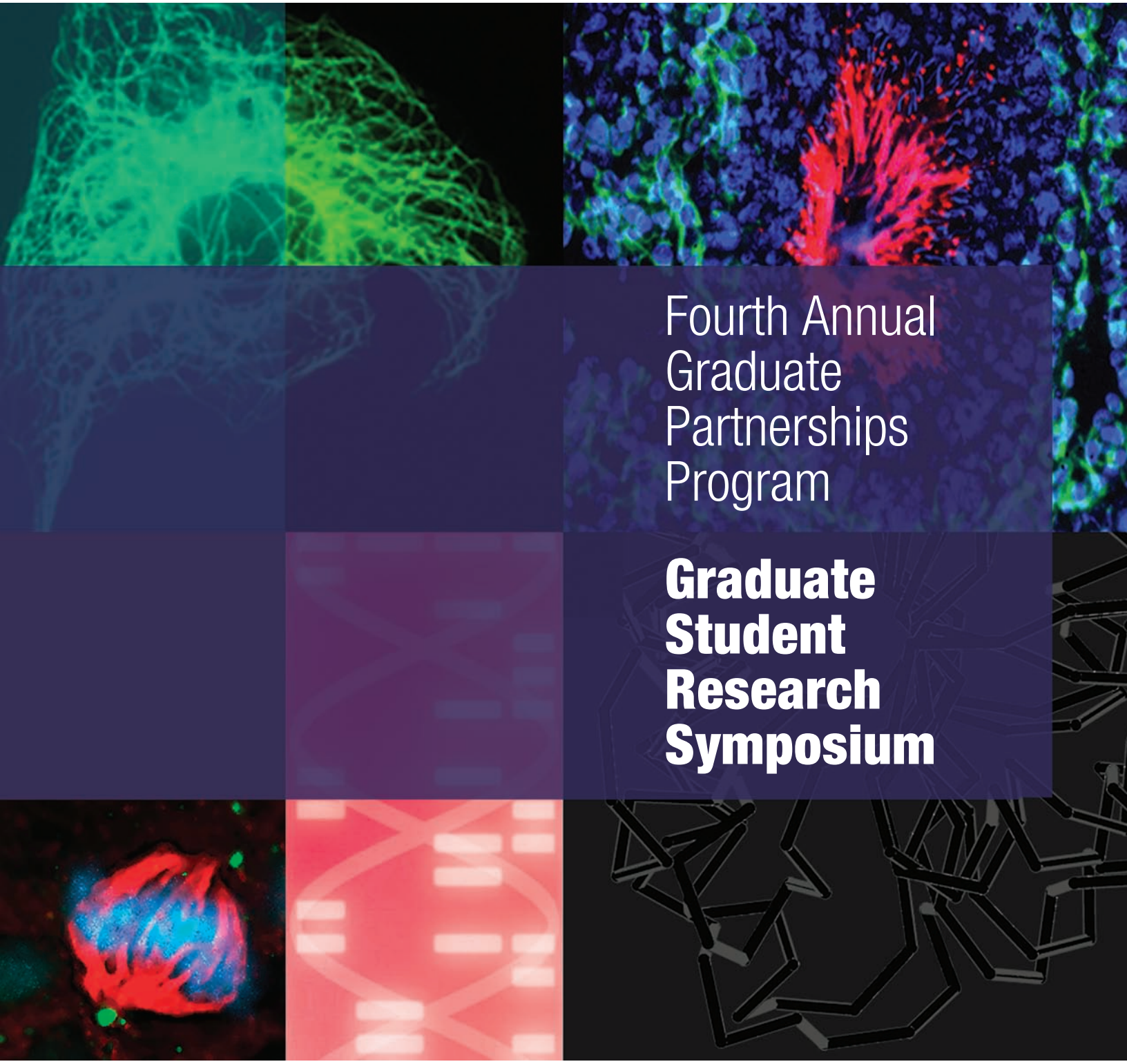


Fourth Annual Graduate Partnerships Program

Graduate Student Research Symposium

Friday, May 4, 2007
Natcher Conference Center

National Institutes of Health

Program and Abstracts

Fourth Annual
Graduate
Partnerships
Program

Graduate Student Research Symposium

Office of Intramural Training
and Education

Foreword

Welcome to the Fourth Annual NIH Graduate Student Research Symposium

When you think about training at NIH, you probably think first of postdocs, postbacs and summer students; however, there are also more than 400 graduate students performing dissertation research at NIH. Graduate students working at NIH are enrolled in graduate programs at over 100 national and international universities — from 21 countries around the world. The Graduate Partnerships Program (GPP), in the Office of Intramural Training and Education (OITE), is the home of graduate students at the NIH. The GPP has many missions: to establish formal partnerships with national and international universities dedicated to quality education in biomedical research; to support the National MSTP Program; to welcome students coming to NIH on an individual basis; and to provide academic support and training for the growing graduate student community. The GPP works closely with the Graduate Student Council (GSC). The GSC serves to represent the intellectual, social and living needs of graduate students at NIH.

Before the GPP was founded, graduate students came to the NIH on an individual basis and were absorbed by their particular Institute. As the number of students increased so did the need to develop a cohesive graduate student community. In the summer of 2000, under the direction of the Office of Intramural Research and the leadership of Dr. Mary DeLong, former GPP Director, the distinct needs of graduate students at NIH were identified. Since then the graduate student population has tripled in size. This annual symposium symbolizes the evolution of the GPP and is our opportunity to share our scientific contributions with the NIH community.

As the grad community grew, so did the symposium. The 2007 symposium is a milestone in many ways. A record number of students submitted posters this year submissions — 101, a 33 percent increase from last year! This year also marks the introduction of Dr. Sharon Milgram as Director of the Office of Intramural Training and Education and of GPP. In the short time that Dr. Milgram has been at the NIH, she has demonstrated an admirable devotion to graduate students. Thanks to her efforts, we have eight travel awards of $500 each to be awarded to the top posters. We are confident that Dr. Milgram's efforts will change the face of training at the NIH.

We are also honored to have an architect of the GPP, Dr. Michael Gottesman, Deputy Director for Intramural Research, as our opening speaker.

We have a great symposium planned and we hope you will enjoy the full day of events.

The 2007 Graduate Student Research Symposium Planning Committee
Kate Callahan, Georgetown University
Emmette Hutchison, Brown University
Philip Wang, University of Maryland, College Park

Acknowledgements:

Over 70 NIH scientists, including NIH postdocs, staff scientists, and principal investigators are judging this year's poster session. Thank you to Dr. Cesar Perez-Gonzales and the NIH Fellows Committee (FelCom) for their support and participation in the poster competition. We would also like to thank the GSC, the GPP Partnership Directors, and the Postbaccalaureate IRTA Committee for their support of the symposium and the graduate community.

Finally, we would like to thank the amazing individuals in the GPP office for helping with every aspect of organizing this symposium. It is due to their hard work and dedication that this symposium has been made possible.

Program

Fourth Annual
NIH Graduate Student Research Symposium

"The Faces of Tomorrow's Science"

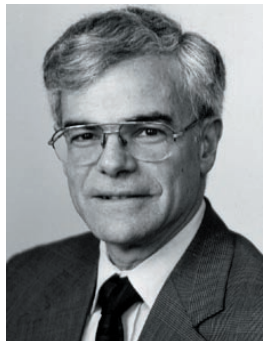
Graduate Partnerships Program
Natcher Conference Center
Balcony B/C and Atrium
May 4, 2007

9:00	Opening	Dr. Michael Gottesman
		Deputy Director for Intramural Research
9:30 – 10:30	Student Oral Presentations – Session 1	
	Kee Chan	*T-Cell Receptor Excision Circles: Application to*
		Newborn Screening for Severe Combined
		Immunodeficiency and Understanding the
		Development of T-Cell Diversity
	Jason Hall	*Intestinal Specific Factors Induce Foxp3+*
		Treg Conversion by Lamina Propria DC
	Megan Cleland	*State of GTPase Cycle Dictates Mobility and*
		Localization of Large Mitochondrial GTPases,
		Mfn1 and 2
	Amrita Ghosh	*Effects of* In Vitro *Culture Conditions Mimicking*
		Gene Therapy Trials on Gene Expression Profiling
		of Human Hematopoietic CD34+ Progenitor Cells
10:45 – 11:30	Keynote Address – *The Future of Training and Education*	
	Dr. Sharon Milgram	Director, Office of Intramural Training and
		Education and the Graduate Partnerships Program
11:30 – 2:30	Light Refreshments	

12:00 – 1:00	Poster Session I	
1:30 – 2:30	Poster Session II	
2:45 – 3:00	Outstanding Mentor Awards	
3:00 – 4:00	Student Oral Presentations – Session 2	
	Brian Capell	In Vitro *and* In Vivo *Effects of Farnesyltransferase*
		Inhibitors for Hutchinson-Gilford Progeria Syndrome
	Catherine Schwartz	*Generation and Characterization of Neural*
		Progenitors and Mature Neurons from Pluripotent
		Stem Cells
	Jeff Blackinton	*RNA Binding Targets of the Recessive*
		Parkinsonism Protein DJ-1 Reveals Involvement
		in Mitochondrial, Oxidative Stress and PTEN/Akt
		Survival Pathways
	Arjun Prasad	*Exploring the Mammalian Phylogeny by Analyzing*
		Large Comparative Sequence Datasets
4:00 – 5:00	Director's Discussion	
	Dr. Sharon Milgram	An informal discussion about career development
5:00 – 6:00	Reception	
	Announcement of Poster Award Winners	

Speakers

Opening Address Speaker



Michael M. Gottesman, MD

Michael Marc Gottesman is Deputy Director for Intramural Research at NIH, and Chief of the Laboratory of Cell Biology, NCI, 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. 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 α- and β-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 anti-cancer drugs. This research has shown that this gene encodes a protein that acts to pump anti-cancer 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.

Dr. Gottesman's professional activities include many active memberships in professional societies and editorial boards. He was elected a Fellow of the AAAS in 1988 and elected to membership in the Institute of Medicine in 2003. He received the Milken Family Foundation Cancer Research Award in 1988, the Rosenthal Foundation Award in 1992, the ASPET Award in 1997, the Public Health Service Commissioned Corps Meritorious Service Medal in 1999, the ISI "World's Most Cited Authors," 1980–2000 (top 0.5 percent), the NIH Director's Award in 2002, Elected to the Institute of Medicine of the National Academies, November 2003, and Department of Health and Human Services (DHHS) Secretary's Award for Distinguished Service, May 2005.

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, and has instituted training programs for disadvantaged students. He has also overseen the creation of loan repayment programs for clinical researchers, a clinical research training program for medical students, and formalized institutional training and mentoring for postdoctoral fellows at the NIH.

Keynote Speaker

Sharon L. Milgram, PhD

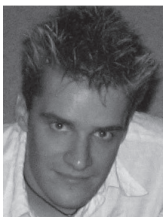
Dr. Milgram is the Director, Office of Intramural Training and Education and the Graduate Partnerships Program at NIH. She is a Senior Investigator in the Laboratory of Kidney & Electrolyte Metabolism in NHLBI and an Adjunct Faculty member in NHGRI. Dr. Milgram's research focuses on cell signaling and protein trafficking in polarized cells, including kidney and airway epithelial cells. In April 2007, Dr. Milgram joined NIH from the University of North Carolina at Chapel Hill where she was Professor of Cell & Developmental Biology and the director of several training programs for undergraduate students, doctoral students, and postdoctoral fellows.

Dr. Milgram received a BS degree in Physical Therapy from Temple University in 1984. After working as a physical therapist for two years she returned to graduate school and was awarded a PhD in Cell Biology & Anatomy from Emory University in 1991. Dr. Milgram completed a postdoctoral fellowship at the Johns Hopkins University until 1994 and then joined the faculty at the University of North Carolina at Chapel Hill.

Student Oral Presenters

Jeff Blackinton
Karolinska Institute, NIA Graduate Partnerships Program

Jeff graduated from Rice University in the spring of 2003. That fall, he entered the NIH-Karolinska Institute Graduate Partnership Program under the mentorship of Mark Cookson of the Laboratory of Neurogenetics at the NIA and Lars Olson of the Department of Neuroscience at Karolinska Institute. In the spring of 2005, he published his first paper in Molecular Brain Research. His research focuses on understanding the cell biology of genetic causes of Parkinson's disease, in particular the protective effects of the protein DJ-1.



Brian Capell
New York University School of Medicine, NHGRI Graduate Partnerships Program

Currently pursuing a joint MD and PhD at New York University, Brian came to the lab of Francis Collins as a Howard Hughes Medical Institute-NIH Research Scholar in July 2004. His research involves Hutchinson-Gilford progeria syndrome and its potential links to normal aging. First author of a 2005 Proceedings of the National Academy of Sciences paper, as well as a December 2006 review in Nature Reviews Genetics, he was a recipient of the HHMI Continuing Support Award as well as the Predoctoral Trainee Award at the American Society of Human Genetics 56th Annual Meeting. He graduated from Boston College in 2000.

Kee Chan
Yale University School of Public Health, NHGRI Graduate Partnerships Program

Kee received her BS and MS in Biology from University of California, San Diego. She is a PhD student at Yale School of Public Health, conducting her project in Dr. Jennifer Puck's lab at NIH. Her specific aims were to develop a newborn screening test for severe combined immunodeficiency (SCID), understand T cell diversity during gestation and the neonatal period, and evaluate the costs and benefits of newborn screening. Her future goals are to evaluate the policy development on innovative medical technologies in children's health by studying biological and psychosocial determinants during perinatal period, infancy, and adolescence.

Megan Cleland
John Hopkins University, NINDS Graduate Partnerships Program

Megan Cleland, a native of Sioux Falls, SD, is currently a second year graduate student in the Johns Hopkins' Cellular, Molecular, Developmental Biology, and Biophysics Program. She received her BS in Biochemistry/Biomedical Science in 2005 from Saint Cloud State University in St. Cloud, MN. Megan first came to NIH in 2004 as a summer student in Brenda Peculis' lab in NIDDK. Currently, she is a member of the Youle lab at NINDS. In December, Megan was awarded the DuPont Teaching Award for her instruction of an undergraduate biochemistry lab. Megan is looking forward to completing her graduate board oral exams, after which she can focus more on her research on mitochondria dynamics as well as take a week off to vacation at a cabin in northern Michigan.

Amrita Ghosh
New Jersey Medical School, NHGRI Graduate Partnerships Program

Amrita attained her BS in Biology from the Massachusetts Institute of Technology in 2002. She worked as a research associate at the Harvard Institutes of Medicine in the laboratory of Dr. Towia Lieberman from 2002–2003 after which she entered into New Jersey Medical School's MD/PhD program at the University of Medicine and Dentistry of New Jersey. In 2005, Amrita began her doctoral research in Dr. Fabio Candotti's clinical gene therapy laboratory at NHGRI. Her research focuses on the evaluation of *in vitro* modeling systems and safety modifications for gene therapy clinical trials.

Jason Hall
University of Pennsylvania, NIAID Graduate Partnerships Program

Jason received his BS in chemical engineering from Northwestern University in 2001. After a brief stint in pharmaceuticals, he left industry to pursue his growing interest in biological research under Dr. Ellen Puré at the Wistar Institute located in Philadelphia, PA. In the fall of 2005 he enrolled into University of Pennsylvania's Immunology Graduate Group/NIH partnership. Now in the second year of his PhD studies, he has recently begun his thesis work under the mentorship of Dr. Yasmine Belkaid, NIAID. His research focuses on antigen presentation in the gut and the factors that govern maintenance of intestinal homeostasis in both non-infectious and infectious models.

Arjun Prasad
George Washington University, NHGRI Graduate Partnerships Program

After a short career as a Systems Administrator, Arjun studied evolution at the University of California at Santa Barbara where he graduated with highest honors in 1999. In the fall of 2000 he joined the laboratory of Eric Green as a technician to assist with the development of comparative genome mapping and analysis techniques. Convinced of the value of a career in science, he entered the Institute for Biomedical Sciences PhD program at George Washington University in 2003 and continues to work with Eric Green applying evolutionary approaches to comparative genomics.

Catherine Schwartz
Karolinska Institute, NIA Graduate Partnerships Program

Catherine graduated from James Madison University in December 2002. In January 2003, she continued her research in Dr. Gina MacDonald's laboratory of biochemistry/biophysics as a research fellow. In July of 2003, she joined the NINDS laboratory of neurophysiology as a postbaccalaureate research fellow under Dr. Jeffery Barker. In the fall of 2004, she entered the Karolinska Institute/NIH GPP under the mentorship of Dr. Mark Mattson (NIA) and Dr. Ernest Arenas (KI). Her research focuses on the differentiation and characterization of dopaminergic neurons derived from stem cells. From her graduate studies, she has authored/co-authored five published manuscripts and one book chapter.

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Abstracts

A Retro-inhibition Approach Reveals the Tumors Cells Are the Primary Target for Rapamycin in Head and Neck Squamous Cell Carcinomas

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Recently, we have shown that that dysregulation of PI3K/Akt/mTOR signaling pathway represents a key event in head and neck squamous cell carcinoma (HNSCC) progression. Of interest, rapamycin and its derivatives, which are currently used in the clinic as immunosuppressants, inhibit specifically mTOR and its downstream molecules involved in protein translation, and cell cycle control. Indeed, we observed that rapamycin effectively blocks mTOR in HNSCC xenografts, thereby causing a reduction in cell proliferation, apoptosis of tumor cells, and a concomitant decrease in the tumor vascular density, leading to tumor regression. However, we could not detect any pro-apoptotic or growth suppressive activity of rapamycin in HNSCC cells *in vitro*. This observation prompted us to investigate whether the antitumoral effects of rapamycin on HNSCC cells *in vivo* are a consequence of the effects of rapamycin on the stromal cells within the tumor microenvironment. By a reverse-pharmacology approach, which involved the lentiviral expression of a rapamycin-insensitive form of mTOR in HNSCC cells, we found that cancer cells are the primary targets of rapamycin. In turn, blockade of mTOR in HNSCC cells leads reduces tumor vascularity by decreasing the expression of pro-angiogenic mediators by a HIF1a-dependent mechanism. **Poster 32**

Firing Rate and Local Field Potential Changes in Basal Ganglia Output during Walking and Rest in the Hemiparkinsonian Rat

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One goal in Parkinson's disease (PD) research is to determine what changes in basal ganglia (BG) output are associated with impaired movement. In PD patients, changes in BG rate and beta frequency activity (10–30Hz) have been observed. To explore correlates of rate and beta activity with movement, firing rate and pattern in the substantia nigra pars reticulata (SNpr), a BG output nucleus, were evaluated in chronic recordings of spike activity and local field potentials (LFP) in a rat model of PD during rest and walking in a novel rotating treadmill. In control rats, SNpr firing rates were typically higher during walking, showing a relationship between rate and movement. PD rats had difficulty with the walking task and showed less modulation of SNpr rate. Rate did not correlate with motor activity, *per se*, however, as PD rats treated with l-DOPA rotated robustly when SNpr firing rates were significantly depressed. In preliminary studies, beta frequency LFP power was higher during rest, relative to walking in both lesioned and control rats. However, beta power in the SNpr was higher in lesioned rats than control during rest. These observations indicate this preparation is useful for gaining insight into the significance of changes in basal ganglia output in PD and effects of potential therapies. **Poster 85**

Molecular Diagnosis for Hereditary Neurological Disease in Mali, West Africa: Social and Research Implications

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Hereditary neurological diseases (HND) debilitate millions of people worldwide. Although a formal genetic diagnosis for HND is available for many people with these conditions, these services are generally not available in the developing world. Additionally, due to the increased frequency of consanguineous relationships in some developing countries, the appropriateness of such services is not well understood. Yet, as genetic advances become a more significant part of health care delivery, minimization of further health disparities between developed and developing countries is an important goal. Therefore, the purpose of this research study is to assess the social and research implications of introducing genetic diagnosis for HND in Mali, West Africa. To this end, the knowledge and beliefs of Malians regarding genetic diagnosis will be queried when subjects with clinically diagnosed HND will receive first-time genetic testing and counseling for their condition. A questionnaire will be adapted, piloted, and administered for this inquiry. Secondly, any families with an autosomal recessive mode of inheritance whose genetic test returns negative for a known mutation for HND will be investigated further for novel candidate regions by using single nucleotide polymorphism array as a means of homozygosity mapping. **Poster 86**

PKA Phosphorylation Activates HIV-1 Vpr Cell Cycle Arrest
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AIDS results from a dramatic loss of CD4+ T-cells during HIV infection. Better understanding HIV-induced cell death could be useful for developing treatment during the critical early stages of HIV infection. Viral protein R (Vpr) is a 14 kD accessory protein that contributes to cell death as well as cell cycle arrest. A direct link between cytopathicity and cell cycle arrest has been suggested but remains unresolved. Mutation of serine 79, S79A, attenuates cell cycle arrest, suggesting phosphorylation at this residue is critical for Vpr function. However, the kinase that phosphorylates Vpr remains unknown. We examined the primary sequence of Vpr and found that serine 79 is part of a putative phosphorylation site recognized by the cAMP-dependent Protein Kinase, PKA. In HIV-infected T-cells, wild-type Vpr associates with PKA by immunoprecipitation. However, binding is greatly reduced by another mutation that abolishes S79 phosphorylation, R80A. Furthermore, a PKA inhibitor attenuated cell cycle arrest, suggesting Vpr function is at least partially dependent on PKA activity. Whether S79 phosphorylation contributes to Vpr cytopathicity is still under investigation. Understanding the mechanism of Vpr activity will help elucidate the mode of HIV cytotoxicity and the consequent development of AIDS. **Poster 70**

Blood Brain Barrier Disruption and RNA Expression following Acute Ischemic Stroke in Humans
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Stroke is the third leading cause of death in the US. This high rate of mortality can be attributed to complications following the primary ischemic event. One complication following acute ischemic stroke (AIS) is blood brain barrier (BBB) disruption which can be observed as delayed gadolinium (Gd-DTPA) enhancement of the cerebrospinal fluid (CSF) space on fluid-attenuated inversion recovery (FLAIR) images. This has been termed hyperintense acute reperfusion marker (HARM) and is associated with hemorrhagic transformation. The pathophysiology of this process remains unknown. The objective of this study is to determine if there is a different RNA expression profile for AIS patients with BBB disruption. RNA will be isolated from whole blood samples and run on Illumina microarray HumanRef-8 v2 BeadChips. Fifty AIS patients with BBB disruption will be matched to 50 AIS patients without BBB disruption and 50 healthy control subjects. MR imaging will be performed using a 1.5 T MR system for FLAIR, DWI, T2* weighted GRE, and PWI and read by blinded reviewers. The expression set will be analyzed for relationships using ANOVA. Hierarchical cluster analysis will be performed on genes of significance. Pathway analysis will be conducted to provide a biologic pathway associated with HARM on MRI. **Poster 61**

Effect of Alanine Substitutions in Sigma70 Region 4 on its Interactions with the Bacteriophage T4 Activator MotA and Co-Activator AsiA
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E. coli RNA polymerase consists of a core and a sigma factor that recognizes host promoter DNA elements; the primary sigma in *E. coli* is sigma70 (s70). During bacteriophage T4 infection of *E. coli*, the T4 proteins MotA and AsiA direct polymerase to T4 middle promoters by a system called sigma appropriation. MotA binds to a DNA element centered at -30 and interacts with the far C-terminal region of s70. AsiA binds to s70 residues in regions 4.1 and 4.2, disrupting its normal contacts with the host -35 element and with the core beta-flap. Structural work in other labs has identified s70 residues that interact with AsiA and the beta-flap. We have investigated how specific mutations within region 4 affect its interactions with AsiA, MotA, and the beta-flap using *E. coli* 2-hybrid assays and native protein gels. We find that AsiA requires the entire region 4 to have a significant interaction with s70, while MotA can interact well with just s70 residues 569-613 (region 4.2 to the end of s70). Our results support biochemical and structural evidence that the tight binding of AsiA to both regions 4.1 and 4.2 remodels s70 region 4, disrupting its interactions with the beta-flap and with DNA. These disruptions then facilitate the interaction of MotA with the MotA binding element and with the far C-terminal end of s70. **Poster 21**

Differential Spread of HSV-1 and HSV-2 Is Influenced by the Latency-associated Transcript
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Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) establish lifelong latency in the host and may periodically reactivate to cause recurrent disease through an unknown mechanism mediated by the latency-associated transcript (LAT), the only gene expressed during latency. HSV-1 may reactivate to cause "cold sores" or ocular herpes keratitis while HSV-2 is associated with recurrent genital herpes. HSV-1 infection of the CNS may manifest as severe necrotizing encephalitis that often results in death or severe neurological sequelae while HSV-2 may cause relatively benign meningitis. Prevailing theories on HSV latency have not addressed mechanisms for these type-specific differences in reactivation or CNS complications. We have determined that HSV-1 and HSV-2 preferentially spread to and establish latency in different regions of the nervous system. Elements within the LAT region influence viral spread into and through the CNS, and also regulate tissue-specific transcription of LAT and ICP0, a viral transactivator involved in reactivation. These data suggest that LAT may contribute to HSV-1 and HSV-2 type-specific differences in reactivation and CNS infection by mediating viral spread and gene expression in specific types of neurons in the peripheral and central nervous systems. **Poster 80**

RNA Binding Targets of the Recessive Parkinsonism Protein DJ-1 Reveals Involvement in Mitochondrial, Oxidative Stress, and PTEN/Akt Survival Pathways
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Parkinson's disease is a major neurodegenerative condition with several rare Mendelian forms. Oxidative stress and mitochondrial function have been implicated in the pathogenesis of PD, but the molecular mechanisms involved remain unclear. DJ-1 mutations are one cause of recessive parkinsonism, but this gene is also involved in cancer by suppressing PTEN-induced apoptosis. DJ-1 responds to oxidative stress and may help maintain mitochondria. We show that DJ-1 binds to specific RNA targets in cells and in the brain including mitochondrial genes, genes involved in glutathione metabolism, and members of the PTEN/PI3K cascade. Pathogenic recessive mutants are deficient in this activity. Oxidative stress causes DJ-1 to dissociate from RNA and neurons deficient in DJ-1 show altered responses to oxidative stress at the protein level. DJ-1 knockout mice show differences in protein expression in brain for DJ-1 targets with aging. Exposing DJ-1 knockout flies to glutathione synthesis inhibitors reveals an increased sensitivity *in vivo*. These data implicate a single mechanism for the pleiotropic effects of DJ-1 in different model systems, namely that the protein binds and regulates specific groups of RNA targets in an oxidation-dependent manner. **Poster 89**

Brain Computer Interface: Early Prediction of Human Intention
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Using single trial magnetoencephalography, we are able to predict the location of the target of a reaching movement between 1.5 and 2 seconds before the movement begins. Subjects, using their right hand, chose to reach to targets in the ipsilateral or contralateral spatial field. Binary classification of the selected target was made with greater than 70 percent accuracy in one subject and with 65 percent accuracy in two other subjects within the time window of 1.5 to 2 seconds prior to movement onset. Classification of target location was not possible in later time windows, suggesting that 1.5sec to 2 sec prior to movement is a crucial period for selection and spatial localization of a target. The best classification accuracy was achieved using these two features: a parietal sensor in the mu frequency band (8–14 Hz); and a centro-parietal sensor in HFO bands of 200–240 Hz. These results regarding the temporal and spatial characteristics of target selection will contribute to our research aim to design an 'automatic' Brain Computer Interface (BCI) that can translate users' implicit intentions directly and effortlessly to control external devices without utilizing the typical channel through the spinal cord and muscles. This translational research shows promise in improving the lives of people with severe paralysis. **Poster 93**

Characterization of Regional Variability and Correlated Processes in BOLD fMRI during Mild Hypercapnia
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Respiratory challenges, such as breath hold-induced hypercapnia, elicit global changes in the levels of oxygenated hemoglobin in the brain, subsequently altering the cerebral hemodynamics. The details of this process can be observed via blood oxygenation level dependent (BOLD) functional MRI, which measures changes in local oxygen concentration. The amplitude of this BOLD signal change varies significantly throughout regions of the brain, although full characterization of the heterogenous whole-brain response has not yet been accomplished. In this work, we explore the significant variance of the BOLD signal timecourses, also using Independent Component Analysis to extract temporally independent spatial maps. Here we present the early results of this analysis, including a summary of the timecourses present, maps of the phase lag in the hemodynamic response to the breathing challenge, and example components differentiated by ICA. Also, the so-called "resting-state" networks (RSNs), generally hypothesized to be strictly neuronal in origin, were easily located within data from our vascular experiments. The implications of a possible relationship between RSNs and vasculature are not yet understood, but hypercapnia experiments may offer a new window into explaining the function of these intriguing networks. **Poster 98**

Gene Expression Profiling during Optic Fissure Closure
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Regulation of optic fissure closure during the 5th week of human gestation is poorly understood. Failure of the fissure to close at this time can lead to uveal coloboma with varying loss of visual function. Using laser capture microdissection, we isolated the margins of the optic fissure in wild-type C57BL/6J mice at three embryonic timpoints which represent the fissure in unfused (E10.5), closing (E11.5) and fused states (E12.5). RNA was isolated from each embryonic timepoint, amplified linearly, labeled and hybridized to Affymetrix MOE 430 2.0 chips. A total of 297 genes were found to be differentially expressed as measured by at least a twofold change over the three days (FDR<.15). Biological significance of this subset was evaluated using Ingenuity Pathways Analysis from which several possible mechanisms emerge: non-canonical Wnt signaling/planar cell polarity, regulation of cell adhesion (e.g., cadherins), integrity of the basement membrane and differential cell proliferation and apoptosis. Real-time PCR and tissue *in situ* hybridization confirm the spatiotemporal expression of several transcripts meeting both statistical and biological indices. The highly regulated expression of these genes leads us to conclude that they may play a critical role in normal closure of the optic fissure. **Poster 15**

Genetic Analysis of Wireless, an ENU-induced Mutation that Disrupts Neural Crest Development
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The neural crest is a transient population of embryonic cells that gives rise to multiple cell types including cranial and sympathetic nerves of the peripheral nervous system, and melanocytes in the skin. To identify additional genes involved in neural crest development we have utilized ENU mutagenesis to screen for altered expression of Sox10, an early marker of neural crest. Using a $Sox10^{LacZ}/+$ reporter assay, we identified a recessive, embryonic lethal mutation, called *wireless*. Wireless mutant embryos lack cranial and sympathetic ganglia, but melanocyte precursors are normal.

Analysis of candidate genes on mouse chromosome 10 identified a point mutation in the epidermal growth factor receptor 3(*ErbB3*) gene, a member of tyrosine kinase receptor family. Targeted disruption of *ErbB3* results in the absence of cranial and sympathetic ganglia, as observed in wireless. However, while protein expression is abolished in null embryos, we found that ERBB3 is expressed in the wireless embryos at E11.5. This suggests that an ENU-induced point mutation is sufficient to impair ERBB3 signaling without impeding protein expression. Our future studies will focus on understanding how this mutation disrupts protein function. **Poster 16**

A Novel Superfamily Binding Diverse Soluble Ligands: Implications for Bacterial Competence, Cell-surface Polysaccharide Binding, Propanediol Degradation, and the Evolution of Vitamin B12 Uptake

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Using sensitive sequence and structure similarity searches, we have identified a novel superfamily of protein domains containing the β-grasp fold that are likely to bind a range of diverse soluble ligands. Domains belonging to this superfamily were found in a diverse set of proteins including animal B12 uptake transcobalamin-like proteins, polysaccharide export proteins, the competence DNA receptor protein ComEA, the cob(I)alamin-generating enzyme PduS, and certain subunits of the respiratory electron transport chain. In order to elucidate the possible functional roles of these domains, we subjected the superfamily to a battery of comparative genomic analyses. The statistically significant findings obtained through these analyses resulted in several functional inferences for the superfamily related to aspects of protein-ligand interaction in the bacterial DNA uptake competence pathway, cell-surface polysaccharide binding, and propanediol degradation. Finally, this investigation provides a possible evolutionary scenario for the origin of vitamin B12 uptake in animals via transcobalamin and transcobalamin-related proteins. **Poster 51**

Nucleolar Transcription Level Regulates Cell Cycle Dynamics of Condensin Distribution in the Yeast Genome

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Chromosome condensation is established and maintained by the condensin complex. The mechanisms governing loading of condensin onto specific chromosomal sites remain unknown. To elucidate the molecular pathways that determine condensin binding to the nucleolar organizer, a key condensin binding site, we analyzed the properties of condensin-bound sites within the rDNA repeat in budding yeast and demonstrated that the bulk of mitotic condensin binding to rDNA is reduced or eliminated when Pol I transcription is elevated. Conversely, when Pol I transcription is repressed either by rapamycin treatment or by promoter shut-off, condensin binding to rDNA is increased. This novel potential role for constitutive and/or periodic repression of Pol I transcription in rDNA condensin loading could to be an important factor in determining the segregation proficiency of NOR-containing chromosomes. A new aspect of this transcriptional control was demonstrated in cells with an episomal NOR, where rDNA is actively transcribed. Genome-wide analysis showed that these calls acquired a new pattern of chromosomal condensin distribution, which changed the condensin requirement for chromosome segregation in budding yeast. **Poster 23**

Understanding the Structure and Function of the L1 Retrotransposon ORF1p

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Human LINE-1 (L1) retrotransposon replicates by reverse transcribing its RNA transcript into genomic DNA and has generated approximately 30 percent of the human genome. An L1 element contains two open reading frames. The second open reading frame encodes for a 149kDa protein with endonuclease and reverse transcriptase activity. The first open reading frame (ORF1) encodes a 40kDa protein containing a coiled coil domain thought to mediate trimer formation between ORF1p. Coiled coil domains are often involved in protein-protein interactions and earlier work in our laboratory showed that the coiled coil domain underwent adaptive evolution. These findings indicate that L1 may have undergone positive selection in response to the host environment, suggesting a possible interaction between L1 and its host. We are using deletion analysis and amino acid substitution to investigate the role of the coiled coil domain and other structural features of ORF1p in retrotransposition, multimer formation, and interaction with host proteins. We will correlate these results with both the biochemical properties and structure of ORF1p, the latter of which we hope to determine by X-ray crystallography. **Poster 2**

***In Vitro* and *In Vivo* Effects of Farnesyltransferase Inhibitors for Hutchinson-Gilford Progeria Syndrome**

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Hutchinson-Gilford progeria syndrome (HGPS) is the most dramatic form of premature aging, characterized by death due to cardiovascular disease at a mean age of 13. HGPS is caused by a *de novo* point mutation in the LMNA gene that produces a mutant lamin A protein, termed "progerin", that is permanently modified by a lipid farnesyl group. It is hypothesized that progerin remains associated with the nuclear membrane due to its farnesyl-anchor, thus disrupting the lamina and overlying nuclear structure, and leading to the numerous nuclear defects characteristic of HGPS, in particular misshapen nuclei. We have previously shown that treatment with farnesyltransferase inhibitors (FTIs) can prevent and reverse this nuclear abnormality in cultured skin fibroblasts. Now we report preliminary results demonstrating that the administration of the FTI, tipifarnib (R115777, Zarnestra®) to a transgenic HGPS mouse model can prevent a cardiovascular phenotype (loss of vascular smooth muscle cells in the media of the large arteries) that is strikingly similar to that seen in HGPS patients. These observations provide encouragement for the clinical trial beginning later this year testing an FTI for children with progeria. **Poster 10**

T Cell Receptor Excision Circles: Application to Newborn Screening for Severe Combined Immunodeficiency and Understanding the Development of T Cell Diversity

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Severe combined immunodeficiency (SCID) describes a group of diseases characterized by absent cell-mediated and humoral immunity. Infants with SCID are healthy at birth, but die of infections unless provided with a functional immune system by hematopoietic stem cell transplantation, enzyme replacement or gene therapy. SCID infants identified by a prior family history and treated early have better survival and lower treatment costs than those recognized only after serious infections. Unfortunately, over 80 percent of infants with SCID today are not identified in the pre-infectious period. Universal newborn screening could remedy this problem, but no screening test for SCID is available. T cell receptor excision circles (TRECs) are circular DNA molecules formed in thymocytes from portions of the T cell receptor genes, which normally undergo VDJ rearrangement to provide a diverse cellular immune repertoire. We found that TRECs can be quantitated by PCR on DNA extracted from the dried blood spots already collected from all babies. Infants with SCID, regardless of genotype, have very few T cells and lack TRECs, while healthy newborns have abundant TRECs. While sensitivity of the TREC test in dried blood spots from infants with SCID was excellent, specificity was limited by failure to amplify TRECs in 1.4 percent of anonymous newborn samples. However, a repeat sample increased specificity > tenfold. Therefore the TREC assay is a promising screening tool with the potential for cost-effective early identification and management of SCID. **Poster 77**

Mechanotransduction and Differentiation in Distraction Osteogenesis

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Distraction osteogenesis (DO) is a form of fracture healing that occurs in bone lengthening. Despite the extensive current use of DO, the mechanisms responsible for the *de novo* ossification involved are not well understood. We hypothesize that mechanical stimulation changes the biological state of osteoblasts (OBs). The activated OBs may secrete soluble signals that enhance the osteogenic differentiation of mesenchymal stem cells (MSCs) to promote bone formation. To test this hypothesis, an *in vitro* loading system that mimics the biomechanics of DO will be developed to mechanically stimulate OBs. Microarray data from the *in vitro* loaded OBs will be compared to microarray data from a mouse DO model. The gene expression patterns will be used to optimize the *in vitro* regimen for osteogenesis and to identify candidate osteogenic molecular signals. We aim to assess the effects of the activated OBs on MSC osteogenic differentiation and to examine the underlying signaling. Elucidation of these mechanisms may provide insights for improving DO and fracture healing in general. **Poster 25**

Identification of Potential Biomarkers for Ovarian Cancer through the Correlation of Pathway Analysis of Gene Expression and Genomic Copy Number Alterations

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Ovarian cancer is an aggressive disease with poor prognosis. Approximately 75 percent of all cases are diagnosed at advanced stages, reflecting the asymptomatic nature of the earlier stages of the disease. It is widely accepted that ovarian surface epithelium plays an essential role in disease etiology, with the most common histological subtypes being serous and mucinous. These subtypes can be further divided into three different categories; benign, borderline and malignant tumors based on possible outcome. Approximately 80 percent of patients with borderline ovarian tumors (BTs) have a favorable prognosis. However, there are approximately 20 percent of patients with BTs that progress to a more aggressive stage of the disease, with a 5-year survival rate less than 50 percent. These two classes of BTs, with favorable or poor prognosis, cannot be differentiated at the histological level. Therefore, a better understanding of the underlying molecular mechanisms that are directly affecting ovarian tumorigenesis is needed. In order to discover biomarkers for early tumor detection and to further understand ovarian tumor progression, we performed array-comparative genomic hybridization (array CGH) of 97 ovarian surface epithelial patient samples that included borderline, primary adenocarcinomas and metastasis of adenocarcinomas stage III.Since chromosomal instability is a distinctive feature of tumor development, genomic technologies such as array-CGH can be used to identify chromosomal copy number alterations.This technology facilitates the discovery of new biomarkers for cancer and other genetic diseases, by identifying areas of gains and losses, which could harbor oncogenes and tumor suppressor genes, respectively. The array-CGH platform consists of high-density oligonucleotides (44K) representing genes and genomic areas evenly distributed along the entire human genome.In order to validate the array-CGH results; we correlated copy number alterations with pathway analysis of gene expression results obtained previously from the same patients. The integration of these two independent methods, array CGH and gene expression microarray, confirmed the possible involvement of several molecular pathways in the tumorigenesis and progression of ovarian cancer. **Poster 33**

State of GTPase Cycle Dictates Mobility and Localization of Large Mitochondrial GTPases, Mfn1 and 2

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Mitochondria are dynamic organelles that are undergoing frequent fission and fusion events. The mitofusions, Mfn1 and Mfn2, mediate mitochondrial fusion. These proteins localize to distinct areas on the mitochondria and have been shown to tether the mitochondria together in both hetero- and homo-dimeric complexes. We have found using FRAP (Fluorescence Recovery After Photobleaching) that mutants of mitofusins display markedly different mobilities on the mitochondrial membrane reflecting changes in complex formation. Mfn2-K109T, a GTPase-inactive mutant decreases FRAP recovery, whereas Mfn2-rasG12V a dominant active GTPase mutant greatly increases FRAP recovery (Mfn2-rasG12V>Mfn2>Mfn2-K109T). These differences are supported by the mitochondrial localizations: Mfn2-K109T is highly focal, while Mfn2-rasG12V is more evenly distributed along the mitochondria. Conversely, Mfn1-K88T, a GTPase mutant, increases FRAP recovery compared to Mfn1 and Mfn1-rasG12V (Mfn1-K88T>Mfn1rasG12V>Mfn1). Thus the submitochondrial localization and mobility on the mitochondrial membrane change as the two mitofusins transit through their GTPase cycles. **Poster 3**

Theoretical Study of the Rhenium-Alkane Interaction in Transition Metal-Alkane Sigma-Complexes
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Metal-alkane binding energies have been calculated for $[CpRe(CO)_2]$(alkane) and $[(CO)_2M(C_5H_4)C\ C(C_5H_4)M(CO)_2]$(alkane), where M = Re or Mn. Calculated binding energies were found to increase with the number of metal-alkane interaction sites. In all cases examined, the manganese-alkane binding energies were predicted to be significantly lower than those for the analogous rhenium-alkane complexes. The metal (Mn or Re)-alkane interaction was predicted to be primarily one of charge transfer, both from the alkane to the metal complex (70 to 80 percent of total charge transfer) and from the metal complex to the alkane (20 to 30 percent of the total charge transfer). **Poster 36**

Family Classification and Functional Annotation of Heme Biosynthesis Pathway Enzymes Using the PIRSF Classification System
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The PIRSF protein classification system (*http://pir.georgetown.edu/pirwww/dbinfo/pirsf.shtml*) reflects evolutionary relationships of full-length proteins and domains. The primary PIRSF classification unit is the homeomorphic family whose members are both *homologous* and *homeomorphic* (sharing full-length sequence similarity and a common domain architecture). Here we present family classification and annotation of enzymes in the heme biosynthesis pathway done with PCS software. Manual curation includes membership, domain architecture, annotation of biological functions, biochemical activities, and analysis of taxonomic distribution. For example, ferrochelatase (PIRSF001457) catalyzes the final step in heme synthesis, and members of PIRSF001457 span all kingdoms of life. 5-aminolevulinate synthase (ALAS; PIRSF000444) is the first enzyme in the heme biosynthetic pathway. Erythroid and housekeeping forms of vertebrate ALAS form tight orthologous groups, which are presumably derived from a gene duplication event. Annotated PIRSF families facilitate standardization of protein nomenclature in the UniProtKB database, classification and annotation of new protein sequences, and insights into the evolution of heme biosynthesis enzymes. **Poster 52**

Interference of Macrophage Migration Inhibitory Factor Expression in a Mouse Melanoma Delays the Angiogenic Switch by Up-regulating Thrombospondin 1
William David Culp, Panagiotis Tsagozis, Michael Ray Burgio, Keri Elizabeth Ramsey, Dietrich A. Stephan, Pavel Pisa, Donita Garland

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Macrophage migration inhibitory factor (MIF), a pleiotropic protein, is associated with solid tumor progression. An interfering MIF RNA (iMIF), and a control interfering RNA (iRNA) were stably introduced into B16-F10 mouse melanoma cells. Gene expression analyses of iMIF and iRNA melanoma cell lines identified a 55- and 87-fold induction of thrombospondin 1 (TSP-1) in iMIF cells. The significant induction of TSP-1 mRNA expression resulted in twofold higher levels of TSP-1 protein in supernatants of iMIF cultures. iMIF cells, subcutaneously injected into C57BL/6 mice, demonstrated delayed tumor establishment, and in day 3 iMIF tumors, intratumoral vasculature was absent. These results define a novel function of MIF as an important regulator of TSP-1 and the angiogenic switch in a mouse melanoma model. **Poster 34**

Detection of Tumor Therapy Response using Hyperpolarized 13C Magnetic Resonance Imaging
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Magnetic resonance imaging of ^{13}C-labeled molecules has, until recently, been unfeasible due to the low sensitivity of the technique. However, the recently introduced method of dynamic nuclear polarization (DNP) offers gains in sensitivity of more than 104-fold, allowing subsecond acquisition of ^{13}C spectral data *in vivo*. Using DNP MRI we have studied the metabolism of hyperpolarized 1-^{13}C pyruvate in EL-4 lymphoma cells and in implanted EL-4 tumors, before and after treatment with the chemotherapeutic drug etoposide. There was significant reduction in lactate dehydrogenase-catalyzed exchange of ^{13}C label between pyruvate and lactate in tumors 24h after treatment. High-resolution ^{13}C MR images of the lactate/pyruvate ratio showed marked reduction in intensity within the tumor following etoposide exposure. The decrease in exchange can be explained by a reduction in the lactate concentration in the tumor, a reduction in cellularity, and possibly decreases in intracellular LDH and coenzyme (NAD(H)) concentrations. The absence of any background ^{13}C signal means that enzyme activity can be imaged specifically. The lack of ionizing radiation, the use of an endogenous metabolite and a single imaging modality makes DNP ^{13}C MRI an attractive potential tool for clinical imaging of early tumor treatment response. **Poster 99**

Investigation and Characterization of Activities Responsible for the Resolution of Double Holiday Junctions during Meiosis I in Saccharomyces cerevisiae
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Meiotic recombination is initiated in early prophase by formation of DNA double-strand breaks (DSBs). The products of meiotic recombination are reciprocal (crossover, CO) and nonreciprocal (noncrossover, NCO) exchanges. COs are required for proper segregation of homologous chromosomes at the first meiotic division (meiosis I), and are produced by the resolution of double Holiday junction (dHJ) recombination intermediate. The protein or proteins that are responsible for dHJ intermediate resolution as COs have not been identified.

Deletion of the meiosis specific transcription factor Ndt80 results in arrest of the cells in late meiosis I prophase with a reduction of CO products and accumulation of dHJ intermediates. We are investigating CO production using a unique feature of *S. cerevisiae*, namely the ability return to growth (RTG) when meiotic cells are transferred to vegetative growth media. Cells complete some meiotic processes but switch to the mitotic cell cycle and produce diploid cells.

We find that upon RTG, *ndt80*Δ cells resolve dHJs and produce CO and NCO products. Interestingly, the resolution of dHJ in these cells parallels the disappearance of Zip1p, a component of the central region of the synaptonemal complex (SC), suggesting a role for the SC breakdown in dHJ resolution. **Poster 63**

Expression of the KIAA0319 Gene from a Haplotype Associated with Developmental Dyslexia
Megan Dennis, Silvia Paracchini, Thomas Scerri, Richard Wade-Martins, Eric D. Green, Anthony P. Monaco

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Developmental dyslexia (DD), or reading disability, is a condition that affects an individual's ability to read and spell in the absence of any profound sensory or neurological impairment, despite adequate intelligence and educational opportunity. Recently, the KIAA0319 gene has been proposed as a candidate for DD susceptibility by two independent association studies. Gene expression analysis has shown that a specific risk haplotype associated with DD susceptibility reduces the expression of the KIAA0319 gene, yet no causal genetic variants have been established. We sought to identify the specific functional variant on the risk haplotype affecting KIAA0319 expression by studying bacterial artificial chromosomes (BACs) containing the risk versus non-risk haplotype. Two BACs containing each haplotype were sequenced, and all genetic variants were catalogued.Variants residing within multi-species conserved sequences (MCSs) were tested for association with DD in a collection of 264 DD-affected families. We are now focusing our functional analyses on variants falling in or near the KIAA0319 promoter region with a deletion series using luciferase-based reporter plasmids constructed from risk and non-risk BACs, with expression studies being performed with a human embryonic kidney cell line (HEK293T) and a neuroblastoma cell line (SHSY5Y). **Poster 62**

Embracing the Complexity of Gene Expression in the Interpretation of Gene Microarrays
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Microarrays and other high-throughput molecular profiling platforms are revolutionizing biomedical research, but it remains a challenge to link the resulting molecular profiles to functional signatures such as those of disease or response to therapy. Previously published analytical methods have proved useful but often do not take into account the complex, interdependent relationships among genes in a given pathway or functional ensemble. For that reason, we have developed the Learner of Functional Enrichment (LeFE) algorithm. LeFE builds non-linear, multivariate, machine-learning models based on ensembles of functionally related genes. The gene ensembles ranked the best are those that contain genes deemed most critical to the machine-learning model when compared with randomly generated sets of negative control genes. Two separate application of LeFE to the analysis of sensitivity to the EGFR agonist Gefitinib and to gene expression in the lung epithelia of smokers and non-smokers yielded results that were highly consistent with established knowledge or that may represent plausible, new findings. We have incorporated the algorithm into a user-friendly web application, LeFEminer, which is freely available at *http://discover.nci.nih.gov/lefe*. **Poster 53**

Small Angle Neutron Scattering Studies of Clathrin Triskelia in Solution Show Evidence of Molecular Flexibility
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Clathrin is a major component in the protein coats of certain post-golgi and endocytic vesicles. At low pH, or in the presence of assembly proteins, clathrin triskelia will self assemble to form a complete clathrin lattice or “basket”. Recently static light scattering (SLS) and dynamic light scattering studies (DLS) of clathrin triskelia in solution showed that triskelia have an intrinsic pucker similar to that shown in a recent high resolution cryoEM structure of a clathrin “basket”. We extend this study by performing small angle neutron scattering (SANS) experiments on isolated triskelia in solution under conditions where baskets do not assemble. Unlike the static light scattering experiments, SANS probes a q-range beyond the Guinier regime probed by SLS. Results of SANS measurements are consistent with light scattering experiments but show a shoulder in the scattering function at intermediate q-values (~0.016 1/A). This feature cannot be fully accounted for by theoretical calculations based on rigid bead models of the triskelia. A dynamic bead-spring model of a triskelion is used to generate a time averaged scattering function. This model adequately describes the experimental data for flexibilities close to previous estimates. **Poster 43**

Mood Congruent Processing Biases in Amygdala Response to Backwardly Masked Emotional Facial Stimuli in Major Depressive Disorder
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The current fMRI study investigated differential amygdala responses to facially expressed sadness and happiness presented outside explicit conscious awareness in healthy controls (HC), currently depressed (dMDD) and remitted (rMDD) patients. 15 HC, 16 dMDD and 14 rMDD subjects viewed face stimuli displaying sad, happy, or neutral expressions during a target face recognition task. For each stimulus, a face was displayed for 27ms and immediately followed by a “masking” face for 107ms. Subjects indicated if the presentation included a target or non-target face. Behavioral results indicated correct detection rate did not differ from false alarm rate ($p=0.80$), establishing efficacy of the masking technique. fMRI results indicated an increased BOLD signal in the amygdala during exposure to unmasked sad and happy faces across groups. When masked *sad* faces were presented, the anterior amygdala BOLD signal was significantly larger in dMDD patients vs HC subjects ($p< 0.05$). In contrast, when masked *happy* faces were presented, anterior amygdala activity was significantly larger in HC subjects vs dMDD ($p< 0.05$) and rMDD patients ($p< 0.05$). These findings suggest the role of automaticity in response to emotional stimuli and intentional biases toward happy faces in HC subjects and sad faces in dMDD. **Poster 94**

Functional Analysis of HIV-1 Envelope Glycoproteins Covalently Linked to a Toll-like Receptor 7/8 Agonist

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The HIV-1 envelope glycoproteins (Env) gp120 and gp41 are the sole virally encoded targets for neutralizing antibodies and as such are a major focus for candidate vaccine design. Previously it was demonstrated that covalent linkage of CpG agonists specific for toll-like receptor (TLR) -9 to gp120 were more efficient at inducing humoral immune responses than unconjugated TLR-9 ligand. Therefore, in parallel with Env-based immunogen design to elicit more efficiently broadly neutralizing antibodies, we are investigating the ability of selected TLR agonists to enhance Env-directed antibody responses. Here we present the biochemical and immunological analysis of gp120 covalently linked to a proprietary TLR7/8-agonist. We show that the ability of the conjugate proteins to stimulate different subsets of human dendritic cells is influenced by the stoichiometry of the coupling. As there are only a few relevant targets for vaccine induced antibodies on gp120 we probe the surface of the conjugate proteins with monoclonal antibodies as well as with CD4 and show that increased linkage may result in partial loss of antigenicity. Our data has implications for vaccine design as the coupling of TLR agonists to Env-based immunogens represents a promising strategy for efficient induction of specific antibody responses. **Poster 71**

ZFP36L3: A Cytosolic, Placental Tristetraprolin Family Member

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The integrity of the placenta, the site of exchange between the mother and fetus during gestation, is necessary for successful fetal development and survival. Initial studies of the recently discovered CCCH tandem zinc finger protein, ZFP36L3 (L3), implicate this protein as a participant in placenta physiology. As a new member of the Tristetraprolin (TTP) family, L3 may function in a similar manner to its relatives by binding to AU-rich elements in the 3´ untranslated region of certain mRNAs causing their destabilization and degradation. Our recent investigation of the subcellular localization of L3 has led to the identification of a functional nuclear localization sequence (NLS) within its conserved tandem zinc finger domain. However, we found that the region corresponding to the nuclear export sequence in the other family members was non-functional. Most importantly, the unique C-terminal repeat domain of L3 overrides the NLS activity, thus preventing nuclear import of L3. We are currently attempting to identify the physiological protein binding partners and mRNA targets of L3. Completion of these studies will aid in understanding the mechanism of action of L3, and perhaps of the TTP family of proteins in general, as well as in the delineation of the role of L3 in the normal physiology of the placenta. **Poster 4**

Inhibition of Laser-induced Choroidal Neovascularization by a Sustained Delivery of an Integrin Antagonist EMD 478761

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Purpose: Age related macular degeneration (AMD) is one of the leading causes of legal blindness in the developed countries. The number of people affected by AMD is rising dramatically due to the fast aging population. The purpose of this study is to develop a sustained delivery method for a novel integrin antagonist, EMD, and to evaluate the inhibitory effects of this integrin antagonist on a laser-induced choroidal neovascularization (CNV) in a rat model.
Methods: Experimental CNV was induced in Brown Norway rats with a diode laser. Polyvinyl alcohol (PVA)-based reservoir type microimplants (A and B) releasing EMD 478761 at different rates were designed. The microimplants measuring 1x1x2 mm consisted of a compressed drug core embedded within a PVA matrix. *In vitro* release rates were measured by an HPLC. In the current study, a sham implant or an EMD microimplant was placed within the vitreous chamber of the right eye and the left eye served as laser control. The rats were sacrificed 1 week or 2 weeks after laser, perfuse with FITC-dextran and choroidal flatmounts were generated. Areas of CNV were examined and quantified.
Results: Both types of EMD microimplants inhibited CNV relative to sham controls in a statistically significant fashion. 7 days after laser, in the eyes that received EMD microimplants A and B, the mean CNV areas of lesions decreased 58 and 60 percent, respectively, as compared to those of sham implant treated eyes. Similarly, 14 days after laser, the CNV areas from microimplant A and B treated eyes were reduced by 63 and 65 percent, respectively, compared to those treated with sham implants. In addition, there was no significant difference between sham implant and laser controls.
Conclusions: EMD microimplants demonstrated antiangiogenic properties in a rat model of CNV. This study provided evidence that EMD may be useful in the treatment of eye diseases associated with neovascularization via long acting sustained release intraocular implants. **Poster 1**

Degradation of Extracellular cAMP Is Required for Differentiation, But Not Chemotaxis of *Dictyostelium* Cells

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Upon starvation, *Dictyostelium* cells enter a developmental program leading to aggregation and the formation of a multicellular organism. The aggregation is guided by cAMP, which acts as a chemoattractant by binding to a family of G protein-coupled receptors. The chemotactic signals are propagated by the highly regulated synthesis and degradation of cAMP. cAMP degradation is controlled by the extra cellular phosphodiesterase PdsA. Cells lacking PdsA do not express receptors or other components required for signal reception and relay. These cells are unpolarized and unable to sense or move toward a cAMP source. The addition of partially purified PdsA to *pdsA*$^{-}$ cells during development allows the cells to differentiate. The localization of signaling molecules in treated *pdsA*$^{-}$ cells is normal and these cells are able to efficiently migrate to a point source of cAMP. Furthermore, treating chemotaxing wild type cells with exogenous PdsA decreases the range and the speed of cells responding to a point source of cAMP. This work shows that signal degradation is required for gene expression in *Dictyostelium* cells and that the role of PdsA during chemotaxis is subtle, possibly involving maintenance of the gradient and prevention of cAMP accumulation. **Poster 7**

Effects of *In Vitro* Culture Conditions Mimicking Gene Therapy Trials on Gene Expression Profiling of Human Hematopoietic CD34+ Progenitor Cells

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Oncogenesis due to viral activation of the LMO2 oncogene has resulted in leukemia among patients treated in gene therapy clinical trials in France (FR) but not in the United Kingdom (UK); we intend to understand the reasons underlying this critical difference. Since retroviral vectors are thought to integrate preferentially into actively transcribing genes, we postulate that cells expressing different genes offer different targets to retroviral integration. We thus set out to study if differences in culture conditions used in the two trials result in activation of different sets of genes. We cultured hematopoietic stem cells in conditions that mimic the two trials. Condition "FR" differed from "UK" in serum composition and IL-3 concentration. Portions of these cells were harvested at Day 2, 3, and 4, representing times of retroviral transductions performed in the trials. Total RNA was extracted, amplified, and hybridized to Affymetrix arrays. Two-way ANOVA analysis was performed comparing signal intensities of each time point, and lists of genes $\geqslant$ threefold upregulated and $\geqslant$ threefold downregulated were compared in FR and UK. Analysis of the results showed differences among molecular pathways in the two culture conditions. Further analysis will be performed to identify differences that may influence future gene transfer practices. **Poster 60**

Mouse Mutants as Models for Human Developmental Malformations: Characterization of the Extra-toes Spotting (XsJ) Mouse

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A major focus in our lab is the study of genetic diseases that include limb malformations. Greig cephalopolysyndactyly syndrome (GCPS) is a malformation syndrome where patients exhibit polydactyly and syndactyly, in addition to craniofacial manifestations of hypertelorism and macrocephaly. GCPS is caused by mutations in the Glioma-associated oncogene-3 (GLI3) transcription factor, which plays a role in Sonic hedgehog (SHH) signaling. The Extra-toes (*Gli3*^{XtJ}) mouse is an excellent animal model for GCPS. Like the human phenotype, *Gli3*^{XtJ} mice exhibit preaxial polydactyly. An additional mouse model for polydactyly, the Extra-toes spotting (*XsJ*) mouse, shares a very similar phenotype with the *Gli3*^{XtJ} mouse and, like *Gli3*^{XtJ}, is inherited in a semidominant pattern. Previous linkage mapping studies have excluded mouse *Gli3* as the *XsJ* gene, and the gene and *XsJ* mutation remains unknown. We hypothesize that since *Gli3*^{XtJ} and *XsJ* mice overlap phenotypically, the *XsJ* gene plays a role in the GLI3/SHH pathway. To identify *XsJ*, we are performing recombination mapping in *XsJ* mice. We have mapped the candidate locus to a 322 kb region on mouse chromosome 7. Here we present a phenotypic characterization of *XsJ*, our genetic analysis, the mapping data, and our plan for developmental analysis of the animals. **Poster 64**

Modulation of Brain Response to Emotional Images by Alcohol Cues
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Alcohol drinkers report that they use alcohol both to enhance positive affect and to reduce dysphoria, and alcohol cues themselves may elicit these effects. We used fMRI to examine brain activation in response to combination images which juxtaposed negative or positive IAPS images with and alcohol or non-alcohol beverages to test the following hypotheses: 1) alcoholics would show more activation to negative than positive images; 2) alcohol cues would modulate the response to negative images in alcoholics; 3) patients with higher anxiety scores would show increased activation to negative pictures, and this correlation may be modulated by alcohol cues.

There are three main findings of this study. First, in the absence of the alcohol cue, alcoholics showed more activation to negative than to positive images. Second, in the alcohol cue condition, there was a decreased difference in activation between the positive and negative images among the alcoholics. Third, in the neutral beverage conditions, anxiety ratings predicted activation in the right parahippocampal gyrus, but not when the alcohol cues were presented. In conclusion, the alcohol cues may have modulated cortical networks involved in the processing of emotional stimuli by eliciting a conditioned response in the alcoholics, but not in the controls, which may have decreased responsiveness to the negative images. **Poster 100**

Large-scale LC-MS/MS Identification of Proteins Excreted in Urinary Exosomes: Gender Differences
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Exosomes are protein-containing vesicles (<100nm) secreted by epithelial cells lining the renal tubules and urinary drainage system. Urinary exosome isolation provides a potential means of enrichment of disease biomarkers. For example, we have already found several proteins that play roles in blood pressure regulation (e.g., ACE, thiazide-sensitive Na-Cl cotransporter [NCC]). However, biomarker studies using tandem mass spectrometry (LC-MS/MS) require prior knowledge of normal variability among human populations. Here we use LC-MS/MS to identify urinary exosomal proteins in humans that vary on the basis of gender. We compared exosomes pooled from the urine of males versus females. Samples were separated by SDS-PAGE followed by in-gel trypsin digestion of contiguous gel slices. The digests were analyzed by LC-MS/MS with quantitation using label-free analysis. A total of 344 proteins were identified after target/decoy analysis predicting a false positive identification rate of 1 percent. Among all proteins, only 16 were identified that differ by at least two-fold. These include Na-glucose cotransporter-2, 14-3-3-β, 15-hydroxyprostaglandin dehydrogenase, and aldose reductase (up in female) as well as Na/K -ATPase a-1, NCC, and Na-Ca antiporter SLC3A2 (up in male). We conclude that there is a high concordance between urinary exosomal proteomes derived from males and females, but that several differences warrant further study. **Poster 50**

An Escalating Dose–Binge Regimen of Methamphetamine (METH) Results in Variable Protection of Monoamine Levels in the Striatum
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Methamphetamine (METH) is an illicit, neurotoxic drug, the use of which has reached epidemic proportions throughout the United States and Asia. In order to study the effects following a chronic exposure to METH, we have developed a dosing regimen for rodents that mimics dosing schedules used by humans. Using HPLC with electrochemical detection, striatal monoamine content was measured following this escalating dose (ED)-binge model of administration. Male Sprague Dawley rats were pretreated with METH or the saline equivalent over 10 d prior to administration of a challenge dose of the drug (8x5 mg/kg, every hr). Following the challenge dose, there was no significant protection afforded by the pretreatment for either dopamine (DA) or its metabolites, HVA and DOPAC. In contrast, the ED-binge regimen afforded protection against METH-induced serotonin (5-HT) and 5-HIAA depletion. This protection was evident at 2 hrs after the final challenge dose, but by 24 hrs and 7 d following the challenge dose, there was no significant difference between pretreated-challenged and drug naïve-challenged animals. These results are consistent with findings that chronic human abusers show DA depletion in their brains. **Poster 90**

Voltage-sensitive Channels and Intracellular Calcium Dynamics in A17 Amacrine Cells
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Reciprocal inhibitory feedback from A17 amacrine cells (A17s) to rod bipolar cells(RBCs) shapes the time course of visual signaling. Recent work has shown that calcium influx triggering this feedback is mediated by calcium-permeable AMPA receptors(CP-AMPARs) and occurs independently of voltage-gated calcium channels (VGCCs). Here we determine the identity and location of voltage-sensitive channels and measure intracellular calcium dynamics in A17s, in an effort to develop a general model of A17 physiology. Depolarizing voltage steps elicit currents mediated by L-type VGCCs and calcium influx that, when visualized, were localized to the soma and to the same dendritic varicosities that receive AMPA receptor-mediated synaptic input. We also detected small, rapidly activating and inactivating, TTX-sensitive currents in A17s that likely reflect a small population of voltage-sensitive sodium channels. CP-AMPARs and VGCCs appear co-localized in dendritic varicosities, suggesting that calcium signaling in these small structures is highly compartmentalized. Further experiments are required to determine the physiological role of VGCCs in A17 varicosities. Possible roles for putative sodium channels in propagating membrane depolarizations through A17 dendrites remains to be determined. **Poster 91**

Sir2 Overexpression Induces Apoptosis through JNK-dependent Pathways in *Drosophila*
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Increased expression of the histone deacetylase sir2 has been reported to extend the lifespan of diverse organisms including yeast, *C. elegans*, and *Drosophila melanogaster*. It has also been shown that lifespan extension via calorie restriction requires Sir2 activity in these organisms. Additionally, a small molecule activator of Sir2, resveratrol, can improve the fitness and survival of simple model systems as well as mice fed high calorie diets. However, recent studies in yeast suggest that the effects of calorie restriction can be Sir2 independent and that Sir2 itself may limit certain measures of longevity. The role of Sir2 in *Drosophila* longevity is also unclear. One sir2 deletion fly has a shorter lifespan while another shows an extension of lifespan under certain conditions. Here, we show that overexpression of sir2 in *Drosophila* does not increase longevity but promotes caspase dependent apoptotis which can be alleviated by expression of anti-apoptotic proteins. The programmed cell death is mediated through the JNK and FOXO signaling pathways and leads to an upregulation of pro-apoptotic genes, reaper, grim, and hid. These results suggest that Sir2 expression can regulate cell survival and death in Drosophila. **Poster 29**

Intestinal-specific Factors Induce Foxp3+ Treg Conversion by Lamina Propria DC
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The intestinal immune system must avoid potentially calamitous responses to ubiquitous antigen, such as commensal bacteria and food antigen, while preserving its capacity to mount powerful immune responses against pathogens. To facilitate this paradox the intestine plays host to several unique populations of cells that possess both effector and regulatory function. Among these are several unusual populations of dendritic cells (DC), which fail to produce IL-12p70 upon microbial stimulation. Still uncertain, is whether these DCs are capable of inducing tolerogenic responses via Foxp3 regulatory T cells (Treg). We have recently perfected a strategy to isolate DCs residing within the intestinal lamina propria (Lp) and assessed their potential to induce Treg conversion, *in vitro*. Here, we present data that LpDC, but not splenic DC (Sp), elaborated high frequencies of Treg when cocultured with polyclonal stimulating anti-CD3 and TGF-beta. Treg generated in the presence of LpDC also displayed several distinct traits, including a more intense profile of Foxp3 expression and upregulation of the integrins, CD103 and alpha4beta7. Separation of LpDCs based on CD103 expression further revealed that in the absence of exogenous TGF-beta the CD103+ subpopulation selectively induced Treg, albeit at low levels, in a TGF-beta dependent manner. Finally, we elucidated the mechanism underlying LpDC induced optimal Treg conversion and block this effect in LpDC cocultures and reciprocally rescue suboptimal levels of conversion in SpDC cocultures. Specifically, we conclude that TGF-beta synergizes with an oxidative mechanism that is prominent amongst epithelial cells and antigen presenting cells in the gut to promote Treg conversion. Thus, we propose that the intestinal immune has evolved a self-contained strategy to promote Treg neo-conversion. These findings may be harnessed to generate or expand Treg with specific homing properties to target tissue pathology while preventing global immunosuppression. **Poster 74**

DNA Structure Conservation in Functional Elements

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A successful tool for elucidating signals in the human genome has been to locate regions under evolutionary constraint. However one of the more interesting results of the ENCODE Pilot Project is that many functional annotations have little sequence similarity. It is possible that other intrinsic properties of DNA are being constrained in ways that may not be evident by examining primary sequence alone. One such possibility is that local DNA structure is evolutionarily conserved. Nucleotide level structure information can be obtained by hydroxyl radical cleavage patterns.

Our method calculates a structural conservation score for every base in the ENCODE region. A key observation of our work has been that different sequences can have similar structures. When equivalent false discovery rates are used comparing sequence constraint to structure constraint, more of the human genome appears to be constrained in structure than in sequence. Importantly experimental annotations appear to be highly enriched for structurally conserved regions. Indeed a number of functional annotations exhibit a higher correlation with structural conservation than with primary sequence conservation. Our results support the idea that functional information can be transmitted by DNA in ways besides primary sequence. **Poster 54**

Effects of Rearing on Aspartamen Consumption in Male Rhesus Monkeys (*Macaca mulatta*)

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Rhesus monkeys (*Macaca mulatta*) reared without mothers typically display heightened responses to aversive stimuli. Yet, little is known about the effects of adverse rearing on responses to appetitive stimuli. In a clinical setting, hyper-responsivity to negative emotional events is often also associated with alterations in reward. In the present study, we assessed preference for a sweet solution in a two bottle preference test in a group of 2-year-old nursery-reared (n=4) and mother-reared monkeys (n=4). Testing took place over five consecutive days in a novel cage and consisted of one hour of free access to two bottles attached to the cage. One bottle contained a 6 percent aspartame solution and the other contained water. T-tests [a=0.05] showed nursery-reared monkeys had a significantly greater preference for aspartame solution than mother-reared monkeys. These findings suggest that adverse rearing experiences may have widespread influences on emotional development—influencing not only aversive emotional systems but also those related to reward. **Poster 87**

The Polycomb Group Protein Bmi-1 Collaborates with H-Ras to Promote Cellular Proliferation and Transformation of Mammary Epithelial Cells *in vitro*, and Development of Poorly Differentiated Mammary Tumors *in vivo*

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The polycomb group protein Bmi-1 is a transcription repressor that regulates self-renewal of normal and cancer stem cells, and prevents senescence through inhibition of cyclin-dependent kinase inhibitors $p16^{Ink4a}$ and $p19^{Arf}$. Originally discovered as a cooperating oncogene with c-Myc in a murine model of leukemia, overexpression of Bmi-1 has since been reported in several cancers, including breast cancer. A role of Bmi-1 in the pathogenesis of breast cancer would be critical in the development of novel biomarkers for diagnosis and treatment of this disease. Our soft agar assays suggest a collaborative role of Bmi-1 with H-Ras in the induction of proliferation, transformation and invasion of mammary epithelial cells (MEC) *in vitro*. We show that overexpression of Bmi-1 alone and in combination with H-Ras in normal MEC (MCF10A) markedly changes cell morphology, increases proliferation, and decreases the apoptotic response to apoptosis-inducing agents. Utilizing xenograft models, we show that while Bmi-1 overexpression alone cannot induce mammary tumors, the combination of Bmi-1 and H-Ras induces development of poorly differentiated aggressive mammary neoplasms. These findings suggest collaboration between H-Ras and Bmi-1 to induce neoplastic transformation of mammary epithelium. **Poster 31**

Evidence for an Abnormal Relationship between IQ and Brain Structure in Patients with Schizophrenia and Their Siblings

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Regional gray matter volume is associated with general intelligence in normal populations (NC). Schizophrenia is associated with impairments in general intelligence (IQ). Patterns of structure and function associated with IQ may vary both patients with schizophrenia (SCZ), and in unaffected siblings of patients with schizophrenia (SIB). WRAT-R reading scores vary from normal measures of IQ as they are preserved in patients with schizophrenia, and may represent premorbid cognitive capacity. We sought to investigate if these scores predicted more "normal" frontal gray matter variance in SCZ and SIBs compared to more traditional measures of IQ. We explored correlations of FSIQ and WRAT on gray matter volume using optimized voxel-based morphometry (VBM) in a large group of NC, SIB, and SCZ. In NCs, we found a main effect of FSIQ in dorsolateral prefrontal cortex, parietal and temporal cortices, not in SCZ and SIB. However, WRAT-R scores did predict more variance in frontal and temporal regions in SCZ and SIB. These data suggest that IQ is not a valid measure of the cortical systems involved in "g" in SIB and primarily SCZ. Instead, WRAT-R does probably monitor brain development related to g, and these scores reveal "premorbid" variance of gray matter volume in SCZ. **Poster 95**

GST Genotypes and Lung Cancer Susceptibility in Asian Populations with Indoor Air Pollution Exposures: A Meta-analysis

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Globally, three billion people are exposed to smoke from heating or cooking with coal, wood, or biomass. These exposures have been associated with increased lung cancer risk. Functional polymorphisms have been identified in the glutathione S-transferase genes, which may alter the risk of lung cancer among individuals as they play an important role in detoxifying carcinogens in these exposures. We performed a meta-analysis of six published studies (912 cases; 1063 controls) evaluating the GSTM1 null, GSTT1 null, and GSTP1 105Val polymorphisms, from regions in Asia where indoor air pollution makes a substantial contribution to lung cancer risk. Using a random effects model, we found that carriers of the GSTM1 null genotype had an increased lung cancer risk (OR=1.31; 95 percent CI=0.95-1.79), especially in regions that use coal for heating and cooking (OR=1.64; 95 percent CI=1.25-2.14). The GSTT1 null genotype was also associated with an increased lung cancer risk (OR=1.49; 95 percent CI=1.17-1.89). Previous meta-analyses suggest at most a small association between the GSTM1 null genotype and lung cancer risk in populations with a vast majority of lung cancer attributed to tobacco. Our results suggest that the GSTM1 null genotype may be associated with a more substantial risk of lung cancer in populations with coal exposure. **Poster 56**

Vaccinia Virus Encoded Protein A26 is Anchored to the Viral Membrane by A27

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During vaccinia virus morphogenesis, the mature virion (MV) is wrapped with virus-modified trans-Golgi membrane. Wrapping is important for virus spread and requires the A27 protein. We observed that the protein encoded by the A26 gene was not expressed in an A27 deletion virus, suggesting A27 is required for A26 stability. The role of A26 in vaccinia virus and variola virus infection is currently unknown. Experiments conducted to investigate the potential interaction of A26 with A27 revealed that A26 is anchored to the MV membrane by A27. Immunoprecipitations showed that A26 forms a complex with A27 (A26/A27) that is not disrupted by SDS and pulls down A27 trimers in both lysate and purified MV. Trimeric A27 is tethered to the MV membrane by A17, an MV transmembrane protein, via an 'anchoring domain.' A26 contains a region that has a 45 percent amino acid identity to the A27 anchoring domain and is required for A26/A27 interactions and interactions of A26/A27 with A17. A26/A27 complex formation does not require A17; however, A26 interacts with A27 trimers only in the presence of A17. Biotinylation experiments showed that A26 is an MV-surface protein associated with MV only in the presence of A27. We are currently investigating the possibility that A26 regulates A27 during MV wrapping. **Poster 81**

Interactive Splice–Site Analysis for Sequence Prediction and Motif Discovery
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SplicePort is a web-based tool for splice–site analysis that allows the user to make splice–site predictions for submitted sequences. In addition, the user can also browse the rich catalog of features that underlies these predictions, and which we have found capable of providing high classification accuracy on human splice sites. Feature selection is optimized for human splice sites, but the selected features are likely to be predictive for other mammals as well. With our interactive feature browsing and visualization tool, the user can view and explore subsets of features used in splice–site prediction (either the features that account for the classification of specific input sequence or the complete collection of features). Selected feature sets can be searched, ranked or displayed easily. The user can group features into clusters and frequency plot WebLogos can be generated for each cluster. The user can browse the identified clusters and their contributing elements, looking for new interesting signals, or can validate previously observed signals. The SplicePort web server can be accessed at *http://www.cs.umd.edu/projects/SplicePort*. **Poster 55**

Pharmacokinetics of Sorafenib in Patients with Metastatic, Androgen-independent Prostate Cancer
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Purpose: To characterize the pharmacokinetics (PK) of sorafenib in patients with metastatic, androgen independent prostate cancer (AIPC) by using an in-house, developed and validated, liquid chromatography tandem mass spectrometric (LC-MS-MS) assay for determination of sorafenib in plasma samples.
Methods: The samples for PK analysis were collected from patients enrolled in phase II study of sorafenib in metastatic AIPC, receiving sorafenib 400 mg orally twice daily continuously in 28-day cycles. The blood samples were collected on day 1 and 2 of first cycle; at baseline, 0.25, 0.50, 1, 2, 4, 6, 8, 12, and 24 hrs after the ingestion of initial doses. The plasma was separated, stored at -80 º C and analyzed with a validated LC-MS-MS method. PK parameters were evaluated by noncompartmental analysis using WinNonlin professional software version 5.0.
Results: The geometric mean for AUC0-12 was 9.76 hr*mg/L (95 percent confidence interval, 6.76-14.09) and for C_{max} was 1.28 mg/L (95 percent confidence interval, 0.88-1.87). The t_{max} ranged from 2 to 12 hr (median 6.01 hrs). PK parameters showed high inter-individual variability comparable with results from phase I studies. The accumulation ratio after second dose ranged from 0.68 to 6.43 (median 1.84); indicating accumulation after multiple dosing, as expected based on sorafenib's half life of 25 to 48 hours. **Poster 44**

Mutations in the Beta Subunit of RNA Polymerase that Affect Bacteriophage T4 Transcription
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T4 is capable of transcribing only after infecting *E. coli*, and directing the host RNA polymerase to phage promoters. Expression of middle RNA from early promoters requires extension of the early RNA into downstream middle genes. My project is to investigate a pathway that expresses middle RNA and does not use known middle promoters. Using a mutant *tabG* strain, I found two mutations that were previously mapped near the beta subunit of RNA polymerase were determined and at very interesting locations. Thus far, I have isolated and analyzed RNA of this mutant strain by primer extension assays. These assays revealed unexpected findings, suggesting that the known phage activator for middle RNA expression is needed for the extension of early RNA. It will be important to investigate whether other T4 middle genes are similarly expressed in these conditions. Given that the extension of T4 early RNA into the T4 middle genes requires transcription elongation by E. coli RNA polymerase, it is reasonable to assume that an interesting regulation of the elongation operates during T4 infection. This mutant RNA polymerase is currently being isolated for *in vitro* transcription analyses for further characterization. These results will be crucial to understanding how T4 early RNAs extend into middle genes. **Poster 22**

Presynaptic Kainate Receptors Bidirectionaly Regulate Thalamocortical Transmission during Development
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Kainate receptors (KARs) are part of the ionotropic glutamate receptor family and are tetramers of GluR5, 6, 7, KA1, and KA2. Recent work from our lab demonstrates that KARs mediate synaptic transmission at the thalamocortical (TC) input in layer IV of developing rodent barrel cortex. Pre-synaptic KARs are also found at this input, and are involved in short-term synaptic plasticity; however the mechanism and consequences of this regulation are unclear. To investigate the regulation of TC transmission by pre-synaptic KARs, we used brain slices prepared from mice aged between post-natal day 3 to 6 and made whole-cell patch-clamp recordings from layer IV neurons in barrel cortex. Bath application of the KAR agonist, kainate, regulated the TC synaptic response in a dose-dependent manner. Pharmacological experiments on wild type and GluR5 or GluR6 KO mice confirmed that the actions of kainate were restricted to KARs rather than AMPARs and reveal a major role for the GluR6 subunit in the pre-synaptic regulation of TC transmission. Furthermore, during high frequency stimulation the pre-synaptic KARs can be activated by synapticaly released glutamate to produce a short-term plasticity. Taken together these results suggest a novel role for pre-synaptic KARs in regulating TC transmission in neonatal barrel cortex. **Poster 92**

Drug Elimination Kinetics in the Eye after Subconjunctival Delivery
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Subconjunctival injections of drugs are commonly performed in patients for the treatment of various ocular disorders. Injections under the conjunctiva form a localized depot, allowing for long-term release of the drug into the eye. While subconjunctival injections have become routine, the rate at which drugs are retained and eliminated from the subconjunctival depot remains unknown due to the difficulty in obtaining drug concentration measurements *in vivo*. Since magnetic resonance imaging (MRI) allows the acquisition of images non-invasively *in vivo*, we performed MRI studies in rabbits after subconjunctival injection of two model drugs, Gd-DTPA and Gd-albumin. The signal intensity values from the images were then converted to concentration levels at various time points to determine the rate of drug clearance from the sub-conjunctival depot. The effect of injection volume on drug clearance rate was also studied by injecting 200 and 600 µl of Gd-DTPA. Results indicate that Gd-DTPA cleared rapidly from the subconjunctival depot with a half-life of 30 minutes while Gd-albumin cleared at a much slower rate and exhibited a half-life of 5.3 hours. MRI offers a better alternative for data acquisition as measurements can be safely acquired *in vivo*, unlike many traditional methods in ocular pharmacokinetics. **Poster 45**

A Comprehensive Study of the Effects of Osteoblasts on the Osteogenesis of Mesenchymal Stem Cells
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The cells, matrix, and soluble factors that surround adult stem cells within the body (known as a “niche”) are thought to maintain the stem cells in their undifferentiated state. Once they leave the niche, they undergo rapid proliferation and differentiation to renew mature tissues. Mesenchymal stem cells (MSCs) can be found in bone marrow and many other tissues. The components of their niche are yet to be determined. Osteoblasts (OBs) potentially play an important role in the MSC niche because they are abundant in the bone marrow and are known to effect hematopoietic stem cells. In order to determine the role of OBs in the differentiation state of MSCs, an *in vitro* coculture system was developed to monitor the osteogenesis of MSCs. Before coculture, MSCs were labeled with a fluorescent dye. After coculture, OBs were separated from MSCs by FACS and the RNA levels of various osteogenic genes were analyzed in the MSCs. The presence of OBs was found to increase the osteogenesis of MSCs. Ongoing studies involve culturing MSCs either with OB matrix or OB-conditioned medium to decipher which component of the OBs is responsible for the effect seen. **Poster 26**

HIV-1 GP120: Conformational Flexibility and Humoral Response to N-linked Glycosylation
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HIV-1 uses a number of strategies to evade the humoral immune response. One strategy is glycan silencing, in which a dense mask of N-linked glycans protects much of the exposed surface of the HIV-1 exterior gp120 glycoprotein from immune recognition. Ironically, this glycan tactic may be exploited to help the immune system recognize the virus more effectively. In this study, computational modeling was used to add N-linked glycan sites to a variety of gp120 immunogens to focus the vaccination response by silencing immunogenic surfaces that elicit non-neutralizing antibodies. Another strategy of HIV-1 evasion is conformational masking, in which antibodies are elicited against conformations that are incompatible with the functional viral spike. To investigate the conformational flexibility of gp120, hydrogen-deuterium exchange was used to probe the flexibility of unliganded gp120 and of gp120 bound to CD4. Results were compared to conformational flexibility inferred from crystal structures of unliganded and CD4-bound gp120. **Poster 47**

Effect of Age and Loading Frequency on Intervertebral Disc Cell Response to Dynamic Compression
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Introduction Intervertebral disc (IVD) degeneration is a common consequence of aging with enormous socioeconomic impact. Cells from anulus fibrosus (AF) and nucleus pulposus (NP) regions have distinct phenotypes and have been shown to be mechanically sensitive. The goal of this work is to evaluate the potential for mechanical stimulation to regenerate extracellular matrix (ECM) using IVD cells from young and mature tissue sources. Methods NP and AF cells were harvested from IVDs removed from mature and young bovine tails and seeded into 3D constructs. Constructs were compressed for 2 hours/day for 7 days at one of three frequencies. Gels were harvested at 7 days or further cultured and harvested on days 14 and 21. Mechanical properties, viability, DNA, GAG and collagen content were determined. qRT-PCR was performed for aggrecan, collagen I and II, MMP-3,-13 and TIMP-1 and-2. Results GAG accumulation tended to increase with higher frequencies of loading and was higher in NP than AF constructs. Mechanical stimulation upregulated gene expression for collagen (AF and NP) and aggrecan (NP). Gene expression was generally greater for young cells than for mature cells. Preliminary data suggest that increased frequency of loading increases mRNA expression and production of ECM proteins such as GAG and collagen. **Poster 14**

Vesicle Trafficking is Essential for the Proper Cellular Distribution of the Adenylyl Cyclase ACA and cAMP Release during Chemotaxis and Streaming
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Intracellular compartmentalization of cAMP and the localization of adenylyl cyclases to cellular domains have been observed in mammalian cells. We have shown that the adenylyl cyclase ACA is enriched at the back of chemotaxing Dictyostelium cells, locally releasing cAMP, and allowing cells to align head to tail, forming streams. Interestingly, we also found that ACA labels intracellular vesicles. Here we investigated whether these vesicles are involved in ACA localization using Fluorescence Recovery After Photobleaching (FRAP). We found ACA recovers uniformly across the bleached area consistent with vesicle delivery to the PM. Moreover, the recovery was directional resulting in an earlier recovery at the back than at the front of the cell. When the cytoskeleton is depolymerized and vesicle trafficking is disrupted using latrunculin A or nocodazole, ACA recovery changes to a pattern consistent with membrane diffusion. Furthermore, ACA vesicles co-localize with microtubules and most strikingly, nocodazole-treated cells have strong streaming defects thereby suggesting that vesicle trafficking is required for cAMP release. Together, our findings establish a role for vesicle trafficking in ACA localization and cAMP secretion. **Poster 18**

HCP1 Polymorphisms and Neural Tube Defect Risk in the Irish Population

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Purpose: Neural Tube Defects (NTDs) are a common congenital malformation affecting 1/1000 pregnancies and occur when the neural tube fails to close properly in embryonic development. NTDs demonstrate complex origins with environmental and genetic components. Maternal periconceptional folate reduces risk of NTD development by 70 percent, while inherited variants in genes involved in folate metabolism may modify a person's risk of having a child with an NTD. HCP1, the gene responsible for intestinal folate absorption was identified recently. HCP1 genetic variation on NTD risk is unexplored.
Methods: We collected DNA from more than 500 Irish NTD family triads and 1,000 mothers with normal births. HapMap linkage disequilibrium data was utilized to select genetic markers that would represent the most functional variation in HCP1. We are genotyping 10 HCP1 variants via matrix assisted laser desorption/ionization time-of-flight mass spectroscopy.
Analysis: The frequency of the variants will be compared between the mothers of cases, cases and controls using family-based association tests. Measures of linkage disequilibrium allele and genotype frequencies in the study population will be compared to the HapMap's CEPH reference population.
Conclusions: HCP1's role in NTD development in the study population will be discussed. **Poster 68**

An Animal Model of Bilateral Pheochromocytoma in ErbB-2 Mice

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Pheochromocytoma (pheo) is a rare adrenal chromaffin cell tumor. Known familial germline mutations with disposition to pheo account for over 20 percent of cases. There is clinical evidence suggesting that enhanced ErbB-2 growth factor receptor signaling may play a role in pheo. In this study, we examined the effect of ectopic expression of an activated isoform of ErbB-2 in transgenic mice on pheo formation. PB-ErbB-2Δ transgenic mice express an activated ErbB-2 growth factor receptor splice variant under the control of the minimal rat probasin promoter. MRI and subsequent histological examinations revealed the presence of phenotypic and biochemical bilateral pheo in the adrenal gland of multiple animals. Tumor suppressor gene *pten* revealed a significant decrease in both the mRNA and protein levels in the pheo tissue versus normal adrenal. Additionally, levels of phosphorylated (activated) AKT were induced. Immunohistochemical analyses revealed the nuclear localization of the cell cycle regulatory protein, cyclin D1, was increased in tumors versus the normal adrenal. These data indicate that the ErbB-2 and AKT signaling pathways along with the cyclin D1/CDK enzyme complex may be important targets for clinical pheo intervention. **Poster 12**

Neural Stem Cell Behavior Regulated By Integrin/Laminin Interaction in the Developing Embryo

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Laminin, an extracellular matrix molecule, and integrin receptors are important regulators of stem cell behavior throughout the body. Given the similarity in regulatory mechanisms between stem cell systems, we hypothesize that laminins and integrins are essential to neural stem cell behavior in the ventricular zone (VZ) during development. Since the expression and role of beta 1 integrin remains poorly understood during development, we first examined expression throughout embryogenesis in the murine cortex. High expression of beta 1 integrin was consistently observed in the VZ. Flow cytometry analysis of neural stem cells derived from Sox2-GFP mice shows a high correlation with beta 1 integrin and sox2. Next, the role of beta 1 integrin on neural stem cell behavior was examined by injecting beta 1 integrin blocking antibodies in utero. We observed proliferation 16 hours after injection and found a greater number of cells proliferating away from the VZ along with detached neural stem cells. Several laminin mutants were also examined and show a similar phenotype. Based on these findings, we propose a model in which beta 1 integrin is responsible for keeping neural stem cells in the VZ. Overall, these data suggest a novel regulatory role for laminin/integrin interactions in the neural stem cell niche. **Poster 17**

Automated 100-Position Specimen Loader and Image Acquisition System for Transmission Electron Microscopy
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We report the development of a novel, multi-specimen imaging system for high-throughput transmission electron microscopy. Our cartridge-based loading system, called the "Gatling", permits the sequential examination of as many as 100 specimens in the microscope for room temperature electron microscopy using mechanisms for rapid and automated specimen exchange. The software for the operation of the Gatling and automated data acquisition has been implemented in an updated version of our in-house program AutoEM. In the current implementation of the system, the time required to deliver 95 specimens into the microscope and collect overview images from each is about 13 hours. Regions of interest are identified from a low magnification atlas generation from each specimen and an unlimited number of higher magnifications images can be subsequently acquired from these regions using fully automated data acquisition procedures that can be controlled from a remote interface. We anticipate that the availability of the Gatling will greatly accelerate the speed of data acquisition for a variety of applications in biology, materials science and nanotechnology that require rapid screening and image analysis of multiple specimens. **Poster 48**

A Central Role for Extended Polypeptide Linkers in Establishing the Spatial Architecture of a Pyruvate Dehydrogenase Complex
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Icosahedral pyruvate dehydrogenases enzymes have an architecture consisting of a central E2 scaffold decorated by a shell of peripheral enzymes that localize at a distance of ~ 90 Å from the core. An important unresolved question is whether the gap between the E2 core and the peripheral enzymes is maintained by protein-protein interactions in the outer shell or by rigidity of the linkers that connect the core to the peripheral subunit binding domain. Using circular dichroism, analytical ultracentrifugation, and solution NMR studies we show that the linker peptide has an extended conformation. Further, based on cryo-electron microscopic analysis of single PDH complexes, we show that the mean distance of outer shell enzymes is independent of the occupancy, providing definitive evidence that the annular gap does not arise from protein-protein interactions in the outer shell. We conclude that the inner linker regions of PDH enzymes serves as a critical element in maintaining the spatial separation required for coupling the decarboxylation of pyruvate in the outer shell to the synthesis of acetyl CoA in the core. **Poster 49**

Gene Expression Profiling of the Inner Ear in the Adult Zebrafish with Massively Parallel Signature Sequencing (MPSS)
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The inner ear is a highly specialized compartment with uniquely differentiated cell types conducting the auditory and vestibular functions. Thus, we assume that there are genes that are specifically expressed in the inner ear and are critical for the auditory and vestibular functions. Here we used a relatively new technique, massively parallel signature sequencing (MPSS), to study the gene expression profile of the inner ear in adult zebrafish. Using MPSS, we profiled gene expression of both the zebrafish inner ear and the zebrafish brain. We generated 45,259 unique transcription tags with a sensitivity to detect the level of transcription as low as three transcripts per million (tpm). By comparing the inner ear MPSS tags to the brain MPSS tags, we were able to identify tags that: 1) are mapped to genes already known to be expressed in the inner ear; 2) are mapped to transcripts previously identified, but with no known expression or function in the inner ear; and 3) can not be mapped to any currently known or predicted transcript. RT-PCR results have verified the tissue-specific expression of some candidate genes in our MPSS data. The preliminary analysis of our data suggests that the MPSS is a powerful technique for large-scale and in-depth gene expression profiling of the inner ear in the adult zebrafish, which will allow us to identify new and potentially exciting genes expressed specifically in the inner ear. **Poster 65**

Developmental Differences in Attention Bias to Angry Faces
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Event-related, functional magnetic resonance imaging (fMRI) was used to compare right amygdala activation in 20 psychiatrically healthy adults and 19 psychiatrically healthy children. Subjects viewed angry/happy/neutral faces pairs during fMRI acquisition. The face pairs were replaced by an asterisk on the right or left side of the screen. The subject's response to the asterisk provided a measure of the spatial attention to the angry and happy faces. Behavioral data shows that both adults and children attend away from the threatening angry face; there is no significant difference between the two groups. Children show greater neural activation in the right amygdala when allocating their attention away from the threatening angry faces. **Poster 101**

Endothelial Cell Matrix Influences Mesenchymal Stem Cell Differentiation into Vascular Cell Types
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Mesenchymal stem cells (MSCs) are multipotential stem cells that have the ability to differentiate into a variety of cell types. We have identified vascular extracellular matrix as a critical regulator of MSC differentiation toward the vascular cell lineages: endothelial cells (ECs) and smooth muscle cells (SMCs). MSCs cultured on macrovascular EC matrix underwent EC differentiation at early time points and SMC differentiation at later time points. MSCs cultured on fixed EC matrix resistant to modification, however, differentiated toward an EC lineage only. Conversely, MSCs cultured on EC matrix pre-modified by MSCs underwent SMC differentiation only. Interestingly, both lanes of cross-talk between stem cell and matrix, i.e., the MSC-induced matrix alterations and the matrix-supported differentiation toward EC and SMC lineages, proved to be facilitated by soluble players. Furthermore, the MSC-induced matrix alterations were found to deplete the factors responsible for EC differentiation yet activate the SMC differentiation factors. Thus, in exposing the framework by which EC matrix regulates MSC differentiation, we have uncovered evidence of a feedback system in which the same MSCs are able to alter the matrix. Exploring such a system will help to elucidate the role of matrix in stem cell differentiation. **Poster 27**

Analysis of Smoking Cessation Patterns Using a Stochastic Mixed Effects Model with a Latent Cured State
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A major problem when studying smoking addiction behavior is that participants make several quit attempts before successfully quit. It is necessary to distinguish transient quitting (temporarily smoking-free but relapse later) from permanent quitting (lifelong smoking-free). We identified and quantified baseline factors associated with permanent quitting using the Alpha-Tocopherol Beta-Carotene (ATBC) Lung Cancer Prevention study dataset (a longitudinal cohort study with 29133 subjects). We modeled the smoking cessation patterns using the discrete-time stochastic mixed-effect model with three states: smoking, transient quitting, and permanent quitting. We found that age was positively associated with probability of making quit attempts ($p<0.001$). However, years of smoking, cigarette and alcohol consumption had inverse association with probability of making quit attempt ($p<0.001$). If the quit attempt was made, more alcohol consumption was associated with higher probability of relapsing ($p=0.005$). Moreover, 5 years in age increased the odds of permanent quitting by 10.2 percent (CI -0.40 percent~21.8 percent; $p=0.06$). Individuals with psychological symptoms were significantly less likely to be successful permanent quitters ($p=0.03$). Thus, baseline risk factors have different effects on different transition probabilities. **Poster 57**

Determining the Activity and Cleavage Specificity of the Individual Components in the Presenilin/g-secretase Complex
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The γ-secretase complex [Presenilin (PS), Nicastrin (Nct), Aph1 and Pen2] cleaves single-pass transmembrane proteins to regulate cell function. To screen for novel mechanisms, I am studying this complex in *Dictyostelium*. Upon starvation, single-celled *Dictyostelium* aggregate and initiate multicellular development resulting in two terminally differentiated cell types. After analyzing several single and double mutations in the PS, Nct, and Aph1 genes, I conclude that all the components are important for proper development, but that loss of Aph1 and Nct together leads to more severe phenotypes than does loss of both PS genes. It is thought that PS is the main enzymatic component of the complex, yet the combined loss of other components causes more severe defects. Potentially, loss of Aph1 and Nct may alter, rather than eliminate PS cleavage specificity or activity, or Aph1 and Nct may function in a novel, (i.e., non-γ-secretase dependent) pathway. Alternatively, γ-secretase activity may be compensated by another protease in the absence of the two PS genes. To explore these mechanisms, I am developing a read-out of γ-secretase activity by expressing tagged substrates of γ-secretase in wild-type and mutant cell lines. Differences in cleavage may explain the developmental anomalies of the various strains. **Poster 8**

Molecular and Functional Analysis of G Protein-coupled Receptor Dimers
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The superfamily of G protein-coupled receptors (GPCRs) makes up the largest class of cell surface receptors in the mammalian genome. Upon binding of extracellular ligands, these receptors mediate a wide variety of physiological functions by activating specific classes of heterotrimeric G proteins. Accumulating evidence suggests that GPCRs exist on the cell surface as dimers and/or higher order oligomers, but the molecular mechanisms underlying this association are not well understood. We are working to elucidate receptor regions and/or particular amino acids involved in GPCR dimerization using the M3 muscarinic acetylcholine receptor as a model. The study involves random mutagenesis of the M3 receptor and testing of the introduced mutations for their effect on receptor dimerization using a recently developed method known as the 'split-ubiquitin method'. Much like classical yeast two hybrid screens, this system monitors the interaction of two proteins of interest but has been developed specifically for membrane proteins. The data from the yeast screen will then be utilized in further studies to assess the role of dimerization in GPCR function. Since GPCRs are excellent targets for drug therapy, the information obtained in these studies should be of considerable therapeutic relevance. **Poster 24**

Defining the Ezrin and Chloride Intracellular Channel 4 Interaction in Metastasis
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Ezrin, a linker between the cytoskeleton and plasma membrane, has previously been show to facilitate the metastatic phenotype in both osteosarcoma and rhabdomyosarcoma. Because ezrin's biology is mediated through protein-protein interactions, we believe ezrin's role in metastasis may be understood through its binding partners. Using affinity chromatography, we have identified the direct ezrin binder CLIC4, a stress-response protein which has been shown to translocate to the nucleus and subsequently cause apoptosis in cells. Data in our lab has shown that ezrin may be assisting metastatic cells in resisting apoptosis. We therefore hypothesize that ezrin is holding CLIC4 out of the nucleus in metastatic cells providing them with such a survival advantage. *In vitro* validation of the ezrin-CLIC4 interaction has been accomplished and we have confirmed co-expression of ezrin and CLIC4 in osteosarcoma. To test our hypothesis, we have begun to track CLIC4 nuclear localization under conditions of stress that may mimic those in metastasis and plan to assess the effect of these stressors on cells during manipulation of ezrin and CLIC4 expression and binding. If the ezrin-CLIC4 interaction proves to be necessary for metastasis, it may be reasonable to consider the interaction as a target for novel cancer therapies. **Poster 35**

Adaptation-dependent Neural Responses to Basic Visual Shapes in Monkey Cortical Area V4
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A physically identical visual stimulus might be perceived differently depending on the patterns an observer has previously viewed. These perceptual changes can occur with inspection times of minutes to less than a second and are typically referred to as 'adaptational aftereffects'. For instance, gazing at a concave outline stimulus for several seconds causes a subsequently presented straight pattern to appear convex and vice versa. This class of phenomena raises questions about the theoretical role of adaptation in normal vision, as well as the neuronal underpinnings of such perceptual distortions.

Here we recorded brain activity of two alert macaque monkeys under conditions of adaptation that give rise to such aftereffects. Specifically, we measured the tuning of cells as well as the power of different frequency ranges in cortical area V4. Although tuning of single units is affected in diverse ways, an analysis of specific neuronal subpopulations suggests a strong link between neural activity in area V4 and the perceptual aftereffect known in humans. **Poster 96**

Recognizing a Person by Face: Dissociating Brain Regions Involved in Perceptual and Conceptual Components of Person Identification
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Recognition of a familiar face affords access to a wealth of perceptual and conceptual information about the identified person, including biographical information. Two possible neural candidates for person identification by face are the fusiform face area (FFA) and anterior inferior temporal cortex (aIT). Here we attempt to dissociate the diverse perceptual and conceptual representations automatically activated when a face is recognized. We measured brain activity with functional magnetic resonance imaging (fMRI) while subjects viewed face images of three levels of familiarity: 1) faces never seen before (new), 2) faces repeatedly viewed previously (seen), and 3) faces repeatedly viewed previously in conjunction with associated names and short biographies (known). Blood-oxygen-level-dependent fMRI measurements were performed at high resolution (1.95 x 1.95 x 2 mm^3 voxel volume) using a 3T scanner (GE) and a custom-made 16-channel head coil. In the two subjects studied so far, we found similar patterns of activity during perception of faces of all three levels of familiarity, including early visual regions and the FFA. We found a greater response to less familiar faces (greatest for new ones) in the FFA. A left aIT region showed a slightly greater response to seen and known faces than to new faces. **Poster 97**

Parenting Behavior and Aggression in Low-income African-American Adolescents
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The goal of this study was to examine the bivariate relations between parenting practices, parenting style, and adolescent aggression. A total of 254 low-income, predominately African-American adolescents (males=135, females=147) completed questionnaires about their aggressive behaviors and their perceptions of a parent/guardian's aggression specific parenting practices and parenting style at two time points. Three variables were used to measure parenting style: social support, psychological control and behavioral control. Bivariate analyses between time 1 parenting variables and time 2 aggression variables revealed a significant association between time 1 aggression specific parenting practices and time 2 overt aggression. Analyses also indicated a significant association between time 1 overt aggression and all four time 2 parenting variables. Similarly, time 1 relational aggression was significantly associated with all four parenting variables at time 2. These study findings suggest that parents may both influence the expression of aggressive behavior and respond to aggressive behavior by changing their parenting strategies. Future research will involve an investigation of the potential bidirectional relations between parenting and adolescent aggressive behavior in this sample. **Poster 59**

Vaccinia Virus A28 Protein is the Target of Neutralizing and Protective Antibodies
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Vaccinia virus, a member of the poxvirus family, is the virus used in the current smallpox vaccine. It is able to infect a wide range of cells, but its mode of entry is not well understood. The vaccinia virus protein A28, located on the mature virion membrane, has recently been shown to be necessary for cell entry. Virions lacking A28, assemble and exit cells normally, have normal morphology, and are able to attach to cells. However, penetration of the core into the cell is blocked. We have expressed and purified a recombinant form of A28 using the baculovirus system. Using this recombinant A28 we made polyclonal rabbit antibodies, which bind not only to the recombinant protein but also to native protein present on the virus. The antibodies are able to neutralizing the entry of vaccinia virus up to 75 percent. In addition this neutralization can be blocked by sequestering the A28 antibodies with rA28 showing that the neutralization is specific to the A28 IgG. Mice immunized intraperitoneal with the A28 antibodies, and then challenged intranasally with vaccinia virus were protected against disease as measured by weight loss. These experiments suggest that the A28 protein is a target for neutralizing antibodies and could be an important component of a smallpox vaccine. **Poster 82**

Learning to Change: Re-Defining the Neural Correlates of Response Selection and Feedback in a Probabilistic Response Reversal Task
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At the heart of social intelligence is the ability to detect subtle changes in communication and act upon these changes rapidly as they occur. This rapid reversal learning is a capacity relevant to socioemotional behavior. Impairments in reversal learning are observed in several psychiatric conditions that are characterized by disinhibition, social inappropriateness and irresponsibility, such as psychopathy. The current study used fMRI to measure BOLD responses in 20 healthy individuals during performance of a novel probabilistic response reversal (PRR) task.

The PRR requires participants to choose between two objects, one associated with reward and the other with punishment. While previous studies have focused on decision making (cue evaluation and response choice), none has allowed the separate investigation of differential patterns of neural activity during each of these processes. The current study controls confounds in previous study designs by separating out the presentation of the stimulus and feedback phases. We hypothesized that structures such as the orbitofrontal cortex and anterior cingulate cortex, involved in computing and planning would be activated specifically during choice selection, and that the anticipation phase would engage prominently limbic structures, particularly the ventral striatum due to its central role in reward prediction. The results are discussed with reference to response reversal and response reversal impairments in specific neuropsychiatric populations. **Poster 88**

Cellular Effects of Viral Proteins Highlight a Link between Mitochondrial Morphogenesis Machinery and Apoptosis
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Apoptosis is a host defense mechanism against viruses. Human cytomegalovirus encodes vMIA, which is targeted to mitochondria and functions as a potent cell death suppressor. The exact mechanism of vMIA action remains unknown. vMIA interacts with Bax, a pro-apoptotic Bcl-2 family member that regulates apoptosis and mitochondrial morphology. Paradoxically, vMIA causes Bax to localize to mitochondria, which normally only occurs during apoptosis, while still preventing cytochrome c release. Recently, a murine CMV homolog of vMIA, m38.5, was identified. Despite little homology to the vMIA Bax-binding domain, m38.5 also causes Bax translocation. To further study Bax targeting to mitochondria, we utilized Bax lacking C-terminal amino acids required for translocation during apoptosis. Surprisingly, BaxDC translocates to mitochondria with expression of either viral protein. The localization of Bax/BaxΔC at the mitochondria increased mitochondrial connectivity, but this effect was lost in cells lacking Mfn2. Bax and Mfn2 colocalize during apoptosis, and Bax elongation in healthy cells occurs through regulation of Mfn2. Thus, vMIA may control both apoptosis and mitochondrial morphology through Bax and Mfn2, further linking proteins involved in maintenance of mitochondrial morphology to regulation of apoptosis. **Poster 13**

Nanotube Confinement Can Stabilize or Denature Protein Helices: Lessons for Helix Formation in the Ribosome Tunnel
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As the ribosome translates mRNA to protein, the newly synthesized peptide is pushed through a tunnel inside the 50S subunit of the ribosome, and out into the cellular milieu. This 'ribosome exit tunnel' is approximately cylindrical in shape with a length of 10 nm and an average diameter of 1.5 nm. Polymer theory predicts that confining a protein in an inert cylindrical tunnel of similar dimensions will stabilize helical conformations. However, experiments on different peptides inside the ribosome tunnel indicate that there is a sequence dependence as to whether or not helices are stabilized. This observation motivated us to ask whether a similar sequence dependence of helix formation can be observed in the simpler case of confinement by carbon nanotubes. To answer this question, we have examined the helical stability of different sequences under carbon nanotube confinement using Langevin dynamics simulations and a coarse-grained representation of the polypeptide. In agreement with polymer theory, entropic stabilization of the helix is observed for all sequences when the nanotube is weakly hydrophobic. However, we find a strong sequence dependence as the hydrophobic character of the nanotube wall increases. For an amphiphilic sequence, the helical stability increases as the hydrophobic character of the nanotube increases. In contrast, the helical stability of a polyalanine sequence decreases as the hydrophobic character of the nanotube increases. Decreasing the size of the `hydrophobic patch' lining the nanotube, which better mimics the chemical heterogeneity of the ribosome tunnel, increases the helical stability of the polyalanine sequence. Finally, we present simulation results of peptides inside a coarse-grained representation of the ribosome tunnel complex. **Poster 37**

Loss of Plasma Membrane Asymmetry: Mechanism and Requirement for Sperm and Egg Fusion
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The transbilayer distribution of lipids across biological membranes is asymmetric. The choline containing lipids are enriched primarily on the external leaflet of the plasma membrane. In contrast, amine-containing glycerophosphalipids like phosphatidylserine (PS) are located preferentially on the cytoplasmic leaflet. Although asymmetry is the rule for normal cells, loss of asymmetry, especially the appearance of PS at the cell surface, is associated with many physiologic and pathologic conditions. Studies with pure phospholipid membranes suggest that anionic phospholipids like PS may promote membrane fusion in the presence of calcium. To test the requirement of PS in cell-cell fusion, we used sperm and egg as a model system. We demonstrated that PS is localized on plasma membrane of sperm head following activation and blocking of PS head group inhibits sperm and egg membranes fusion. It is generally assumed that mechanism of loss of asymmetry is protein mediated however exact mechanism remains elusive. To explore the transfer of PS across the membrane we have developed instrumentation to electrically and optically monitor a vertically oriented Montal-Mueller lipid bilayer. Using these instruments we have produced and observed asymmetric lipid bilayers and showed that this process is mediated by the lateral diffusion of anionic lipids through the lipidic pores. **Poster 38**

The Clathrin-independent Endocytic Pathway and H-RAS: Interplay of Trafficking and Signaling
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Ras is a small GTP binding protein that is involved in major cellular processes among them is membrane trafficking and actin reorganization. Here, we demonstrate that H-Ras traffics with the constitutive clathrin-independent endocytic pathway and that expression of the active H-Ras (G12V) mutant induces macropinocytosis, a stimulated form of the clathrin-independent pathway in HeLa cells. H-Ras co-localized with the clathrin-independent cargo molecule, MHCI, but not Transferrin and with the Arf6 Q67L vacuoles, indicating that H-Ras internalize through the clathrin-independent pathway. Using live cell imaging and biochemical assays we show that Arf6 Q67L alters H-Ras trafficking and hence reduces the levels of H-Ras-GTP by 50 percent following EGF stimulation. Conversely, expression of H-Ras (G12V) stimulated the clathrin-independent pathway and caused the formation of macropinosomes. Our previous studies demonstrated that expression of EFA6, an Arf6 GEF, also induces macropinocytosis. In order to examine the potential interplay of Arf6 and H-Ras in macropinocytosis we depleted cells of Arf6 and found that Arf6 is required for the H-Ras (G12V)-induced macropinocytosis. Altogether, our work demonstrates a new trafficking route for H-Ras and an alternative mechanism for H-Ras induced macropinocytosis involving Arf6. **Poster 19**

Exploring the Mammalian Phylogeny by Analyzing Large Comparative Sequence Datasets

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The ongoing generation of prodigious amounts of genomic sequence data from many vertebrates is providing unparalleled opportunities for establishing phylogenetic relationships among mammals. The size and complexities of such comparative sequence datasets allow smaller and more difficult branches to be resolved, but also present unique challenges, including large computational requirements and the negative consequences of systematic biases. We have analyzed a large dataset of high-quality genomic sequence that we generated from >40 vertebrates orthologous to a 1.9-Mb region of the human genome that encompasses the CFTR gene. To understand the characteristics and challenges associated with phylogenetic studies of such a large dataset, we partitioned the sequence data in several ways, analyzed the data with several phylogenetic reconstruction algorithms, and attempted to control for possible sources of systematic error. Our analyses of this unusually large dataset yielded well-supported trees largely in agreement with other recent multi-gene studies. Though some clades remain difficult to resolve even with these large amounts of data, some controversial associations (such as the placement of guinea pig in the rodents or the rooting of placental mammals) are very strongly supported. **Poster 69**

Genetic Risk Factors for Congenital Heart Defects: An Investigation of Genes from the Folate Metabolic Pathway

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Congenital heart defects (CHD) are the leading cause of deaths attributed to birth defects. CHD are presumed to be multifactorial in origin, having a genetic basis potentially influenced by environmental factors. The occurrences of certain CHDs are reduced by periconceptual folic acid administration and with dietary folic acid supplementation. We hypothesized that polymorphisms in folate pathway genes could be genetic risk factors for CHD. Four SNPs, MTHFR 677C>T; MTHFD1 R653Q; RFC1 R27H; and TCII P259R, were genotyped in 2883 children diagnosed with a CHD by age two and ethnically matched controls born in New York state between 1997–1998. Cases with known chromosomal defects were not included. Cases were subdivided into five clinical categories — atrial septal (ASD), conotruncal, obstructive, ventricular septal (VSD) and single ventricle — and stratified by ethnicity to minimize genetic heterogeneity. Allele frequencies were significantly different between ethnic groups. The 1998 cases showed association in African Americans between MTHFD1 653RR with ASD (OR=1.7 [1.1-2.6, p=0.02]), and MTHFR 677CC with VSD (OR=2.1 [1.1-3.8, p=0.03]). TCII 259PP was associated with conotruncal defects in Hispanics (OR 4.4 [1.5-13.2, p=0.007]). RFC1 R27H was not associated with CHD. The 1997 CHD cases were used to replicate our findings. To date (with greater than 95 percent of genotyping completed), the 1997 cohort does not confirm the associations found in the 1998 sample for either conotruncal defects or ASDs. These data suggest that these polymorphisms in folate pathway genes do not contribute to the genetic etiology of CHD. **Poster 66**

Involvement of Transcription in Somatic Hypermutations of Antibody Genes

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Germinal centre B lymphocytes undergo somatic hypermutation, a genetic diversification process used to generate high-affinity antibodies with different isotypes. The mutation pathway is initiated by activation-induced cytidine deaminase (AID) protein, which is expressed only in B cells. It is not clear what targets mutation to the immunoglobulin loci. Genetic evidence indicates that both promoter and enhancer transcription elements are required for hypermutation, implying that transcription might somehow orchestrate AID recruitment. However, the role of transcription in hypermutation has not been characterized. To study targeting, I am taking a fundamental approach to determine the pattern of transcription in the mutating switch region compared to the non-mutating constant gene. We generated mutational map and transcriptional profile of the 5 Kb switch region spanning the Sμ [Mυ] region promoter through the Cμ [Mυ] gene. Our results show that the mutation pattern complies with transcription pattern of the switch region, suggesting a role for transcription in targeting AID to these regions specifically. **Poster 75**

The Role of Toll-like Receptors in Activating Humoral Memory Responses
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Humoral immune memory (HIM) is composed of both long-lived plasma cells (PC) that maintain serum antibody levels in the absence of antigen and memory B cells (mB) that respond rapidly to antigen challenges to give rise to new PC. The cellular and molecular basis of the activation of HIM is poorly understood. Here we explore the role of TLR4, TLR9 in the activation of mB in the NP-KLH immunized mouse model. We followed HIM responses by measuring the IgG production, the frequency of NP-specific PC, and the frequency of NP-binding mB (B220+ IgM- IgD- CD138-) in the spleen. Purified NP-specific mB cultured *in vitro* with CpG ODN or LPS alone yield similar numbers of high affinity, NP-specific, IgG-secreting PC. IgM/D+ NP-specific B cells purified from the same mice differentiated into low affinity, NP-specific, IgM-secreting PC, especially with LPS. Congruent with these observations, both subpopulations of B cells had similar expression level of TLR9, but a higher TLR4 levels in IgM/D+ B cells than mB. Boosting *in vivo*, CpG ODN or LPS alone failed to increase the frequencies of high affinity PC and mB, but NP-KLH + CpG ODN or LPS were effectively activating HIM responses. Taken together, these results indicate that while TLR agonists can directly act on mB to promote their differentiation *in vitro*, both antigen and TLR agonists are required for effective activation of B cell memory responses *in vivo*. **Poster 79**
This work was supported by grants from NIH and UMD to Wenxia Song.

The Compliant Lever and Pivot (CLAP) Model for Myosin V's Mechanics
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Myosin V is mechanistically fascinating molecular motor which serves *in vivo* as an actin based cargo transporter. A recent explosion of experimental data on myosin V has provided a rather clear picture of on how it operates. Structurally, myosin V has two motor domains connected to unusually long levers which are tethered via a coiled-coil. These long levers and the motors' high actin affinity allow the molecule to take multiple steps per diffusional encounter along actin in a hand over hand manner. Myosin V's most extraordinary feature is its ability to transmit intermolecular strain between its motors and coordinate their chemo-mechanical cycles. This synchronization has been shown to decelerate detachment of the leading motor and accelerate detachment of the trailing motor, effectively generating a strong forward bias on the molecular motion. The mechanistic details of this communication pathway remain largely unresolved. Our understanding of this feature is limited by the fact that the current paradigm for modeling molecular motors (based on biochemical kinetics and thermodynamics) is not able to address mechanical questions. In this work we demonstrate how recent advances in single molecule biophysical experimental techniques and molecular structure determination can be used to build new types of quantitative models which provide a more mechanistic view of how molecular motors function. A new model for myosin V is presented which we refer to as the Compliant Lever and Pivot (CLAP) model. The CLAP model is based on a physical analysis of single molecule experimental data on the step size of myosin V and mutants with truncated levers. The novel analysis provides several unique insights into previously unresolved features of the molecule. Intuitively, the CLAP explains myosin V's funny walk. **Poster 39**

Spatial and Temporal Heterogeneity in Timing and Seasonal Rates of Hospitalizations Associated with Influenza and Respiratory Syncytial Virus (RSV) in Ten U.S. States, 1989–2003

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Geographical differences in the influenza hospital burden have not been studied in the United States, as hospitalization rates have only been estimated at the national scale or for small communities. Further, the relative contribution of influenza and RSV to respiratory hospitalizations is unclear and may be confounded by spatial heterogeneity in viral activity. We analyzed ten State Inpatient Databases from the Healthcare Cost and Utilization Project, representing 34 percent of the U.S. population to quantify spatio-temporal heterogeneity in weekly virus-associated hospitalizations between 1989 and 2003. We focused on hospitalizations with a specific code for influenza or RSV (primary or any-listed) and explored peak timing by season, state and virus. We found that neither influenza nor RSV consistently peaked before the other, even when controlling for dominant influenza subtype (P=0.94). Variability in timing of influenza epidemics was more important between seasons than between states (R^2=0.45 for season and 0.13 for state). By contrast for RSV, there was more variability between states than seasons (R^2=0.37 for state and R^2=0.16 for season). Additionally, we used Serfling regression analyses to calculate seasonal hospitalization rates of 38 and 23 for influenza-associated and RSV, respectively per 100,000 persons. **Poster 58**

STAM Adaptor Proteins Interact with COPII Complexes and Function in ER-to-Golgi Trafficking

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Signal transducing adaptor molecules (STAMs) are involved in cytokine signaling, and they form complexes with a number of endocytic proteins. Here we demonstrate that STAM adaptor proteins also colocalize prominently with the Golgi apparatus and profoundly regulate Golgi morphology. Upon STAM overexpression the Golgi becomes extensively fragmented and dispersed, but when STAMs are knocked down the Golgi becomes highly condensed. Under both scenarios, trafficking of VGVG-GFP to the plasma membrane is markedly inhibited. In addition, recovery of Golgi structure after brefeldin A treatment in STAM knockdown cells is substantially impaired. Finally, we show that STAM proteins interact with COPII proteins at ER exit sites, and loss of STAM proteins causes a clustering of exit sites that likely gives rise to the highly condensed Golgi. Thus, in addition to their roles in cytokine signaling, STAMs have prominent roles in ER-to-Golgi trafficking, likely at the level of recruitment of the Sec13/Sec31 cage, as well as maintenance of Golgi morphology. **Poster 20**

Simultaneous Imaging of NAD+ and NADH using CARS and 2-photon Fluorescence Microscopy

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The redox balance of cells and organelles is of great biological interest; in particular, the relative concentrations of reduced and oxidized nicotinamide in mitochondria are of great value for example. NADH is fluorescent, and both UV-induced and 2-photon fluorescence has long been used to monitor it. NAD^+, however, is nonfluorescent. To quantify NAD^+, we have built a CARS microscope driven by a picosecond Ti: sapphire oscillator and optical parametric oscillator. The characteristic ring breathing mode of the nicotinamide moiety of NAD^+ near 1032 cm^{-1} is driven by the difference between two NIR beams, resulting in red anti-Stokes emission near 675 nm.

The picosecond pulsed NIR beam also weakly excites two photon fluorescence of NADH (but not NAD^+). While the fluorescence has wideband excitation and is retained, the CARS signal is lost when detuning ~1nm or when either NIR beam is interrupted. To simulate our eventual imaging target (mitochondria), we prepared DPPC LUVs (large unilamellar vesicles) loaded with millimolar levels of NAD^+, NADH or their mixture. These vesicles yielded signals for CARS only, fluorescence or both, respectively. The images were clearly spherical vesicles of the correct size. Calibration of the count levels with power and schemes to subtract background signals will be discussed. **Poster 40**

Regulation of Cbl-c Ubiquitin Ligase Activity
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Cbl proteins are ubiquitin ligases (E3s) which regulate tyrosine kinases. There are three mammalian family members: Cbl, Cbl-b, and Cbl-c. All have a highly conserved N-terminus composed of 1) the Tyrosine Kinase Binding (TKB) domain (which consists of a Four Helix bundle (4H), an EF hand calcium binding motif, and a Src homology 2 (SH2) domain), 2) the catalytic RING-Finger domain (RF) and, 3) the linker region. Bacterially produced GST-Cbl-c has minimal activity in an *in vitro* assay. In contrast, a bacterially produced GST protein containing only the RF of Cbl-c is highly active *in vitro*. Further analyses reveal an inhibitory region composed of the EF hand and SH2 domains of the TKB which, when removed, restores E3 activity. Computer models suggest that Y341 in the linker region is hydrogen-bonded to T190 in the EF hand and W228 in the SH2 domain. Introduction of a charged residue in the linker enhances the E3 activity of Cbl-c. The computer model predicts that such a charge would disrupt the hydrogen-bonding leading to a conformation change. Src is known to phosphorylate Y341 resulting in an increase of Cbl-c E3 activity. Together, these data suggest a structural regulation of Cbl-c E3 catalytic activity. Ongoing studies are investigating further the molecular mechanisms of the autoinhibition. **Poster 5**

Huntingtin Interacts with Argonaute Proteins, Localizes to P Bodies and Contributes to Post-transcriptional Gene Silencing
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Huntington's disease (HD) is a dominant autosomal neurodegenerative disorder caused by an expanded poly-glutamine tract within the amino-terminus of the Huntingtin (Htt) protein. Being that HD is a monogenic proteinopathy, many groups have focused on the identification of Htt interactors; however the precise cellular function of Htt remains controversial. In order to gain insight into Htt function and uncover potential HD pathogenic mechanisms, we have taken a biochemical approach to purify Htt with endogenous interacting proteins. Mass spectrometric analysis of the purified proteins identified several previously reported interacting proteins; additionally we reproducibly identified Argonaute proteins. This novel Htt interaction appears to be RNase insensitive and has been verified by glycerol gradient sedimentation followed by immunoprecipitation. Co-localization of endogenous and transfected proteins demonstrates Htt to be present in P bodies where it seems to play an important role in their maintenance. Additionally, cells with reduced Htt levels demonstrate compromised siRNA/miRNA dependent silencing. These data lead us to suggest that the previously reported transcriptional deregulation observed in HD may be in part attributed to mutant Htt's role in post-transcriptional gene silencing. **Poster 6**

Generation and Characterization of Neural Progenitors and Mature Neurons from Pluripotent Stem Cells
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Human embryonic stem cell (hESC)-derived dopaminergic neurons are potentially useful for understanding and treating Parkinson's disease through cell replacement therapy, drug screening, and as a model to study mechanisms of neural degeneration or regeneration. Despite the potential benefits hESCs hold, they can be unstable and difficult to maintain. We have, therefore, used NTera2 human embryonic carcinoma stem cells which have several advantages over hESCs. We have previously shown that NTera2 cells express characteristic human pluripotent stem cell markers, possess global gene expression profiles similar to several hESC lines, and can differentiate into functional dopaminergic neurons. Here we demonstrate that a population of neural progenitors can be isolated from NTera2 cells by flow cytometry, which can then be used to generate mature neuronal phenotypes. We have characterized the derived neural progenitors and mature neurons by global gene expression microarray analysis and immunochemistry. Therefore, the NTera2 cell line can serve as a useful model system to elucidate mechanisms involved in dopaminergic neuron development and to evaluate transplantation-based therapies in Parkinson's disease models. **Poster 28**

A Genome-wide Association Study of Sporadic Amyotrophic Lateral Sclerosis

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Background: Amyotrophic lateral sclerosis (ALS) is a rapidly progressive, fatal neurodegenerative disorder of unknown etiology. While it is clear that 5 percent of ALS is familial in nature, the role of genetic factors in the more common sporadic form of the disease has not been well defined.
Objective: We sought to identify genetic variants associated with an increased or decreased risk for developing ALS in a cohort of American sporadic ALS cases and to replicate these findings in a separate cohort of Italian sporadic ALS cases.
Design/Methods: We performed genome-wide SNP genotyping in a publicly available samples of American sporadic ALS cases (n = 276), and American neurologically normal controls (n = 276), and replicated the study in Italian sporadic ALS cases (n = 276) and Italian neurologically normal controls (n = 276). More than 550,000 unique SNPs were genotyped in each sample using the Illumina Infinium II HumanHap550 SNP chip.
Findings: More than 525 million genotypes were produced in 1,104 participants. The results of the genotypic and allelic association tests will be presented. All raw genotypes have been made freely available on the internet and represent the first publicly accessible SNP data for ALS cases. **Poster 67**

Formation of Virus-like Vesicles through Assembling of Proteolipid Domains

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Creation of “negative” membrane curvature is imperative for budding of enveloped viruses. This process is driven by the interaction of a specialized matrix protein with the host cell membrane. The mechanism of this budding machinery still remains unknown.

We reconstituted a minimal budding machine using the matrix (M) protein of Newcastle Disease Virus (NDV) and model membranes. Upon adsorption onto large unilamellar liposomes, M protein deformed the vesicle membrane and ruptured it. On giant liposomes M protein adsorption produced negative curvature and consecutive formation of small vesicles. In both lipid systems the membrane deformation are caused by formation of lipoproteic domains. Measurements of the electrical admittance of a small membrane patch revealed the details of the budding event: gradual capture of the membrane into a growing bud, rapid closure of the bud retrieving an area of membrane, comparable to the area of the NDV envelope.

Our results suggest that M protein interact with the membrane, creating lipid–protein associations which impose negative curvature, and, in consequence, production of vesicles comparables in size to that created by the whole virus. **Poster 41**

The Effectiveness of Fast Adaptation as Cochlear Amplifier

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Outer hair cells are critical for the sensitivity and the frequency selectivity of the mammalian ear. This function of these cells is attributed to their motile response to mechanical stimuli either at the hair bundles (fast adaptation) or at the cell body (electromotility). Here we attempt to evaluate the effectiveness of fast adaptation by estimating their mechanical work in response to steady sinusoidal stimulation with small amplitudes and then comparing the work with the viscous loss at the gap between the tectorial membrane and the reticular lamina. We found that “twitch”, which is re-closure of the transducer channel due to Ca entry, leads to a gain in the mechanical energy, whereas “release,” which is relaxation due to Ca entry, does not. Our calculation leads to a frequency limit, up to which fast adaptation can counteract the viscous drag. The limiting frequency that we obtained was rather low compared with the auditory frequency. We discuss the implications of the discrepancy. **Poster 42**

Increased Expression of the Parkinson's Disease Associated Kinase PINK1 by Modulators of the PTEN Pathway
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Early onset Parkinson's disease can be caused by mutations in several genes, including parkin, DJ-1 and PINK1. Prior to mutations being associated with parkinsonism, PINK1 was originally described as one of several genes that are transcriptionally transactivated by PTEN. PINK1 is a mitochondrial kinase and pathogenic mutations in PINK1 are loss of function mutants. Little is known about the cellular role of this protein but it has been shown to protect neurons against mitochondrial damage. Previous studies have shown that $PPAR_g$-agonists, such as Troglitazone, can induce PTEN expression in a concentration and time dependent manner by decreasing activity of Casein Kinase 2 (CK2), a negative regulator of PTEN. We hypothesized that PINK1 mRNA could be induced by modulators of PTEN expression and the PI3K/Akt pathway. Using qRT-PCR, we show that $PPAR_g$-agonists and inhibitors of PI3K, which antagonize the function of PTEN, induce PINK1 mRNA expression levels. No induction was seen in cell lines stably expressing a short hairpin RNA to PINK1. We are currently exploring the functional consequences of this induction, but these data delineate the signaling pathways by which PINK1 expression is controlled. These results suggest that PINK1 may serve as a feedback modulator of the pro-apoptotic activity of PTEN. **Poster 30**

Vaccinia Virus A43R Gene Product Contributes to Virulence
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Vaccinia virus (VV) is a member of the *Orthopoxvirus* genus of the family *Poxviridae*. VV has a linear double stranded DNA genome with ~200 open reading frames (ORFs). The A43R ORF is predicted to encode a type 1 transmembrane protein. The present study was initiated to characterize and determine the role of A43. We constructed a panel of recombinant VV with a V5 epitope-tag on A43, a deletion or stop codon mutation of A43 and an A43 revertant. Expression of A43V5 was shown by SDS-PAGE followed by Western blotting with a V5 monoclonal antibody. A time course experiment indicated that the protein is synthesized at late times and that DNA replication is required. Treatment with peptide N-glycosidase F and endoglycosidase H caused increased electrophoretic mobility of A43. A43 was shown by confocal microscopy to localize in the Golgi complex during infection, consistent with the sensitivity to endoglycosidase H. Although dispensable for virus replication and spread in tissue culture cells, initial mouse studies indicated that expression of A43 is important for full virulence. We suggest that A43 might retain a host immune response protein in the Golgi complex. **Poster 83**

Using Non-invasive Multi-spectral Imaging to Quantitatively Assess Tissue Vasculature
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This research describes a non-invasive, non-contact method used to quantitatively analyze the functional characteristics of tissue. Multi-spectral images collected at several near-infrared wavelengths are input into a mathematical optical skin model that considers the contributions from different analytes in the epidermis and dermis skin layers. Through a reconstruction algorithm, we can quantify the percent of blood in a given area of tissue and the fraction of that blood that is oxygenated. Imaging normal tissue confirms previously reported values for the percent of blood in tissue and the percent of blood that is oxygenated in tissue and surrounding vasculature, for the normal state and when ischemia is induced. This methodology has been applied to assess vascular Kaposi's sarcoma lesions and the surrounding tissue before and during experimental therapies. The multi-spectral imaging technique has been combined with laser Doppler imaging to gain additional information. Results indicate that these techniques are able to provide quantitative and functional information about tissue changes during experimental drug therapy and investigate progression of disease before changes are visibly apparent, suggesting a potential for them to be used as complementary imaging techniques to clinical assessment. **Poster 73**

Association of Vaccinia Virus Fusion Regulatory Proteins with the Multi-component Entry/Fusion Complex
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The A56R and K2L gene products of vaccinia virus (VACV) form a heterodimer (A56/K2) and have a regulatory role in membrane fusion as deletion of either causes infected cells to form large syncytia. Here we showed that syncytia formation requires proteins of the entry fusion complex (EFC), which is essential for virus-cell fusion and low pH-triggered cell-cell fusion. This suggested A56/K2 prevents fusion via an interaction with the EFC. To test this hypothesis, we made a panel of mutant VACV that have a tandem affinity purification tag attached to A56, K2 or the A28 EFC protein. Interaction between A56/K2 and the EFC was shown by their co-purification from detergent-treated lysates of infected cells and identification by mass spectrometry and Western blotting. In addition, a purified soluble transmembrane-deleted form of A56/K2 interacted with the EFC. Tagged A56 did not interact with the EFC in the absence of K2 nor did tagged K2 interact with the EFC in the absence of A56. The finding that both A56 and K2 are required for efficient binding to the EFC fits well with prior experiments showing that mutation of either A56 or K2 results in fusion of infected cells. Therefore, A56/K2 may interact with the EFC of progeny virions on the cell surface to prevent their fusion back into already infected cells. **Poster 84**

Peptide-MHC Class II Trafficking to Antigen Presentation Compartments
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In antigen presenting cells, MHC class II (MHC-II) alpha-beta dimers associate with invariant chain (Ii) that escorted MHC-II out of the ER and into endocytic antigen processing compartments via the plasma membrane (PM). When Ii is completely degraded in these compartments, MHC-II is loaded with antigenic peptides and peptide-MHC-II (pMHC-II) complexes traffic to the PM. Once on the PM, pMHC-II can internalize and once again enter antigen processing compartments and exchange antigenic peptides. Surprisingly, the machinery regulating endocytosis and transport of pMHC-II from the PM is not known. The goal of this study is to examine which molecules that are involved in the internalization of pMHCII from the PM. By expressing mutants that interfere with clathrin-mediated endocytosis and by treating cells siRNA targeting clathrin and AP-2, we hope to unravel how pMHC-II traffics from the PM. Our studies show that internalization of pMHC-II from the PM is AP-2 and clathrin-independent and follows a pathway marked by the GTPase Arf6. We are currently examining if ubiquitin might play a role of the internalization of pMHC-II. **Poster 76**

Synaptic Adhesion-like Molecules (SALMs) Promote Neurite Outgrowth
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Cell adhesion molecules (CAMs) have a variety of functions in the CNS, and are critical for synaptic development and maintenance. The synaptic adhesion-like molecules (SALMs) are a family of CAMs that interact with NMDA receptors and function in the regulation of synaptic morphology. Comprised of five members, SALMs 1-5, the structure of the SALMs include a single TM domain, leucine-rich repeats, an IgC2 domain, a FNIII domain, and a PDZ binding motif present in SALMs 1-3. SALM1 has been shown to function in neurite outgrowth, yet the roles of other SALMs in this phenomenon are unknown. We investigated the roles of the SALMs in neurite outgrowth by performing a detailed analysis of total outgrowth, process number, mean process length, and branches. Hippocampal cultures were transfected with SALM cDNA at DIV4 and analyzed 48 hours later. SALMs localize throughout the cell body and are concentrated at tips of neurites, growth cones, and cell junctions. SALMs 1-4 promote increases in total outgrowth and branches. Increases in branch number mediated by SALMs 1-3 are dependent on PDZ interactions. SALM2 promotes increases in axon number and branches, while SALM4 promotes increases dendrite numbers. Using deletion constructs, we are investigating how the various domains contribute to neurite outgrowth. **Poster 11**

Anthrax Lethal Toxin Induces IRF-1 Expression in Human Endothelium
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The interferon regulatory factor (IRF) family of transcription factors plays a central role in regulating innate and adaptive immune responses. IRF-1 is typically expressed in endothelial cells and other immune cells in response to pro-inflammatory cytokines such as interferon-γ and tumor necrosis factor-γ (TNFγ). We investigated the effect of anthrax lethal toxin (LT), a key virulence factor of *Bacillus anthracis*, on the expression of IRF-1 and VCAM-1, an IRF-1-regulated gene, in primary human endothelial cells. LT alone or in combination with TNFγ induced a concentration-dependent increase in IRF-1 mRNA that correlated with increased expression and nuclear localization of IRF-1 protein as determined by real-time PCR and western blot. LT-induced activation of IRF-1 led to minor increases in VCAM-1 mRNA and protein on unstimulated cells, and dramatically enhanced VCAM-1 expression on cytokine-stimulated cells as indicated by cell surface ELISA and immunofluorescence microscopy. Interestingly, LT did not activate NFγB or other transcription factors that possess binding sites in the enhancer region of the VCAM1 gene. Altering the expression of key transcription factors involved in host response to infection, such as IRF-1, may represent a novel mechanism by which LT contributes to anthrax pathogenesis. **Poster 78**

A Search for Genes that Regulate Nuclear Morphology in Budding Yeast
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Maintenance of nuclear morphology is crucial for cell function. However, the mechanism governing nuclear shape remains elusive. The nucleus of the budding yeast is round, while yeast cells lacking the *SPO7* gene (*spo7*D), a regulator of phospholipid biosynthesis, exhibit a single nuclear protrusion, referred to as a flare. Interestingly, flares in spo7D cells are limited to a nuclear region occupied by the nucleolus and are absent from nuclear regions containing the bulk of the DNA, which remains round and compact. This observation suggests the existence of a molecular connection inhibiting flare formation at the interface between DNA and nuclear membrane. To isolate genes responsible for such a molecular connection, we have taken a two pronged approach. First, we reasoned that inactivation of this molecular connection, in conjunction with elevated levels of membrane synthesis, will result in cell death due to severe alteration of nuclear structure. Thus we performed a screen for EMS generated mutations that lead to lethality when combined with *spo7*Δ. Second, we are carrying out a screen for peptides that, when over expressed, disrupt the molecular connection causing lethality in a *spo7*Δ background. Both of these methods should reveal mechanisms that regulate nuclear morphology in budding yeast. **Poster 9**

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