T cell hyperactivity, respectively, could promote accelerated CVD, either as single or bicongenics. To test this, we transferred control (B6) or experimental (B6.Sle1, B6.Sle3, or B6.Sle1.3) bone marrow into lethally irradiated low density lipoprotein deficient (LDLr) mice and placed them on a high fat Western diet 16 weeks after transplantation. We found that all 3 experimental groups had decreased serum cholesterol and no difference in serum triglyceride compared to LDLr.B6 mice. Autoantibody titer assessment showed that Sle1.3 had significantly increased anti-dsDNA titers compared to control mice. There were no differences in antibody titers against ß2GP1 or cardiolipin. Flow cytometric analyses of splenocytes revealed increased T cell activation and decreased regulatory T cells in Sle1 and Sle1.3 mice. However, oil-red-O staining revealed decreased lesion area in experimental mice compared to the control mice. These data present an interesting finding in which the combination of Sle1and Sle3 may have disparate and unlikely effects on the progression of SLE-mediated atherosclerosis. These data also suggest that B cell hyperactivity, mediated by Sle2, may play a larger role in SLE-accelerated atherosclerosis than anticipated. [Funding: NIH NRSA 1F31HL105024 (NSW) and NIH 1R01HL088364 (ASM)]

MICROBIOLOGY AND VIRUSES

2.45 **Parvovirus Minute Virus of Mice Induces an ATM-p53 Mediated DNA Damage Response That Enhances Viral Infection**

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Infection by DNA viruses can elicit DNA damage responses (DDRs) in host cells. In some cases the DDR presents a block to viral replication that must be overcome, and in other cases the infecting agent exploits the DDR to facilitate replication. Using western blots and immunofluorescent assays we have found that infection with the autonomous parvovirus minute virus of mice (MVM) results in the activation of a DDR, characterized by the phosphorylation of H2AX, Nbs1, RPA32, Chk2 and p53. These proteins are recruited to MVM replication centers, where they co-localize with the main viral replication protein, NS1. The DDR is seen following infection of both human and murine cell lines and requires replication of the virus. Using mutant cell lines and specific kinase inhibitors, we show that ATM is the main transducer of the DDR in the normal murine host. ATM inhibitors restrict MVM replication and ameliorate virus-induced cell cycle arrest, suggesting that DNA damage signaling facilitates virus replication, perhaps in part by promoting cell cycle arrest. Furthermore, characterization of the downstream effects of p53 activation revealed the proteasome mediated down-regulation of p21, suggesting that a p21-independent mechanism is involved in the virus-induced cell cycle arrest and that p21 up-regulation might be detrimental to viral replication. Subsequently, we found that MVM downregulates the mitotic cyclin B1 thereby preventing infected cells from progressing into mitosis and allowing for continued viral replication in G2-arrested cells. Overall, our studies show that MVM carefully navigates and, via a novel mechanism, exploits the cellular DNA damage response machinery early in infection to enhance its replication in host cells. [Funding by NIH grants AI21302 and AI46458]

2.46 **Adenovirus E1B55K Protein Blocks Interferon-Induced Inhibition of Viral Replication in Normal Cells**

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The Adenovirus type 5 (Ad5) early protein E1B55K has been shown to possess multiple early- and late-phase functions. A large body of research performed in transformed cell lines previously concluded that a central role of E1B55K is repression of the p53 tumor suppressor protein, via targeted degradation and transcriptional silencing. These findings led to the development of E1B55K-null Ad5 mutants as therapeutic oncolytic agents. However, recent studies have cast doubt on this canonical role of E1B55K in the context of normal cells. Using microarray analyses our lab observed that the p53 transcriptional program is not inhibited by E1B55K in human foreskin fibroblasts (HFFs). Unexpectedly, we instead observed repression of genes associated with immune responses. In this report we identify E1B55K as a repressor of the anti-viral interferon (IFN) response in HFFs and normal bronchial epithelial cells. Pretreatment of cells with type I IFN led to a specific defect in E1B55K-null Ad5 yield and DNA replication, as measured by plaque assay and qPCR respectively, despite robust expression of viral early genes as observed by western blot. Transcripts of several IFN-stimulated genes were shown by RT-PCR to be repressed by wild-type Ad5 but not by E1B55K-null viruses. Furthermore, using Ad5 mutants unable to assemble the E1B55K-containing viral E3 ubiquitin ligase, which mediates E1B55K-targeted protein degradation, we showed that this function was not required for inhibition of the IFN-response. This work represents the first report of a novel role for E1B55K in modulating host cell immune responses in primary human cells, and has several important implications for the development of Ad5-derived oncolytics and vectors for gene therapy.

2.47 **A UDP-glucose Sensitive Rheostat for Cell Size**

Jenny An-Chun Chien and Petra Levin, Washington University in St. Louis

Bacterial cells increase their size in a carbon-dependent manner. Cells cultured in carbon-rich medium can be up to twice the size of their counterparts cultured in carbon-poor medium. To understand this fundamental aspect of cell biology, we used genetic approach combined with fluorescence microscopy and biochemical analysis, and identified that information about carbon availability is transmitted directly to the division machinery via changes in the intracellular concentration of nucleotide sugar UDP-glucose by the diacylgycerol-glucosyltransferase UgtP during cell division in B. subtilis. Under carbon-rich conditions, when the intracellular concentration of UDP-glucose is presumably high, UgtP inhibits assembly of the cell division protein FtsZ, delaying maturation of the division apparatus and increasing cell size. Conversely, during growth in carbon-poor medium, where intracellular UDP-glucose levels are likely to be low, UgtP is sequestered in punctate foci and division proceeds unimpeded reducing average cell size. Data from electron microscopy further suggest that UgtP interaction with itself and FtsZ are mutually exclusive phenomena. Similarly, increasing FtsZ expression three-fold during growth in carbon-poor medium results in rapid disassembly of UgtP puncta, indicating that the excess FtsZ disrupts interactions between UgtP monomers. These data support a model in which substrate dependent changes in UgtP's affinity for itself and FtsZ serve as a rheostat, precisely coordinating increases in carbon availability with increases in cell size. This model sheds light on our understanding of cell size homeostasis and provides a tantalizing link between cell growth, cell envelope morphogenesis and cell division.

2.48 **The Effect of the Cellular SUMOylation Pathway on KSHV Infection and Replication**

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Kaposi sarcoma-associated herpesvirus (KSHV), a lymphotropic gammaherpesvirus, is the causative agent of Kaposi sarcoma, the most common AIDS-associated tumor and is associated with B-cell malignancies. Post-translational modifications of proteins are critical in the control of numerous cellular processes. Sumoylation is a reversible process of covalently attaching the SUMO (small ubiquitin-related modifier) moiety to a target protein and is involved with multiple cellular processes and viral infections. The role of the SUMOylation pathway in viral infection is still not well understood, however recent studies indicate a role for SUMO during herpesvirus infection. The KSHV protein K-bZIP, is a SUMO E3 ligase specific for SUMO-2 and -3. K-bZIP catalyzes the SUMO modification itself, p53 and Rb and is involved with RTA-mediated transactivation of viral and cellular promoters. The viral protein K10 is covalently conjugated to SUMO-1 and -2, contributes to disruption of host cell defenses by disrupting PODs and plays a critical role in B-cell malignancies. We hypothesize that SUMOylation plays a critical role in the lifecycle and oncogenesis of KSHV. We will examine whether knocking down Ubc9 (the only E2 enzyme involved in mammalian SUMOylation) will alter the global SUMOylation patterns of cellular and viral proteins that theoretically alter viral replication kinetics. In addition, proteomic analysis will define the SUMOylated proteins in virally infected cells. The results of these studies will reveal the roles of SUMOylation in viral replication and lead to further understanding of how the SUMOylation of specific viral and/or cellular proteins regulate viral replication and oncogenesis. [This study is funded by the Department of Microbiology at SUNY Upstate]

2.49 **Dissecting the Role of Trafficking Machinery in the Influenza A Life Cycle Reveals Novel Restriction Factors**

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Influenza A replication primarily takes place in the lung epithelium and involves extensive transport of viral RNA and proteins across cellular compartments. To identify the genes involved in influenza trafficking, we used RNAi to silence 488 genes associated with vesicle trafficking and nuclear-cytoplasmic transport in primary human lung epithelial cells and challenged them with A/PR/8/34 influenza. The screen identified 24 candidate host factors and 36 candidate restriction factors. Known factors such as EPN1, NXF1, XPO1, and RUVBL2 were among the strongest identified hits, validating the experimental design. Of the candidate restriction factors, several novel potent factors were associated with the same human disorder. Depletion of these newly identified restriction factors increased influenza entry, but not MLV or arenavirus entry, suggesting that these factors act at an entry step involving late endosomal compartments. Subsequent imaging experiments using dye-labeled influenza demonstrated that the genes act upstream of viral fusion with late endosomes. Consistent with a potential role for these genes in controlling viral infection in patients, cells isolated from human patients and mice with homozygous coding mutations in these genes permitted significantly higher viral replication than control cells. This study thus led to the identification and characterization of novel influenza entry restriction factors associated with a human disorder. [Funded by U01 AI074575 (NH) and a Ford Foundation Predoctoral Fellowship (MD)]

2.50 **Probing the Starvation Stress Response in the Malaria Parasite, Plasmodium falciparum**

Shalon E. Ledbetter, and Dan Goldberg, Washington University in St. Louis

Like all eukaryotes, P. falciparum requires amino acids in order to grow efficiently. Most of its amino acid needs are met through the degradation of hemoglobin in red blood cells (RBCs), however the one amino acid that the parasite must acquire from an exogenous source is isoleucine (Ile). Our lab has observed that parasite growth stalls at the trophozoite stage when grown in culture medium lacking Ile, however, growth can be recovered upon re-supplementation, suggesting that a growth regulation mechanism has been activated. It is known that in higher eukaryotic organisms, nutrient starvation triggers the phosphorylation of the translation effector eukaryotic initiation factor 2 alpha (eIF2a), a regulatory response that ultimately results in growth inhibition. Using Western blot analysis, we have detected the phosphorylation of parasite eIF2a in P. falciparum cultures starved for isoleucine, suggesting the conservation of this critical regulatory stress-response pathway. Furthermore, the parasite-specific nutrient responsive eIF2a kinase was identified using a genetic approach. Although DNA replication and parasite schizogony is effectively inhibited during isoleucine starvation, we have observed that starved parasites exhibit some metabolic activity in that marked morphological changes occur and hemoglobin degradation appears to continue during prolonged starvation. Amino acid starvation in higher eukaryotes can result in the upregulation of genes involved in amino acid biosynthesis. Given that Plasmodium is auxotrophic for all amino acids, we are investigating the unique starvation-associated transcriptional events that occur downstream of eIF2a phosphorylation in this persistent parasite. [NIAID T32]

2.51 **Inhibition of Ebola Virus Entry by a C-peptide Targeted to Endosomes**

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Ebola virus (EboV) and Marburg virus (MarV) (filoviruses) are the causative agents of severe hemorrhagic fever. Infection begins with uptake of particles into cellular endosomes where the viral envelope glycoprotein, GP, catalyzes fusion between the viral and host cell membranes. This fusion event is thought to involve conformational rearrangements of the transmembrane subunit (GP2) of the envelope spike that ultimately result in formation of a six-helix bundle by the N- and C-heptad repeat regions (NHR and CHR) of GP2. Infection by other viruses employing similar viral entry mechanisms (such as HIV-1 and SARS-CoV) can be inhibited with synthetic peptides corresponding to the native CHR sequence ('C-peptides'). However, previously reported EboV C-peptides have shown weak or insignificant antiviral activity. To determine if the activity of a C-peptide could be improved by increasing its intracellular concentration, we prepared an EboV C-peptide conjugated to the arginine-rich sequence from HIV-1 Tat that is known to accumulate in endosomes (tat-Ebo). We found that this peptide specifically inhibited viral entry mediated by filovirus GP proteins and infection by authentic filoviruses. We determined that antiviral activity was dependent on both the Tat sequence and the native EboV CHR sequence. Confocal microscopy with FITC labeled tat-Ebo revealed that the peptide enters cells and localizes to late endosomes. Mechanistic studies suggested that the peptide acts by blocking a membrane fusion intermediate. We believe that this peptide serves not only as as a model for future therapeutics against filoviruses, but also as a useful tool to study the EboV fusion reaction. [R01 AI088027]

2.52 **Interaction Between the Human Cytomegalovirus Proteins UL94 and UL99 is Required for Viral Assembly**

Stacia L. Phillips and Wade Bresnahan, University of Minnesota

The mechanisms by which the HCMV virion is assembled are poorly understood. However, several viral tegument proteins are known to be essential for assembly of the virus particle. In an effort to identify protein-protein interactions that may be involved in the processes of virion assembly, we designed a yeast two-hybrid screen among HCMV virion proteins. Our screen revealed 17 novel interactions including an interaction between the tegument proteins UL94 and UL99. Using co-immunoprecipitation analysis we demonstrate that UL94 and UL99 interact at late times during infection and that this interaction also occurs in the absence of other viral proteins. We hypothesize that the interaction between UL94 and UL99 is essential for HCMV replication. UL99 has been shown to be an essential tegument protein that is required for secondary envelopment of virions in the cytoplasm while the function of UL94 is unknown. To investigate the function of UL94 during infection, we constructed a UL94-null mutant virus. This mutant is completely defective for replication, demonstrating that UL94 is also an essential tegument protein. Using immunofluorescence staining of infected cells we show that during wild-type infection, UL94 co-localizes with UL99 to the juxtanuclear viral assembly compartment. In contrast, UL99 displays aberrant localization in cells infected with the UL94-null mutant, suggesting that UL94 is required for the proper localization of UL99 during infection. Finally, we used mutagenesis to show that the interaction between UL94 and UL99 is required for viral replication. Taken together, we have identified UL94 as an essential viral gene and demonstrated that the interaction between the UL94 and UL99 tegument proteins is essential for HCMV replication. [Funding sources NIAID 5RO1AI059340-05 and NIDCR T32DE007288]

2.53 **Host immune status affects the regulation of colonization by Candida albicans in the GI tract**

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The yeast Candida albicans colonizes the GI tract of most humans, yet is capable of causing severe disease when a host becomes immunocompromised. C. albicans is a major nosocomial pathogen: further understanding of how Candida colonizes the GI tract may eventually lead to better diagnosis and treatment options. Using a murine model of GI tract colonization, we investigated the role of EFG1, a transcription factor which regulates cellular morphology and virulence. We observed that an efg1- mutant initially had increased colonization in the GI tract, yet this mutant was also more susceptible to the immune response than wild type Candida, which led to a reduction in colonization. In a host which lacks T-cells, the efg1- mutant also initially hyper-colonized but levels remained high over time in this host, demonstrating that the reduction in the population over time in a healthy host is due to a T-cell dependent process. In addition, EFG1 is differentially expressed over time between healthy and T-cell deficient hosts. We propose that, through EFG1, Candida may respond to environmental signals in the GI tract which differ between healthy and immunocompromised hosts, leading to large changes in the transcription profiles between cells colonizing different hosts. These data show that EFG1 is a key regulator of colonization of C. albicans in the GI tract: the absence of EFG1 allows Candida to colonize at high levels, one of the first steps towards disease progression, yet this phenotype is balanced by an increased susceptibility of these cells to the immune response. Regulation of EFG1 through the sensing of changes in the host environment, including immune status, may determine the balance between colonization and pathogenesis of C. albicans. [NIAID]

MITOSIS/CHROMOSOMES/CELL CYCLE

2.54 A Novel Role of Human APE1/Ref-1 and Its Acetylation in Structural Maintenance of the Genome

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While AP-endonuclease has a central role in repair of both endogenous and environmentally induced oxidative and alkylation damage in the genomes of all organisms, the mammalian enzyme (APE1) has distinct additional functions as an activator of several transcriptional factors (TF's), which requires its non-conserved N-terminal domain. We have shown that human APE1 is acetylated by p300 at N-terminal Lys 6 and 7, which increases its interaction with TFs and enhances their functions. Both acetyl acceptor K6/7 residues and the enzyme active site H309 in APE1 are independently essential for cell survival. Based on our preliminary observations that acetylated APE1 (AcAPE1) level was cell-cycle dependent and that APE1 depletion blocked cell cycle progression, we investigated the functions of AcAPE1 in cell cycle progression. Confocal microscopy analysis of synchronized fibroblasts showed that AcAPE1, unlike unmodified APE1, is exclusively nuclear in interphase cells and is associated with the chromatin during mitosis. AcAPE1 colocalizes with phospho-histone H3 (Ser 10), and interacts with and colocalizes with condensin core protein SMC2, and centromere protein A (CENPA) in an acetylation-dependent manner. Upon APE1 depletion, CENPA localization on the mitotic chromosome becomes diffuse and disorganized suggesting that APE1 is involved in condensin-dependent regulation of CENPA localization. Together these observations suggest that APE1 acetylation is critical for mitotic progression, where it is likely to have a structural role in maintaining the mitotic chromosomal architecture, in addition to maintaining the proper distribution of CENPA during the mitosis. These studies have unraveled a novel, structural function of APE1 in cell cycle-dependent genome dynamics that is regulated by its N-terminal acetylation. (Research supported by USPHS grant R01 ES08457)

2.55 **Asymmetrical localization of a lysine deacetylase to the bud neck and the daughter spindle pole body**

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Acetylation of lysine residues provides cells a means of dynamic regulation similar to other post-translational modifications. While most of the progress so far was made on histone or nuclear protein acetylation, little is known about how acetylation can also act on many cytoplasmic proteins. In a localization profiling study of all ten S. cerevisiae histone deacetylases (HDACs), Hos3p displays a unique cytoplasmic localization at the mother-bud neck and the spindle pole body. This localization pattern significantly suggests novel in vivo roles of Hos3p other than histone deacetylation. A microscopy screening was carried out to search for mutants with defective localization of Hos3p at the bud neck and revealed septins and five other genes as hits. Interestingly, all of these genes fall into a signaling cascade termed the morphogenesis pathway, which monitors cellular growth to the cell cycle. Using yeast genetics, we found that localization of Hos3p to the bud neck is likely due to a physical interaction to one of the hits. However, localization of Hos3p to the daughter spindle pole body is independent on this hit gene. To be targeted to this site, the N-terminus of Hos3 determines the asymmetry while the interaction domain with the unknown spindle pole body component lies within the C-terminus of Hos3. Interestingly, Hos3 could be recruited to both spindle pole bodies in dyn1? mutant that displayed misaligned spindle, which suggested a potential role of Hos3 in regulating the mitosis exit network. Finally, truncated Hos3p localizes to the mitochondria, and such association is likely through mitochondria import. This is a surprising observation of which the biological role could be inducing mitochondria-wide deacetylation under certain stresses. The exact functions and substrates of Hos3 are still under investigation.

REGULATION OF GENE EXPRESSION

2.56 **Regulation of the Histone Demethylase LSD1 by the Glucocorticoid Receptor**

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Nuclear receptors (NRs) regulate a wide range of physiological processes by mediating cellular hormone action often through regulation of gene expression. NR transcription regulation has been found to involve dynamic changes in chromatin structure. Histone methylation plays a fundamental role in regulating chromatin structure and transcription. The histone demethylase LSD1 has been shown to act as both a transcriptional co-repressor with histone H3K4me2 demethylase activity, and a co-activator with H3K9me2 demethylase activity. We hypothesize that histone modifying enzymes, like LSD1, can be regulated by the proteins they interact with. NRs are likely regulatory candidates given their duel roles in transcription activation and repression. A better understanding of the molecular mechanisms regulating histone demethylases and their roles in NR function will provide insight into potential methods to manipulate gene expression and hormone action, which have broad therapeutic potential for human disease. Here we show by tandem-affinity purification that LSD1 is part of the Glucocorticoid Receptor (GR) complex. Using microarray analysis, we show that LSD1 is an important co-regulator of many glucocotorticoid target genes, and by ChIP show that LSD1 directly binds to GR target genes. We further elucidate the mechanism by which LSD1 can act as both a co-repressor and co-activator by demonstrating that the GR, as well as multiple other NRs, inhibit LSD1 H3K4me2 demethylase activity in vitro via the NR activation domain. We conclude by proposing a model where LSD1 interaction with the NR activation domain inhibits LSD1 H3K4me2 activity and thus facilitates its activity against H3K9me2. [Funding sources: NIH and Harvard Medical School]

2.57 **miR-29 Regulation of ECM and Smooth Muscle Phenotype**

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Objective: Investigate the role of miR-29 in modulating fibrosis associated genes and smooth muscle phenotype. Methods: Genome wide profiling was performed for IMR-90 cells treated with Antisense LNA oligos to knockdown miR-29. David (EASE) programs were used for array data analysis. 3'UTR Luciferase Assays confirmed miR-29 direct targets. qRT-PCR and Western Blots confirmed miR-29 targets and smooth muscle marker mRNA and protein expression. ISH and IHC localized miR-29, KLF4, myocardin and SMA expression in lung tissue. Results: miR-29 expression was significantly reduced in fibrotic lungs, inversely correlating with expression levels of profibrotic target genes, and severity of fibrosis. miR-29 is preferentially expressed in cells of mesenchymal origin, such as in subsets of interstitial cells of the alveolar wall, pleura and at the entrance of alveolar ducts, known sites of pulmonary fibrosis. Further, miR-29 directly controls a large number of genes associated with pulmonary fibrosis in vitro. In addition, high miR-29 expression was found in subepithelial SMCs of conducting airways and SMCs associated with blood vessels. Multiple smooth muscle markers were down-regulated when miR-29 was reduced in lung fibroblasts and SMCs in vitro. Further, we have proven KLF4, a negative regulator of MYOCD and smooth muscle marker genes, is a novel direct target of miR-29 that is up-regulated in miR-29 KD cells and co-localizes with miR-29 in the perivasculature. Reduced SMA-related genes in miR-29 KD SMCs are accompanied by increased collagen expression. Conclusion: Our findings suggest a role for miR-29 in regulating ECM gene expression as well as modulating smooth muscle phenotype, factors involved in remodeling in fibrotic lung injury. [RO1HL081800, PO1HL47049, RO3HLO95415]

2.58 **pH Homeostasis is Critical in Managing Oxidative Stress and Iron Metabolism**

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V-ATPases are highly conserved proton pumps that acidify multiple organelles and contribute to overall cellular pH balance. Mutations in the yeast V-ATPase (vma mutants) result in an accumulation of reactive oxygen species and hypersensitivity to exogenous oxidative stress. Our earlier microarray screen revealed TSA2 as the only antioxidant gene to be upregulated in vma2? mutant. Multiple AFT1-regulated genes were also upregulated, consistent with perturbed iron homeostasis. This suggested a connection between pH, redox, and iron regulation. We developed GFP biosensors to monitor expression of iron-dependent AFT1-regulated genes and the redox regulator TSA2. Inhibition of V-ATPase activity via the specific inhibitor concanamycin A in wild-type cells resulted in coordinate upregulation of both AFT1- and TSA2-driven biosensors within 30 minutes. A multi-copy suppressor screen, to identify genes that suppress sensitivity of vma2? mutants to exogenous H2O2 when overexpressed, identified lipid biosynthesis, DNA repair, and mitochondrial genes. These suppressors generally downregulated the TSA2- but not AFT1-driven biosensors. Concurrently, when measured for metal levels, vma2? cells had sufficient levels of iron. However, if these mutants were supplemented with iron, both, AFT1- and TSA2-driven expression decreased. This suggests that the V-ATPase mutants have defective iron signaling and proposes a new link between TSA2 and iron homeostasis. In all, we are finding revealing connections between pH homeostasis, iron metabolism, and resistance to oxidative stress that are likely to be relevant in all eukaryotic cells. [NIH RO1 GM50322]

2.59 **Bryonolic Acid Transcriptional Control of Inflammatory and Antioxidant Genes in Macrophages**

Tonibelle N. Gatbonton-Schwager, Gregory P. Tochtrop, John J. Letterio, Case Western Reserve University

The aim of this study is to characterize the mechanism of the anti-inflammatory activity of bryonolic acid (BA) and validate its use as a tool to understand the pleiotropic effects of triterpenoids. We show that BA reduces the inflammatory mediator nitric oxide through suppression of expression of the inflammatory enzyme iNOS in LPS-activated RAW 264.7 macrophage cells. In addition, BA robustly induces the antioxidant protein, heme oxygenase-1 (HO-1) in vitro and in vivo in an Nrf2-dependent manner. Further analysis of the Nrf2 target genes yielded a non-typical triterpenoid gene expression profile in BA treated macrophages selectively regulating antioxidant genes which include NQO-1, CAT, GCLC and GR. These findings are significant because this is the first study to show a mechanism for the anti-inflammatory activity of BA through suppression of iNOS and induction of HO-1. The distinct expression profile of BA on the Nrf2 target genes validates the use for this molecule as a tool to explore the role of the triterpenoid scaffold on its anti-inflammatory and chemopreventive properties. Understanding how selective gene modulation is controlled by the triterpenoid skeletal structure will aid in the design of selective therapeutics that minimizes undesirable effects typical of targeting pathways with multiple downstream targets. [Ruth L. Kirschstein, Silber S. Fellowship, The Reuter Foundation].

2.60 **Evolution of the Hox Gene fushi tarazu in Arthropods**

Alison M. Heffer and Leslie Pick, University of Maryland

Hox genes are evolutionary conserved transcription factors, best known for their roles in determining segment identity in insects. fushi tarazu (ftz) is a rapidly evolving Hox gene that has changed from an ancestral homeotic gene to a pair-rule segmentation gene in Drosophila. During arthropod evolution, ftz has undergone three changes thought to contribute to this switch in function. ftz gene expression during embryogenesis changed from Hox-like to seven stripes in Drosophila. Additionally, in the protein sequence an LXXLL motif was acquired and a YPWM motif was lost. These two changes switched the protein partner that Ftz interacts with and thus switched the DNA binding targets that Ftz regulates. We have mapped these sequence and expression changes through 550 million years of arthropod evolution by isolating and sequencing ftz genes and examining their expression patterns in a range of non-model arthropods. We find that while the segmentation LXXLL motif was stably acquired once at the base of holometabolous insects, the homeotic YPWM motif has degenerated in several arthropod lineages, and several of these "degen-YPWMs" retain homeotic potential in Drosophila. Additionally, expression in the crustacean Artemia revealed that ftz is expressed as a weak Hox gene during naupliiar development. Together, studying the sequence and expression pattern changes of ftz during arthropod evolution will provide a better understanding of the flexibility allowed for evolution of a regulatory gene in an otherwise highly-conserved complex of genes. [Research supported by National Science Foundation]

2.61 **Mechanism of FACT Action during Transcription through Chromatin by Pol II**

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Nucleosome survival during transcription is essential for cell viability and normal aging, and nucleosomal barrier is involved in gene regulation. FACT (Facilitates Chromatin Transcription) is an elongation factor and histone chaperon that stimulates transcription through chromatin by RNA polymerase II (Pol II) and nucleosome survival in vivo. The mechanism of FACT action is unknown. Previously we have shown that transcription through chromatin by Pol II is accompanied by formation of a small intranucleosomal DNA loop (Ø-loop) that couples nucleosome survival and Pol II progression. Here we show that FACT strongly facilitates transcription through chromatin and nucleosome survival in vitro through interaction with H2A/H2B dimers. Analysis of the effect of FACT on nucleosomal pausing suggests that FACT affects efficiency of formation of the Ø-loop-containing intermediate. Taken together, the data suggest that FACT directly affects formation of the Ø-loop by competing with the H2A/H2B-DNA interactions and facilitating DNA uncoiling from the histone octamer. [This work was supported by NIH GM58650 grant to V.M.S.]

2.62 **Regulation of MicroRNA-Mediated Repression: Enter Ribosomal Proteins**

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MicroRNAs (miRNAs) participate in numerous cellular processes and, when dysregulated, contribute to oncogenesis. MiRNAs bind to 3' untranslated regions of target mRNAs, leading to post-transcriptional repression by mechanisms that are incompletely understood. To discover factors required for miRNA activity, we performed an RNAi screen using a clonal reporter cell line that provides a sensitive readout of miRNA repression of translation initiation. We show that knockdown of ribosomal protein genes (RPGs) associated with either the small or large ribosomal subunit preferentially increases polysome association, translation, and stability of miRNA-targeted mRNAs relative to untargeted mRNAs. Global analyses of total and polysomal mRNAs by RNA-Seq indicated substantial overlap in the sets of genes exhibiting increased or decreased polysomal association following knockdown of Argonautes or RPGs, suggesting similarity in affected pathways. Genes with increased polysomal association were enriched in predicted miRNA target sites. Notably, dissociation of repressive Ago-containing miRNA complexes from target mRNAs was observed following RPG knockdowns. Together, our data show that the levels of RPGs alter the translation rates of miRNA-targeted mRNAs relative to untargeted mRNAs by modulating miRNA-mediated repression of translation initiation. Our findings may explain why RPG down-regulation predisposes to cancers in model organisms and in patients with bone marrow failure syndromes characterized by RPG mutations. [Distinguished Young Scholars Award from the W.M. Keck Foundation to C.D.N].

2.63 **Cooperative Wnt signaling regulates lung progenitor differentiation**

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Epithelial-mesenchymal signaling regulates the formation of most endodermally derived organs, but how signaling by multiple ligands is integrated to guide endoderm development is poorly understood. We show that two Wnt family ligands, Wnt2 and Wnt7b, cooperatively induce TCF-dependent transcription in mesenchymal cell types without increasing b-catenin stabilization. Combined loss of Wnt2 and Wnt7b in the embryonic lung causes severe hypoplasia and branching defects consistent with perturbed distal progenitor development, while loss of either Wnt2 or Wnt7b alone causes only mild defects. Taken together, these data indicate that the interaction between Wnt2 and Wnt7b is critical for lung development. To identify factors mediating this cooperation, we performed a screen utilizing a Library of Pharmacologically Active Compounds (LOPAC), the results of which revealed that PDGF pathway activity is required for cooperative Wnt2/Wnt7b signaling. Moreover, treatment with recombinant Pdgf protein enhances signaling by Wnt2 in mesenchymal cell types. Since we have previously shown that Wnt7b is both necessary and sufficient for Pdgfr expression in the lung mesenchyme, these data suggest a model in which Wnt7b potentiates Pdgf signaling that subsequently enhances canonical Wnt pathway activation downstream of Wnt2. Acknowledgement: This work is supported by NIH RO1-Hl-087825

2.64 **Critical Role of a Novel Transcription Co-Regulator in Mammary Gland Development and Morphogenesis**

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Recent studies of genome-wide transcription suggest that transcription elongation may represent a common step for mammalian gene regulation. Negative Elongation Factor B (NELF B, also known as COBRA1) acts as an integral subunit of a complex that stalls RNA polymerase II at the promoter proximal regions of several estrogen-dependent genes. Interestingly, reduced expression of NELF B is highly correlated with metastatic breast cancer and reduced patient survival rate. The objective of this project is to understand the functions of Nelf B in mammary gland biology. In this study we employed MMTVCre to delete Nelf B in the luminal epithelium of the mouse mammary glands. These mice exhibited defective ductal development and lactation. Flowcytometry analysis of mammary epithelial cells (MECs) using established cell surface markers revealed an increase in luminal progenitor population in Nelf B deleted glands. Surprisingly, Nelf B deleted progenitor population demonstrated a reduced colony forming ability, suggesting proliferation defect. Ex vivo deletion of Nelf B from MECs and subsequent transplantation revealed the absolute requirement of Nelf B for regenerative ability of mammary stem cells. Global gene profiling experiment performed on sorted MECs revealed the activation of stress response pathways in Nelf B deleted MECs. At present I use mouse genetics and epigenetic approaches to study the contribution of different stress response pathways in phenotypes observed in Nelf B deleted glands. These studies will shed light on the mechanisms by which Nelf B regulates the normal development of mammary gland and also its potential synergistic interaction with other key molecules in breast cancer development. (DoD predoctoral award (BC083034) to SJN and NIH grant (DK64604) to RL)

2.65 **HER-2 Signaling Affects SRC-3 Phosphorylation and Gene Regulatory Potential within the Cistrome**

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Steroid Receptor Coactivator-3 (SRC-3/AIB1) is a potent transcriptional coregulator for nuclear receptors and other transcription factors and is often amplified or overexpressed in tumors. The growth promoting effects of SRC-3 involve its ability to integrate extracellular signals into discrete patterns of gene expression. SRC-3 is regulated by various post-translational modifications, including multiple phosphorylations that define a combinatorial code that tailors SRC-3 activity in response to specific signaling stimuli. HER-2 is a member of the ErbB receptor tyrosine kinase family of growth factor receptors and is often associated with cancer. Clinical studies show that breast cancer patients with tumors expressing high levels of both HER-2 and SRC-3 have reduced disease-free survival, and SRC-3 phosphorylation is influenced by HER-2. The current study seeks to define the effect of HER-2 signaling events on SRC-3 activity in breast cancer cells. HER-2 knock down using siRNA affects multiple kinase pathways, as evidenced by decreased AKT and c-Raf phosphorylation. HER-2 knockdown causes the isoelectric point of SRC-3 to shift, suggesting that post-translational modification of SRC-3 is actively altered by HER-2 signaling. Reduction of SRC-3 phosphorylation in HER-2-depleted cells also was evident using a phospho-specific antibody to SRC-3. Expression microarray analysis revealed that many genes are altered by HER-2 knockdown. Parsing the microarray with SRC-3 ChIP-seq data identified genes that are coordinately regulated by a HER-2/SRC-3 signaling axis. Collectively, this work describes transcriptomic and cistromic mechanisms for oncogenic cooperation between HER-2 and SRC-3 and identifies key genes that contribute to proliferation of breast cancer cells. [NIH R01 HD08818 and The Welch Foundation]

2.66 **Identifying Novel Gata4-regulated Molecular Networks during Cardiogenesis**

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The gata4 gene encodes a transcription factor with multiple defined roles in cardiogenesis. The gene is expressed in all three layers of the heart, and has been associated with numerous aspects of cardiac morphogenesis including valve formation, primitive tube looping, chamber outgrowth, and regeneration. We found that gata4 can also function in a non-cell-autonomous manner to modulate specification of cardiac progenitors. It is currently unclear how this single gene controls developmental programs relevant to multiple tissues and developmental stages, but presumably it involves the integration of distinct sub-regulatory networks. A principal challenge in identifying these networks is obtaining pure populations of specific cells where these networks operate. We are exploiting transgenic zebrafish reporter strains coupled with next generation sequencing to gain insight into these molecular programs. Next generation sequencing has the advantage of identifying novel transcripts with greater precision than previous multiplex expression technologies. We compared transcriptome profiles of purified cardiomyocytes isolated from wild-type zebrafish embryos and gata4 morphants. Using this approach, the gata4-regulated genetic network in the myocardium has been elucidated. We demonstrate differential expression of known targets such as the cyclins, as well as previously unknown targets including specific matrix metalloproteinases. Network modeling of gata4-regulated genes in the myocardium suggests a role in the development of the adjacent epicardium. Utilizing a conditionally expressed dominant negative gata4 transgene, we confirm the requirement of myocardial gata4 function and epicardial specification. [NIH Training Grants GM07491 and 1T32HD060600-01A1]

2.67 **The Her7 Node Asymmetrically Regulates the Hes6 Hub to Modulate the Segmentation Clock Network**

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The zebrafish segmentation clock is profoundly complex due to multiple signaling pathways that interact within an oscillatory gene network. The primary genes of this mechanism are the hairy/enhancer of split-related (her) family, encoding bHLH proteins. These genes undergo cycles of expression and repression when the Her proteins dimerize and inhibit their transcription. Oscillations are achieved through a delayed negative feedback loop whose period is determined by production and degradation rates of her mRNAs and proteins. Zebrafish have five her genes expressed in partially overlapping, oscillating patterns and hes6, expressed in a gradient linking the clock to posterior growth of the tail. Loss of function analysis suggests that while her genes have distinct roles in somitogenesis, there is some functional overlap. This redundancy has made it difficult to elucidate the developmental roles of these genes. Therefore, we used biochemical techniques to provide an unexplored dimension of this process. First, we examined dimerization of Her proteins in vitro and in vivo. Then, we analyzed the ability of the dimers to bind putative binding sites in the her7 enhancer. These parameters enabled direct comparison to existing mathematical models and a better interpretation of loss of function synergies. Our resulting model suggests that Hes6 is the hub of the dimer network and Her7 dimerizes with Hes6 but is unique in that it does not homodimerize. These characteristics give the her7 node uneven impact on the hes6 hub by indirectly affecting the balance of dimers in the network. Further, genetic data indicate that it is loss of Her7 regulation of the Hes6 hub, rather than loss of Her7 DNA binding, that produces the her7 loss of function phenotype. [T32 GM007499, R01 HD045738-06].

SIGNALING

2.68 **The Role of Calcium/Calmodulin-dependent Protein Kinase II Signaling in Neuronal Excitotoxicity**

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Excitotoxic calcium signaling during ischemia induces functional and structural changes in calcium/calmodulin-dependent protein kinase II (CaMKII). At the onset of insult, CaMKII activity is significantly increased. Using a variety of pharmacological inhibitors of CaMKII, we determined that preventing this aberrant increase in activity affords neuroprotection in cultured cortical neurons exposed to excitotoxic glutamate stimulation. However, we also identified that prolonged inhibition of CaMKII resulted in increased neurotoxicity and sensitivity to excitotoxic insult. Prolonged inactivation of aCaMKII has been observed following excitotoxic insult as the kinase undergoes an activity-dependent aggregation termed self-association. Thus, we aimed to identify the mechanisms underlying self-association in order to subsequently verify the negative role this process plays in neuronal viability. Interestingly, bCaMKII has been shown to be resistant to self-association. Using genetic sequencing information, in combination with the crystal structure, we functionally screened mutations within the autoregulatory domain of aCaMKII that mimic bCaMKII. These mutants were tested in an in vitro light scattering assay and an in situ cell-based approach whereby aggregation of GFP-CaMKII mutants was examined following stimulation using live-cell fluorescent imaging. Mutation of S272C/H273Q in aCaMKII rendered the kinase resistant to self-association in all assays. We are now examining neuronal viability when wild-type aCaMKII is knocked down in neurons and S272C/H273Q-aCaMKII is expressed and neurons are subjected to excitotoxic glutamate insult. Together, these data suggest that aberrant kinase activity- both activation and self-association- contribute to excitotoxicity. [Stark Fellowship]

2.69 **Gbetagamma-mediated activation of p110beta is required for transformation**

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Class IA PI3-kinases are activated downstream of RTKs and GPCRs. p110beta is unique among the class IA catalytic subunits in being activated downstream of GPCRs by binding to Gbetagamma subunits, however the region of binding, mechanism of activation, and significance in p110beta signaling are not well understood. Using sequence alignment, we identified a segment of the p110beta C2-helical domain linker that showed low homology with p110alpha and p110delta. Replacing this region in p110beta with the corresponding region from p110delta results in a mutant that is normal for basal and phosphopeptide stimulated kinase activity, but defective for Gbetagamma-mediated activation. Moreover, peptides derived from this site block Gbetagamma-mediated activation of p110beta both in vitro and in vivo. The Gbetagamma-regulated site in p110beta is near the helical domain-nSH2 interface in the p110/p85 dimer, suggesting that Gbetagamma might activate p110beta by disrupting this inhibitory contact. This was confirmed by showing that p85 and p110beta mutants that disrupt the nSH2-helical contacts lead to loss of Gbetagamma activation. To examine the role of Gbetagamma signaling to p110beta in vivo, we assayed cells expressing wild type or mutant p110beta. Mutation of the Gbetagamma binding lead to reduced rates of wound closure in wound-healing assay and loss of transforming potential. Moreover, a myristylated peptide from the Gbetagamma binding site in p110beta blocks p110beta-mediated transformation in NIH3T3 cells and inhibits growth of PC3 prostate cancer cells, which require p110beta. Our study defines the mechanism and significance of Gbetagamma-mediated activation of p110beta, and provides an approach for targeting Gbetagamma-p110beta interactions in cancer. [This work is funded by NIH and NCI grants]

2.70 **A Beneficial Role of Apoptotic Cells in Muscle Development**

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During many developmental processes excess cells are produced, of which only a few are selected for further maturation to become part of the tissue. The rest die and are cleared by neighboring healthy cells or resident professional phagocytes. Currently, it is believed that dead cells are a nuisance that must be removed so that they do not leak ‘self’ antigens into surrounding tissue. We have recently made an unexpected discovery, however, that in the context of muscle development, dead cells may play a beneficial role. To explore to role of apoptotic cells in myoblast differentiation, we utilized the mouse myoblast cell line, C2C12, which can be induced to fuse in vitro. We observed that a fraction of C2C12 cells die as early as four hours after adding differentiation medium. Interestingly, blocking cell death during differentiation effectively inhibits myoblast fusion; moreover, the cell death appears time sensitive since blocking cell death at later time points durring differentiation did not impair myoblast fusion. Remarkably, addition of dying myoblasts to cultures where cell death has been blocked could restore fusion. Importantly, the dying cells, but not the supernatants, positively promoted myoblast fusion. Since apoptotic cells expose ‘eat me’ signals on their surface, we addressed the role of phosphatidylserine (PS), a key eat me signal exposed on dying cells. By adding a soluble decoy molecule that binds and blocks PS/receptor interaction, we observed that PS recognition was required for fusion. We are currently determining how apoptotic myoblasts drive differentiation via PS recognition, and the role played by known PS receptors in modulating the downstream signaling pathways during myoblast differentiation. [Funding Agency: NIH; 2RO1 GM064709 8/1/2007-7/31/2011]

2.71 **Win55, 212-2 Decreases Plasma Triglycerides in Cannabinoid Receptor Type 2 (CB2) Deficient Ldlr-null Mice**

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Objective: Exogenous cannabinoid receptor stimulation with Win55, 212-2 has been shown to reduce atherosclerosis in ApoE-null mice by a mechanism thought to be mediated by CB2. However, the involvement of CB2 in this process has not been confirmed genetically using CB2 deficient (CB2-/-) mice. Therefore, we investigated the effect of Win55, 212-2 on atherosclerosis in CB2 deficient hyperlipidemic low-density lipoprotein receptor null (Ldlr) mice. Methods and Results: Ldlr-/- (WT) and CB2-/-Ldlr-/- double knockout mice (n=8) were fed an atherogenic diet for 8 weeks with administration of Win55, 212-2 (1mg/kg) daily over the last two weeks. Aortic lesion area was not significantly affected by Win55, 212-2 treatment in either genotype. No change in total plasma cholesterol in response to Win55, 212-2 treatment was observed, but surprisingly total plasma triglycerides were significantly decreased in CB2-/-Ldlr-/- mice given Win55, 212-2, but not in similarly treated WT mice. Immunohistochemical staining showed no significant differences in lesional content of macrophages and smooth muscle cells between genotypes or in response to Win55, 212-2 treatment. Lesional apoptosis, collagen content, and elastin fiber fragmentation were also unchanged in response to Win55, 212-2 or genotype. Conclusion: Win55, 212-2 does not alter plaque area or cellular composition, but reduces total plasma triglyceride levels in CB2 deficient Ldlr-null mice. This result suggests that in CB2-null mice, Win55, 212-2 may activate a pathway involved in plasma triglyceride clearance or metabolism that is normally suppressed in WT mice by a CB2-dependent mechanism. [This work was supported by the National Institutes of Health (grant No. HL085137 to Dr. Thewke).]

2.72 **An Important Role for Akt3 in Platelet Activation and Thrombosis**

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The Akt family of serine/threonine kinases includes three isoforms, Akt1, Akt2 and Akt3. Prior studies have reported that Akt1 and Akt2, but not Akt3, are expressed in platelets. We show that Akt3 is expressed in substantial amounts in platelets. Unlike Akt1 and Akt2 that are important in platelet secretion and aggregation induced by nearly all tested platelet agonists, Akt3-/- mice selectively exhibit impaired platelet aggregation and secretion induced by low concentrations of thrombin receptor agonists and thromboxane A2, but not VWF and collagen. Akt3-/- platelets exhibit a significant reduction in thrombin-induced phosphorylation of glycogen synthase kinase 3ß (GSK-3ß) at Ser9, which is known to inhibit GSK-3ß function. This result suggests that Akt3 is important in inhibiting GSK-3ß. Accordingly, treatment of Akt3-/- platelets with a GSK-3ß selective inhibitor rescued the defect of Akt3-/- platelets in thrombin-induced aggregation, suggesting that negatively regulating GSK-3ß may be a mechanism by which Akt3 promotes platelet activation. Importantly, Akt3-/- mice showed significant retardation in FeCl3-induced carotid artery thrombosis in vivo. Thus, Akt3 plays an important role in platelet activation and in in vivo thrombosis, and the role of Akt3 in platelets appears to be distinct from that of Akt1 and Akt2.

2.73 **Interception of Stress Signaling by the ASK1/IKK Signaling Node**

Mary C. Puckett, Erinn Goldman, Lisa Cockrell, Fadlo Khuri, Haian Fu, Emory University

Reactive oxygen species (ROS) play pivotal roles in normal cell function and disease pathology. The apoptosis signal regulating kinase 1 (ASK1), which can initiate pro-apoptotic signaling cascades through activation of JNK and p38, is a key mediator of ROS signaling. Improper regulation of ASK1 has been implicated in a wide array of diseases, from cancer to neurodegenerative disease. Conversely, nuclear factor ?B (NF-?B) protects cells again ROS by decreasing JNK activation. However, the mechanism of crosstalk between NF?B mediated survival and JNK mediated apoptosis remains unclear. We identified a direct, transcription-independent mechanism of cell death inhibition by an upstream regulator of NF?B, the inhibitor of ?B kinase (IKK). We demonstrated IKK can phosphorylate ASK1 S967, a molecular switch suppressing ASK1 activity. Through endogenous immunoprecipitations and time-resolved fluorescence resonance energy transfer (TR-FRET), we established IKK directly interacts with ASK1. To elucidate the relationship between ASK1 and IKK, the interface was identified and the consequences on ASK1-mediated signaling were studied. The ASK1/IKK interface was dissected through deletional mutagenesis and protein complementation. ASK1 activity was assessed by H2O2 induced apoptosis, neurite outgrowth and phospho-status under several IKK states. IKK prevented stress-induced dephosphorylation of ASK1 and inhibited ASK1 mediated apoptosis. Our study reveals an IKK/ASK1 signaling node that negatively regulates apoptosis in response to pro-survival conditions. Manipulation of this interaction offers a promising strategy to develop targeted agents for ASK1 and IKK related diseases, such as cancer, inflammatory diseases and neurodegenerative disorders. [PhRMA Foundation, NIH T32 GM008602]

2.74 **Snf1-related Kinase Enhances Cardiomyocyte Metabolism through Tribbles Homolog 3 Downregulation**

Amy K. Rines, Michael A. Burke, Hossein Ardehali, Northwestern University

Background: Snf1-related Kinase (SNRK) is an AMP-activated kinase family member with unknown function and substrates. We found that SNRK is upregulated in hearts from ischemic cardiomyopathy patients, and gene array suggested SNRK alters metabolic gene expression. Thus, we hypothesized that SNRK regulates cardiomyocyte metabolism. Results: SNRK knockdown reduced ATP by 23.34±4.86% in neonatal rat cardiomyocytes (NRCM) compared to control siRNA, and SNRK adenovirus increased ATP 20.67±2.37% relative to GFP control. SNRK knockdown reduced oxygen consumption at baseline and with the mitochondrial uncoupler carbonyl cyanide 3-chlorophenylhydrazone, while SNRK overexpression increased oxygen consumption. Mitochondrial content was unchanged, but tetramethylrhodamine ethyl ester staining increased, suggesting that SNRK maintains mitochondrial membrane potential. Furthermore, the extracellular acidification rate was reduced 19.47±1.36% with SNRK knockdown and increased 12.57±1.10% with overexpression, signifying that SNRK enhances glycolysis. SNRK also increased lactate, glucose uptake, and hexokinase and glucose transporter mRNA. Additionally, palmitate oxidation decreased 69.39±6.81% with SNRK knockdown and increased 96.47±9.69% with overexpression, and SNRK increased acetyl-coA carboxylase phosphorylation. To determine a mechanism, we conducted a yeast two hybrid screen with SNRK bait. Tribbles homolog 3 (TRB3) was identified as a SNRK-interacting prey. In NRCM, SNRK decreased TRB3 protein, and TRB3 knockdown rescued the effects of SNRK knockdown on ATP, mitochondrial membrane potential and glycolysis. Conclusions: Our results show that SNRK decreases TRB3 to enhance cardiomyocyte metabolic flux. [Supported by American Heart Association Grant #10PRE3730013]

2.75 **The Phagocytic Receptor Jedi Signals Engulfment through the Tyrosine Kinase Syk**

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Approximately 50% of neurons in the developing peripheral nervous system undergo apoptosis as part of a normal pruning process. These neuronal corpses need to be cleared in order to prevent an inflammatory response and the possible development of autoimmunity. We recently demonstrated that apoptotic neurons in the developing mammalian dorsal root ganglia (DRG) are cleared by satellite glial cell (SGC) precursors, and Jedi-1 is a novel engulfment receptor involved in this process. The signaling pathway activated by Jedi-1 is currently unknown. However, Jedi-1 is homologous to the engulfment receptor Draper in Drosophila, which is phosphorylated by Src42A then bound by Shark. We found that Src Family Kinases (SFKs) can phosphorylate Jedi-1 on the intracellular NPXY and ITAM motifs. Tyrosine phosphorylation of Jedi is detectable in E13.5 DRG lysates, during the time of massive developmental apoptosis. We also found that Jedi-1 interacts with spleen tyrosine kinase (Syk), a mammalian homolog of Shark, by co-immunoprecipitation, and the interaction between Jedi-1 and Syk is promoted by SFK overexpression. In a DRG co-culture engulfment assay, Syk overexpression increases engulfment, whereas Syk knockdown decreases engulfment. These results suggest that Jedi leads to engulfment by signaling through SFKs and Syk. [NINDS NS064278]

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Quick Check ☑ Exit Survey – How did we do?

Our goal was to provide you with an enjoyable and valuable experience at the NIH.

Please take a moment to provide us with your feedback. Feel free to write in any additional comments or suggestions that you may have.

1) How did you learn about the NIH National Graduate Student Research Conference?

☐ Graduate school training director
☐ Graduate research advisor
☐ Professional meeting
☐ Email flier
☐ Other (*please specify*)

2) How much did you know about the research laboratories (Intramural Research Program) at the NIH **prior to attending** the Conference?

☐ A lot ☐ A little ☐ Nothing (*skip to Ques. 3*)

Had you considered doing postdoctoral training at the NIH **prior to attending** the Conference?

☐ Yes ☐ No

3) Did the Conference meet your expectations? ☐ Yes ☐ No ☐ Yes and No
Please explain.

_____

_____

4) Would you recommend the Conference to your colleagues? ☐ Yes ☐ No

5) Please rate the following **activities** and the **usefulness of the abstract book** on a scale of 1 to 5 (*1=Poor, 2=Fair, 3=Good, 4=Very Good, 5=Excellent*).

1	2	3	4	5	
☐	☐	☐	☐	☐	NIH 101
☐	☐	☐	☐	☐	First Evening – *"Dine Arounds"*
☐	☐	☐	☐	☐	Breakfast with NIH Training Directors
☐	☐	☐	☐	☐	Concurrent Scientific Sessions – *Senior NIH Postdocs*
☐	☐	☐	☐	☐	Poster Sessions
☐	☐	☐	☐	☐	Lunch with NIH Postdoctoral Fellows and NIH Investigators
☐	☐	☐	☐	☐	Resources for Postdoctoral Fellows at NIH
☐	☐	☐	☐	☐	Keynote Scientific Presentation – *Dr. Elaine Ostrander*
☐	☐	☐	☐	☐	Former Trainee Panel
☐	☐	☐	☐	☐	Interviews (if applicable)
☐	☐	☐	☐	☐	Usefulness of Abstract Book

6) How could the Conference **application process** be improved?

_____

_____

7) How could the Conference **travel and hotel arrangements** be improved?

_____

_____

8) How many potential postdoctoral mentor/laboratory contacts did you make **BEFORE** arriving to the Conference?

☐ 0 ☐ 1–2 ☐ 3–5 ☐ 6–10 ☐ >10

9) How many potential postdoctoral mentor/laboratory contacts did you make **DURING** the Conference?

☐ 0 ☐ 1–2 ☐ 3–5 ☐ 6–10 ☐ >10

10) If offered a postdoctoral position at the NIH, what is the likelihood that you would accept it?

☐ Very likely ☐ Not likely ☐ No answer at this time

☐ Likely ☐ Very unlikely

11) Would you recommend postdoctoral training at the NIH to your colleagues? ☐ Yes ☐ No
Please indicate why or why not?

_____

_____

12) Are you a member of an under-represented minority group? ☐ Yes ☐ No
If yes, please check all that apply:

☐ Black or African American ☐ American Indian or Alaskan Native

☐ Hispanic ☐ Native Hawaiian or Other Pacific Islander

☐ Other (*please specify*:) _____

Please share any additional comments or suggestions on how we might improve the Festival.

_____

_____

_____

_____

Thank you for taking the time to help us improve the Festival. If you would like for us to follow-up with you regarding any of your responses, please print your name below.

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