FIFTH ANNUAL NIH GRADUATE STUDENT RESEARCH SYMPOSIUM

THE FACES OF TOMORROW'S SCIENCE

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U.S. Department of Health & Human Services

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ABSTRACTS

Lactate Transport in the Human Retinal Pigment Epithelium
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The high glycolytic activity at the inner retina results in high lactic acid deposition at the subretinal space (SRS). The RPE transports excess lactate out of the SRS (to the choroid) with MCT1 (apical membrane) and MCT3 (basolateral membrane) to maintain the pH and osmotic condition necessary for optimal photoreceptor function. A recent study illustrated the importance of lactate transport in the RPE by showing that MCT3 knockout mice have altered visual function. H/Lac co-transport by MCT1 was inhibited by niflumic acid and pCMBS. Proton-entry via MCT1 activated the Na/H exchanger at the apical membrane, which alkalinized the cell to return the RPE to its resting pH. Perfusing lactate to the basolateral bath caused an alkalinization that can be reversed into an acidification by exposing the apical membrane to either niflumic acid or pCMBS. Functional interaction between MCT1, carbonic anhydrase II (CAII) and NBC1 (Na/HCO3 co-transporter) has been described. However, blocking NBC1 with DIDS at the apical membrane did not affect MCT1 activity. Inhibiting CAII with dorzolamide enhanced the pH and electrical responses to apical or basolateral lactate, which is indicative of an increased MCT1 and AE2 activity. Our data suggest that MCT1 do not functionally interact with NBC1 or CAII in the RPE. **Poster 2**

Investigating the mechanism of *fim* gene activation in *Bordetella pertussis*
Kimberly Baxter, Qing Chen, Scott Stibitz, Deborah Hinton

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Bordetella pertussis is a Gram-negative bacterium that infects the human respiratory tract and causes pertussis (whooping cough). Three virulence genes, *fim3*, *fim2*, and *fimX*, encode adhesive proteins that allow the bacterium to stick to epithelial cells. The *fim* genes are regulated by the *B.pertussis* transcription factor BvgA as well as by the length of poly-cytosine tracts within the *fim* promoters. However, the mechanism of this regulation is not understood. I determined the *in vivo* transcription start sites for *fim3*, *fim2*, and *fimX*. I also established an *in vitro* system using *E.coli* RNA polymerase, *B.pertussis* BvgA, and *fim* DNA that recapitulates the regulation of BvgA activation *in vivo*. Previous work by our collaborating lab found that one of the BvgA binding sites in *fim3* overlaps a promoter element typically bound by the RNA polymerase sigma subunit. This finding suggests that BvgA activation at *fim3* occurs through a mechanism that differs from the well-characterized prokaryotic activation systems. To investigate the role of sigma and BvgA at the *fim* promoters, I performed *in vitro* transcription experiments using wild type and mutated forms of polymerase. My results suggest that certain residues of sigma are required for BvgA activation, but not those residues typically implicated in transcription activation. **Poster 43**

Massively Parallel Sequencing of MicroRNAs in Neuroblastoma
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18285502, 18639376, 18392026

MicroRNAs (miRNAs) are an important class of gene expression regulators that play a critical role in cancer biology. With the advent of next-generation sequencing technologies, millions of miRNAs can be sequenced in parallel and molecule counts can be directly observed. We applied Illumina sequencing technology to characterize miRNA expression in Stage 4, MYCN-amplified Neuroblastomas, which are associated with very poor prognosis despite advances in treatment. Sequencing yielded 4.1 million high-quality reads. A custom analysis pipeline revealed the expression levels of 508 known mature miRNAs and a novel miRNA candidate gene. Two miRNAs, let-7f and let-7a, display extremely high expression, accounting for ~50 percent of the miRNA in the samples. These miRNAs have been linked to other cancers and are associated with important players in carcinogenesis. The expression of let-7f, let-7, and the novel miRNA are being confirmed by qRT-PCR. Enrichment analysis was performed on the predicted targets of the most highly expressed miRNAs. Cell differentiation, nervous system development, and transcriptional regulation are among the most significantly enriched biological processes. Our results give the most direct, comprehensive look at miRNA expression in Neuroblastoma to date. **Poster 20**

Revising the Heterochromatin-Mediated Repression of FXN Gene in Friedreich's Ataxia Patient Cell Lines

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Friedreich's ataxia (FRDA) is an early onset, and frequently fatal genetic disorder characterized by ataxia, diabetes and hypertrophic cardiomyopathy. It is caused by a GAA-repeat expansion in intron 1 of the FXN gene that decreases the yield of full-length FXN transcript. This results in a deficit of frataxin, a protein essential for mitochondrial function.

In the curreWe have previously shown that expanded alleles are hypermethylated and associated with marks of heterochromatin. To understand the effect of these epigenetic changes, we measured the levels of exon1 and 2 of the FXN gene in the presence and absence of 2 histone deacetylase inhibitors.

In the curreThe amount of exon 1 produced in the FRDA cell lines was similar to normal cells, while levels of exon 2 were significantly lower. This suggests that repeat expansion affects transcription elongation not initiation. Splitomicin, an inhibitor of Class III histone deacetylases, affected both initiation and elongation. In contrast, a previously studied compound, IVB, that inhibits Class I, II, and IV histone deacetylases, had much smaller effect that was limited to an effect on transcription initiation. Our results suggest that Class III histone deacetylases, in particular, may be useful therapeutic targets for treatment of FRDA. **Poster 44**

Genome-Wide Interactions of the Glucocorticoid Receptor with the Chromatin Landscape

Simon C. Biddie, Sam John, Myong-Hee Sung, Thomas A. Johnson, Peter J. Sabo, Robert Thurman, John A. Stamatoyannopoulos, Gordon L. Hager

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Transcription factor (TF) interactions with DNA occur in the context of an organized chromatin landscape. The glucocorticoid receptor (GR), part of the nuclear receptor family of transcription factors, activates or represses genes in a hormone-dependent fashion. GR is known to induce local chromatin remodeling at model genes. Using massively parallel signature sequencing, we characterized genome-wide occupancy of GR binding events (ChIP-Seq) and measured changes in local chromatin transitions using DNaseI as a probe for chromatin accessibility (DNase-Seq). Genome-wide analysis in multiple cell lines, a mammary adenocarcincoma cell line and pituitary corticotroph cell line, demonstrate that GR invariably binds to regions of accessible chromatin. GR binding occurs at constitutive or GR-mediated local chromatin transitions. We also observe that these events are concordant with cell-specific DNaseI hypersensitive sites. The cell-specific conjunction of binding events with chromatin transitions reveals an important mechanism for cell-specific expression of GR responsive genes. Computational and bioinformatic analysis of GR binding events in multiple cell lines will be discussed. We propose that GR binding at sites local chromatin transitions are common mechanisms for all GR interactions with chromatin *in vivo*. **Poster 21**

Visible and Invisible: NMR Studies on the Exchange of Human α-Synuclein on the Phospholipid Membrane

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In dopaminergic neurons, α-synuclein (αS) partitions between a disordered cytosolic state and a vesicle-bound conformation. Membrane association of αS likely mediates its role in synaptic regulation but is also implicated in the amyloid pathways of Parkinson's disease. We use solution NMR to spy on the "invisible" membrane-bound conformation of αS via its readily observed unfolded state. In the presence of lipid vesicles, αS signal is attenuated proportional to its membrane partitioning. The exchange rate is slow on the NMR timescale, and relaxation data reveals the binding kinetics. Membrane affinity of αS varies with marked sequence dependence: greatest effect is noted for the N-terminal 25 residues, and weak interactions persist even for the C-terminal tail widely reported not to associate with the membrane. The profile shows stretches of residues that bind in tandem, identifying four distinct bound species of αS. NOE measurements reveal extensive *i* to *i* ± *n* crosspeaks, evidencing a large molecular weight (>150 kDa) helical aggregate in exchange with the visible monomer. Paramagnetic relaxation studies are consistent with transmembrane orientation of the helices. Our conditions do not lead to fibrilization, and we believe our model is relevant to the native role of αS at the pre-synaptic termini. **Poster 69**

The Role of SMN Regulation in Muscle as it Applies to Spinal Muscular Atrophy
Katherine Bricceno, Barrington Burnett, Terence Partridge and Kenneth Fischbeck

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Spinal muscular atrophy (SMA) is a progressive neuromuscular disease affecting the anterior horn cells of the spinal cord. SMA is caused by a mutation in the survival of motor neuron (SMN) gene which leaves patients deficient in the ubiquitously expressed SMN protein. While SMN is known to function in snRNP assembly, mRNA processing and neurite outgrowth, it remains unknown how the deficiency of SMN results in SMA. Motor neuron degeneration is the hallmark of SMA disease pathology, but the role of tissue-specific defects cannot be excluded. It remains unclear if the SMA phenotype is due mainly to SMN deficits in motor neurons, in muscles and peripheral tissues or in a combination of these tissues. Recent research has found defects the neuromuscular junctions of SMA mice. However, muscle weakness preceded the cellular defects. This inconsistency hints at an intrinsic muscle defect. Previous work showed SMN-deficient myoblasts have defects in their development to myotubes. Presently, we are investigating the role of SMN in the differentiation of proliferating muscle cells to myotubes. Understanding the regulation of SMN in muscle and the effect of this regulation will allow us to better characterize the role of muscle in the SMA phenotype and elucidate parallel roles in other tissues involved in SMA. **Poster 83**

Developmental consequences of maternal immune activation in the rodent
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Maternal infection during pregnancy is associated with neurodevelopmental psychiatric disorders, including autism and schizophrenia. This association is thought to be mediated by the maternal immune response characterized by the production of proinflammatory cytokines within the maternal-fetal unit. Although cytokines were first described in terms of their role in the inflammatory response, there is evidence that they influence normal, central nervous system (CNS) developmental processes, such as glial and neuronal commitment, survival, proliferation and differentiation, axodendritic outgrowth, synaptogenesis, and neurotransmitter function. It is therefore possible that altered cytokine expression in response to maternal immune activation could have deleterious effects on brain and subsequent behavioral development through their ability to directly influence specific neurodevelopmental processes, or more likely by altering the expression of genes known to regulate CNS development.

In the current study, I investigated the hypothesis that maternal exposure to the bacterial endotoxin lipopolysaccharide (LPS), which stimulates a strong innate immune response, will change the postnatal (PN) developmental profiles of social and exploration behaviors of offspring born to these dams, which were evaluated on PN days 25, 30, 35, 45, and 55. Offspring born to LPS-exposed dams displayed a significant reduction in social and exploration behaviors across development. The mechanisms by which maternal LPS alters the behavioral trajectory of offspring is currently under investigation. **Poster 52**

Structure and Function of the ORF1p from the LINE-1 Retrotransposon
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L1 is an autonomous retrotransposon that copies its RNA transcripts, as well as those from SINEs and nuclear genes, into genomic DNA. Evolutionary and population genetic studies showed that at times L1 has been deleterious enough to be subjected to negative selection. Nonetheless, L1 has thrived in mammalian genomes generating approximately 40 percent of mammalian DNA. Despite the profound affect of L1 on mammalian hosts, little is known about many aspects of L1 biology. How L1 activity is regulated, the biochemical steps involved in L1 replication, and the interactions of L1 with its host are largely unknown.

In the curreAn L1 element contains two open reading frames. The second open reading frame (ORF2) encodes for a 149kDa protein with endonuclease and reverse transcriptase activity. The first open reading frame (ORF1) encodes a 40kDa protein that contains a coiled coil domain thought to mediate homo-trimer formation between ORF1p monomers. 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 1**

Control of Cytoskeletal Dynamics by Cell Fate Specification Pathways
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Gastrulation is the period of morphogenesis when the three germ layers are correctly positioned in the embryo. The genetics and cell lineages of *C. elegans* are well established, and its transparent body is easy to image, making it an ideal model to study gastrulation at the cellular level. In *C. elegans* the two endoderm precursor cells (E cells) move from the outside of the embryo to the inside, where they will form the intestine. Myosin is recruited to the apical surface of the E cells, where its light chain is phosphorylated, causing apical constriction of the actin cytoskeleton. Six surrounding cells fill the gap left behind by the ingressing E cells. Three of the six surrounding cells form dynamic Arp2/3-dependent actin-rich protrusions on their apical surfaces. These three cells are all descendents of a mesodermal (MS) precursor cell. We are using a combination of genetics, live cell imaging and fixed cell imaging to test which cell fates are necessary for these protrusions to form. Our goal is to identify links between cell fate specification and the organization of the cytoskeleton. **Poster 77**

Neurochemical Identity Governs Cholinergic Phenotype Across Hippocampal Basket Cell Networks
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9310461; 17920013

Basket cells (BCs), by releasing GABA perisomatically onto pyramidal cells, synchronize pyramidal cells during gamma (40 Hz) oscillations. Two major BC subtypes are present in the hippocampus, cholecystokinin (CCK) and parvalbumin (PV) BCs. These two inhibitory networks are thought to play different roles in the hippocampus; PV BCs provide rigid precision clockwork whereas CCK BCs are tuned by neuromodulatory input conveying mood. In neocortex, CCK and PV BCs possess distinct cholinergic phenotypes; however, cholinergic neuromodulation of hippocampal CCK and PV BCs is poorly understood. To examine differential cholinergic modulation in BC subtypes, we performed whole-cell recordings in hippocampal slices from GAD65-GFP or PV-GFP transgenic mice, morphologically identifying CCK and PV BCs, respectively. We conclude that CCK and PV BCs are both highly responsive to cholinergic neuromodulation. Each BC subtype possesses distinct cholinergic neuromodulatory specializations capable of being tuned by neuromodulatory input. **Poster 53**

Exploring the Role of Cis-Elements in the Partitioning of Human Papillomavirus Genomes
Sandra Chapman, Alison McBride

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"High-risk" Human papillomavirus (HPV) infection is associated with malignant progresion and is responsible for virtually all cases of cervical cancer. The virus infects the highly proliferative cells of the basal epithelial layer and the viral genome is maintained at a constant copy number for many cell divisions. Thus, the virus has developed a mechanism to allow maintenance and partitioning of its genome during host cell division. Therefore it is valuable to identify the mechanism by which the HPV genome is able to persist in the infected cell through multiple rounds of cell division. The HPV genome contains many elements that control viral transcription and replication, but it is not known whether these elements play a role in genome maintenance. These elements are required for transcription of the viral genes necessary for genome replication and so it has been difficult to distinguish the effect of these multi-functional cis-elements between transcription, replication and genome maintenance. To circumvent this, we have developed a very efficient complementation assay in primary human keratinocytes whereby subgenomic fragments or genomes mutated in specific cis-elements are transfected together with wild-type genomes to express the necessary viral proteins for replication. Identification and characterization of these essential elements will allow us to develop anti-viral strategies to disrupt persistent viral infection before they progress to malignancy. **Poster 29**

Exploring Gene-Gene Interactions in a Genome Wide Association Study of Prostate Cancer: Novel Methods and Implications

Julia Ciampa, Kevin Jacobs, Merideth Yeager, Peter Kraft, Sholom Wacholder, Kai Yu, Laufey Amundadottir, David Hunter, Robert Hoover, Gilles Thomas, Stephen Chanock, Nilanjan Chatterjee

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BACKGROUND: Single nucleotide polymorphisms (SNPs) that recent genome wide association studies link to prostate cancer may affect disease risk through genetic interactions.

METHODS: Data are from the ~4000 cases and ~4000 controls of Cancer Genetics Markers of Susceptibility (CGEMS) follow-up I. They were analyzed for multiplicative interactions between each of ~27000 SNPs with main effect $p<0.05$ in the initial CGEMS scan and SNP(s) in each of 9 established susceptibility regions. We used standard logistic regression and methods that can gain power by exploiting an assumption of gene-gene independence in the underlying population.

RESULTS: Q-Q plots suggest methods that rely on gene-gene independence inflate type-I error even when loci under study are on separate chromosomes. A novel empirical-Bayes (EB) method that exploits the independence assumption in a data adaptive fashion was more robust. EB analyses identified 11-35 SNPs with interaction $p<0.001$ for each susceptibility region. No SNP reached genome-wide significance (alpha=1.85E-6), but several top SNPs are noteworthy for their biology.

CONCLUSION: Our study provides empirical evidence of bias in analyses that assume gene-gene independence in the underlying population. Our EB analysis identifies novel SNPs to follow-up for genetic interactions. **Poster 22**

Ferrochelatase Deficiency Contributes to Development of Erythropoietic Protoporphyria in Mice Lacking Iron Regulatory Protein 2

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15831703

Iron-regulatory proteins (IRP) 1 and 2 regulate protein expression by binding to iron-responsive elements present in the mRNA transcripts of genes important for iron metabolism such as ferritin and transferrin receptor. We previously demonstrated that mice lacking IRP2 (IRP2-/-) exhibit an atypical anemia, characterized by a low hematocrit, microcytosis, and elevated RBC protoporphyrin IX levels, but with normal serum transferrin saturation and increased serum ferritin levels. To investigate the cause of elevated protoporphyrin IX levels in the blood of IRP2-/- mice, we developed an assay to measure activity of ferrochelatase, the terminal heme biosynthetic enzyme, in tissues of IRP2-/- mice. Ferrochelatase activity in bone marrow and spleens of IRP2-/- mice was significantly decreased to 59 percent and 50 percent of control, and was paralleled by a decrease in ferrochelatase protein levels. Although ferrochelatase protein levels were decreased in spleen, ferrochelatase mRNA levels were increased, suggesting a post-transcriptional regulatory mechanism involving either diminished protein synthesis or enhanced protein degradation. Current in-vitro studies in our lab using mouse erythroleukemia cells as are directed at determining the molecular mechanism of ferrochelatase regulation during iron-deficient erythropoiesis. **Poster 3**

How Yeast Resumes the Cell Cycle upon Return to Growth from Meiosis
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We are studying a unique feature of S. cerevisiae, namely the ability return to growth when meiotic cells are transferred to vegetative growth media. The return to growth assay is done with cells lacking Ndt80, a transcription factor that is necessary for the exit from the pachytene stage of meiosis. Cells lacking Ndt80 arrest with fully replicated but unsegregated chromosomes. Homologues (parental homologous chromosomes) are tightly paired by the synaptonemal complex, and are linked at the DNA level by unresolved double Holliday junctions.

We find that upon return to growth, ndt80Δ cells retain high viability and abandon all meiotic features. Double strand breaks are rapidly repaired and synaptonemal complex rapidly breaks down. In addition, double Holliday junctions are completely resolved by 2.5 hours, releasing connections between homologues and thus preparing the cells for mitotic division. In contrast to meiosis, where double Holliday junctions are mostly resolved as crossovers, double Holliday junctions are resolved in favor of noncrossovers, further reducing the potential for connection between homologues. At this time, daughter cell bud emergence occurs, followed by a mitosis-like nuclear division that produce two diploid cells. Unlike in mitosis however, bud emergence and growth are not accompanied by chromosome replication.

The unique phenotypes that are seen upon return to growth, suggest a well-established program that provides rapid adaptation of the cells. This adaptation ensures high viability of the cells and genome stability. **Poster 45**

Role of microRNA 135 (miR135) in the Development of Hippocampal Neurons
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1. Ornitología Neotropical 15 (suppl.). 215-222. 2004
2. PMID:18584181

MicroRNAs are small RNAs (21-25 nucleotides) that interact with specific binding sites at their mRNA targets to trigger the degradation of mRNA or inhibit the translation. MicroRNAs have emerged as important molecules for gene expression regulation in various cellular functions. We investigated the role of microRNA 135 (miR135) in neurons, considering that many of its predicted targets are related with synaptic function or neuronal development. Here we show that overexpression of miR135 in cultured hippocampal neurons decreases the levels of its target proteins complexin (a key protein in synaptic vesicle release) and rap2 (a small GTPase which has been shown to control axonal growth). Morphological analyses show that miR135 overexpression increases axonal length. However this overgrowth is inhibited when miR135 is cotransfected with either complexin or rap2. In addition, the expression of miR135 and its target proteins is inversely correlated during the development of hippocampal neurons. Our data suggest that miR135 plays an important role in the early development of neurons by controlling the expression of complexin and rap2. **Poster 54**

Functional Characterization of Human and Mouse Sphingosine Kinase 1 and 2 Using Short Hairpin RNA
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Sphingosine-1-phosphate (S1P) is a lipid second messenger and an autocrine/paracrine mediator generated by two sphingosine kinase isoforms: sphingosine kinase 1 and 2 (SphK1 and SphK2). Mast cells (MC) react to specific antigens (Ag) to release a variety of mediators involved in the allergic response. Upon MC activation by Ag, SphK1 and SphK2 are activated and produce S1P. Studies using mouse models with genetic deletions in SphK1 or SphK2 have shown that SphK2 is critical in influencing MC degranulation, cytokine production, and calcium responses, but showed no role for SphK1. However, studies using siRNA for SphK1 and SphK2 in cord blood derived human MC have shown a predominant role of SphK1 in the degranulation response and chemotaxis towards antigen, while SphK2 is important for cytokine production.

Short hairpin RNA (shRNA) will be used as an alternative approach to knockdown expression of SphK1 and SphK2 in $CD34^+$ human MC and bone-marrow derived mouse MC to determine whether functional differences between these kinases in mouse versus human MC are attributable to the type of MC population, the experimental conditions, or the species of origin. In addition, a genetic analysis of the cells' genome will be done to find any variations that may be caused by the loss of SphK gene expression. **Poster 30**

An important role for STAT3 in T cell-dependent colitis

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1. PMID: 11588011
2. PMID: 16648838
3. PMID: 17363300

The STAT family of transcription factors regulate the cytokine signals necessary for driving T cell differentiation, proliferation and survival. STAT3 in particular promotes the differentiation of T helper 17 (Th17) cells, which are important for defense against extracellular bacteria and fungal infections1. Conversely, Th17 cells have also been implicated in several diseases such as RA, MS, psoriasis, uveitis, and the human IBDs. *In vitro*, the conditions that promote murine Th17 differentiation inhibit the differentiation of regulatory T cells (Tregs), which suppress inflammation2. In the absence of STAT3; however, there is induction of Tregs under Th17 conditions3, suggesting that manipulation of STAT3 in T cells may alter inflammatory disease *in vivo*. These studies aim to dissect how STAT3 regulates mouse T cell responses in the intestine during both homeostasis and chronic inflammation. We have found that STAT3-deficient naïve T cells are non-pathogenic when transferred into a Rag-/- host in a model of chronic colitis. This is associated with both a reduced expansion of T cells and elevated numbers of Treg cells within the colonic lamina propria. Future studies will aim to deduce the mechanism of STAT3-dependent pathology in colitis and how STAT3 is involved in protective immunity in the intestine. **Poster 31**

Prion and Amyloid Formation by *Candida glabrata and Candida albicans* Ure2 Proteins

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Saccharomyces cerevisiae has three established prions: [PIN+], [PSI+], and [URE3], each a self-propagating amyloid. [URE3] is the prion form of Ure2 protein, an important regulator of nitrogen catabolism. The N-terminus of Ure2p has been shown to be both necessary and sufficient for forming the prion [URE3], and so is referred to as the prion domain (PD). The amino acid sequence of Ure2p PD can be shuffled and still form prions, showing that amino acid content, not sequence, is critical for prion formation. One reason for the prion forming ability could be the Q/N richness of the Ure2p PD. The Ure2 protein is found in several yeast species with the greatest homology in the C-terminal nitrogen regulation domain. There is a high degree of variability in the N-terminus, but some of the homologues are Q/N rich. *Candida albicans* and *C. glabrata* Ure2 proteins were found to complement *S. cerevisiae* Ure2p. Preliminary data show *C. albicans* Ure2 is able to form prions, while *C. glabrata* Ure2 does not. We have found both that *C. albicans* and *C. glabrata* Ure2 N-terminal regions can form filaments, though *C. glabrata* Ure2p is less prone to form amyloid. We hypothesize that the difference between the ability of *C. albicans* and *C. glabrata* to form prions is due to structural variation in the amyloid of the proteins. **Poster 4**

Mood-congruent Attentional Biases in Amygdala Response to Masked Sad and Happy Faces Resolve with Antidepressant Treatment in Major Depressive Disorder

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The current fMRI study investigated amygdala responses to emotional faces presented outside explicit conscious awareness in healthy controls (HC), currently depressed (dMDD) and remitted (rMDD) subjects with MDD. 25 HC, 22 dMDD and 16 rMDD subjects viewed faces displaying sad, happy, or neutral expressions during a novel backward masking task. Ten dMDD subjects completed the task before and after sertraline antidepressant treatment. Prior to scanning, subjects were shown two target faces and instructed to respond based on the presence of a target. For each item, a "masked" face was displayed for 27ms and followed by a "masking" face for 107ms. EPI images were acquired on a GE 3T scanner. ANOVA analyses revealed an interaction between trial type (SN-NN, HN-NN) and group in the amygdala ($p<0.05$). T-tests for the SN-HN comparison showed greater amygdala activity in dMDD vs. HC ($p<0.01$) and rMDD vs. HC ($p<0.01$). T-tests indicated for SN-NN, the amygdala response was greater in dMDD vs. HC ($p<0.01$). In contrast, for HN-NN, amygdala activity was greater in HC vs. dMDD ($p<0.05$). Paired t-tests showed the amygdala response for SN-NN was greater in MDD subjects in the pre vs. post treatment scans ($p<0.05$). In addition, MDD subjects showed a greater response to HN-NN following treatment ($p<0.05$). **Poster 55**

Effect of Aerobic Exercise on Testosterone and Sex Hormone Binding Globulin in Breast Cancer Survivors: A Randomized Controlled Trial

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High levels of testosterone (T) and low levels of sex hormone binding globulin (SHBG) are common in obese postmenopausal women, are associated with an increased breast cancer risk, and may be associated with poor prognosis in breast cancer survivors. No published study has examined the impact of physical activity on T and SHBG in breast cancer survivors. We examined the effects of a 6-month controlled aerobic exercise intervention vs. usual care on T and SHBG concentrations in 75 postmenopausal breast cancer survivors who completed chemotherapy and/or radiation therapy. Baseline and 6–month serum hormones were assayed simultaneously at the study's end. After 6 months, exercisers experienced a greater increase in SHBG (+0.26 nmol/L) than usual care participants who experienced a decrease (-13.55 nmol/L) (p=0.04). Exercisers had no change in total T (+0.03 ng/mL), but usual care participants experienced a slight increase (+0.40 ng/mL), though this difference was not statistically significant. Total T decreased among adherent exercisers (-0.06 ng/mL) and increased among nonadherent exercisers (+0.14 ng/mL) (p=0.02). Moderate-intensity aerobic exercise produces favorable changes in testosterone and SHBG.

Sex hormones may partly explain the observed association between physical activity and survival in breast cancer survivors. **Poster 46**

Investigating Signaling Complexes Downstream of SLAM Family Receptors

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The SLAM family of immunoregulatory receptors consists of CD150, CD229 (Ly9), CD244 (2B4), CD84, NTBA (Ly108) and CD319 (CRACC), which are expressed on immune cells. Most of these receptors are self-ligands. SLAM receptors have cytoplasmic ITSMs (Intracellular tyrosine based switch motifs), which bind the adaptor SAP. SAP recruits and activates the FynT kinase, resulting in receptor phosphorylation and downstream signaling. Defective SAP expression is associated with X-linked lymphoproliferative disease, characterized by dysregulated responses to EBV, lymphoproliferative disorders, defective germinal center formation and hypogammaglobulinemia. We wish to dissect signaling downstream of Ly108 and CD84, which are highly expressed on thymocytes and the Tfh subset, which is important for humoral responses. We have found that CD84 affects germinal center formation and that CD84 and Ly108 participate in T:B cell interactions. Differential expression of Ly108 isoforms is associated with lupus susceptibility in mice. We have generated Tandem affinity purification (TAP) constructs of 3 Ly108 isoforms and CD84 for affinity purification of protein complexes. We have generated stable clones expressing Ly108 in EL-4 mouse thymoma cells and are optimizing stimulation protocols and purification methods using these clones. **Poster 32**

A Prospective Study of One-Carbon Metabolism Biomarkers and Risk of Renal Cell Carcinoma

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No studies have examined biomarkers for nutrients involved in one-carbon metabolism and risk of renal cell carcinoma (RCC). We conducted a nested case-control study within the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study, a prospective study of Finnish male smokers aged 50-69. Prediagnostic folate, vitamin B_6, vitamin B_{12}, cysteine, riboflavin and homocysteine concentrations were measured in fasting serum from 224 incident RCC cases and 224 controls (matched on age and date of serum collection). Conditional logistic regression was used to calculate odds ratios (ORs) and 95 percent confidence intervals (CIs), adjusted for smoking, body mass index, leisure physical activity, and dietary intake of fat, protein, methionine, and energy. Serum folate tended to be inversely associated with RCC (highest vs. lowest quartile, OR=0.67, 95 percent CI 0.37-1.20, P-trend=0.19). When modeled as a threshold effect, subjects with folate status below deficiency (≤3 ng/ml) had a significantly increased RCC risk (OR=1.68, 95 percent CI 1.06-2.65). The other one-carbon metabolism biomarkers were not associated with RCC. This study in male smokers suggests that inadequate folate status may be associated with an increased risk of RCC, and further exploration is warranted in other epidemiologic studies that include women and non-smokers. **Poster 23**

Dissociable Effects of Prefrontal and Anterior Temporal Cortical Lesions on Stereotypical Gender Attitudes.

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A previous study in 7 patients with ventral prefrontal lesions has shown abnormalities in social attitudes using a well-established measure of gender stereotypes, the Implicit Association Test (IAT). Here, we investigated the differential effects of ventromedial prefrontal (vmPFC) and ventrolateral prefrontal (vlPFC) cortical lesions in a larger sample of 154 patients. In addition, we investigated the role of the superior anterior temporal lobe (aTL), recently shown to represent conceptual social knowledge. First, we used a linear regression model to determine the association between performance on the IAT and extent of damage in each brain region. We found that larger lesions in either the vmPFC or the superior aTL were associated with *increased* stereotypical attitudes, whereas larger lesions in the vlPFC were associated with *decreased* stereotypical attitudes. Second, we grouped patients by lesion location and compared their performance on the IAT with that of healthy volunteers. Compared to controls, patients with lesions in either the vmPFC or the superior aTL showed *increased* stereotypical attitudes, whereas patients with lesions in the vlPFC showed *decreased* stereotypical attitudes. The functional contributions of these regions in social attitudes are discussed. **Poster 56**

Functional Comparisons Between Human and Avian Influenza Virus PB2 Proteins

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The first recorded cases of humans infected with an H5N1 avian influenza A virus occurred in 1997 and since then more than 300 human infections have been confirmed, and more than 60 percent were fatal. How these viruses were able to infect humans and cause severe disease remains unknown. One factor that has been determined to play a role in the viruses' ability to overcome host-range restriction and be virulent in animal models is the viral PB2 polymerase subunit, however the mechanism remains unknown. Previous work has shown that the PB2 proteins from two human influenza viruses localize to the mitochondria as well as the nucleus of the host cell. Here we demonstrate that the PB2 proteins from avian and human influenza viruses differ in their ability to localize to the mitochondria and that this localization is determined by amino acid nine of the PB2 protein. An alignment of PB2 protein sequences shows a conserved difference between avian and human influenza viruses at this position, suggesting a link between mitochondrial localization and the host-range of these viruses. Immunoprecipitation assays have revealed an interaction between the PB2 protein and the mitochondrial type I interferon inducer, MAVS, thereby suggesting a role of the mitochondrial PB2 protein in the regulation of interferon expression. **Poster 33**

Dendritic Integration and Reciprocal Inhibition in the Retina
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The mammalian retina is capable of signaling over a vast range of mean light levels (~10^{10}). Such a large dynamic range is achieved by segregating signals into contrasting pathways and utilizing excitatory and inhibitory neural circuits. The goal of this study was to elucidate subcellular mechanisms responsible for shaping dendritic computation and reciprocal inhibition within the retinal circuitry.

Amacrine cells make up a unique class of inhibitory interneurons which lack anatomically distinct input and output structures. Although these interneurons clearly play important roles in complex visual processing, there is relatively little known about the ~30 subtypes. A17 amacrine cells have been shown to shape the time course of visual signaling in vivo. Intuition might suggest that a wide field (~400 µm) interneuron, such as A17, would provide long range lateral inhibition or center surround inhibition. However, using multi-discipline approaches, we have uncovered multiple mechanisms which underlie dendritic integration and synaptic transmission in A17 that allow it to respond with a high degree of synapse specificity. Additionally, these mechanisms work in concert with post-synaptic mechanisms to extend the dynamic range of reciprocal inhibition in the inner retina. **Poster 57**

Fluorescence Activated Cell Sorting: A Novel Method to Study Neurons Selectively Activated During Context-Specific Cocaine Sensitization
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We hypothesize that learned associations between specific stimuli are encoded in a pattern of sparsely distributed neurons called "neuronal ensembles". In context-specific locomotor sensitization, rats learn to associate cocaine stimuli with stimuli in the drug administration environment. When cocaine-activated neuronal ensembles in nucleus accumbens are specifically lesioned, rats no longer express the learned response to cocaine. Therefore, the cells that make up these neuronal ensembles are important for behavior.

We are developing a method for identifying neuroadaptations in these cells using c-fos-lacZ transgenic rats. Electrical activation of neurons turns on the c-fos promoter, leading to transcription of the lacZ gene and labeling of the activated cells with f3-galactosidase. Fluorescence Activated Cell Sorting (FACS) is then used to purify the highly activated cells expressing f3-gal. We have separated f3-gal-positive from -negative neurons and extracted mRNA from sorted cells. We are currently using quantitative PCR to characterize the different molecular changes occurring in these cells. For the first time, we can examine the unique molecular neuroadaptations in selectively activated neuronal ensembles that mediate learned associations. **Poster 58**

Initial Characterization of *Drosophila Melanogaster* Myosin XVIII
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11206129, 12082124, 10733906

Myosins are molecular motors that move along actin filaments within a cell by means of ATP hydrolysis. A wide range of physiological processes rely on the activity of myosins including cytokinesis, cell migration, vesicular trafficking, development and muscle contraction. To date, over 35 classes of myosins have been identified according to sequence similarity.

Little is currently known about the class XVIII myosins other than the presence of two isoforms (A and B) in vertebrate macrophages. However, research has suggested that the human myosin XVIIIB isoform may be critical in tumor suppression, while XVIIIA seems to require domains in addition to its motor domain to bind with actin. Myosin XVIII from Drosophila melanogaster has not yet been studied and was originally identified through sequence analyses of the drosophila genome.

The goal of this research is to characterize the cell biological and biochemical properties of the drosophila myosin XVIII isoforms. Sequence analysis reveals six alternatively spliced isoforms that can each be isolated from cDNA pools at different developmental stages. FISH data suggests that the isoforms may play a role in immunity, which is reminiscent of the other studied class XVIII myosins. Cellular localization of the longest isoforms as well as in vitro kinetic characterization of truncated constructs is currently being performed. **Poster 5**

Abnormal Formation and Remodeling of Type I Collagen Homotrimers Fibrils

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Insufficient or abnormal synthesis of the α2(I) chain results in replacement of normal type I collagen by α1(I) homotrimers in various pathological conditions, e.g., cancer, glomerular sclerosis, and several forms of Osteogenesis Imperfecta. To understand how α1(I) homotrimers may contribute to pathology, we investigated murine and human homotrimers and their mixtures with the corresponding heterotrimers. *In vitro*, type I collagen fibrillogenesis revealed distinctive nucleation mechanism and fibril morphology of α1(I) homotrimers, and the segregation of homo- and heterotrimers at a subfibrillar level [1]. Treatment of homo- and heterotrimer fibrils with rhMMP-1 revealed preferential heterotrimer degradation and cleavage-resistant homotrimer fibrils. More interestingly, rhMMP-1 showed the same binding affinity to homo- and heterotrimers, but the difficult opening of homotrimer triple helix necessary for presenting the unwound chains to the catalytic site. In mixtures, rhMMP-1 degraded the normal type I heterotrimers before noticeable cleavage of the homotrimers. We hypothesize that the subfibrillar segregation of homotrimers and selective proteolytic degradation of the heterotrimers may alter tissue remodeling, resulting in accumulation of MMP-resistant homotrimer fibrils. **Poster 70**

Caregiving and Serotonin Transporter (*5-HTT*) Gene Variation Interact to Influence Anxiety-like Behaviors in Rhesus Macaque Neonates (*Macaca mulatta*)

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Children with inhibited temperaments or a history of unresponsive caregiving are at risk for developing anxiety disorders. Mediation of anxiety-like behaviors by caregiving has been demonstrated in rodents. The current study examines caregiving and serotonin transporter gene polymorphism (rh*5-HTT*LPR) influences on anxiety-like behavior in neonatal rhesus macaques. Using median scores, infants were identified as receiving high or low ventral contact/nursing (VCN) and grooming (GR). Infants were genotyped for rh*5-HTT*LPR variation from whole blood. 84 infants were classified into VCN (high: 30 *l/l*, 14 *l/s*; low: 14 *l/l*, 28 *l/s*) and GR (high: 23 *l/l*,19 *l/s*; low:19 *l/l*, 23 *l/s*) groups. Factorial ANOVAs on a number of anxiety-like behaviors revealed a genotype x caregiving interaction for vocalizations ($F(1,92)=4.392$, $p<.05$, partial $\eta^2=.044$), where low VCN *l/s* vocalize less than *l/l* infants ($p<.05$). A main effect of genotype was identified for motion ($F(1,92)=5.958$, $p<.05$, partial $\eta^2=.073$) such that *l/s* display less activity than *l/l* subjects. Main effects of GR revealed that low GR infants display less motion ($F(1,92)=10.998$, $p<.001$, partial $\eta^2=.222$) and greater passivity ($F(1,92)=4.985$, $p<.05$, partial $\eta^2=.047$). This study indicates a role for caregiving and genetics on early anxiety-like behaviors. **Poster 47**

The Putative Dopamine D3 Receptor Antagonist PG-01037 Inhibits the Rewarding Efficacy of Methamphetamine: Examining the Progressive-Ratio Reinforcement Schedules and Brain Stimulation Reward

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Methamphetamine (METH) is a potent psychomotor stimulant and a major drug of abuse worldwide. There are no effective medications available for the treatment of METH addiction. Like other drugs of abuse, METH produces strong rewarding effects by elevating extracellular dopamine (DA) in the brain reward-circuit. Previous research indicates that acute administration of selective DA D3 receptor antagonists significantly inhibits cocaine self-administration under a progressive-ratio (PR) reinforcement schedule. There is no published data on DA D3 antagonist efficacy against the more potent and addictive METH. The PR reinforcement model imposes incrementally increasing work demand in order to receive a single infusion of drug; It is considered an index of the appetitive value of a reinforcer. The present investigation examined the effects of PG-01037, a novel D3 receptor antagonist, on METH self-administration under a PR reinforcement schedule and METH-enhanced BSR. Acute administration of PG01037 (3, 10, 30 mg/kg i.p) dose-dependently lowered (10, 60, 70 percent, respectively) the break-point for METH self-administration under a PR schedule of reinforcement but had no effect on sucrose self administration. These findings suggest D3 antagonism may be effective in attenuating the rewarding effects drugs of abuse such as METH without affecting natural rewards. Furthermore, PG-01037 (10, 30 mg/kg, i.p.) dose dependently attenuated METH-enhanced brain stimulation reward thresholds. However, the high dose of PG-01037 (30 mg/kg) alone resulted in a significant inhibition of BSR, indicating an aversive like effect of the drug. The mechanism underlying the aversive-like effects produced by this high dose remains to be determined, but one possible explanation is that PG-01037 is no longer highly selective for D_3 receptors at that dose and may be affecting D_2 or 5HT receptors as well. Whatever the underlying mechanism, we caution that these inhibitory effects on BSR shown by the high-dose PG-01037 alone may translate into dysphoric effects in humans. If true, PG-01037's anti-methamphetamine clinical utility may be dose-limited. **Poster 59**

Early Determinants of Endogenous Hormones in Migrant Populations

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Lifetime exposure to endogenous hormones is linked to breast cancer. Current research looks at prenatal exposure to hormones as an early life factor of cancer risk. However, evidence from Bangladeshi women who migrated to the UK suggests that there is a postnatal developmental component to variation in adult hormone levels that occurs before puberty. Little research has looked at adrenarche (puberty of the adrenal gland) and its role in reproductive hormones. This cross-sectional project will explore adrenarche as a critical period for setting adult hormone levels. Healthy girls (n=490) aged 5-16 will be recruited from 3 populations: 1) Bangladeshi residents, 2) Bangladeshi migrants to Britain, and 3) British residents in similar communities. The study will assess androgen levels in relation to chronological age, age at migration, and time since migration. To determine populational differences in timing of adrenarche, effects of growth rate, body shape, diet and exercise will be explored. We hypothesize that androgens will be lower and rise at a later age in Bangladeshi residents and older migrant girls due to immunological stressors experienced in Bangladesh. Study results could have public health implications for migrant populations concerning earlier puberty and elevated breast cancer risk. **Poster 81**

The Role of the Viral Polymerase Subunit PB1 in the Evolution of Influenza Pandemics
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Influenza A virus (IAV) pandemics cause considerable morbidity and mortality. IAV has a stable natural reservoir in waterfowl, and reassortment of genome segments between human and avian IAVs has resulted in the generation of pandemic viruses. Previous analyses of pandemic IAVs have shown that the polymerase basic protein-1 (PB1) has been avian-derived in each of the last three pandemics. Further, while PB1 exists in evolutionary stasis in the avian host, it is subject to positive selection and consequently accumulates nonsynonymous mutations during human circulation. These observations suggest a role for PB1 in determining the fitness of pandemic viruses. Thus, avian and pandemic PB1s may possess enhanced enzymatic activity compared with human interpandemic PB1s.

We determined the relative activities of avian, interpandemic, and pandemic PB1s isolated between 1940 and 1968, using a transcriptional activity assay. When expressed in the context of an interpandemic IAV ribonucleoprotein complex, avian and pandemic PB1s demonstrated two- to fourfold greater activity than interpandemic PB1s. Efforts to confirm this with reverse genetics are ongoing. These findings significantly inform our understanding of the forces contributing to the rise of pandemic influenza. **Poster 34**

Identification and Characterization of Novel Binding sites in mutants of ClpX in Escherichia coli
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Controlled degradation of cytoplasmic proteins is a universally conserved activity that serves to remove misfolded or otherwise damaged proteins and is essential for survival of bacteria under conditions of severe stress. The Clp (caseinolytic protease) proteases, ClpXP and ClpAP, from prokaryotic cells, have provided a wealth of information about the mechanism of activity and functions of ATP-dependent proteases. In *Escherichia coli*, the Clp proteases are responsible for the degradation of a number of important regulatory proteins and for eliminating several classes of abnormal proteins. While much is known about the degradation signals in substrate protein recognition by Clp proteases, there is limited knowledge about the structure and properties of the sites in ClpX at which substrates bind. Previous work has suggested that the loops within the central chamber of ClpX contribute to the binding of some substrates; how ClpX recognizes different substrates and how specific substrate binding at these sites occurs is not known. In this study, the binding sites of mutant ClpX are being investigated utilizing the degradation tag SsrA. Using a phenotypic marker, *sulA*, fussed to mutant *ssrA*, I am screening for mutants of ClpX that confer the ability to recognize altered recognition tags that are no longer recognized by wildtype ClpX. Random mutations of *clpX* should result in identification of a ClpX mutant that has acquired the ability to efficiently bind and degrade mutagenized SulA-SsrA. Once the binding site has been identified we will use additional rounds of mutagenesis to fully characterize the nature of ClpX-substrate interaction. Characterization of this activity could pave the way for synthesis of drugs that act as protease inhibitors in bacterial cells, therefore eliminating an escalating problem of antibiotic resistance. **Poster 78**

Quantitative Bedside Kaposi's Sarcoma Imaging

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Early assessment of therapeutic effectiveness of drugs used for Kaposi's sarcoma lesions in AIDS patients can save lives and decrease cost. We have developed a non invasive, non contact multi-spectral imaging system, which uses a selection of physiologically significant wavelengths in the near infrared spectrum, to provide quantitative measures of therapeutic effectiveness.

We have shown previously that our method is able to follow functional parameters, such as blood volume and blood oxygenation, as a measure of disease progression or regression. However, due to different physiological restrictions such as the curvature of the human body and or the presence of hair on the skin surface these assessments were semi-quantitative.

To make our measurements fully quantitative, and free of these artifacts, we have developed different strategies including improved registration, curvature correction and improved calibration and image analysis techniques.

These methods allow us to approach bedside quantitative objective imaging technologies, which will enhance cancer detection and treatment methods in a vulnerable population. Future work will include continuing developments of the system and diversification of the subject base to include imaging of other subcutaneous abnormalities or malignancies. **Poster 71**

Drug-Resistant Antibacterial Natural Products Isolated from the Marine Sponge, *Siliquariaspongia sp.*

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***J. Angew. Chem., Int. Ed.* 1998, 37, 2162–2178**
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There is a continuing need for bioactive compounds to combat the rise of drug-resistance among pathogens. Marine natural products have proven to be a rich source of unique structures with biological activities, and many marine natural products or their synthetic derivatives are currently in preclinical and clinical trials. In particular, lithistid demosponges have yielded structurally diverse natural products, with antitumor, anti-HIV, antibacterial, and antifungal activities. As part of an effort to identify new antibiotics, a Fijian specimen of the sponge *Siliquariaspongia* was studied. Six new antibacterial fatty acids were isolated and their structures established using a combination of 2D NMR and ESI-MS techniques. These natural products are fourteen carbon, unsaturated, dibrominated fatty acids, with terminal modifications including the addition of a glycine, glycinamide, or azirine ring. Several of these compounds inhibited the growth of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA), revealing an interesting structure-activity relationship. The most potent compound inhibited the growth of *S. aureus* and MRSA with an IC_{50} of approximately 4 μg/mL. **Poster 72**

Computational Investigation of Large Intergenic Regions in *Escherichia coli*: A Screen for Small RNAs

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The era of high-throughput sequencing produced a wealth of gene annotation in hundreds of organisms including *Escherichia coli*. Despite this extensive annotation effort, there are still 16 intergenic (IG) regions in *E. coli* of ≥700 bp in length where small ORFs (< 50 amino acids) or genes for small non-coding RNAs (sRNAs) may have been overlooked. In bacteria, most sRNAs regulate gene expression by base pairing with mRNA targets and altering translation and stability. Since the discovery of these regulatory sRNAs, more and more computational biologists have turned their attention to the prediction of these sRNAs in bacterial intergenic (IG) regions, but it is clear that not all genes have been found.I am using a combination of algorithms to predict sRNAs together with tiling expression microarray data to re-examine the large *E. coli* IGs for the presence of additional genes. These approaches indicate additional genes will be found in these IGs. Next I will carry out Northern blot and primer extension analysis to detect the predicted RNAs as well as construct deletion knockouts to determine their functions. **Poster 6**

Enhanced A3 Adenosine Receptor Selectivity of Multivalent Nucleoside-Dendrimers

Athena M. Klutz , Zhan-Guo Gao, John Lloyd, Asher Shainberg, Kenneth A. Jacobson

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A novel method of delivering small molecule ligands to G protein-coupled receptors (GPCRs) is through covalent coupling to dendrimer carriers, which are highly branched macromolecules that can be bound to multiple ligands and targeting agents. Adenosine receptors (AR), members of the Class A family of GPCRs, are involved in cardioprotection. A_1AR agonists, such as ADAC (hA_1 K_i = 10.4 nM), have anti-arrhythmic and anti-epileptic properties. In order to test dendrimer-ligand delivery at GPCRs, an appropriately functionalized ADAC derivative, N-(2-aminoethyl)-ADAC, with a terminal aminoethylamino group was synthesized and found to be equipotent at the A1AR and the anti-inflammatory A_3AR. This derivative was coupled through amide bonds to a fluorescently-labeled generation 2.5 (G2.5) polyamidoamine (PAMAM) dendrimer. In radioligand binding and functional cAMP inhibition assays, this conjugate had a K_i of 2.4 nM and 100-fold selectivity for the A_3AR. This multivalent agonist was useful in microscopic imaging of cells expressing the A_3 receptor but did not bind cells lacking the receptor. Thus, this is the first example showing that it is feasible to enhance the pharmacological profile of a GPCR ligand based on conjugation to a nanocarrier and the precise structure of the linking group. **Poster 73**

Impaired p53 Binding to Importin: A Novel Mechanism of Cytoplasmic Sequestration Identified in Oxaliplatin-resistant Cells

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The level and localization of p53 is tightly regulated. Following DNA damage, p53 translocates to the nucleus to initiate cell cycle arrest and/or apoptosis. Alterations in this process can lead to malignant transformation. It is thought that p53 has three putative nuclear localization signals (NLSI,-II,-III) and that NLSI mediates p53 nuclear translocation while NLSII and NLSIII appear to be optimal. The KB cell line selected for resistance to oxaliplatin was found to have increased levels of p53 predominantly in the cytoplasm that is larger in size. This longer protein ($p53^{420}$) is the result of a frameshift mutation caused by a single nucleotide deletion in the sequence encoding amino acid 382. We investigated possible explanations for the cytoplasmic sequestration of $p53^{420}$ and studied the role of NLSII and NLSIII in p53 nuclear import. We found that $p53^{420}$ is able to tetramerize, bind to dynein, transactivate genes. We concluded that neither NLSII nor NLSIII are essential for p53 nuclear localization and that the reduced nuclear accumulation of $p53^{420}$ is not the result of increased p53 nuclear export. However, the association of $p53^{420}$ with importin was found to be impaired. We identified a novel mechanism of p53 inactivation by cytoplasmic sequestration as a result of impaired importin binding. **Poster 74**

Vesicular Trafficking is Required for Chemoattractant Delivery at the Trailing Edge of Rapidly Migrating Cells

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Chemoattractant signaling induces the polarization and directed movement of cells secondary to the activation of multiple effector pathways. In addition, chemotactic signals can be amplified and relayed to proximal cells via the synthesis and secretion of additional chemoattractant. The mechanisms underlying such remarkable features remain ill-defined. We show that the asymmetric distribution of adenylyl cyclase (ACA) at the back of *Dictyostelium* cells, an essential determinant of their ability to migrate in a head-to-tail fashion, requires vesicular trafficking. This trafficking results in a local accumulation of ACA containing intracellular vesicles and involves intact actin and microtubule networks, and *de novo* protein synthesis. We also show that migrating cells leave behind ACA-containing vesicles, likely secreted as multivesicular bodies and presumably involved in the formation of head-to-tail arrays of migrating cells. We propose that similar compartmentalization and shedding mechanisms exist in mammalian cells during embryogenesis, wound healing, neuron growth, and metastasis. **Poster 7**

The Effect of Proteasome Inhibitors on Spinal Muscular Atrophy
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Spinal muscular atrophy (SMA), an autosomal recessive neuromuscular disorder, is one of the leading inherited causes of infant mortality. SMA results from a deletion of the survival of motor neuron-1 gene and a consequent deficiency in the SMN protein. Although a second nearly identical gene called *SMN2* is retained in humans, the *SMN2* gene product is alternatively spliced, producing an SMN isoform that is highly unstable. A promising avenue for treatment of SMA involves increasing levels of existing functional SMN protein produced by the *SMN2* gene. The SMN protein is degraded through the ubiquitin proteasome pathway (UPP) and is tagged by a chain of ubiquitin molecules, identifying it for proteolysis by the 26S proteasome complex. Proteolytic sites in the 26S proteasome function by specific mechanisms, rendering it possible to synthesize drugs that selectively inhibit it. One of the aims of this study is to elucidate the molecular mechanism underlying SMN protein degradation, with particular attention to the factors modulating its targeting by the UPP. We have acquired novel toxic proteasome inhibitors from Santhera Pharmaceuticals and are currently studying the effect of these inhibitors on SMN protein levels and on the SMA disease phenotype in SMA model mice. **Poster 84**

Identification of a Triage Factor for Posttranslational Membrane Protein Insertion into the ER
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Tail-anchored (TA) membrane proteins possess a single transmembrane domain (TMD) close to the C terminus that mediates post-translational insertion into intracellular membranes. The TMD of these proteins is specifically recognized by a cytosolic targerting factor termed TRC40. Once captured by TRC40, the TA protein is delivered to the membrane for insertion. How hydrophobic TA proteins are successfully chaperoned to TRC40 after release from the ribosome is unknown. To address this problem, we have reconstituted TA protein capture by TRC40 in an *in vitro* system. Using this system, we demonstrate the existence of a factor necessary for efficient capture of substrate by TRC40. Fractionation led to the identification of a complex containing the multi-domain protein Bat3. Bat3 was shown to interact with ribosomes, TA substrates, and TRC40. Immunodepletion of Bat3 from the *in vitro* reconstituted system led to significantly less efficient TA protein capture by TRC40. We propose that the Bat3 complex interacts with TA substrates at the ribosome and delivers them to TRC40. Thus, Bat3 represents a triage factor for posttranslational membrane protein insertion. **Poster 8**

Development of Neural and Behavioral Mechanisms Underlying Attention Orientation Toward Social-Emotional Cues
Kara Lindstrom, Amanda E. Guyer, Karin Mogg, Brendan P. Bradley, Nathan A. Fox, Monique Ernst, Eric E. Nelson, Ellen Leibenluft, Christopher S. Monk, Daniel S. Pine, Yair Bar-Haim

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Orientation towards or away from threatening situations and the ability to attend to and interpret positive social cues are crucial to functioning in social contexts. However, the developmental progression of the neural architecture supporting attention orientation towards negative or positive social-emotional stimuli is relatively unknown. The present study examined normative maturation of attention biases to positive and negative affective stimuli in a psychiatrically healthy sample (9 to 40 years) using functional magnetic resonance imaging paired with a dot-probe task. The dot-probe task yields a behavioral index of attention bias towards or away from an emotional cue (happy or angry face). Behavioral response data indicated a positive correlation between age and happy face bias, such that older participants showed greater bias to attend to happy relative to neutral faces. Angry face bias scores did not correlate with age. Younger participants demonstrated greater activation in the left cuneus and left caudate during trials in which the target appeared at the neutral face location and decreased activation in these areas during trials in which the target appeared at the happy face location. These findings suggest that children exhibit greater activation than adults in certain brain regions sub-serving reward when attention is modulated by positive stimuli. **Poster 60**

Joint Modeling of Longitudinal Intercourse Patterns and Time-to-Pregnancy
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BACKGROUND: Most epidemiologic models of time-to-pregnancy (TTP), a proxy measure of fecundity, do not incorporate dependency on intercourse patterns on fertile days around ovulation.

OBJECTIVE: Our goal was to assess the effects of determinants, both observed (e.g. smoking, parity) and unobserved (e.g. hormones, stress) on TTP and intercourse patterns jointly, and determine if the two are dependent.

METHODS: We developed a joint model using association models for intercourse pattern and a discrete survival model for TTP. We incorporated a continuous latent variable to represent unmeasured hormones or stress that generate dependency between intercourse and TTP. We analyzed data from the NY State Angler Cohort study of couples trying to conceive (n=82).

RESULTS: Intercourse acts on consecutive days were found to be dependent (CI (1.1,1.2)). Significant ($p<0.05$) increases were seen in the odds of intercourse on a weekend compared to a weekday (OR CI (1.4,2.2)). Similar significant increases were seen in probability of conception, for parous couples compared to nulliparous (CI (0.7,2.1)) and for couples who had intercourse on days prior to ovulation compared to those who did not (CI (0.2,1.5)).

CONCLUSIONS: TTP and intercourse patterns are dependent and impacted by determinants both observed and unobserved. **Poster 24**

The Biophysical Basis for the Dynamic Localization of Myosins in Actin Protrusions
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I use quantitative analysis of live and fixed cells to investigate a physical model that describes the active localization of myosins and their cargo inside actin protrusions during steady-state conditions. The proposed mechanism of localization is through the interplay of free diffusion and directed motion, which is driven by coupling to treadmilling actin filaments and by directed motion due to myosin motor activity. The resulting localization of both the molecular motors and their cargo is calculated, and is found to have an exponentially decaying profile. The active plus-end localization of proteins that influence actin polymerization reactions at the tips of actin protrusions is a simple mechanism the cell can use to control the length of its protrusions, and these results suggest that 'snapshot' images of immunolabeled and transfected cells can reveal information about the temporal dynamics of these processes, which may not be observable in 'real-time'. **Poster 9**

Intravital Two-photon Microscopy for Studying Uptake and Trafficking of Fluorescent Molecules in Live Rodents
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Endocytosis and membrane trafficking have been studied mainly in two-dimensional cell cultures. Although these systems provide a valuable tool to investigate molecular machineries, they do not reproduce the characteristics of the cells in their native tissues. Non-linear imaging techniques such as two-photon microscopy have been increasingly used to visualize tissues *in vivo* because of reduced photodamage, increased imaging depth and the capacity to detect broad spectrum of fluorescent probes as well as endogenous molecules that provide information on the tissue organization. Here we describe an experimental system based on intravital two-photon microscopy for studying endocytosis in live animals. The rodent submandibular salivary glands were chosen as model organs since they can be exposed easily and imaged without compromising their function. Furthermore, the submandibular glands are amenable to pharmacological and genetic manipulations enabling the study of membrane trafficking at the molecular level. We show that systemically injected molecules such as fluorescently conjugated dextran and bovine serum albumin diffuse out of the vasculature and are rapidly internalized by the supporting cells within the parenchyma of the glands. The cells that internalized fluorescent dextran were labeled by antibody directed against vimentin suggesting their fibroblastic nature. As revealed by time-lapse imaging, the early endosomes undergo homotypic fusion in the periphery of the cells, and within 30-40 minutes reach the late endosomal/lysosomal compartments in the perinuclear area. Notably, heterotypic endosomal-lysosomal fusion was not observed, instead, material from the lysosomes appeared to exchange with endosomal cargo before undergoing fusion with the lysosomal structures. Last, we show that injection of Latrunculin A or Cytochalasin D into the salivary gland via the major excretory duct disrupts the filamentous actin cytoskeleton and markedly reduce the levels of fluorescent dextran uptake. In summary, we demonstrate for the first time that sub-cellular organelles can be dynamically imaged in live animal for extended periods of time. Furthermore, this study establishes that a combination of intravital two-photon microscopy as an imaging technique and the submandibular salivary glands as a model organ can be successfully utilized to study the molecular machinery regulating membrane traffic in live animals. **Poster 10**

Grade- and Stage-specific Age incidence patterns of serous ovarian epithelial cancer among different racial and ethnic groups in the United States
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18317228, 15111296

Molecular and epidemiologic studies suggest that low- and high-grade serous ovarian epithelial cancers (sOEC) have distinct etiological pathways. However little is known about patterns in tumor grade among different racial/ethcic groups in the US. Data on sOEC were obtained from SEER for non-Hispanic Whites (NHW), Hispanic Whites (WH), African Americans (AA), and Asian Pacific Islanders (API), ages 20-84 for the period 2000-2005. Age-adjusted and age-specific rates were calculated and stratified by race/ethnicity, stage, and/or grade. There were a total of 11,569 cases; of which 79.8 percent were NHW, 9.2 percent WH, 5.4 percent AA, and 5.3 percent API. The age-adjusted rate ratio for high-grade to low-grade sOEC was 2.85. Age-specific rates were similar for all groups except WH. There was significant age interaction with tumor grade ($p<0.001$ for both early and late stage). When age-specific rates were stratified by stage and grade, there was a highly significant age interaction in WH compared to WNH (reference) in the late-stage/high-grade stratum ($p<0.0001$). Grade appears to play an important role in late-stage sOEC, particularly among WH. Though age incidence patterns by grade were generally similar among all groups in late-stage sOEC, the age incidence pattern for high-grade sOEC within late-stage was distinct for WH. **Poster 25**

***Dictyostelium* has a functional presenilin/gamma-secretase that regulates cell differentiation**

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The gamma-secretase complex [Presenilin (PS), Nicastrin (Nct), Aph1 and Pen2] cleaves single-pass transmembrane proteins to release intracellular domain moieties that regulate a variety of signaling pathways. Proteolytic activity seems to reside within PS. In humans, mutations in PS alter specificity in cleavage of Amyoid Precursor Protein (APP) and can cause Alzheimer's disease. I have chosen to screen for novel functions of PS and to examine the role of the different complex components in the model eukaryote *Dictyostelium*. *Dictyostelium* grow unicellularly, but upon starvation, initiate a multicellular developmental cycle that results in two terminally differentiated cell types, spore and stalk cells. *Dictyostelium* has two genes that encode PS-like proteins and one each for Nct, Aph1, and Pen2. Although the genes in *Dictyostelium* display significant similarity to their mammalian counterparts, they are nonetheless highly diverged. To assess a definitive functional association with gamma-secretase activity, we expressed the mammalian substrate APP in WT and mutant variants of *Dictyostelium*. APP processing was observed in WT cells, but not in strains lacking PS, Nct, and Aph1. Additionally, all the gamma-secretase components are required for proper development, specifically in differentiation of spore cells. **Poster 11**

Synaptic Vesicle Endocytosis is Triggered and Modulated by Calcium

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Pub Med ID (PMID) numbers ONLY are preferred. Otherwise, provide only brief references: journal name, date, and page numbers.

After synaptic vesicles release neurotransmitter by fusing with the plasma membrane, membrane is retrieved and used to form new vesicles, in order to replenish the vesicle pool and maintain synaptic transmission. This retrieval process is called endocytosis. While it is known that calcium influx is the trigger for fusion, it is unclear whether endocytosis also has a trigger, or whether it is simply molecularly coupled to the fusion process. We addressed this issue at the calyx of Held, a large presynaptic terminal in the mammalian central nervous system, using capacitance recordings to monitor endocytosis. We observed that dialysis of low levels of free calcium into the calyx could evoke vesicle fusion with negligible endocytosis. We also saw a strikingly reduced rate of endocytosis after stimulation when we reduced the post-stimulus rise in intracellular calcium levels. The endocytic rate increased with stimulus intensity to levels 3 orders of magnitude faster than the lowest stimulus-induced rate. Because these changes were calcium-dependent and not related to the amount of vesicle fusion, our data suggest that calcium triggers and modulates endocytosis, separately from fusion. An overshoot was seen after strong stimulation, which may serve to retrieve vesicles stranded during low-calcium conditions. **Poster 61**

BDNF-TrkB Signaling Regulates PKMξ In the Maintenance of Long-Term Potentiation

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***Science*. 2007 Aug 17;317(5840):951-3.**
***Science*. 2006 Aug 25;313(5790):1141-4.**
***J Neurosci*. 2005 Feb 23;25(8):1979-84.**

Long-term potentiation (LTP) is believed to be the cellular mechanism underlying learning and memory. LTP is divided into two phases: early phase LTP (E-LTP), which requires post-translational modification of pre-existing proteins, and late phase LTP (L-LTP) that requires protein synthesis. It is apparent that orchestration of various signaling pathways regulates the maintenance of LTP. Previous studies indicate both brain-derived neurotrophic factor (BDNF) and protein kinase C (PKC) are essential in sustaining late phase LTP. BDNF is a major activity dependent secretory neurotrophic factor. By binding to a receptor tyrosine kinase Trk B, BDNF triggers multiple intracellular signaling pathways to ensure long lasting synaptic efficacy. Recent studies have shown that an autonomously active isoform of PKC, PKMξ , is the only molecule proven to be both necessary and sufficient during the persistent memory storage.

Because both BDNF and PKMξ regulate surface AMPA receptor expression, and are essential for late-phase LTP and long term memory, we hypothesized that PKMξ may be part of the BDNF-TrkB signaling pathway. To test this hypothesis, we examined the role of PKMξ in BDNF-dependent L-LTP. First, by applying forskolin, a chemical inducer of BDNF-dependent L-LTP on hippocampal slices derived from wild type mice we showed increased expression of PKMξ in the CA1 region. Similar treatment on slices prepared from BDNF knock out mice failed to increase the levels of PKMξ suggesting that BDNF is essential for PKMξ expression. Second, to test whether TrkB signaling is involved in BDNF induced PKMz expression, we used the TrkB (F616A) knock in mice and the chemical genetic approach, where TrkB activity is regulated by applying the inhibitor 1NMPP1. Similar to the result from BDNF knockout model, we did not observe an increase in PKMξ expression. Together, these results suggest that BDNF-TrkB signaling during L-LTP regulates PKMξ expression. To further demonstrate that PKMξ is downstream of BDNF-TrkB signaling in BDNF-dependent L-LTP, we used the pseudosubstrate (regulatory domain of PKC or ZIP peptide) to inhibit PKMξ in the maintenance phase (1hr after the induction of L-LTP). Only ZIP, but not the control scrambled peptide blocked BDNF-dependent L-LTP. These results suggest that PKMξ is regulated by BDNF-TrkB signaling and is essential for the sustenance of BDNF-dependent L-LTP. **Poster 85**

Induction of ISGF3 in Response to Interferon Gamma in Human Cells

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A549 cells, a human lung epithelial carcinoma, are resistant to the antiproliferative effects of type-I and type-II IFNs at concentrations greater than 100ng/mL. However, they both induce antiviral protection at 107 (for IFN-α2c) and 108 IU/mL, respectively. Western blot analysis revealed that these cells signal through the Jak/Stat pathway in a different manner when treated with IFN-α or -γ, yet they induce many of the same downstream proteins. Phosphorylated Stat1 (Y701) peaked at 2h when treated with IFN-α, remaining at low levels for up to 48h. Cells treated with IFN-γ showed the same trend until 15h, when an increase in pStat1 was detected with a second peak at 24h. Additionally, antiviral activity was delayed from 6h to 24h at an IFN-γ concentration inducing IC50 (0.1ng/mL). Gene expression microarray analysis following IFN-γ treatment indicated increased levels of antiviral proteins normally associated with a type-1 IFN response, such as Mx1 and PKR. Induction of these genes by type-I and type-III IFNs was ruled out using both neutralizing antibodies in biological assays and qRT-PCR. Yet the ISGF3 complex was isolated by co-immunoprecipitation after IFN-α and -γ treatment, each inducing comparable amounts at 24h. However, the role of ISGF3 induction in these cells is still unclear. Further studies are in progress to determine the conditions for the delayed formation of ISGF3 in these cells, and to ascertain its biological significance in the cellular response to IFN-γ. **Poster 35**

Changes in Lipid Levels During the Menstrual Cycle

Sunni L. Mumford, Enrique F. Schisterman, Brian W. Whitcomb, Jean Wactawski-Wende, Maurizio Trevisan, Richard W. Browne, Kathleen Hovey

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Lipid levels are known to be associated with cardiovascular events, and some studies have shown cyclical variation in lipids across the menstrual cycle. Results have been mixed due to small samples and poor timing of lipid measurements to menstrual cycle phase. This study evaluated the association between serum lipid and hormone levels across the normal menstrual cycle among 259 healthy, regularly menstruating women enrolled in the BioCycle Study. Blood samples were collected at 16 points across 2 menstrual cycles, with collection scheduled to occur during specific phases of the cycle and timed using fertility monitors. Linear mixed models adjusted for age, BMI, and smoking status were used for this analysis. Total cholesterol and low-density lipoprotein (LDL) cholesterol were lower during the luteal phase as compared to the follicular phase, and high-density lipoprotein (HDL) levels were highest around ovulation. Lower total cholesterol and LDL levels, and higher HDL levels, were associated with higher estradiol levels during the luteal phase. Cyclical variations in lipid and hormone levels have potential implications on the design of studies in reproductive aged women and studies of chronic risk factors should account for hormonal variability. **Poster 26**

Ranking 96 Object Images by Their Activation of FFA

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The fusiform face area (FFA) is a region in human inferior temporal cortex that has been shown to respond most strongly to faces. Previous imaging studies only assessed category-average activation as they grouped stimuli into predefined natural categories. Here we ask whether there are particular non-face object images that elicit a strong FFA response or particular face images that elicit a weak response. To address these questions, we rank 96 particular object images by the activation they elicit in FFA.

BOLD fMRI measurements were performed using a 3T scanner. First, FFA was defined conventionally at varying sizes using a separate block design experiment. Then, the activation in those voxels in response to 96 different object photos was measured. FFA responses to the 96 object images were ranked according to response amplitude.

Group results indicated that single-image activation of both left and right FFA was stronger for face images than most other object images (average choice probability = .92). This result was clearest for maximally face-selective FFA voxels. Right FFA responses were stronger and more robust against increasing size than left FFA responses. Activation in the parahippocampal place area (PPA) and early visual cortex (EVC) did not rank faces before most other object images. **Poster 62**

Children with Prepubertal Hyperandrogenism due to Classic Congenital Adrenal Hyperplasia or Familial Male Precocious Puberty are at Risk for Psychopathology

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BACKGROUND: The effect of exposure to excess androgen during fetal and childhood development on the risk of mental illness is unknown. We investigated the current and lifetime prevalence of psychiatric disorders in children with genetic causes of hyperandrogenism.

METHODS: Pediatric patients (aged 9 to 18 years) with the diagnosis of classic congenital adrenal hyperplasia (CAH) or familial male precocious puberty (FMPP) followed at the NIH Clinical Center were invited to undergo a semi-structured interview, The Kiddie Schedule for Affective Disorders and Schizophrenia-Present and Lifetime Version (KSADS-PL), to determine present and lifetime history of psychiatric disorders. Patients were also assessed for anxiety, depression, substance use and behavior problems. Data were gathered from 2002 to 2007.

FINDINGS: We evaluated 63 patients (48 CAH, 15 FMPP) and 15 (31 percent) patients with CAH and 8 (53 percent) patients with FMPP met the criteria for at least one current psychiatric diagnosis. Attention-deficit-hyperactivity-disorder (ADHD) was the most common diagnosis and was present in 12.5 percent of CAH patients and 40 percent of FMPP patients.

INTERPRETATION: Patients with genetic causes of excess androgen in early childhood are at risk for psychopathology, most notably ADHD. **Poster 12**

Familial Haplotyping and Innovative Protein Modeling During Identification of the Gene Responsible for Schnyder Corneal Dystrophy

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Schnyder Crystalline Corneal Dystrophy (SCCD, MIM #121800) is characterized by deposition of cholesterol and lipids in the corneas of affected patients. It is a relatively rare disease that is inherited as an autosomal dominant trait with high penetrance. Deposition of cholesterol and lipids in the cornea leads to progressive loss of vision in patients and many possess abnormally high serum cholesterol levels. Analysis of several large families allowed linkage to be established to chromosome 1p34-36 and this was subsequently narrowed to a 1.6 Mbp region containing approximately 30 genes. Despite extensive sequencing by several independent groups, no mutations were identified. Close inspection of individual haplotypes that defined the published region of linkage revealed a typographic error misrepresenting a single patient's affected status in the family that allowed the centromeric boundary to be determined. Re-assessment resulted in dramatic changes to the familial haplotype and subsequent revision of the SCCD critical region. Two groups independently identified mutations in the *UBIAD1* gene as causal for SCCD in 2007. Innovative analysis of membrane protein structure revealed a useful model to examine protein function. Haplotypes and protein modeling are presented. **Poster 48**

Probing the Surface of *Plasmodium falciparum*-Infected Erythrocytes with DNA Aptamers

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Shangguan, PNAS 2006, p. 11838-43.

As part of its complex lifecycle, *Plasmodium falciparum* invades a variety of human and mosquito cell types. However, all the symptoms that define a clinical episode of malaria are attributed to the intracellular blood stages. How the parasite manipulates the host red cell surface to allow for selective nutrient uptake and other survival functions are unresolved questions. High throughout proteomics confirms the presence of multiple-novel parasite proteins on the infected erythrocyte surface but the technology is limited by the lack of specific molecular probes. The development of nucleic acid affinity reagent (DNA aptamers) capable of distinguishing between developmentally related surfaces, diseased and non-disease cells, and proteins that differ only in conformation suggests that similar reagents can be targeted to the surface of parasitized erythrocytes. We will test the hypotheses that: 1) DNA aptamers can specifically recognize molecules on the surface of *P.falciparum* infected red cells, 2) binding of the selected aptamers to these cells can be used to modulate functional properties of infected red cells, and 3) specific aptamers can be used to identify and characterize important ligands on the host red cell surface. It is expected that these studies will help elucidate the molecular complexity of this host-parasite interface and provide novel targets for clinical intervention. **Poster 36**

Local and Distant Elements Regulate Tissue-Specific Expression of ANK-1 Gene Transcripts

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Ankyrin, a protein connecting the red blood cell (RBC) membrane to the RBC skeleton, has isoforms arising from two tissue-specific promoters (erythroid,1E; brain/muscle,1B) and a ubiquitous promoter (1A). We hypothesize that promoter choice is driven by chromatin architecture. We identified DNase hypersensitive sites (HS) immediately 5' to 1E (5'HS) and two adjacent HS (3'HS1, 3'HS2) located ~6kb downstream of 1E, in all cell types. Barrier elements are associated with HS and are found at boundaries between hetero- and euchromatin. We found that 5'HS and 3'HS1+2 are barrier elements that prevent transgene silencing in both cells and transgenic mice. Chromatin Conformation Capture (3C) demonstrated the formation of a loop-like structure in which 5'HS and 3'HS2 are brought into physical proximity only in erythroid cells. 3'HS1 binds the erythroid-specific transcription factor, NF-E2, which increases transcriptional activity when adjacent to the ANK-1E promoter only in erythroid cells. In agreement with the 3C results, chromatin immunoprecipitation revealed that the both distal ends of the ANK-1E region bind Brg-1, USF-1, GATA-1, and RNA Pol II. Our current model involves the formation of an erythroid-specific loop at the ANK-1E promoter mediated by GATA-1 and NF-E2. **Poster 49**

Quantification of CREB Binding Protein (CBP) Immunolabeling in Hippocampal Neurons of a Rat Model of Cognitive Aging

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CBP is a transcriptional co-activator that increases gene expression. One of the mechanisms by which CBP acts is through its histone acetyltransferase activity, modulating histone conformation and hence accessibility to the DNA. Several genetic models with lower expression levels of CBP have been shown to have deficits in memory tasks, which can be rescued by histone deacetylase inhibitors. These studies point to the involvement of histone modifications in the processes of learning and memory. Since normal aging is accompanied by memory impairments and is associated with changes in the expression of a broad constellation of genes implicated in synaptic plasticity and other functions, we hypothesize that these alterations may be coupled with a more general dysfunction of transcription regulation mediated by chromatin remodeling. Here, we quantified the intensity of CBP immunolabeling in the hippocampus of young (6 m.o.) and aged (24 m.o. or more) rats. The aged group was divided into aged-impaired and aged-unimpaired based on performance on the Morris water maze, a measure of spatial memory. Additionally, we stereologically estimated the number of neurons labeled for CBP in all groups. The results from this study will illuminate the contributions of epigenetic mechanisms to normal cognitive aging. **Poster 63**

Probing α-Synuclein Membrane Binding with Tryptophan Fluorescence

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Understanding how environmental factors affect the conformational dynamics of α-synuclein (α-syn) is of great importance because the accumulation and deposit of aggregated α-syn in the brain are intimately connected to Parkinson's disease etiology. In the current study, we employed anionic phosopholipid small unilamellar vesicles (SUVs) to investigate membrane induced protein conformational changes. Unstructured to α-helical transitions were monitored by circular dichroism (CD) spectroscopy, while steady-state and time-resolved fluorescence measurements of single Trp containing variants report on distinct membrane interactions at four different sites (W4, W39, W94, W125). For all variants, a saturable transition occurred approximately at a ratio of 10 α-synucleins per vesicle. Interestingly, Trp residues at positions 4 and 94 were the most sensitive probes for vesicle-protein interaction (W4˜W94>W39>W125). Our data suggest that lipid-protein interactions occur even with no observed secondary structural changes. **Poster 75**

Genome-Wide Analysis of EKLF Occupany in Erythroid Chromatin Reveals 5', 3', and Intragenic Binding Sites in EKLF Target Genes

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Erythroid Kruppel-Like Factor (EKLF) binds a consensus motif (NCNCNCCCN), first identified at the beta-globin gene promoter. Knowledge of other EKLF target genes is limited, as EKLF-deficient (-/-) mice die by embryonic day 15 (dE15) of anemia. Analysis of dE14 -/- fetal liver erythroid progenitor cells revealed a maturation failure. Bioinformatic analyses showed >3000 dysregulated genes, with an affected cell cycle pathway centered on E2F2. We confirmed decreased E2F2 mRNA and protein; demonstrated accumulation of -/- cells at G0/G1; verified EKLF binding to the E2f2 promoter; and demonstrated EKLF-dependent chromatin remodeling of the E2f2 locus. To identify genome-wide EKLF-dependent effects, we are combining chromatin immunoprecipitation and massively-parallel signature sequencing (ChIP-seq). Our analyses revealed 531 sites of direct EKLF association. Of unique sites, 119 (22 percent) were located ≥10 kb from the nearest RefSeq gene (*intergenic*); 78 (14.6 percent) were <10 kb from a gene (*adjacent*); while a plurality (222; 42 percent) were within RefSeq coordinates (*intragenic*). Among the cell cycle genes, the majority (59 percent) of EKLF-bound sites were intragenic. Based on these observations we propose that occupancy of intragenic sites, and not only adjacent promoters, may facilitate activation of EKLF target genes. **Poster 50**

Does Clathrin-Independent Endocytosis Plays a Role In Capillary Lumen Formation?
Natalie Porat-Shliom, Yoel Kloog, Julie G. Donaldson

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During vasculogenesis, new blood vessels arise from precursor endothelial cells. Their ability to form a lumen is increasingly recognized as a critical event in establishing a functional vascular system. Although the mechanism underlying capillary lumen formation is poorly understood, several models have been proposed. One model suggests that lumen development arises from pinocytic vesicle fusion followed by exocytosis to generate a continuous tube. We have been studying a clathrin-independent endocytic (CIE) pathway in HeLa and COS7 cells that could be the source of these vesicles. Here we show that Human Umbilical Vein Endothelial Cells (HUVEC) have clathrin-independent endocytic pathway that has a larger and distinct endosomes. These endosomes contain the Major Histocompatibility Complex I protein (MHCI), a CIE cargo, but not Transferrin. Furthermore, we demonstrate that HUVECs have high levels of endogenous Arf6, a small GTP binding protein, associated with CIE. We speculate that this unique endocytic pathway is the key to understanding the mechanism of lumen formation in this cell system and plan to examine this using 3D cell culture and live cell imaging. **Poster 13**

What Can Genomic Datasets Tell Us About Primate Phylogenetics?
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Large comparative genomic datasets are becoming increasingly common for a wide number of species. Contained within those DNA sequences is a wealth of information about the evolutionary history of species. One event that should leave its impression in genomic sequence is speciation. Although DNA sequence has been used to determine the history of speciation in primates with notable success, some relationships, particularly within the new world monkeys (Platyrrhini), remain difficult to resolve. We are developing large genomic sequence resources as part of the NISC Comparative Sequencing Program, and applying phylogenetic techniques to resolve some of those relationships and better understand patterns of sequence evolution in the primates. We have found that incongruence among genomic regions is more prevalent than we previously thought, even in taxa that are already well resolved. Among the new world monkeys incongruence is widespread. The source of this incongruence is still unclear, though incomplete lineage sorting may play a role. Using techniques of parallel computation on large Linux clusters we are finding new approaches to analyze these large genomic datasets to learn about the evolutionary history of ourselves and our close relatives. **Poster 27**

Correlation of Transcription and Somatic Hypermutation in the Switch Region Preceding the IgM Constant Gene
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After B cells are stimulated with antigen, antibodies are diversified by somatic hypermutation of variable genes and class switch recombination of constant genes. Both processes are initiated by an enzyme called activation-induced cytidine deaminase (AID) that converts deoxycytidine to deoxyuracil on single-strand DNA. However, the mechanism for targeting AID to the immunoglobulin locus is not known. Since transcription is required for hypermutation and switching, it may play a role in bringing AID to the locus. We hypothesize that AID travels with RNA polymerase II (pol II), and when the polymerase pauses, AID is deposited onto the single-strand DNA. This predicts that there will be more mutations where RNA polymerase molecules are located. We studied polymerases and mutations in the switch region before the constant gene encoding IgM, because AID can be induced *in vitro* in murine splenic B cells with mitogens and cytokines. We then determined the location of the polymerases and compared it to the location of mutations. Using a nuclear run-on assay, we found that the amount of trans-cripts flanking 7switch region. Consistent with this, a ChIP assay with anti-pol II antibodies indicated that pol II molecules were indeed more dense in 500 bp before and after the core region than in the rest of the switch region. Interestingly, when compared to the mutation data, the results show that upstream of the switch core, there is high pol II activity and a high frequency of mutation, whereas downstream of the core, there is high pol II activity but a very low frequency of mutation. Based on these results, we propose that pol II molecules pause when they encounter secondary structure in the G-rich core region, and AID produces mutations in the single-strand DNA. After the transcription complex slowly passes through the core region, AID dissociates and mutation is ablated. This theory can be tested using ChIP analyses with anti-AID antibodies to see if AID is present in DNA after the core region. **Poster 37**

The Agonists of TLR4 and 9 Are Sufficient to Activate Memory B Cells to Differentiate into Plasma Cells *in vitro* but Not *in vivo*

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18641311

Memory B cells can persist for a lifetime and be reactivated to yield high affinity, isotype switched plasma cells. The generation of memory B cells by Ag immunization requires adjuvants that generally contain TLR agonists. However, requirements for memory B cell activation and the role of TLRs in this activation are not well understood. In this study, we analyzed the response of memory B cells from immunized mice to TLR9 and 4 agonists CpG oligodeoxynucleotides (ODN) and LPS. Mouse memory B cells express both TLR9 and 4, and respond to both CpG ODN and LPS *in vitro* by differentiating into high affinity IgG secreting plasma cells. In contrast, neither CpG ODN nor LPS alone is sufficient to activate memory B cells *in vivo*. Ag is required for the clonal expansion of Ag-specific memory B cells, the differentiation of memory B cells to high affinity IgG secreting plasma cells, and the recall of high affinity Ab responses. The Ag-specific B cells that have not yet undergone isotype switching showed a relatively higher expression of TLR4 than memory B cells, which was reflected in a heightened response to LPS, but in both cases yielded mostly low affinity IgM secreting plasma cells. Thus, although memory B cells are sensitive to TLR agonists *in vitro*, TLR agonists alone appear to have little affect on B cell memory *in vivo*. **Poster 38**

Visualizing NAD^+ and Water using Coherent Anti-Stokes Raman Spectroscopy

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12080137, 12904580, 11171993

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. NADH is *fluorescent* and has been monitored both by UV-induced and 2-photon fluorescence. To quantify the *nonfluorescent* NAD^+, we have built a Coherent Anti-Stokes Raman Spectroscopy microscope. We use it to excite the ring breathing mode of the nicotinamide moiety of NAD^+ near 1032 cm^{-1}, resulting in anti-Stokes emission at 675nm. For possible simultaneous monitoring, one of the two different input laser pulses concurrently (weakly) excites two photon fluorescence of NADH (but not NAD^+). To simulate mitochondria, we prepared DPPC LUVs (large unilamellar vesicles) loaded with millimolar levels of NAD^+ and/or NADH. The NAD+ vesicles yielded signals for CARS **only** (2p NADH emission was absent). We have also used our instrument to visualize *water* in vesicles and live Hela cells using the O-H stretch at 3340 cm^{-1}. The resulting anti-Stokes signal appears at 510nm. In live cells, a quick switch of the surrounding media to D_2O produces a rapid decrease of the cell interior water signal. Since the O-D stretch is centered at 2800 cm^{-1}, that vibrational mode is not significantly excited at a frequency difference of 3340 cm^{-1}. **Poster 76**

Phosphorylation by Src Leads to Activation of Cbl-c by Decreasing Binding Affinity with UbcH5b

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15117950, 14661060, 10362357

Cbl proteins are RING-finger (RF) ubiquitin ligases (E3s). There are three mammalian family members: Cbl, Cbl-b and Cbl-c. All have a conserved N-terminus, a catalytic RF and a C-terminal proline rich domain. The N-termini of Cbl and Cbl-b inhibit their respective E3 activity. However, the mechanism is unknown. An *in vitro* auto-ubiquitination assay with various deletion mutants of Cbl-c confirmed that, like c-Cbl and Cbl-b, the N-terminus of Cbl-c inhibits its E3 activity. Phosphorylation of Cbl-c at tyrosine 341 by Src leads to an increase in activity. This phosphorylation is both necessary and sufficient for activation of Cbl-c E3 activity. An activating mutation that mimics phosphorylation (Y341E) was used to determine the mechanism by which the N-terminus inhibits and by which phosphorylation activates the protein. Kinetic data indicate that the Cbl-c WT binds to the ubiquitin-conjugating enzyme (E2) with a higher affinity than Cbl-c Y341E. The process of poly-ubiquitination requires the release and rebinding of an E2, thus the slower release of the E2 from Cbl-c WT leads to a lower catalytic rate. In conclusion, the N-terminus increases the affinity of Cbl-c for the E2 and inhibits activity. Phosphorylation of Y341 on Cbl-c by Src lowers the affinity for the E2 and increases the E3 activity. **Poster 14**

CReMM, a Novel CHD Protein Involved in Polymerase I Transcription

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The novel CHD (chromodomain-helicase-DNA binding) protein (CHD9 or CReMM) was recently identified as a chromatin remodeling protein expressed in murine osteoprogenitor cells. CReMM contains numerous signature motifs associated with CHD proteins including: chromodomains, a SNF2-like ATPase domain and a DNA binding domain.

To study the cellular localization of CReMM, we performed immunofluorescence (IF) experiments using antibodies targeted to various epitopes of the protein. We demonstrate that CReMM has a broad subcellular localization (both in nucleus and nucleoli). The nucleolar form shows partial or complete co-localization with pol I and fibrillarin. Depletion of the nucleoli foci by Actinomycin D treatment, implies that CReMM is involved with rDNA transcription.

We confirmed the specificity of the antibody, using recombinant protein as a competitor for intracellular IF. The results showed a dramatic depletion of the nucleolar staining pattern, further confirming the unique localization of the CHD protein. Using high resolution tiling arrays, we show CReMM interactions with specific sites at the rDNA locus.

In light of recent studies that link nucleolar function to stem cell and cancer cell proliferation, we are pursuing to further study of CReMM as a novel nucleolar chromatin remodeling protein. **Poster 15**

Cellular Physiology in Living Muscle: Application of Real-Time Motion Correction to *In-Vivo* Two-Photon Microscopy

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16734715, 15596503, 18089951

We have developed a real-time motion-correction device to control a 2-photon excitation laser scanning microscope. Using this tool we are able to overcome drifting motion, a major barrier to time-course imaging of living perfused tissue. An *in vivo* mouse muscle preparation permitted control of muscle tension, contraction excitation, and measured force generation. The mice were anesthetized and the tibialis anterior (TA) muscle exposed. The microscope objective was coupled to the tissue using a isotonic optical gel with no coverglass. The exogenous dye ANEPPS-8 was used to stain endothelial cells of vascular structures for registration of tracking algorithm. Within the muscles the sarcomere lengths and mitochondrial energy state could be directly accessed for both slow and fast twitch muscle fibers in the same field, along with the regional capillary flow. This quantitative muscle model was used to examine the effects of various physiological perturbations, and provides a unique *in vivo* insight into factors that affect the oxidative capacity of heart and skeletal muscle. **Poster 28**

Interaction of Human Papillomavirus Type- 8 E2 Tethering Protein with Mitotic Chromosomes

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During persistent papillomavirus infection of the host, the viral E2 protein tethers the viral genome to the cell chromosome, ensuring maintenance and segregation of the viral genome during cell division. Our aim is to understand the molecular mechanism by which HPV-8 E2 maintains and segregates the viral genome. E2 protein consists of transactivation domain linked by a flexible hinge region to a DNA binding and dimerization domain. The hinge and DNA binding domains are crucial for association with mitotic chromosomes. We have identified a 16 amino acid region within the hinge that is both necessary and sufficient for mitotic chromosome binding, when linked to the DNA binding domain. This region confers on E2 the ability to bind to ribosomal DNA loci and colocalize with the ribosomal transcription factor, UBF. The sequence shows similarity to other viral tethering proteins such as EBNA1 and LANA from EBV and HHV-8 respectively. We have identified specific residues within this 16 amino acid region that are required for chromosome binding and are potential sites for post translational modifications such as phosphorylation and methylation. Characterizing the interaction of E2 protein with mitotic chromosomes will enable development of anti-viral therapies to eliminate viral genomes from infected cells. **Poster 39**

Utilizing Selenocysteine Technology for the Generation of Bi-specific Antibodies and Antibody Fragments for Cancer Therapy

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Monoclonal antibodies (mAbs) employ low toxicity, high specificity, long serum half-lives, and various effector functions. However, mAbs have fallen short in some clinical trials due to poor anti-tumor effects. Small synthetic molecules, providing enhanced tumor potency, are rapidly cleared from the blood. Thus, hybrids combining mAbs and small synthetic molecules are highly specific, potent, and maintain long circulatory half-lives.

We have previously developed a means to site-specificically conjugate small molecules and mAbs via an unnatural amino acid, selenocysteine (sec). Sec is cotranslationally inserted by re-coding the stop codon UGA. Sec's selinum group (pKa 5.2) provides superior nucleophillic activity and therefore unique reactivity with small molecules derivatized with electrophilic groups in the presence of a selenate source at low pH.

Using the sec technology and a mammalian expression system, we have generated bispecific Abs for CD3 (component of the T cell receptor) and NKG2D (NK cell activating receptor). The C-terminal ends of each Ab encode a sec moiety conjugated to a peptidomimetic, LLP2A, that binds with high affinity to integrin a4B1 on malignant cells. Additionally, we have generated a mouse Fc fragment encoding a C-terminal Sec for usage in a mouse model of B-CLL. **Poster 16**

Cyclic Hydrostatic Pressure Suppresses Hypertrophy in Mesenchymal Stem Cell Chondrogenesis

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TGF-β-induced chondrogenesis in bone marrow-derived MSCs is usually accompanied by hypertrophy, which occurs normally during endochondral bone formation, but is undesirable in the engineering of cartilage grafts. Physiologic levels of hydrostatic pressure have been shown to augment MSC chondrogenesis. In this study, we investigated the potential of cyclic hydrostatic pressure to direct MSC chondrogenesis and inhibit hypertrophy via altering the time course of hydrostatic loading. MSC pellets were differentiated for 20 days with TGF-β3 and treated with either: no load (NL), daily load (L), daily load on days 1-10 (L/NL), or days 11-20 (NL/L). After 20 days, gene expression of chondrogenic markers type II collagen and aggrecan was highest in Group L/NL. Loading on days 11-20 in Groups NL/L and L tended to decrease gene expression of these chondrogenic markers as well as hypertrophic markers Indian hedgehog, type X collagen, and bone sialoprotein compared to their controls (Groups NL and L/NL, respectively). Our results indicate that cyclic hydrostatic pressure enhances chondrogenesis during the beginning stages of MSC differentiation. However, when applied during later stages of the differentiation process (days 11-20) hydrostatic pressure inhibits both chondrogenesis and hypertrophy of MSCs. **Poster 17**

Regulation of NMDA receptor endocytosis by differential combinations of NR2 subunits

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Background References (3 max):
PMID:15254094; 14529712

Synaptic transmission depends on the type and number of receptors localized at synapses. The modulation of the NMDA receptor abundance at excitatory synapses is crucial for neuronal development as well as in learning and memory, however the underlying molecular mechanisms are unclear. NMDA receptors are tetramers, composed of obligatory NR1 subunits and one or more NR2A-D and NR3 subunits, which assemble in the endoplasmic reticulum (ER) to form functional channels. The functional diversity of NMDA receptors arises from the assemblies of different NR2 subunits. It is well known that there are endogenous NMDA receptors composed of diheteromeric assemblies (NR1/NR2A, NR1/NR2B) as well as triheteromeric assembies (NR1/NR2A/NR2B). Although we have previously shown that NR2A and NR2B have distinct endocytic motifs and that they undergo differential post-endocytic sorting, the relative contribution of each subunit to the trafficking of a triheteromeric complex is not understood. Therefore, we designed an approach based on the dimeric feature of the alpha and beta chains of the human class II major histocompatibility complex (MHCII), to study the relative contributions of the C-termini of NR2 subunits to endocytosis and post-endocytic sorting of NMDA receptor complexes. The MHCII alpha chain must oligomerize with the MHCII beta chain to exit the ER and traffic to the cell surface. Thus, we created chimeras of alpha/beta chains with the NR2A /NR2B C-termini. We found that the endocytic trafficking of NMDA receptors is dependent on different combinations of NR2 subunits. Homodimeric alpha-beta-NR2A chimeras enter the degradation pathway and colocalize with late endosomal markers, whereas the alpha-beta-NR2B chimeras predominantly traffic through the recycling pathway and colocalize with recycling endosomal markers, consistent with our previous results using monomeric chimeras of NR2 C-termini (TacNR2A and TacNR2B). Interestingly, heteromeric alpha-NR2A-beta-NR2B chimeras preferentially sort to recycling endosomes. These data support a dominant role for the NR2B C-terminus in regulating the trafficking of NR2A/NR2B complexes. Future studies will focus on defining the precise sequence determinants in the NR2A and NR2B C-termini that regulate the differential trafficking and subcellular sorting pathways of diheteromeric and triheteromeric NMDARs. **Poster 79**

***Chlamydia trachomatis* Polymorphic Membrane Protein D is an Oligomeric Autotransporter with Higher-Ordered Structure**

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PMID: 16446444, PMID: 17506669, PMID: 15759043

Chlamydia trachomatis is a globally important obligate intracellular bacterial pathogen that is a leading cause of sexually transmitted disease and blinding trachoma. Effective control of these diseases will likely require a preventative vaccine. *C. trachomatis* polymorphic membrane protein D (PmpD) is an attractive vaccine candidate as it is conserved among *C. trachomatis* and is a target of broadly cross-reactive neutralizing antibodies. We show here that immunoaffinity-purified native PmpD exists as an oligomeric autotransporter with a distinct 23 nm flower-like structure. 2D BN/SDS-PAGE analyses showed oligomers were comprised of mature PmpD (p155) and an N-terminal p73 passenger domain (PD) and a C-terminal p82 translocator domain. We also show that PmpD undergoes an infection-dependent cleavage step late in the growth cycle that yields a soluble extended PD (p111) that was processed into a p73 PD and a novel p30 fragment. Interestingly, soluble PmpD peptides possess putative eukaryotic-interacting functional motifs implicating potential secondary functions within or distal to infected cells. Collectively our findings suggest PmpD is a multifunctional virulence factor important in chlamydial pathogenesis and could represent novel vaccine or drug targets for the control of human chlamydial infections. **Poster 40**

Functional NMDA Receptors at Growth Cones of Young Hippocampal Neurons

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N-methyl D-aspartate receptors (NMDARs) are a class of ionotropic glutamate receptors that are involved in regulating neuronal migration and neurite outgrowth/differentiationearly in development, but the mechanism underlying such functions is unknown. Here, we show that NMDARs are present and functional at growth cones of young hippocampal cultures. Immunocytochemistry shows that native and transfected NMDAR subunits are expressed on the surface of axons and growth cones in young (DIV4, days *in vitro* 4), but not older (DIV14), neurons. Whole-cell recordings of DIV4 neurons patch-clamped at the soma or growth cones reveal NMDAR-mediated currents in response to pressure application of NMDA (100μM) at axonal growth cones. To further investigate NMDAR function at growth cones, neurons were loaded with the fluorescent calcium-sensitive indicator, Fluo-4 AM, and calcium transients at growth cones were measured. We find that calcium enters the cell at growth cones, and application of NMDAR antagonists blocks this influx. We are currently investigating the dynamics of this calcium influx through pharmacological manipulations, and we are planning to perform time-lapse imaging in conjunction with the release of caged glutamate to examine morphological effects of NMDAR activity at specific regions of growth cones. **Poster 64**

Anthrax Lethal Toxin Alters the Transcriptional Control of Pro-Inflammatory Gene Expression in Human Endothelium

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Anthrax lethal toxin (LT), a key virulence factor of *Bacillus anthracis*, enhances cytokine-induced VCAM-1 expression on primary human endothelial cells suggesting a causative link between dysregulated adhesion molecule expression and the poor immune response and vasculitis associated with anthrax. Here, we show that LT amplification of TNF-induced VCAM-1 expression is driven transcriptionally by the cooperative activation of NF-kappaB and IFN regulatory factor-1 (IRF-1) and occurred despite efficient inhibition of AP-1 binding activity. We also report that LT enhancement of NF-kappaB activity correlated temporally with increased IKK activity and phosphorylation of IkappaBalpha and the p65 subunit of NF-kappaB. Consistent with these data, LT enhanced transcription of the NF-kappaB and IRF-1 regulated gene, *CD40*. Conversely, LT inhibited transcription of the AP-1 dependent gene, *CCL2*. These data suggest that LT can differentially modulate pro-inflammatory gene expression by dually regulating the activity of key transcription factors. Together, these findings provide new mechanistic insight on how LT may disrupt the host response to anthrax. **Poster 41**

What keeps the yeast nucleus hangin' round? Idenifying genes that affect nuclear shape in S. cerevisiae.
Micah Webster, Joe Campbell, Orna Cohen-Fix

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PMID: 16467382

Nuclear shape is regulated in yeast and higher eukaryotes. Yet mechanisms governing nuclear shape remain elusive. The nucleus in budding yeast is round, while yeast lacking the SPO7 gene (spo7del), an inhibitor of phospholipid biosynthesis, exhibit a single nuclear protrusion that we call a flare. Flares develop in a 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 tether inhibiting flare formation at the interface between DNA and nuclear envelope. We reasoned that inactivation of this molecular tether, in conjunction with elevated levels of membrane synthesis, would result in cell death due to severe alteration of nuclear structure. Thus, to identify genes responsible for this tether, we are performing a screen for mutations that lead to lethality when combined with *spo7del*. Through a secondary visual screen, we have identified *spo7del* synthetic lethal mutants with abnormal nuclear shape. While the screen is ongoing, we have confirmed that the *DBP7* gene affects *spo7del* nuclear shape and preliminary data suggest that the *RPL7a* gene affects *spo7del* nuclear shape, as well. Both genes suggest a role for ribosomal proteins in maintenance of nuclear morphology. **Poster 18**

Hexosamine Signaling is Linked to Mitochondrial Movement
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PMID: 16887820, 16717129, 18592415

The dynamic movement and localization of mitochondria is of special importance for neuronal cells with varying high-energy demands. Neuronal cells specifically require mitochondrial localization, not just in the cell soma, but also at terminus of long axonal projections. The adaptor protein-complex that assists in mitochondrial movement/localization is beginning to be characterized, but much remains to be uncovered. One key factor is the *Drosophila* protein Milton that functions as an adaptor for mitochondria binding to kinesin microtubule-motors; in the absence of Milton, mitochondrial movement and localization is disrupted. This highly conserved Milton protein is also identified as an interacting protein for O-linked N-acetylglucosamine (O-linked GlcNAc) transferase (OGT). The ubiquitous and highly conserved OGT protein is part of the nutrient responsive Hexosamine Signaling Pathway (HSP) that performs a dynamic post-translational modification of nucleocytoplasmic proteins at serine/threonine residues, much like phosphorylation. The signaling pathway is involved in a myriad of intracellular processes including translation, transcription, insulin resistance, cell cycle regulation, and apoptosis. However, the role of OGT in mitochondrial movement/localization has not been explored. The current study examines the role of Milton (and related adaptor proteins) and O-GlcNAcylation in cellular processes, including mitochondrial movement/localization in the simple genetic models systems *Drosophila* and *C. elegans*. We hypothesize that O-GlcNAcylation plays a regulatory role in mitochondrial movement in neurons and that it is necessary for proper neuronal function. Therefore, changes in expression of nutrient responsive O-GlcNAc cycling enzymes will either modulate or inhibit mitochondrial movement. Understanding the interaction between adaptor proteins, microtubule motors and mitochondria may give us clues about the basis of mitochondrial movement as well as insights into the physiological consequences of its dysfunction in neurodegenerative diseases such as Huntingon's disease and Alzheimer's disease. **Poster 80**

Brain-Derived Neurotrophic Factor Val[66]Met Polymorphism Differentially Affects Regional Cerebral Blood Flow during Working Memory and Rest

S-M Wei, A Padmanabhan, K.V. Roe, P.D. Kohn, B Kolachana, D.R. Weinberger, K.F. Berman

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The Val[66]Met single nucleotide polymorphism (SNP) in the human BDNF gene influences hippocampal function, with the met allele being associated with abnormal hippocampal recruitment during episodic and working memory. To further investigate the effects of this BDNF SNP on hippocampal and cortical function, we used $H_2{}^{15}O$ PET to assess regional cerebral blood flow (rCBF) as a function of BDNF genotype during rest and during a working memory task.

During rest, BDNF met carriers showed increased rCBF in bilateral hippocampal and parahippocampal regions (p=.005). Analysis of the working memory data showed increased rCBF for met carriers in the right hippocampus during higher working memory load, indicating a failure to appropriately suppress hippocampal activity. The connectivity analyses indicated that during working memory, met carriers exhibited positive functional connectivity between right hippocampus and a DLPFC/parietal network, rather than the expected negative relationships observed in val homozygotes (p<.005), who also exhibited positive covariance with contralateral hippocampus. These findings define a genetically-modulated neurophysiological hippocampal phenotype in met carriers, namely inefficient neuronal processing that requires increased cellular activity in order to meet system-level demands. **Poster 65**

Systematic Mutantional Analysis of a "Fast Evolving" Telomeric Protein

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PMID: 18772376, PMID: 12510197, PMID: 9275195

Many of the proteins involved in telomere-capping (which distinguishes chromosome ends from double strand DNA breaks) are conserved between *Drosophila* and other eukaryotes. However, the unique structure of the *Drosophila* telomere which, unlike those in most organisms, does not require a specific sequence for capping, has also lead to the emergence of new (species-specific) telomeric proteins. This context provides an opportunity to study the evolution of a new protein to participate in a conserved function. The HP1/ORC associated protein (HOAP), known to be intimately involved in telomere-capping as its absence results in telomere-fusions, was initially recognized in a screen for fast evolving genes. Because most research focuses on highly conserved proteins, this class of genes (predicted to constitute ˜20 percent of the genes in the *Drosophila*) has been underrepresented in functional studies. We propose a detailed study of HOAP through mutagenesis of the endogenous locus. We will use the highly efficient gene targeting method (SIRT) to generate a spectrum of mutants in order to elucidate structurally important elements in the protein. A better understanding of how HOAP evolved to protect chromosome ends might provide new insight into the inherent function of the telomere. **Poster 82**

Social and Cognitive Development in Preterm Infants
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PMID: 9835078; PMID: 12169077; PMID: 16818525

Around 12 percent of live births in the UK and US are premature (i.e. born before 37 completed weeks of gestation). A substantial volume of research has focused on documenting the variety of developmental delays and impairments shown by these preterm infants, with considerable focus on the buffering effect of sensitive and responsive parenting on later cognitive outcomes. However, very little is known about the development of joint attention (JA), imitation and language in the context of parent-preterm infant interactions. This study will measure aspects of the parent-child interaction and JA, imitation and language at 5, 13 and 18 months (adjusted for prematurity) in infants with varying gestational age (GA) at birth. We hypothesize that the development of these skills will be delayed in infants born at younger GA, and this delay will be related to less optimal parenting and attentional impairments in the infant. In term infants, it has been suggested that the shared origins of JA, imitation and language is the infant's ability to see others as intentional agents. This study will be among the first to investigate intention understanding in preterm infants and provide a better understanding of why preterm infants are at risk for low cognitive performance. **Poster 66**

Neuronal avalanches imply maximum dynamic range in neuronal networks
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Recent experiments have demonstrated that spontaneous neural activity both *in vitro* and in intact brains has statistical properties expected near the critical point of a phase transition. This phenomenon, called neuronal avalanches, raises the question, why might the brain operate at a critical point? Theories and models predict optimized computation and information processing, but experimental support for these ideas is lacking. Here, we tested the prediction that dynamic range is maximized at a critical point, by using brain tissue grown on microelectrode arrays (MEA) and a simple numerical model. We varied the conditions of synaptic coupling and measured spontaneous neural activity and response to electrical stimuli. We found that under conditions which result in neuronal avalanches, the dynamic range was maximized. **Poster 67**

Characterization of MTOC Repositioning in T-Lymphocytes
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Engagement of the T-cell receptor (TCR) by an antigen presenting cell (APC) is known to result in the formation of an Immunological Synapse (IS) at the site of contact between the two cells. Along with active recruitment and activation of TCR signaling complexes at the IS, the microtubule organizing center (MTOC) has been shown to reorient to a position adjacent to the synapse. Here we utilize an optical trap to bring the APC to the T cell, and observe spatially and temporally the dynamics of MTOC reorientation to the IS. Currently we are studying the MTOC reorientation in relation to the formation of subdomains within the IS, and in the future, we will address the signaling process as well as the motor proteins essential for MTOC translocation and normal function of the T cell. **Poster 19**

Identification of Factors that Promote Remyelination by Adult CNS Precursor Cells

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In its most basic sense, inflammation is the body's mechanism to protect against injury and promote repair. Yet, many chronic diseases are associated with and caused by inflammation. Multiple sclerosis (MS) is characterized by areas of chronic demyelination where remyelination seen in early stages of the disease has failed. Inflammation associated with MS has two opposing effects; it causes the demyelination that characterizes the disease but also promotes subsequent remyelination. This latter effect can be examined by retinal transplantation of oligodendrocyte precursor cells (OPCs). Intravitreous injections of zymosan have been found to stimulate inflammation in the retina and enhance myelination by transplanted cells. Using this model, tissue and RNA samples of retina were collected comparing zymosan and saline injections over the full inflammatory response. A microarray gene screen was conducted, and the inflammatory response assessed via histology. Candidate factors were then identified and tested for their effects on OPC proliferation and differentiation, and myelination in cerebellar slice cultures. Two factors were found to promote OPC differentiation and myelination *in vitro*. Future studies will be conducted to validate the potential of these factors to promote and support myelination *in vivo*. **Poster 68**

Examination of Cytolytic Defects in SAP-Deficient Mice and XLP Patients

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X-linked lymphoproliferative syndrome (XLP) is a massive immune dysregulation often triggered by infection with Epstein-Barr virus (EBV). It is commonly caused by mutations affecting the small adaptor molecule SAP, which binds to the cytoplasmic tail of the SLAM family members, including SLAM and 2B4. Evidence suggests a role for 2B4 in cytotoxicity against EBV-infected targets. Our lab has also found abnormalities in immune synapse formation in $SAP^{-/-}$ $CD4^{+}$ cells. We hypothesize that SAP and 2B4 are necessary for the cytolytic immune synapse and successful killing of target cells. To examine these questions, we are evaluating $CD8^{+}$ T cells (CTLs) from XLP patients and $SAP^{-/-}$ mice. While CTLs from $SAP^{-/-}$ mice show normal levels of both antigen-specific and anti-CD3 mediated cytotoxicity against T cell targets, they have slightly impaired killing of and lower frequencies of conjugate formation with activated B cells. Studies of XLP CTLs also show normal anti-CD3 mediated cytotoxicity. However, we observed clear defects in conjugation with and killing of EBV-transformed B cell targets. We will use microscopy to evaluate the defects in cytotoxicity against EBV-specific targets.
Poster 42

NOTES