

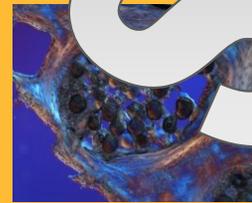
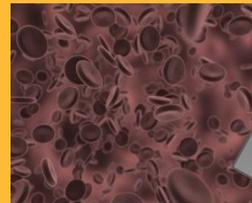
**Session 1 Presenters**

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**UCLA** Minor in Biomedical Research



Minor in Biomedical Research

**POSTER SYMPOSIUM**

Location

Date

Time

Session 1, Time

Session 2, Time

Hosted by the  
College of Letters and Sciences  
Division of Life Sciences  
and the  
Minor in Biomedical Research

## ABSTRACTS

Alphabetical by Author

### **Genetic Screen for Retinal Degeneration Mutants in *Drosophila melanogaster***

By AMETHYST ALAYARI, Alex Tonthat, Jackie Mao, Jeffrey Kho, Sam Bounds, Sampat Sindhar, Stephanie Jensen, Tiffany Mao, ZAFAR GILL, and Frank Laski

Retinal degeneration (RD) diseases such as Age-Related Macular Degeneration and Retinitis pigmentosa are major causes of blindness in humans. For this reason, we have worked to identify potential genes involved in the onset of RD using *Drosophila melanogaster* as a model organism. The primary goal for this research is to identify genes that result in retinal degeneration when knocked-down in the photoreceptor neurons of the retina. This will aid in our understanding of the biological underpinnings of retinal degeneration, and potentially identify homologues of these genes that are involved in human RD. The genetic screen uses an eye-specific RNAi knockdown of a specific gene in the photoreceptors of the *Drosophila* eye for two weeks before dissection and analysis. The goal for our screen is to study 5,000 genes, investigating their roles in retinal homeostasis in *Drosophila melanogaster*. We are currently halfway through our project and have isolated over 50 RD mutant phenotypes resulting from genetic knockdown. We are currently undergoing additional experimentation in order to further investigate the role of these genes and to confirm the positive results we have observed in the general RNAi screen. Specific RD mutants and their phenotypes will be discussed.

## ABSTRACTS

Alphabetical by Author

### **Contribution of Neuroimaging to the Study of Body Dysmorphic Disorder**

By ALEX ZAI, Sarah Madsen, Teena Moody, Jamie Feusner

Body dysmorphic disorder (BDD) is a severe psychiatric condition in which individuals are preoccupied with perceived defects in their appearance. Little is known of the pathophysiology or neurobiology of BDD. Previous small studies of brain morphometry have produced discrepant results in cortical and subcortical structures. To investigate morphometric abnormalities in the largest sample to date, we compared white matter (WM) volumes from high-resolution T1 magnetic resonance images of 49 unmedicated participants with BDD to brain volumes of 44 controls. In addition, we compared volumes in specific regions of interest including the inferior frontal gyrus (IFG), right amygdala, and total gray matter and white matter and examined correlations with symptom severity. Statistical analysis revealed that there was no significant difference in WM volumes between groups, which supports our previous findings. However, there was a significant negative correlation between HAMA scores and total gray matter (GM) volumes ( $p = 0.00458$ ), which suggests that BDD sufferers with less GM are more anxious than those with lower total GM volumes. Further volumetric comparisons between BDD and healthy control subjects will be conducted and these findings may assist in identifying salient regions for further investigation of the pathophysiology underlying the clinical symptoms, and potentially could be incorporated into biomarker algorithms to help identify those at risk for BDD, and/or predict a treatment response.

## ABSTRACTS

Alphabetical by Author

### **Role of Notch1 in Mediating the Effects of Oxidized Phospholipids in Atherosclerosis Progression.**

By HELEN YU, Anais Briot, and Luisa Iruela-Arispe

Atherosclerosis is a common disorder that involves hardening of the arteries due to the accumulation of lipids. High doses of oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (OxPAPC) induce increased permeability of human aortic endothelial cells (HAECs) *in vitro* and increased pro-inflammatory signaling, suggesting it contributes to the disease onset. OxPAPC treatment on donor HAECs also leads to a decrease in NOTCH1 expression (unpublished data), a protein crucial in vascular development and homeostasis. Given this data, we hypothesize that OxPAPC-induced permeability and/or pro-inflammatory signals could be mediated by a decrease in Notch1 signaling in the endothelium. To test our hypothesis, we knocked down NOTCH1 to see if decreasing NOTCH1 expression alone can produce the same effects as OxPAPC treatment. We then used Western Blot and quantitative real-time polymerase chain reaction (qPCR) to analyze our data and observed a small but significant increase in *Interleukin-8 (IL-8)* mRNA levels. In addition, we performed preliminary experiments to optimize the readout of HAEC monolayer permeability. Finally, we aimed to elucidate the mechanisms by which OxPAPC leads to a decrease in NOTCH1 expression and signaling. In particular, we observed that OxPAPC-induced IL-8 upregulation and MAPK/ERK1/2 pathway were unlikely to be responsible for NOTCH1 downregulation.

## ABSTRACTS

Alphabetical by Author

### **Bone Morphogenetic Protein Signaling Can Modulate Gene Transcription in the Absence of Smad4 in the Bone Growth Plate**

By TENI ANBARCHIAN, Diana Rigueur, YooJin Lee and Karen Lyons

Osteoarthritis (OA) is a disease of joint degradation and a leading cause of disability in the U.S. To learn more about OA, it is essential to study the pathways that regulate bone formation, such as the bone morphogenetic protein (BMP) signaling pathway. BMP signaling can be transduced via transcription factors Smads 1/5/8. The phosphorylated Smads then associate with Smad4 protein and translocate into the nucleus to modulate expression of BMP target genes. Studies have shown that mice lacking Smads 1/5/8 have severe skeletal defects and die rapidly after birth. However, loss of Smad4 in cartilage is compatible with life and leads to mild skeletal abnormalities. These data hint at a separate, Smad 1/5/8-dependent, Smad4-independent BMP signaling pathway. To test the hypothesis that Smad4 is not required for all Smad 1/5/8 signaling, chondrocytes from Smad4 knock-out mice were evaluated for expression of BMP target genes. Quantitative reverse transcription PCR (qRT-PCR) and immunostaining revealed that upon BMP stimulation and in the absence of Smad4, Indian Hedgehog (IHH), a BMP target gene, is expressed in lower amounts and in ectopic locations compared to the wildtype cells. These results suggest that Smad4 may not be required for the BMP Smad 1/5/8-dependent gene expression. To confirm these findings, transcription and translation levels of additional BMP targets are being evaluated. Better understanding of molecular pathways involved in bone and cartilage development may lead to future therapeutic treatments for OA.

## ABSTRACTS

Alphabetical by Author

### **Lamina Neuron Targeting in the Visual System of *Drosophila***

By MIRIAM BEYDER, Wael Tadros, and S. Lawrence Zipursky

**Abstract:** The molecular mechanisms underlying the formation of synaptic connections are still poorly understood. The *Drosophila* visual system is an ideal system in which to address this question due to its amenability to genetic and molecular techniques. Within the fly medulla, second order lamina neurons target to five (M1-M5) distinct synaptic layers. Here we conducted a genetic screen addressing the targeting of lamina neurons L1 and L2, which normally target to the M5 and M2 layers respectively. We employed mosaic analysis with a repressible cell marker (MARCM) to assess the effects of large chromosomal deletions, or deficiencies on the targeting of these two neurons. This approach permits the generation and visualization of individual homozygous mutant lamina neurons surrounded by heterozygous tissue. Mutant lamina neurons were identified by variations in morphology, projections to inappropriate layers, and expansion into surrounding columns of the medulla. To date, we have identified one deficiency, *Df(3R)Exel6204*, which results in a distinct morphological and targeting phenotype. L1 neurons homozygous for the above deficiency had M1 terminals that were either reduced or expanded into adjacent columns. Their M5 terminals, on the other hand, often expanded into the adjacent columns. Ultimately, we hope to identify the gene responsible for this and other phenotypes found in the screen. This research will further the understanding of existing pathways involved in neuronal wiring through the identification of novel factors.

## ABSTRACTS

Alphabetical by Author

### **Three-Dimensional Printed Sucrose Preforms for Aqueous-Based Scaffold Fabrication**

By Shannon Wongvibulsin, Stephanie Reed, and Benjamin Wu

With the decreasing cost and increasing availability of three-dimensional printing (3DP) for tissue engineering purposes, the field has grown in its ability to address a variety of injured tissues by employing 3DP scaffolds individualized to the project's unique needs. While scaffold fabrication with 3DP allows for production of intricate structures, 3DP technology is currently limited by the materials compatible with the 3D printer. To overcome this limitation of 3DP, this study advances direct molding techniques in which the structures printed become preforms for post-processing and infusion with the desired scaffolding material. For this project, sucrose was selected as the sacrificial material because it is compatible with the 3D printer and also easily dissolved away with water during the scaffold fabrication process when the sucrose is no longer necessary for structural purposes. However, many polymer solutions desired for the fabrication of the final scaffold are aqueous-based and result in the premature disintegration of the sucrose preform. In this study, we develop a post-processing procedure to produce a preform structure and employ an injection infusion technique to obtain the positive structure composed of the desired polymer. This process can also allow for the production of small-scale features to be obtained through controlled shrinking throughout scaffold fabrication. With this method, structures with complex architecture can be modeled with computer-aided design (CAD), 3D printed with sucrose, post-processed, and infused with aqueous-based polymers and used as constructs for tissue engineering purposes.

## ABSTRACTS

Alphabetical by Author

### **The Role of Hypoxia in the Regulation of Systemic Larval Growth in *Drosophila melanogaster***

By DANIEL M. WONG and Julian A. Martinez-Agosto

The coordination of environmental and genetic cues determines the final size of a mature organism. In *Drosophila melanogaster*, low oxygen availability, or hypoxia, systemically decreases larval growth and size, but the molecular mechanism through which hypoxia does so remains poorly characterized. We show that Sima, which is the

alpha subunit of the *Drosophila* hypoxia-inducible factor (HIF), accumulates in the nuclei of larval fat body cells during hypoxia and

systemically reduces larval growth when overexpressed in this same tissue. Additionally, hypoxia causes the retention of *Drosophila* insulin-like peptides (Dilps) in a small cluster of medial neurosecretory cells in the larval brain. Likewise, Dilps accumulate in these neurons upon Sima overexpression in the larval fat body, suggesting that an endocrine signal emanates from the fat body and is relayed to the brain to regulate Dilp secretion.

Forced membrane depolarization and neurosecretion of Dilps from these insulin-producing cells under hypoxic conditions does not result in a rescue of larval growth, but ubiquitous activation of insulin signaling during hypoxia results in larval resistance to hypoxic growth restriction. Our findings suggest that insulin resistance is an adaptive response to low oxygen availability that may mediate the pathophysiology of many conditions associated with systemic or local micro-hypoxia, including obstructive sleep apnea, obesity, and diabetes.

## ABSTRACTS

Alphabetical by Author

### **Identifying the genes for Selective Autophagy of Endoplasmic Reticulum in *Saccharomyces cerevisiae***

By Vivian Chen, Sebastian Schuck, and Peter Walter

The endoplasmic reticulum (ER) is a large membranous organelle whose functions include protein folding and lipid biosynthesis. The accumulation of misfolded proteins in the ER, termed ER stress, threatens proper ER function and thus cell survival. In response, cells produce more ER-resident protein folding machinery and expand the ER membrane. Part of the expanded ER compacts into tight whorls that are imported into lysosomes for degradation. This stress-induced selective autophagy, termed ER-phagy, may remove damaged portions of the ER and thus help mitigate ER stress. We aim to identify genes essential for ER-phagy through a genetic screen in *Saccharomyces cerevisiae*. We mutagenized a strain expressing a GFP-tagged ER-phagy reporter, visually screened 12,000 mutants and identified 126 mutants unable to degrade the reporter, likely due to ER-phagy defects. Of these, 21 mutants remained after secondary screening by flow cytometry. These 21 mutants through complementation analysis created nine independent groups suggesting nine different genes have been found. One group has been sequenced and identified ynm3 as the mutant gene. To determine if ynm3 truly participates in ER-phagy, ynm3 was knocked out and we found that ER-phagy was defective, demonstrating that the screen was successful in identifying ER-phagy regulatory genes. Future experiments include determining the defective genes through whole-genome sequencing and employ biochemical assays to establish their roles in ER-phagy. Thus, this study will further the mechanistic understanding of ER-phagy and its physiological functions.

## ABSTRACTS

Alphabetical by Author

### Isolation of Amyloid $\beta$ -Protein Oligomers

By Joseph L. Conovaloff, Eric Y. Hayden and David B. Teplow

Amyloid  $\beta$ -protein ( $A\beta$ ) forms fibrils in the brain that were long believed to be the direct cause of Alzheimer's Disease (AD). However, recent evidence supports the "oligomer cascade hypothesis" in which oligomers may be the proximate pathologic agents of AD. Using rapid, zero-length, *in situ* chemical cross-linking (PICUP; photo-induced cross-linking of unmodified proteins), to stabilize the oligomer state, enabled the isolation and study of pure  $A\beta$ 40 oligomers. While individual  $A\beta$ 40 oligomers have been isolated and thoroughly characterized,  $A\beta$ 42, the more neurotoxic and physiologically implicated isoform, has been difficult to purify using the same procedures. Recent data suggest that  $A\beta$ 42 with amino acid substitutions Phe<sup>10</sup> and Tyr<sup>42</sup> biophysically and biochemically behaves similarly to wild-type  $A\beta$ 42. Using a novel step-wise method, we have obtained isolated  $A\beta$ 42 [F10, Y42] oligomers, which remain stable for characterization studies. In this method, the cross-linked protein sample was fractionated by SDS-PAGE, excised, fractionated by SDS-PAGE with urea, excised and electro-eluted. The addition of 25% dimethyl sulfoxide (DMSO) to the cross-linked protein solution as well as 6M urea to a second polyacrylamide gel improved the percent purity of the electro-eluted oligomer samples. The resulting isolated individual oligomers can be used for further biophysical and biochemical study. As there are numerous diseases that involve amyloid proteins, this process of isolating oligomers could be used for isolating oligomers of other amyloid proteins.

## ABSTRACTS

Alphabetical by Author

### Identifying Small Molecule Activators of GDP Bound RAB5

By Joshua Weinreb, and John Colicelli

RAS is a GTPase and also one of the first oncogenes identified. The human genome encodes more than 170 RAS-related GTPases that participate in virtually all cell functions. When bound to GTP, these proteins adopt an "active" conformation with high affinity for downstream effector molecules. Upon GTP hydrolysis, GTPases convert to an "inactive" conformation with low affinity for downstream effectors. We hypothesize that some small molecules can activate a GDP-bound GTPase, not by releasing GDP but by allosterically inducing a conformational change that mimics the high affinity for downstream effectors normally associated only with a GTP-bound GTPase. I am testing this hypothesis by developing an assay to identify small molecule activators of RAB5, a GTPase that regulates receptor endocytosis in mammalian cells. Constitutively activated RAB5 may lead to increased degradation of receptor tyrosine kinases (RTKs) and thereby reduce RAS activity, which could be therapeutic for solid tumors of epithelial origin. I have created and isolated my desired protein and plan to test the functionality of my protein in the coming quarters.

## ABSTRACTS

Alphabetical by Author

### Functional Characterization of a Novel *Toxoplasma* Inner Membrane Complex Protein

By Kevin Wang, and Dr. Peter J. Bradley

Apicomplexans are obligate intracellular parasites that cause substantial medical and veterinary disease worldwide. One unique aspect of these parasites is their unusual method of cellular division, *endodyogeny*, in which daughter parasites are formed inside of the mother and ultimately consume the mother to release the daughter cells. Construction of daughter parasites is dependent upon an internal membrane system known as the *inner membrane complex* (IMC). The IMC is composed of membrane stacks supported on a network of intermediate filaments that grows throughout endodyogeny. While the IMC is critical for parasite replication, little is known about the protein components of the IMC and how they function in division.

In this project, we investigated the role of an uncharacterized gene (TGME49\_018240) whose characteristics make it a good candidate to be a novel IMC protein. BLAST analysis revealed that TGME49\_018240 lacks identifiable homology to known proteins, suggesting it plays a unique role in *Toxoplasma*. Using a newly developed gene tagging approach, we confirmed that TGME49\_018240 is indeed a novel member of the IMC in *Toxoplasma*, denoted IMC18.

To further assess the function of the *IMC18* we created a knockout that replaced the gene with a drug resistance marker through homologous recombination. The knockout was successful which suggests that IMC18 is not essential to parasite survival or that there is an additional protein that rescues the parasites from dying. We are now performing pull-down experiments coupled to mass spectroscopy to identify IMC18's interacting partners in order to get a clearer understanding of *Toxoplasma*'s IMC.

## ABSTRACTS

Alphabetical by Author

### Monobodies Evolved by mRNA Display as Novel, In Vitro Alternatives to Antibodies

By JONATHAN DIEP, C. Anders Olson, and Ren Sun

Antibodies have become essential recognition tools for molecular biology research. Allelic variation, post-translational modifications, and alternative splicing, however, have made the range of targets in the proteome far exceed the number of commercial antibodies currently available. In vitro selection techniques, such as mRNA display, represent alternative methods for accelerating the generation of antibody mimics called monobodies. We sought to demonstrate in vitro applications for monobodies in immunoassays using monobodies that were evolved by mRNA display to the model targets: the maltose binding protein of *Escherichia coli* and human immunoglobulin G. Subcloning vectors were designed and constructed for the expression of monobodies either with tags for enzymatic biotinylation by BirA or as alkaline-phosphatase fusions for detection. Monobodies linked to streptavidin-labeled horseradish peroxidase via the C-terminal biotin enabled antibody-free target detection by western blot. In antibody-free enzyme-linked immunosorbent assays (ELISAs), monobodies with alkaline-phosphatase fusions showed a limit of detection of 100 pg. Monobodies, therefore, exhibit comparable performance to commercial antibodies on western blots and ELISAs. This validation of in vitro applications for monobodies evolved by mRNA display suggests that monobodies represent stable, expressible, and economical alternatives to traditional antibodies.

## ABSTRACTS

Alphabetical by Author

### **Characterization of Chromomethylase 2 and its Role in Gene Silencing through the RNA-Directed DNA Methylation Pathway**

By Truman Do, Hume Stroud, Steven E. Jacobsen

5'-Cytosine DNA methylation is an epigenetic mark that is crucial for the regulation of gene expression such as transposon silencing, imprinting, differential expression, and chromatin structure. In a mammalian context, it occurs almost exclusively on CG dinucleotides. In plant systems, specifically *Arabidopsis thaliana*, however, cytosine methylation occurs in all contexts: CG, CHG, CHH (in which H = A, T, or C). RdDM (RNA-directed DNA methylation) is a process in which 24nt-long siRNAs (small interfering RNAs) are generated in order to sequence-specifically target DNA methylation. While the proteins responsible for de novo and CHG methylation have been characterized, the mechanism of CHH methylation remains unclear. The goal of our project is to further characterize the pathway that is involved in controlling the establishment and maintenance of DNA methylation. Specifically, by knocking out *Cmt2* (Chromomethylase2), a homolog of the well-characterized *Cmt3* (Chromomethylase3), we demonstrate that it is uniquely responsible for CHH methylation. Our results show that in *cmt2* mutant background, not only is there a near-complete loss of CHH methylation genome wide, but there is also significant decondensation of chromocenters independent of *Cmt3* and *Drm1/2*.

## ABSTRACTS

Alphabetical by Author

### **Molecular Characterization of the Developing Hypoglossal Nucleus**

By ERIC Y. WANG, Albert Y. Han, Bennett G. Novitch

The hypoglossal nucleus directs tongue movement, which allows for critical survival behaviors such as breathing, swallowing, and vocalization. The specification of cranial motor neuron identity during embryonic development is controlled by a variety of signaling molecules and transcription factors. Much is known about the early formation of cranial motor neurons but the mechanisms by which they acquire specific motor pool identities such as the hypoglossal nucleus are unclear. However, the formation of distinct neuronal identities belonging to different motor columns has been well characterized in the spinal cord and may provide a clue to the development of cranial motor neurons. One transcription factor that is critical for spinal cord development is *Foxp1*, which directs neural progenitors to become limb-innervating motor neurons of the lateral motor column. Our preliminary findings show that *Foxp1* and other transcription factors found in the spinal cord are also expressed in distinct subpopulations of the hypoglossal nucleus. We have characterized the expression of these markers in the developing mouse hindbrain (E13.5, E15.5, E17.5, P1). We plan to examine the functional outcome of removing these genes using transgenic mouse models and assess the silencing effects on cranial nerve integrity and axon trajectory as well as muscular control and behavior.

## ABSTRACTS

Alphabetical by Author

### **The Cytoskeleton Regulator Cdc42 Plays a Discrete Role in L3 neuron-specific Pathway that Regulates Layer Specificity in *Drosophila***

By C.Y. KIMBERLY TSUI, Matthew. Y. Pecot, Yi Chen, and S. Lawrence Zipursky

Neuronal networks require precise synaptic connections; however, molecular mechanisms underlying the assembly of such networks are still poorly understood. One key strategy to forming such networks is to form specific connections within separate layers. During targeting, axon growth cones are constantly being remodeled by dynamic cytoskeleton regulation. In the *Drosophila* medulla, lamina neuron axons target to discrete layers using different guidance mechanisms to form precise synaptic connections. Lamina neurons L1, L3 and L5 initially target to a common domain in the outer medulla using N-Cadherin adhesion and Semaphorin-1a (Sema-1a)/ Plexin A (PlexA) repulsion. However, only L3 segregates from that region and retracts into its final target layer M3. Here we explore the role of cytoskeleton regulator Cdc42 in L3 targeting. We discovered that Cdc42 is required in precise L3 targeting. In Cdc42 null L3 neurons, growth cones terminates at the correct layer but filopodia extending from the growth cones mistargets into deeper layers in the medulla. This phenotype resulting from loss-of-function of Cdc42 indicates that it takes part in an L3 specific pathway to mediate filopodia dynamics and control layer specificity. Moreover, Cdc42 mutant L3 neurons generates a similar phenotype as Sema-1a mutants, suggesting Cdc42 may act downstream of Sema-1a. These genetic analyses reveal that different neurons that use same guidance molecules for initial targeting can reach their final target layer by the expression of different cytoskeleton regulators.

## ABSTRACTS

Alphabetical by Author

### **The Effects of Growth Inhibition Reduction on Chronic Cortical Map Plasticity After Traumatic Brain Injury**

By Bibi Eghtedari

After traumatic brain injury (TBI), endogenous levels of growth inhibitory molecules are upregulated mostly as a result of the major glial inflammatory cascade that occurs as part of the wound healing process in order to reduce the effect of injury in uninjured brain regions. These molecules promote a non-growth permissive environment that blocks axonal sprouting, which limits the degree of neural plasticity and functional improvement that can occur. Previous studies have shown that the reduction of one such growth inhibitory molecule, chondroitin sulfate proteoglycans (CSPGs), with the bacterial enzyme chondroitinase ABC (cABC), led to an increase in axonal sprouting. In the current study, we sought to determine a possible linkage between axonal sprouting and the amelioration of forelimb reaching deficits seen in cABC-treated animals post TBI, by measuring c-Fos expression as a correlate of neural activation, by means of immunohistochemistry. It has been demonstrated that at 7 days post injury, cABC enzyme treatment increases c-Fos expression in the rodent brain. We explored the potential for a more chronic effect of cABC enzyme on cortical neuronal activation. We found that in 3 out of the 6 animals treated with cABC, c-Fos expression was significantly increased at 28 days post injury. By increasing structural plasticity through axonal sprouting to circumvent damaged brain regions, the hope is that we can produce more normal patterns of functional activation in order to promote improved behavioral outcome.

## ABSTRACTS

Alphabetical by Author

### Using Change Blindness to Study the Effect of Visual Attention in Visual Area V4

By Daniel J. Foster, Fabrice Arcizet, James W. Bisley

Visual attention is necessary to perceive and interact with our world; when we do not attend something, we are often completely unaware of it. This lack of perception is exemplified in change blindness tasks in which we are unable to detect a difference between two scenes separated by a blank screen, even though the change may be huge. Past studies have suggested that any behavioral effects of attention, including change blindness, are due to a slight modulation of neural activity in visual area V4; the current study seeks to test this hypothesis. Subjects are trained in a simplified change blindness task comprised of one, two, four, or eight objects on a screen. While attention can be focused with one object on the screen, attention must be spread with more than one object. Given the results of past studies we expect to see a change in the neural activity of V4 depending on the degree to which attention is spread; high activity should correlate with focused attention and lower activity with spread attention. Data from one animal demonstrated no modulation in V4 suggesting that V4 is not responsible for the behavior associated with attention. I will present training data from the second animal. Once training is complete we will test the neural activity in V4 from this animal.

## ABSTRACTS

Alphabetical by Author

### Odor-Driven Host-Seeking Behaviors of Parasitic Nematodes

By ANASTASSIA TSELIKOVA, Michelle Castelletto, Ryo Okubo, Spencer Gang, Elissa A. Hallem

Parasitic nematodes infect a significant part of the world's population, causing disease and draining financial resources. The biology of *Caenorhabditis elegans*, a free-living nematode, has been studied extensively; however, the mechanisms behind the olfactory host-seeking behaviors of its parasitic relatives are not well-understood. Examining these mechanisms will give us a better understanding of how the parasites target their hosts. Using chemotaxis assays, we compared the responses of six different parasitic nematode species and the free-living *C. elegans* to a panel of chemically diverse odors. Chemotaxis indices were then calculated to quantify the results. We show that each species has its unique preferences to certain odors, though different parasitic species that share host preferences have more similar chemotaxis indices. We also ran chemotaxis-carbon dioxide assays on the rat parasite species *Strongyloides ratti*, to mimic the mixture of odors that a host may give off. The attractive response phenotype was reduced in the presence of carbon dioxide, a by-product of the worms' host animals. We also compared the responses of different *C. elegans* life stages to a panel of odors to examine potential changes in olfactory mechanisms over the organism's lifetime. Some odors showed significantly different sensory valences between the *C. elegans* dauer and adult stages. We demonstrate that the odor preferences are both host-specific and species specific; moreover, odor combinations lead to a change in the parasites' responses. Understanding the variations between the olfactory mechanisms of the different parasites can lead to novel ways of combatting them.

## ABSTRACTS

Alphabetical by Author

### Functional Characterization Of RBFox Interaction in Pre-mRNA Splicing

By Diana Tran, Andrey Damianov<sup>1</sup>, Douglas L. Black<sup>1,2</sup>.

Through decisions to include or exclude particular exons, the highly regulated process of alternative splicing allows the generation of different isoforms from a given pre-mRNA transcript. A number of RNA-binding proteins, such as heterogeneous nuclear ribonucleoproteins (hnRNPs) are key players in the regulation of splicing. The RNA-binding *feminizing on X* (RBFox) proteins is another family of regulators controlling cell type-specific alternative splicing through binding the sequence UGCAUG within introns adjacent to the alternative exon. We have found that RBFox proteins are components of a novel complex along with hnRNP M. Hence, the possibility arises that RBFox may regulate exons lacking UGCAUG's through recruitment by other RNA binding proteins of this complex. To test this, we have made an hnRNP M-dependent minigene Dup51-WT, and we show that RBFox-3 enhances the effect of hnRNP M on its splicing. Since we found that the splicing of Dup51-WT is affected by the density of the cell culture, we tested this with two other RBFox-dependent minigenes, DupE33 and DupE9\*. We found that culture density affects RBFox-mediated activation of exon E33, but not the silencing activity on exon E9\*.

## ABSTRACTS

Alphabetical by Author

### Identification of the Interaction Sites between *Drosophila* Proteins Rabex — 5 and Rabaptin — 5 by Yeast—Two—Hybrid Method

By Julia T. Garcia, Imilce Rodriguez, Fernandez, Esteban C. Dell'Angelica

In eukaryotes, intracellular protein trafficking is fundamental for the function and survival of the cell. The Rab family of small GTPases are key regulators of this process by acting as switches that control vesicle budding, transport and fusion. Rab5 is a small GTPase that regulates vesicle trafficking involving the early endosome. Rabex---5, a guanine exchange factor (GEF) is critical for the activation of this GTPase. In mammals, Rabex---5 forms a complex with Rabaptin---5, which in turn is an effector of Rab5. This complex is known to enhance Rab5 activity. Previous studies using the human proteins had identified the interacting domains between Rabex---5 and Rabaptin---5; however, the specific amino acid residues required for the interaction need to be identified to gain a better understanding about the complex formation. The objective of this project is to uncover the specific interaction sites between Rabex---5 and Rabaptin---5 from *Drosophila melanogaster* using the yeast---two---hybrid (Y2H) method. Results from the experiments confirmed that the domains that are important for the interaction between both proteins in mammalian cells are similarly functionally conserved in its *Drosophila* homologues. Specific amino acid residues in Rabex---5 that seem to play a critical role in the interaction between this protein and Rabaptin---5 in the in vitro system had been also identified. These results will be used to further investigate the biological relevance of these amino acid residues in the interaction between these two proteins *in vivo*.

## ABSTRACTS

Alphabetical by Author

### **Utilizing small molecules to study presequence-degrading protease (PreP)**

By Jisoo Han, Juwina Wijaya, Carla M. Koehler

The mitochondrion is involved in a variety of critical processes in the cell; it produces energy used by the cell in the form of ATP, and participates in signaling and metabolism of the cells. Defect in mitochondria can result in various metabolic, and neurodegenerative diseases, including Parkinson's and Alzheimer's disease (AD). The majority of mitochondrial proteins are translated in the cytoplasm as preproteins. Preprotein contains N-terminal targeting sequence that directs them to mitochondria. N-terminal targeting sequence is recognized by mitochondrial translocation machineries, allowing preproteins to be translocated into the appropriate mitochondrial compartment. For a matrix-targeted protein, once the preprotein is in the matrix, matrix-processing peptidase (MPP) cleaves the targeting sequence to allow the protein to fold and function properly. The cleaved targeting sequence is further degraded by matrix-residing Presequence-degrading Protease (PreP) and exported out by out of the mitochondria to prevent damage to the mitochondria. Interestingly, PreP has been linked to AD due to its ability to degrade amyloid-beta peptide. In this study, we utilized a high-throughput screening approach to find small molecule inhibitors of PreP. Characterization of these molecules will allow us to use the small molecules as probes to further understand the function of PreP in general and to provide insight into the link between PreP, mitochondria, and AD.

## ABSTRACTS

Alphabetical by Author

### **Fabrication of self-cleaning ultrafiltration membranes using a su-perhydrophilic, self-doping polyaniline additive**

By James A.T. Temple, Brian T. McVerry, Xinwei Huang, Eric M.V. Hoek, Richard B. Kaner

Here we report a scalable drop-in technique towards the fabrication of self-cleaning ultrafiltration membranes. A self-doped polyaniline derivative was blended into polysulfone (PS) ultrafiltration (UF) membranes to enhance hydrophilicity, known to promote anti-fouling properties. Polyaniline, in its base form, was first sulfonated with fuming sulfuric acid, yielding sulfonated polyaniline with a 0.5 degree of sulfonation confirmed by XPS. The SPANi polymer was de-doped and dissolved in the pre-cast solution containing polysulfone at varying concentrations. During the phase inversion process, the SPANi is re-doped and precipitated within the PS membrane films in a facile one-step method. Composite membranes containing increasing amounts of SPANi were compared to the pure PS membranes to determine changes in performance, hydrophilicity, and antifouling characteristics. The composite membranes exhibit similar fluxes as the pure PS membrane and maintain rejection properties similar to current UF membranes. Captive bubble contact angle measurements displayed an increase in membrane hydrophilicity with the increasing amounts of blended SPANi. During flux decline and recovery experiments, SPANi/PS composite membranes showed higher flux recovery than the pure PS membrane, with the champion composite membrane regaining 95% of its original flux by washing with water, demonstrating a high resistance to irreversible fouling.

## ABSTRACTS

Alphabetical by Author

### **The Effect of a Conditional *Dnmt1* Null Mutation on Murine Germ Line Development *In Vivo*.**

By SARA K. TAYLOR, Serena A. Lee, and Amander T. Clark

DNA methylation in mammals is required for genome stability, regulation of imprinted genes, and embryonic development. DNA methylation is maintained through each cell division by DNA methyltransferase 1 (Dnmt1). Embryonic progenitor germ cells called primordial germ cells (PGCs) are specified from the epiblast at embryonic day (e) 6.5 following *Blimp1* expression, which works to repress the somatic program. PGCs begin as a methylated population but undergo global demethylation, which is completed by e13.0 to e14.0. The significance of this methylation in PGC specification and identity is unknown. To address this, we generated a conditional *Dnmt1* null mutation in the mouse germ line using a Cre recombinase driven by the *Blimp1* promoter and analyzed embryonic PGCs at e9.5. Deletion of *Dnmt1* specifically in *Blimp1*-expressing cells does not prevent the generation of Oct4+ presumptive PGCs when compared to the negative control embryos. Precocious expression of later stage germ cell marker MVH is detected in some but not all Oct4+ presumptive PGCs in conditional *Dnmt1* null mutant embryos. This preliminary data suggests a possible role for *Dnmt1* in repressing later stage germ cell genes in mouse germ line development.

## ABSTRACTS

Alphabetical by Author

### **The role of carbonic anhydrase in social motility of *Trypanosoma brucei***

By WALTER HARDESTY, Edwin Saada, Kent L. Hill

*Trypanosoma brucei* is a protozoan parasite that causes African sleeping sickness, a disease of high mortality rates in sub-Saharan Africa. *T. brucei* has a complex lifecycle that alternates between a tsetse fly vector and a mammalian host. In the insect vector, migration through several tissues is required for maturation into mammalian-infectious forms. However, little is known about how surface contact impacts parasite behavior because African trypanosomes have been traditionally studied in suspension culture. The insect form of *T. brucei* engages in social motility (SoMo) when cultivated on semisolid agarose surfaces. It is found that SoMo is affected by changes in CO<sub>2</sub> levels and regulated by flagellar adenylate cyclases (ACs), which catalyze the synthesis of cAMP. In many organisms, ACs function as CO<sub>2</sub> sensors and rely on the activity of carbonic anhydrases (CA), which are enzymes that interconvert CO<sub>2</sub> and bicarbonate. Here the role of the *T. brucei* CA as a mediator of cAMP signaling and social motility is investigated. To test this hypothesis, a knockdown of CA was first generated using tetracycline-inducible RNA interference, and cumulative growth data was collected on four clonal KD lines during uninduced and induced conditions, which indicated that CA was not required for viability. qPCR and SoMo assays are in progress to assess KD level and its impact on social motility. These studies could elucidate the role of CA as an early step in cAMP signaling, thereby providing a novel target for therapeutic agents.

## ABSTRACTS

Alphabetical by Author

### **Glia in Cell Culture Insert Support Neurons *in vitro***

By SAADIA HASAN, Klara Olofsdotter Otis, and Kelsey C. Martin.

The study of neuron-specific factors requires pure neuronal cultures. However, the absence of glia can affect the physiology of neurons negatively. This study compared three different treatments of neuronal cultures in order to find a treatment that reversed the deleterious effects of removing glia from neuronal cultures. The first culture, the control, consisted of a mixture of plated neurons and glia. The second culture consisted of plated neurons and glia treated with an antimetabolic agent, cytosine arabinoside, to kill the glial cells. Lastly, the third culture consisted of plated neurons with a neuron and glia plated cell culture insert, containing a permeable bottom to allow passage of secreted factors such as modulatory proteins. To compare the effects of the different conditions on synaptic activity, miniature excitatory post-synaptic currents (mEPSCs) were recorded using a whole-cell voltage clamp and the number of synapses was monitored using immunocytochemistry. Voltage-clamp data suggested a significant increase in mEPSCs frequency in the insert condition compared to control, indicating that the neurons were healthy and had robust synaptic activity in the presence of the insert. Immunocytochemistry experiments showed restoration of the number of synapses as well as postsynaptic and presynaptic components in the insert to control levels. This indicated that the insert could rescue the effects of removing glia, although the insert increased synaptic function as compared to control levels.

## ABSTRACTS

Alphabetical by Author

### **Therapeutic Targeting of dCTP Biosynthesis and Replication Stress Response in Acute Lymphoblastic Leukemia**

By Lisa Ta

Acute lymphoblastic leukemia (ALL) is an aggressive cancer known to affect 15% and 20% of pediatric and adult cancers, respectively. Despite intensive treatment, 15-20% of patients treated do not achieve complete remission or eventually relapse due to developed resistance. Therefore, recurrent ALL poses a significant challenge that requires new strategies to overcome chemotherapy resistance. We have recently developed a novel therapeutic approach aimed at impeding the biosynthesis of deoxycytidine triphosphate (dCTP), a critical nucleotide for DNA replication and repair in cancer. We demonstrate that co-targeting the novo pathway (DNP) and nucleoside salvage pathway (NSP), the two sole pathways in dCTP biosynthesis, leads to replication stress and DNA damage in chemotherapy resistant ALL cells. Due to a robust replication stress response (RSR) following this combination, apoptosis is modest; however, additional pharmacological inhibition of the RSR results in enhanced tumor cell death. These results indicate that combined targeting of dCTP biosynthesis and the replication stress response can be effective in the treatment of chemotherapy resistant ALL.

## ABSTRACTS

Alphabetical by Author

### **Effect of Tissue Transglutaminase on Fibroblast-Mediated Contraction of 3D Collagen Hydrogels**

By MICHAEL J. RALE, Onika D.V. Noel, Julia Mack, and M. Luisa Iruela-Arispe

Synthetic materials like polyethylene glycol, and natural components like collagen, have allowed hydrogels to model a variety of three-dimensional, extracellular environments. These have also spurred advances in regenerative medicine as investigative models for structures like the collagen scaffolding of the aorta. However, gel contraction and degradation can limit the use of hydrogels in long-term, co-culture experiments. We hypothesized that the addition of tissue transglutaminase (tTG2), a crosslinking enzyme, would structurally enhance natural polymer hydrogels, stabilizing conditions for long-term 3D culture. To examine the effect of transglutaminase on contraction, human dermal fibroblasts (HDFs; 100,000 per gel) were encapsulated in 2.5 and 5 mg/ml collagen hydrogels with 0, 20, or 200 ng/ml transglutaminase. Imaging and gel area measurements were taken over seven day periods to quantify the percent contraction for each condition. The results show that 5.0 mg/ml collagen hydrogels treated with transglutaminase exhibit reduced contraction when compared to the null treatment. This effect appears increased with increasing tTG2 concentrations. However, the levels of tTG2 used in this study seemingly have no effect on 2.5 mg/ml hydrogels suggesting the need for greater tTG2 levels in lower concentration gels. More importantly, the results offer a path for slowing the amount of gel contraction over a seven day period, preserving the advantages hydrogels offer for our future 3D, co-culture studies of cell behavior.

## ABSTRACTS

Alphabetical by Author

### **Treatment of a mouse model of multiple sclerosis with highly specific estrogen receptor- $\beta$ ligand, WAY 200070**

By Ervin Herrera, Timothy Yoo, Spencer Moore, and Seema Tiwari-Woodruff

Multiple sclerosis (MS) is an autoimmune neurodegenerative disease which affects the central nervous system (CNS) and is characterized by motor, sensory, and cognitive deficits. Current therapies for MS come mostly in the form of immunomodulatory drugs, which are not directly neuroprotective. Our lab has shown that the estrogen receptor  $\beta$  (ER $\beta$ ) ligand DPN has a direct neuroprotective effect in mouse models of MS. WAY200070 has 200-fold selectivity for ER $\beta$  over ER $\alpha$ , compared to the 70-fold selectivity of DPN. Our aim was to investigate the potential neuroprotective effects of prophylactic and therapeutic treatment with WAY200070 in a chronic mouse model of MS, experimental autoimmune encephalomyelitis (EAE). Treatment with WAY200070 both prior to and after disease onset improved EAE clinical scores. Immunohistochemistry of brain and spinal cord sections from WAY200070-treated mice showed improved myelin density, and increased mature oligodendrocyte and axon numbers compared to vehicle-treated group. WAY200070 treatment also improved corpus callosal axon conduction. While WAY200070 treatment had no effect on the peripheral immune response, it decreased CNS inflammation. Together, these findings strongly support a direct neuroprotective effect of WAY200070 treatment in a chronic mouse model of MS. Therefore, WAY200070 should be further investigated as a potential treatment option for MS.

## ABSTRACTS

Alphabetical by Author

### Characterizing Derlin-2-Dependent Retrotranslocation of Cytotoxic Distending Toxin and Ricin

By EMILY J. KIM, Aria Eshraghi, and Kenneth A. Bradley

Toxins derived from bacteria and plants are capable of directly altering cellular processes of the host. A subset of these toxins must be trafficked in a retrograde manner through the host cell Golgi apparatus and endoplasmic reticulum (ER) before reaching their intracellular target. Such toxins include cytolethal distending toxins (CDTs), which must enter the nucleus to cause double-stranded DNA breaks, and ricin, which damages host ribosomes, inhibiting protein synthesis. Several Gram-negative, CDT-expressing bacteria have been associated with various human infections. Ricin, extracted from the castor bean, is a potent weapon of bioterrorism. Though retrograde trafficking is necessary for CDTs and ricin to take effect, the details of how they translocate across the host cellular membrane have yet to be elucidated. We focus in particular on the requirement to exit the ER lumen, and we hypothesize that CDTs and ricin usurp ER-associated degradation (ERAD) to cross the ER membrane and access the cytosol. ERAD is a cellular process by which misfolded proteins in the ER are retrotranslocated into the cytosol, where they are degraded by proteasomes. We have determined that CDT and ricin intoxication require Derlin-2 (Derl2), a component of ERAD. The presented data demonstrate that CDT and ricin retrotranslocation is independent of the interaction between the C-terminus of Derl2 and the ATPase p97, an interaction which is necessary for the retrotranslocation of misfolded proteins. This indicates a novel Derl2-dependent ERAD pathway exploited by retrograde-trafficking toxins.

## ABSTRACTS

Alphabetical by Author

### The Effects of Colony Stimulating Factor 1 Receptor (CSF-1R) Activation on Prostate Tumor Cell Invasion and Metastasis

By BILL QUACH, Marcus Ruscetti, and Hong Wu

Prostate cancer is the second leading cause of cancer-related death in American men. While localized prostate cancer is treatable, metastatic, castration-resistant prostate cancer usually leads to death. It is unclear why some tumors metastasize, while other remain localized and indolent. Elevated CSF-1R expression has been demonstrated in numerous carcinomas, including prostate cancer, and is correlated with high Gleason scores, metastatic disease, and poor overall survival in patients. Recently, by combining conditional activation of the *Kras* oncogene and deletion of the *Pten* tumor suppressor gene in the prostate epithelium, our lab has created the *Pten*-null;*Kras*-activated mouse model, which develops distant macrometastasis. At the primary tumor site, we have also observed an epithelial to mesenchymal transition (EMT) phenotype, which could contribute to tumor cell dissemination and metastasis. Isolation and gene expression analysis of laser-captured EMT regions revealed up-regulated CSF-1R expression. In addition, CSF-1R expression is diminished when mTOR and MAPK are pharmaceutically targeted, suggesting that CSF-1R is activated through these pathways. Upon treatment with a selective CSF-1R kinase inhibitor, GW2580, the migration of prostate tumor cells isolated from our mouse model was significantly reduced *in vitro*. These data demonstrate that CSF-1R may be upregulated during the EMT process and involved in metastasis, providing efficacy for the use CSF-1R inhibitors as a treatment for metastatic prostate cancer.

## ABSTRACTS

Alphabetical by Author

### **Investigating the Role of Numb and Numbl like in Motor Neuron Development**

By CHALISA PRARASRI, Caroline Pearson, and Bennett Novitch

Numb (N) and its closely related homolog, Numbl like (NL) are adaptor proteins shown to play a role in regulating cell functions involved in neural development. My studies have indicated that N/NL are expressed by all motor neurons (MNs) in the embryonic mouse spinal cord (SC). Conditional double knockout (cKO) mice with N/NL missing in MNs display irregular ventral SC morphology and aberrant MN nucleic migration through the ventral horn. To determine how N/NL interact with cell machinery to keep MN soma in place within the SC, we investigated four possible causes of this aberrant MN nucleic migration: 1) the mutant SC morphology causes the basal lamina to be disrupted 2) the mutant SC morphology causes boundary cap cells (BCCs), which keep MN nuclei in the SC, to be mispositioned 3) loss of N/NL in MNs disrupts the signaling between BCCs and MNs 4) N/NL directly prevent nucleokinesis by interacting with components of the cytoskeletal machinery. Immunohistochemical and in situ hybridization analyses suggest that the aberrant nucleic migration observed in N/NL cKO MNs cannot be accounted for by a disruption of the basal lamina or disruption of the signaling between MNs and BCCs. Therefore, we are currently investigating whether N/NL directly interact with nucleokinesis components and block movement of MN nuclei. This research is significant because MNs require sensory innervation from the brain and other neurons in the SC in order to function properly, making the location of the cell body critical.

## ABSTRACTS

Alphabetical by Author

### **Dystrophin and Utrophin Expression Require Sarcospan: Loss of Alpha7 Integrin Exacerbates a Newly Discovered Muscle Phenotype in Sarcospan-null Mice**

By ALLAN W. KWOK, Jamie L. Marshall, Eric Chou, Jennifer Oh, Dean J. Burkin, and Rachele H. Crosbie-Watson

Three adhesion glycoprotein complexes stabilize the sarcolemma, protect against contraction-induced damage, and include both the dystrophin- and utrophin-glycoprotein complexes (DGC and UGC) and the alpha7beta1 integrin complex. While the tetraspanin-like protein sarcospan is a core component of both the DGC and UGC, its role within muscle is assumed to be inconsequential because its loss in mice results in an apparently normal phenotype. However, we performed a rigorous analysis of aged sarcospan-null mice and found that loss of sarcospan decreases both DGC and UGC levels, leading to impaired laminin-binding activity. Moreover, sarcospan-deficient muscle is more susceptible to eccentric contraction-induced injury despite an increase in levels of alpha7beta1 integrin. To genetically test whether integrin compensates for the lack of sarcospan, we generated mice deficient in sarcospan and alpha7 integrin (DKO) and examined muscle histology along with levels of the adhesion glycoprotein complexes. Muscle regeneration and fibrosis were exacerbated in DKO diaphragm muscle and were similar to muscle biopsies from patients with Duchenne muscular dystrophy. Expression of the DGC and UGC, laminin-binding, and Akt signaling were all negatively impacted in DKO muscle, resulting in severely diminished specific force properties. Our data demonstrates that sarcospan and integrin regulates DGC levels and that these interactions are necessary for extracellular matrix attachment and force development.

## ABSTRACTS

Alphabetical by Author

### **Increased Risk in RA: Evaluation of Polymorphisms of HDL-associated Haptoglobin Gene and Paraoxonase Gene**

By Cindy La, Ani Shahbazian, Srinivasa Reddy, and Christina Charles Schoeman

Cardiovascular disease (CVD) is the major cause of mortality in patients with rheumatoid arthritis (RA) who die 3-18 years earlier compared to the general population. HDL (high density lipoprotein) functions in cholesterol efflux and reduces the risk for atherosclerosis, a condition that results in the hardening of the arteries when cholesterol plaques build up in arterial walls. The goal of this project is to study the role of HDL associated proteins that may serve as biomarkers of CV risk in RA patients. In particular, our project will focus on genotyping methods to understand the function of two proteins associated with HDL: Haptoglobin (Hp) and Paraoxonase (PON).

Recent evidence suggests that certain polymorphisms of Hp and PON may be linked to cardiovascular complications in certain patient cohorts. We hypothesize that Hp and PON genotypes may function as independent risk factors for atherosclerotic development in RA patients. We have successfully genotyped 246 RA patients for the Hp gene with results showing 42.2% Hp 2-2, 39.8% Hp 1-2 and 17.8% Hp 1-1. We have also genotyped 18 RA patients for the PON gene, with results showing 72.2% QQ, 22.2% QR, and 5.6% RR. Currently we are conducting carotid ultrasounds on these patients to measure levels of carotid plaque development. These preliminary studies will allow for association studies between genotypes of HDL associated proteins—Haptoglobin and Paraoxonase—and the level of carotid plaque found in these RA patient cohorts.

## ABSTRACTS

Alphabetical by Author

### **Cellular Dynamics and demethylation of the developing germ line genome**

By Kevin Nee, Joseph Hargan Calvopiña, John Vincent, Amander T. Clark

Primordial germ cells (PGCs) experience dramatic changes to their epigenome during embryogenesis, undergoing initial genome-wide demethylation at murine embryonic day e8.5. Subsequent demethylation imprinting control centers, known as phase 2 of epigenetic reprogramming in the developing germ line occurs during murine days e10.5-e13.5 and is not well understood. Recent research has suggested that demethylation occurs passively via cellular division of PGCs. Traditionally PGCs have been difficult to study as single cells in culture without a somatic environment. Compounding the problem, genetic mutants that are generated for genes that affect PGC development often result in embryonic lethality. In order to investigate the mechanisms of germ line demethylation, we developed an *in vitro* organ culture model of the murine e10.5 aorta-gonadal-mesonephros (AGM) in order to study PGC demethylation. After demonstrating that PGCs in the organ culture develop identically to *in vivo* PGCs, this work's preliminary data suggests that demethylation may occur through a replication-dependent mechanism.

## ABSTRACTS

Alphabetical by Author

### Microarray Data Implicates Vascular Endothelial Growth Factor Receptor 2 in Multiple Ox-PAPC Signaling Pathways

By WILL MINTEER, Sangderk Lee, and Judith Berliner

Atherosclerosis is a severe chronic inflammatory disease of the vascular endothelium that has been shown to promote coronary heart diseases and stroke, causing more deaths in the U.S. than all cancer-related deaths combined. Oxidized phospholipids, such as oxidized 1-palmitoyl-2-arachidonoyl-sn-glycerol-3-phosphocholine (**Ox-PAPC**), accumulate in atherosclerotic plaque regions, alter vascular permeability, and trigger an inflammatory signaling cascade in cultured human aortic endothelial cells (**HAECs**). Vascular endothelial growth factor receptor 2 (**VEGFR-2**) is activated by Ox-PAPC in HAECs and has been implicated in Ox-PAPC associated cytoskeletal rearrangement and pro-inflammatory IL-8 expression. The aim of this study was to investigate shared signaling pathways of Ox-PAPC and VEGFR-2. We obtained microarray data from HAECs treated with Ox-PAPC alone and HAECs co-treated with a chemical inhibitor of VEGFR-2 (Tyrphostin-**SU1498**). Over 76% of genes up-regulated by Ox-PAPC and over 73% of genes down-regulated by Ox-PAPC showed SU1498 sensitivity. SU1498 sensitive transcripts were matched to groups of similarly regulated genes from previous analyses of donor responses to Ox-PAPC. These groups, called modules, were analyzed using DAVID software to obtain GO category clusters for each module. VEGFR-2 was implicated in many Ox-PAPC pathways including cytokine production, unfolded protein response, actin cytoskeleton organization, cell cycle regulation, and transcription factor activity. In conclusion, by comparing our microarray transcript data with systems analyses, we identified several target genes and important Ox-PAPC pathways mediated by VEGFR-2.

## ABSTRACTS

Alphabetical by Author

### Oxidative Stress and Inflammation in Mice with Disrupted Daily Rhythms

BY Danh Le, Analyne Schroder, and Christopher Colwell

The circadian system drives daily rhythms in biological and behavioral processes including locomotor activity. At the same time, activity is able to feed back to the circadian system and alter its output. This study aims to determine whether scheduling of activity at the wrong time of day will disrupt the circadian system that leads to oxidative stress and pathology. C57B6 mice under 12:12 light/dark conditions, will be provided either no wheels or daily running wheel access during the first 6 hours of the light period (ZT0-6), a time when mice are normally asleep. Studies suggest that mice utilize the wheel during this time, leading to disruptions in daily rhythms of heart rate, body temperature and activity. Our hypothesis is that disruption of circadian rhythms as a result of improperly scheduled wheel access will induce inflammation and oxidative stress in the heart and hippocampus as well as cause abnormal physiology. Over a 15-week period of scheduled wheel access, weight and blood pressure will be monitored. At the end of the study, mRNA levels of inflammatory and oxidative stress genes such as SOD1, catalase, and IL-6 will be measured using real-time PCR, whereby an increase in expression is expected. This study should determine whether improperly timed activity can have negative consequences on health and perhaps even accelerate disease.

## ABSTRACTS

Alphabetical by Author

### **Elucidating the developmental maturity of pluripotent stem cell derived neural progenitor cells**

By Kimberly Loo, Xavier Gaeta, Michaela Patterson, William Lowry

Human pluripotent stem cells (hPSCs) have the potential to differentiate into many cell types, yet it is not known how similar the process of PSC *in vitro* development reflects the *in vivo* process. Recent work from the Lowry lab has found that both human embryonic stem cells and human induced pluripotent stem cells make cells that are more similar to cells found only very early in fetal development. From this work, the Lowry lab identified a set of 105 genes whose expression appears to distinguish mature tissue derived cells from those generated from hPSCs. Among some of the differences observed between hPSC progeny and their respective natural counterparts are genes expressed only in early embryos, such as LIN28, DPPA4, and TCF7L1. Furthermore, a second set of genes fails to be properly induced during *in vitro* differentiation. These “maturity” or “specification” genes include NFIX, HOPX, and ZFP3. Both findings suggest incomplete specification or maturity of the PSC-derivatives. We hypothesize that gene manipulation experiments will be able to bring PSC derivatives closer to their natural counterparts on a global transcriptome level. In attempts to make PSCs that more accurately reflect their natural postnatal tissue derived counterparts, experiments were performed using lentivirus infections aimed to overexpress several maturation/specificity genes in PSC derived neural progenitor cells (NPCs). Real-time reverse-transcription PCR (qRT-PCR) was used to quantify gene expression levels at the RNA level, while immunofluorescence was used to quantify expression levels at the protein level. Further, a functional assay was performed to evaluate the effects of the gene manipulations by determining whether the cells have a higher propensity to generate neuronal or glial cells.

## ABSTRACTS

Alphabetical by Author

### **ORF10 of Murine Gammaherpesvirus-68 Inhibits Type I IFN Signaling**

By Harding Luan, Yong Hoon Kim, Danyang Gong, Leming Tong, Ronika Sitapara Leang, Jennifer Truong, Sara Shu, Emily Duong, Ren Sun, Ting-Ting Wu

Epstein-Barr Virus (EBV) and Kaposi’s Sarcoma-Associated Herpesvirus (KSHV), the two human gammaherpesviruses, are associated with a number of major malignancies including several cancers. Over time, herpesviruses have developed mechanisms to evade immune detection by hosts. However, these evasion strategies are difficult to study in human gamma-herpesviruses due to the lack of an effective small animal model. Therefore, we study viral innate immune evasion using a murine homolog of EBV and KSHV known as murine gamma-herpesvirus 68 (MHV-68). We found that ORF10, which is conserved among gamma-herpesviruses, blocks type I interferon (IFN) signaling. To demonstrate the importance of ORF10 in viral infection, triple stop codons were inserted in the predicted open reading frame. Replication of the mutant virus in culture was moderately attenuated in the presence of IFN alpha. We also studied the underlying molecular mechanisms for the anti-IFN activity of ORF10. ORF10 was shown to reduce the protein level of type I IFN receptor chain 1 (IFNAR1) without affecting the transcript level. Furthermore, ORF10 interacts with RAE1, which plays a role in the mRNA export pathway. We assess the role of RAE1 by constructing a mutant ORF10 that cannot interact with RAE1 and then measuring its effect on IFNAR1 protein level. We hypothesized that the mechanism for the evasion strategy of ORF10 is through intercepting the export of IFNAR1 mRNA, thereby reducing the synthesis of IFNAR1 protein, and preventing cells from responding to type I IFNs.