37 Garo Akmakjian

Dr. Julian Schroeder

Regulating Heavy Metal Homeostasis in Arabidopsis: OPT3 Affects Metal Toxicity Responses and Cadmium Distribution

Heavy metals are essential biochemical co-factors in all biological systems. Heavy metals, however, can be toxic when present in excess, and some heavy metals, such as cadmium, are toxic even in trace amounts. Plants represent a major entry point of cadmium into the food supply, but the processes by which cadmium is transported and distributed in plants are poorly understood. In a screen for heavy metal accumulating Arabidopsis mutants, we discovered that a plant mutant named opt3-2 (Oligopeptide Transporter 3) accumulates significantly large amounts of cadmium in seeds relative to wild-type. opt3-2 also over-accumulated cadmium in roots but under-accumulated cadmium in leaves. A key metabolite for detoxification of cadmium is the tripeptide glutathione (GSH). GSH distribution in opt3-2 was also perturbed, showing a significant under-accumulation of GSH in the roots but an over-accumulation of GSH in aerial tissues. OPT3 is expressed in the plasma membrane of companion cells (phloem), and the loss of OPT3 constitutively up-regulates the iron deficiency response in roots but not leaves. Shoot-specific expression of OPT3 successfully complements the root phenotype in opt3-2, suggesting that OPT3 is responsible for mediating shoot-to-root metal status signalling. Microarray analysis identified several deregulated transporters in opt3-2 that confer Cd sensitivity in yeast that may be responsible for the heavy metal distribution phenotype. In planta characterization of these transporters provides a means of understanding the mechanisms of phloem-mediated Cd transport and seed loading.

38 Angelita Ashbacher

Dr. Elsa Cleland

Evaluating the Effects of Projected Precipitation Shifts on South Coastal Chaparral Communities

The objective of this study is to evaluate the role of precipitation as a habitat filter in native chaparral communities of Southern California. Precipitation in the region is projected to decline overall while the frequency of extreme weather events such as El Nino are expected to increase. The overall decline in precipitation may restrict the number of successful ecological strategies leading species to converge on a set of functional traits such as low specific leaf area. Traits which allow species to tolerate extended periods of drought, may prove restrictive during high rainfall years. Novel species may then be able to exploit resources and space within the community leading to a divergence in traits. To test this, we used a trait-based approach to evaluate both within species responses and community level responses to varying levels of precipitation. Rainout shelters were used to achieve rainfall manipulations. Five levels of rainfall were applied to experimental plots following each winter rain event. Treatment levels were 0%, 50%, 100%, 150% and 200% of ambient rainfall with five replicates. Evergreen and Deciduous seedling as well as established mature shrubs were evaluated for trade-offs in major plant functional strategies, seedling establishment and survivorship in response to treatment. We found expected

trends for specific leaf area between major functional strategies. (p = 2.2e-16) Evergreen species generally had a lower specific leaf area than both deciduous and annual herbaceous species. There was no significant difference in specific leaf area between deciduous and annual herbaceous species. Overall, specific leaf area increased with increasing rainfall (p =.015). Additionally, specific leaf area for each species showed highly individualistic responses to soil moisture (p = 2.2e-16). Seedling survivorship showed a non-linear response to increasing rainfall (p =.0003). Under drought conditions, seedling survivorship was generally low regardless of functional group. Survivorship peaked under ambient conditions with a drop under high rainfall conditions. Deciduous species showed higher rates of survivorship than evergreen species overall (p = 0.084). Survivorship at the species level was highly species specific (p = .005) with no clear trend within major functional groups. Interestingly, we did not find any clear ecological trade offs in survivorship between major functional strategies under varying rainfall conditions. This data highlight the challenges in using a trait-based approach to evaluate community level responses to shifting rainfall conditions.

39 Lindsay Bailey

Dr. Marty Yanofsky

miRroring the importance of the post-transcriptional regulation in Arabidopsis fruit

In the plant research field, molecular and genetic studies mainly rely on a well-characterized member of the Brassicaceae family, Arabidopsis thaliana. The attributes that Arabidopsis possesses make it an excellent system to study developmental processes. Interestingly, data obtained from Arabidopsis have been used as a platform to start new research expeditions in other plant species. Using Arabidopsis, our group is combining genetic and molecular approaches with next generation technologies to study fruit morphogenesis. We have discovered that miRNA post-transcriptional regulation is critically important for fruit development. I will provide recent data supporting the importance of miRNA regulation during fruit development.

40 Eiri Daren

Dr. James Nieh

Sublethal doses of the pesticide imidacloprid alters honey bee (Apis mellifera) response threshold and communication, potentially affecting colony health

Much attention on honey bee declines has focused on the sublethal effects the pesticide, imidaclorpid, has on honey bee behavior. How it affects individual foragers and their ability to communicate known food sources or their preferences for nectar is unknown. The proboscis extension reflex (PER) assay has been used extensively to study how imidacloprid affects a bees ability to learn. Here, we use the PER assay to determine an individual's response threshold. Bees treated with the pesticide have higher response thresholds, thus responding less often to high concentrations of sucrose than control bees. Additional experiments show that individuals treated with imidacloprid forage and recruit less than those treated with the control. This increased preference for sweeter sucrose concentrations and their

decreased foraging and recruitment behavior may limit the intake of nectar and could contribute to a colony's decline.

41 Anish Dhamija

Dr. Brinda K. Rana

Does Accounting for Antihypertensive Medication Alter Genetic Association Studies on BP?

Hypertension is a chronic disease that is a risk factor for impaired cognition, stroke, congestive heart failure (CHF), and kidney failure among other diseases. Previous family studies suggest that the effects of antihypertensive medication may confound (1) the correlation between both body-mass index (BMI) and blood pressure (BP) and (2) heritability estimates of blood pressure. Identifying a model to correct for BP-lowering medications is important for applications in twin analysis of BP, and the relationship of BP to other phenotypes, such as BMI. We assessed blood pressure and antihypertensive medication status in 1,237 male twins (age 51-60 years) from wave 1 of the Vietnam Era Twin Study of Aging (VETSA). We used three approaches to adjust BP measurements for antihypertensive treatment. These approaches include (1) the addition of a fixed value of 10 mmHg and 5 mmHg to measured systolic and diastolic BP, respectively, for subjects on any antihypertensive medication, (2) an incremented addition of mmHg to BP based on the number of medications used, and (3) the addition of mmHg according to a specific antihypertensive drug class. We used the classical twin design to compute the heritability of the corrected BPs. We also assessed the relationship between BP traits and other traits measured in VETSA. Adjusting for antihypertensive treatment did not significantly affect the heritability of BP measurements in the VETSA data. However, we did find that addition of mmHg for antihypertensive treatment resulted in higher correlations between BP and other traits, such as BMI. Our data suggest that not correcting for antihypertensive medications may not greatly impact the results of twin analysis when investigating blood pressure in a cohort of middle-aged men. However, adjusting for antihypertensive medication may provide more power to detect relationships between BP and other traits, and it may have different effects on heritability as participants get older. In addition, we analyzed the demographics to compare the prevalence of hypertension, the blood pressure medications prescribed, and BMI in each region across the United States. The United States was partitioned into regions by (1) geographic location, (2) political affiliation, and (3) varying stress levels. The prevalence of hypertension was significantly different between the regions of high stress and regions of low stress, further suggesting a correlation between stress and hypertension.

42 Clinton Edwards

Dr. Jennifer Smith

A GLOBAL ASSESSMENT OF CORAL REEF HERBIVORES: Evidence for Fishing Effects on the Biomass of Different Taxonomic and Functional Groups

Abstract Effective resilience and restoration strategies for threatened and degraded coral reefs will be most useful if realistic management targets can be accurately determined. Because coral reef herbivores

consume fleshy algae and help promote coral abundance, numerous recent studies have called for the need to protect herbivores as a mechanism for maintaining or rebuilding reef resilience. However, little is known about the natural variability in herbivorous fish abundance within and among reefs and how fishing may alter herbivore biomass or community structure. Here we conduct a global meta-analysis on the variability in biomass of key taxonomic and functional groups and we examine how these values compare between fished and unfished locations at global and regional scales. Data were obtained through an exhaustive search of peer reviewed literature and collaborative efforts between SIO and US governmental monitoring programs. The resulting dataset is comprised of greater than 700 estimates of biomass from 107 sites from around the globe. We resolved the herbivore guild into functional subgroups and analyzed the biomass of these groups separately. Biomass of herbivores at unfished and fished sites was 46.26.3 and 20.11.1 g/m^2, respectively and was independent of regional effects. The functional subgroup analysis shows significantly higher biomass levels of the scraper/ excavator and browser sub-guild in unfished sites relative to fished sites (SE: 5.51.7 and 0.150.05 g/m^2, BR: 7.04 ±0.94 and 2.14 ±0.35; respectively) and was also independent of regional effects. These results suggest that over-exploitation of fish resources has large impacts on the herbivorous fish assemblage; with disproportionate effects in the scraper/excavator and browser sub-guilds. Current evidence suggests that scraping and excavating species are especially important in maintaining low fleshy algal abundance and promoting crustose coralline algae and coral recruitment while browsers intensely crop fleshy macroalgae, supporting the view that restoration strategies must maintain subgroups in proper ratios. Given the important role that herbivores play in maintaining the balance between algal and coral cover these results have significant implications for the development of management strategies to improve the resilience and restoration of the world coral reefs.

43 Hamid Ehsani-Nia

Dr. Tony L. Yaksh, Ph.D.

Effects of Spinal TLR-3 Activation in Non-Neuronal Cells of Rats on Pain Processing

Toll-Like Receptor- 3 (TLR-3) is a receptor of the innate immune system which dimerizes and activates upon binding to Double Stranded RNA (dsRNA). The role of TLR-3 is to bind to non-self dsRNA (usually indicative of viral infection) or self dsRNA (produced by tertiary ssRNA structures of necrotic cells) and signal nociceptive cascades. TLR-3 receptors exist upon glial cells in the brain and spinal cord. These glial cells are known to play an important role in facilitating spinal pain processing. Accordingly, we hypothesized that activation of these spinal TLR-3 by dsRNA would cause glial activation and initiate an enhanced response to noxious stimuli. There are four aims in this study: i) Examine the magnitude and time course of effects of spinal TLR3 activation with an intrathecally (IT) delivered TLR3 agonist (Poly I:C) on tactile and thermal sensitivity, ii) determine the effects of this spinal agent on ongoing diurnal activity; iii) examine the effects of spinal agents (minocycline, pentoxifylline, valdecoxib, and ketorolac) thought to block the activation of glial systems and cyclooxygenase products on pain behavior. And, iv) immunohistochemically define astrocyte and microglia activation motifs in specific regions of the spinal cord at 1 day and at 4 days after intrathecal injection of Poly I:C. In rats (male Holtzman 300 grams) with chronically placed lumbar IT catheters, IT Poly I:C displayed a significant sensitivity to light touch

(tactile allodynia: TA) with little change in thermal sensitivity that persisted for 4-7 days. These displayed a modest reduction in nocturnal activity during the early period. Rats receiving IT minocycline, pentoxifylline, and ketorolac prior to IT Poly I:C showed significant decreases in the persistent pain state. Rats injected with Poly I:C and sacrificed at day 1 showed substantial increases in astrocyte and microglia activation in regions of the superficial lamina and deep lamina of the dorsal horn as well as in the ventral horn. Activation of astrocytes and microglia diminished in rats sacrificed at day 4. Rats injected with IT minocycline or pentoxifylline, prior to Poly I:C and sacrificed at day 1 showed normal levels astrocyte and microglia activation, indicating an inhibition of the nociceptive cascade. Rats treated after to Poly I:C and sacrificed at day 1 showed no change in TA and no change in glial activation These results indicate that activation of TLR3 can initiate a persistent cascade of facilitated pain processing , mediated at least in part by a glially related cascade.

44 Rita Hanna

Dr. Åsa B. Gustafsson

Bnip3 Interacts with LC3 to Induce Selective Removal of Endoplasmic Reticulum and Mitochondria via Autophagy

Bcl-2 family proteins are known to regulate mitochondrial integrity and apoptosis. More recently, they have been found to play a role in regulating autophagy. Autophagy is a process involved in removing excess or damaged organelles. Bnip3 is a pro-apoptotic BH3-only protein which is known to cause mitochondrial dysfunction and cell death. We have previously discovered that Bnip3 is a potent inducer ofmitochondrial autophagy. In this study, we have investigated the mechanism by which Bnip3 promotes removal of mitochondria via autophagy. Bnip3 contains a C-terminal transmembrane(TM) domain that is essential for homodimerization and pro-apoptotic function. Here, we show that Bnip3 homodimerization is also a requirement for induction of autophagy. Several Bnip3 mutants that do not interfere with its mitochondrial localization but disrupt Bnip3 homodimerization failed to induce autophagy when overexpressed in HeLa cells. In addition, the TM domain is important in targeting Bnip3 to membranes and we discovered that endogenous Bnip3 localized to both mitochondria and the endoplasmic reticulum (ER). To investigate the effects of Bnip3 at mitochondria or ER on autophagy, Bnip3 was targeted specifically to mitochondria or ER by substituting the Bnip3 TM domain with that of Acta or cytochromeb5, respectively. Interestingly, Bnip3 induced significant autophagy in cells from both sites in cells. Moreover, we discovered that removal of mitochondria and ER by autophagy appeared to be mediated via binding of Bnip3 to LC3 on the autophagosome. Ablation of the Bnip3-LC3 interaction had no effect on the induction of autophagy by Bnip3 but significantly reduced removal of mitochondria and ER by autophagosomes. Thus, our data suggest that the Bnip3 homodimer functions as an autophagy receptor to ensure removal of both mitochondria and ER.

45 Naseem Khorram

Dr. David Broide

Alternaria induces STAT-6 dependent acute airway eosinophilia and epithelial FIZZ1 expression that promotes airway fibrosis and epithelial thickness

The fungal allergen, Alternaria, is specifically associated with severe asthma, including life-threatening exacerbations. To better understand the acute innate airway response to Alternaria, naïve WT mice were challenged once intranasally with Alternaria. Naïve WT mice developed significant BAL eosinophila following Alternaria challenge when analyzed 24 hours later. In contrast to Alternaria, neither Aspergillus nor Candida induced BAL eosinophilia. Gene microarray analysis of airway epithelial cell brushings demonstrated that Alternaria-challenged naïve WT mice had a 200 fold increase level of expression of “Found in Inflammatory Zone 1” (FIZZ1/Retnla), a resistin-like molecule whose increased expression in airway epithelium was confirmed by qPCR. Lung immunostaining confirmed strong airway epithelial FIZZ1 expression present as early as 3 hours after a single Alternaria challenge that persisted for at least 5 days and was significantly reduced in STAT6-deficient, but not PAR-2-deficient mice. Importantly, direct administration of recombinant FIZZ1 to naïve WT mice lead to airway eosinophilia, peribronchial fibrosis, and increased thickness of the airway epithelium. Thus, Alternaria induces STAT-6 dependent acute airway eosinophila and epithelial FIZZ1 expression that promotes airway fibrosis and epithelial thickness. This may provide some insight into the uniquely pathogenic aspects of Alternaria-associated asthma.

46 Chang Kyung Kim

Dr. Eyal Raz

STAT3 Suppress Invasion of Intestinal Tumor via Down-regulation of SNAI

Signal transducer and activator of transcription 3 (STAT) is a transcription factor that is involved in growth factor signaling, angiogenesis and apoptosis. It has been suggested that constitutive activation of STAT3 in tumor cells contribute to inducing cell proliferation and preventing apoptosis. Due to the role of STAT3 in tumor progressions and metastasis in nearly 70% of solid and hematological tumors, STAT3 has been implicated as a prominent molecular target for cancer treatment. We deleted STAT3 in APCmin/+ mice, which the mice develop polyps confined in mucosa layer within 20 weeks after birth, to determine whether STAT3 has similar impact in Colorectal cancer. However, STAT3 deletion induced invasion of tumor intestinal epithelial cells (IEC) in APCmin/+ mice. STAT3 negatively regulates an epithelial-mesenchymal transition (EMT) inducer SNAI via spontaneous degradation. Furthermore, the deletion in STAT3 expression in colorectal cancer cell lines induced more invasiveness, but deletion in both STAT3 and SNAI suppressed CRC cell invasions. Therefore, STAT3 is necessary for GSK3β-mediated ubiquitination and proteasomal degradation of SNAI.

47 HyeRi Kim

Dr. Eyal Raz

ATG16L1 suppresses IL-1β signaling via down-regulating p62

Frequently, the patients with IBD, inflammatory bowel disease, have ATG16L1 gene mutation, which is an essential component of autophagy formation. Based on this information, we can conclude that autophagy is important in IBD suppression. Then, we examined how loss of ATG16L1 function affects signal transduction pathways initiated by a pro-inflammatory stimulus. First, we checked which signal transduction pathway is influenced by loss of ATG16L1 function. Our data shows that the deletion of ATG16L1 will significantly influence the amplification of IL-1β signal transduction cascades. Based on our result, the influence of ATG16L1 gene occurs in upstream of its cascades; the increase in p62 expression amplifies IL-1β signaling in ATG16L1 knockout cells. When p62 interacts with TRAF 6 in the signal pathway, it will activate TRAF 6 by bringing multiple TRAF 6 together. Therefore, the increase of p62 will result stronger inflammatory response. The ATG16L1 is required for ubiquitination and degradation of p62. Then, we further explored the E3 ubiquitin ligase, Cullin-3, that brings ubiquitin to p62. In order to activate Cullin-3 to transfer ubiquitin to p62, Cullin-3 has to get neddylated, where ATG16L is essential for neddylation of Cullin-3. Based on these data, ATG16L1 suppresses a pro-inflammatory signal through down-regulation of p62 via Cullin-3 neddylation. This foundation can potentially suggest new target for pathologies due to p62 over-expression.

48 Vaibhav Konanur

Dr. Kathleen French

Identifying and characterizing leech neurons labeling for GABA

Inhibition plays an important role in neuronal circuits that produce dynamic processes such as establishing oscillatory rhythmic behaviors, tuning sensory systems to enhance contrast, and maintaining spatio-temporal firing coordination. For example, in the leech, Hirudo verbana, it has been shown that inhibitory connections shape many behaviors. In many circuits, inhibition is provided by GABA, which is typically found in interneurons. In contrast to vertebrate systems, leeches also have GABAergic motor neurons that provide central as well as peripheral, inhibition. We probed leech ganglia using immunohistochemistry to determine which of the 400 neurons in each ganglion along the ventral nerve cord are labeled by antibodies against GABA. We saw staining in several as yet uncharacterized neurons, and we are now characterizing these neurons. We have used several different antibodies against GABA and found that all of them reliably label the same set of cells. However, we were surprised to find that some leech inhibitory motor neurons that have previously been shown to be GABAergic were not labeled. Using dot blots, we determined that indeed the antibodies specifically recognized GABA. We currently cannot explain why these well-characterized inhibitory neurons do not label. We have begun to characterize the electrophysiological properties of the newly identified antibody-labeled neurons. We found that stimulating one of these neurons (cell 116) has an inhibitory effect on a known excitatory motor neuron (cell DE-3). Stimulating cell 116 hyperpolarized the membrane potential of cell DE-3 and decreased its firing rate. When we hyperpolarized cell 116, cell DE-3 depolarized, and its firing rate increased. In all electrophysiological experiments, we verified the identity of cell 116 by filling the cell with a fluorescent dye and then processed the tissue with the GABA antibody to look for colocalization of the dye and the antibody labeling. We found that this neuron has a morphology similar to other known motor neurons, and we will continue to explore its role in producing behavior.

49 Benjamin Lewin

Dr. Dong-Er Zhang

The mechanism of AE9a regulation of ALOX5

AML1-ETO (RUNX1-RUNX1T1) is a fusion of the proteins AML1, a DNA binding hematopoietic transcription factor on chromosome 21, and ETO, a transcriptional repressor on chromosome 8, resulting from the t(8;21)(q22;q22) chromosomal translocation, one of the most common chromosomal abnormalities in acute myeloid leukemia (AML), which is present in ~10% of AML cases and up to 40% of the French-American-British M2 subtype. Although full length AML1-ETO is not leukemogenic in various mouse models, a splice isoform AML1-ETO9a (AE9a) is highly leukemogenic. The ALOX5 gene encodes a lipoxygenase, a class of enzymes along with cyclooxygenases and P450 epoxygenase, responsible for the conversion of arachidonic acid into eicosanoids. Eicosanoids, such as prostaglandins and leukotrienes, are pro-inflammatory molecules that have also been implicated in cancer development. In particular, the ALOX5 gene has been shown to be necessary for function of leukemic stem cells (LSCs) in chronic myeloid leukemia (CML), a hematological malignancy closely related to AML. Through differential gene analysis, our lab has shown that ALOX5 expression is upregulated in AE9a-leukemic mice cells. Microarray analysis of AE9a stably transfected K562 cells not only revealed upregulated ALOX5, but also upregulated c-JUN. c-JUN is known to regulate gene expression through interactions with the transcription factor Sp1, which is also involved in the transcription of ALOX5. c-JUN has also been shown to be involved in the regulation of ALOX12, a homolog of ALOX5. We believe that AE9a may be acting through c-JUN to upregulate ALOX5 expression and plan to investigate c-JUN's role in AE9a's influence over Alox5 expression. We have shown upregulation of c-JUN in AE9a stably-expressing K562 cells compared to control K562 cells. Upon knock down of c-JUN by shRNA in both 293T and K562 cells we not only see lower expression of c-JUN but also observe lower ALOX5 expression. We have also used a luciferase reporter to show upregulation of ALOX5 expression in K562-AE9a cells compared to the control. These results support the hypothesis that AE9a upregulates ALOX5 expression through c-JUN.

50 Sunny Lu

Dr. Nai-Wen Chi

Comparative binding of RXXPDG sequences to pentavalent ANK repeats in tankyrase

Tankyrase-1 is a poly(ADP-ribosyl) polymerase (PARP) with a 20 ankyrin repeat (ANK) domain that interacts with a variety of unrelated proteins found in the nucleus and cytoplasm. This protein binds to a diverse set of TNKS substrates to facilitate PARsylation at the PARP domain located at the C-terminal end of TNKS. The tankyrase ANK domain interacts specifically with proteins bearing the amino acid motif RXXPDG, including Axin, TRF1 (telomere-repeat binding factor-1), and IRAP (insulin-responsive aminopeptidase). Sequence analyses suggest that the ANK domain developed via gene duplication from five separate ANK subdomains called ARCs (ANK repeat clusters). Consequently, the ANK domain likely contains five separate binding sites for RXXPDG-containing proteins. The specific binding avidity of each

ARC to individual RXXPDG-containing proteins remains to be elucidated. A better understanding of ARC interaction and binding specificity will shed light on whether adjacent ARCs may be used by TNKS to juxtapose multiple partners. It will also define subsets of TNKS partners that compete for binding to the same ARCs. Here I have purified each of the five ARCs of TNKS using the bacterial overexpression vectors that I have constructed. I also applied affinity precipitation and immunoblot analyses to compare a set of 18 RXXPDG-containing sequences in terms of their pattern of affinity toward the 5 ARCs. I found that all 18 RXXPDG proteins can bind to at least 2 ARCs, typically ARC-IV and –V, while some variations of the RXXPDG motif can bind to 4 ARCs. These findings suggest that competition for tankyrase can favor stronger binding of one protein over another possibly for poly(ADP-ribosyl)ation activity.

51 Jennifer Lundergan

Dr. Jane C Burns

The Presence of TGFβ-Receptor III in Kawasaki Disease

Transforming growth factor ß (TGFß) is a multifunctional cytokine that has important roles in cardiovascular remodeling, cellular differentiation, and proliferation, as well as T cell regulation, all processes that are important in Kawasaki Disease (KD), the most common cause of acquired heart disease in children. The TGFß superfamily co-receptor III (TGFßRIII) mediates ligand binding in the TGFß pathway, but also has roles in regulation of the endothelial mesenchymal transition and cardiovascular development. Evaluation of TGFßRIII through RTPCR and ELISA showed a significant down regulation of mRNA expression levels ($p<0.05$) with simultaneous up-regulation of protein levels ($p<0.05$) during the acute phase of KD. These results suggest that TGFßRIII may contribute to KD pathogenesis, possibly by altering TGFß-2 signaling.

52 Jen Nguyen

Dr. Amy Kiger

Dynamin in Disease: a Fly model for a Human Centronuclear Myopathy

Myofibers are large, multinucleated cells compartmentalized to perform specialized muscle functions. Small, disorganized myofibers and central nuclei characterize human centronuclear myopathy (CNM), a disease independently associated with mutation of three genes. Due to their common association, MTM1, AMPH2 and DNM2, which encode a lipid phosphatase, a membrane tubulator and a mediator of membrane fission, are predicted to jointly function in membrane trafficking by an unknown mechanism important for myofiber organization. We have previously found that mtm, the fly homolog of MTM1, maintains cell compartmentalization during abdominal myofiber remodeling, and that mtm mutant muscle exhibits hallmarks of human CNM. Specifically, we discovered that mtm is required for normal integrin trafficking and myofiber attachments, and that similar integrin defects arise in human CNM. To investigate the basis for DNM2-related CNM, we explore muscle-specific roles for shibire (shi), the single Dynamin in flies. We found that proper levels of shi are required in Drosophila muscle for animal viability during development. Muscle-targeted depletion of shi, like mtm, resulted in missing or

detached abdominal myofibers, although with a distinct effect on integrin-mediated muscle attachments. Additionally, integrin localization was also perturbed in muscles overexpressing GFP-tagged shi. We also observed GFP:Shi in puncta localized to the plasma membrane and to internal rings. In mtm-depleted muscles, GFP:Shi accumulated with integrin on abnormal endosomal-related compartments associated with defective PI(3)P turnover and disrupted integrin trafficking, indicating a potential site for shared Mtm and Shi functions. These and ongoing studies reveal a shared role for mtm and shi in integrin trafficking important in myofibers and relevant to understanding and treating human muscle disease.

53 Vishnu Parthasarathy

Dr. Shelley Halpain

The Role of Actin Dynamics in Soluble Amyloid-ß Induced Glutamate Receptor Endocytosis

Alzheimer's Disease (AD) is the most common form of dementia with a prevalence rate of 60-80% in adults over the age of 65. It is characterized by a progressive decline in cognitive and memory functions, which have been attributed to soluble, oligomeric forms of Amyloid-b (sAß) and its effect on synaptic function. sAß attenuates synaptic plasticity by inhibiting long-term potentiation (LTP) and enhancing long-term depression (LTD) of glutamatergic transmission (Klyubin et al, 2005) along with inducing memory and learning dysfunction in animal models (Clearly et al., 2005).Although various studies show the effects of sAß on synaptic structure and function, the molecular mechanisms behind these changes remain unknown. We show a role of actin dynamics in sAß induced glutamate receptor endocytosis in Alzheimer's disease. Actin is the primary structural component of dendritic spines and has been implicated in receptor stability at the synapse. Characterization of surface glutamate receptor subunit levels in the presence of sAß and through modulation of actin show that sAß induces severing of f-actin through activation of cofilin, which in-turn is responsible for glutamate receptor endocytosis. This model displaying a decrease in excitatory synapse function in the presence of sAß could present a mechanism behind the early memory loss and cognitive deficits witnessed in Alzheimer's patients.

54 Freyr Petursson

Dr.Terketaub

Activation of AMP-Activated Protein Kinase (AMPK) inhibits Biomechanical Injury-Induced Catabolic Reponses of Articular Cartilage Chondrocyes

AMP-activated protein kinase (AMPK) is a regulator of energy homeostasis and cellular metabolism. AMPK has been shown to exert anti-inflammatory effects in part by inhibition of NF-kB. Biomechanical injury is a known risk factor for osteoarthritis development; We therefore tested whether biomechanical injury alters AMPK activity. We showed mechanical injury increases the catabolic response chondrocytes, evident by an increase in NO production, and GAG release. We also found that biomechanical injury decreased p-AMPK-activity. We therefore tested the effect of AMPK activators in

response to biomechanical injury. Activation of AMPK attenuated the catabolic response indicated by a statistically significant decrease in GAG and NO release.

55 Michael Pham

Dr. Colin Jamora

Mechanism of E-cadherin mediated stem cell differentiation

E-cadherin is a transmembrane protein that mediates intercellular adhesion through binding catenin family proteins to the actin cytoskeleton. E-cadherin plays a role in differentiation but the precise mechanism is unknown. To determine which domain of E-cadherin is sufficient to induce differentiation, chimeric E-cadherin constructs were expressed in keratinocytes, cultured primary skin cells. Luciferase reporter gene assays showed that all constructs that have the p120-catenin binding domain and maintained α-catenin association induced differentiation but not E-cadherin constructs that lack either component. This suggests that sequestering of p120-catenin and association of α-catenin by E-cadherin is sufficient to induce differentiation.

56 Cheryl Philipsen

Dr. Chris van Schie Phd

Phosphoproteomics of Arabidopsis thaliana defense mechanism

Plants have to cope with various abiotic and biotic stresses in every environment. Plants lack the ability to move away from the stress which led to a diverse range of survival and defense techniques to deal with the (a)biotic stress conditions. Two defense mechanisms are triggered upon the entering of a pathogen into a plant. One is triggered by the recognition of pathogen associated molecular patterns (PAMPs) (e.g. flagellin) by transmembrane pattern recognition receptors (PRRs). The other mechanism is triggered upon effector recognition (e.g. AvrRpm1) or activity on targets of these effectors recognized by R-proteins. The Pseudomonas syringae – Arabidopsis thaliana system serves as a model for studying plant diseases, due to a wide variability of strains with mutations in either PAMPs or effector secretion distinction can be made between PTI and ETI regulated defense responses. Recent transcriptomic and proteomic analyses have shown proteins regulated during both mechanisms. e.g. PEN3, MPK3, MPK6, AHA1, ADL8 and ERF13. Because protein phosphorylation is a key post-translational modification and is important in defense signaling, it makes phospho-proteins perfect candidates for analyzing aberrant cell processes. Current liquid chromatography mass spectrometry (LC-MS) techniques for scanning the full proteome of the organism have low efficiency, is expensive and time consuming. Multiple reaction monitoring (MRM) is emerging as a cost-effective, high throughput method with increased sensitivity. In contrast to shotgun proteomics, MRM is directed at a predetermined set of proteins rather then the entire proteome. This increases the speed and accuracy of the analyses. Several steps were tested in the pipeline of a MRM assay and it is shown that organic solvents used for protein extraction is sufficient as well as a 1D ESI-LC MS analyses for analyzing phospho-peptides. However, due to inefficient ionization and signal suppression in phospho-peptides and low abundance in biological samples, problems arise

during sample preparation due to loss of phospho-peptides. Preliminary results show improved results using hot SDS as extraction buffer. Using MRM, results show out of 46 peptides 17 synthetic peptides are detectable in a complex mix of peptides and 5 peptides show promising results when analyzed in a complex biological sample using MRM. Future plans are directed towards faster analyses of data and improving MRM assays.

57 Wu Scott

Dr. Martin Yanofksy

Unraveling the Fruit Development miRstery

Arabidopsis thaliana is the model organism par excellence in plant research. The large portfolio of genetic, molecular and bioinformatic tools make Arabidopsis suitable to explore morphogenetic processes. Our research team has been focused on the analysis of fruit development in Arabidopsis for the last two decades. Whereas a lot of effort has been invested to ascertain the transcriptional regulatory mechanisms controlling this process, the post-transcriptional layer of control has been overlooked. Our recent findings have revealed the big impact that microRNA(miR)-guided posttranscriptional control has during fruit development. The data gained from our studies will open the door on further understanding the gene regulatory networks that orchestrate fruit patterning.

58 Tyler Sloan

Dr. Karl Willert

Defining Microenvironment Cues that Regulate Stem Cell Gene Expression

Human pluripotent stem cells (hPSCs) have the potential to become any type of cell in the body, a property that can be exploited to generate appropriate cells for cell replacement therapies of diseased, damaged, or dead tissues. A major challenge in the study of hPSCs is to direct their differentiation into the cell type of interest. The best way to achieve this is to mimic the events that control embryonic development by manipulating the cellular microenvironment. Using this technique, we can direct cells toward a certain lineage. One major component of the cellular microenvironment is WNT proteins, which control and regulate cellular development. In Humans, WNT genes encode 19 secreted lipid modified signaling molecules that interact with cell surface receptors encoded by the FZD (1-10) and ROR (1 & 2) genes. The interaction between WNTs, ROR, and FZD is still undefined. If we can characterize these WNT-receptor interactions, we will be able to generate a better protocol for the directed differentiation of hPSCs. Currently, the Willert laboratory has developed methods for the isolation and purification of WNT proteins. My approach will be to construct, express, and purify fusion proteins that carry the WNT binding area knows as the cysteine-rich domain (CRD) of various WNT receptors. After purification these recombinant proteins can be used to (1) study WNT-receptor interactions, and (2) interfere with WNT signaling in cell-based assays. To date, I have constructed vectors that carry the WNT binding domain of FZD7 and ROR2 fused to the constant region (Fc) of the immunoglobulin heavy chain. Additionally I am constructing a similar vector carrying the WNT binding

domains of FZD5 and FZD10. Using an affinity purification method, I have successfully purified FZD7-Fc fusion protein and I am currently characterizing the ROR2-Fc fusion protein. In a cell-based WNT reporter assay I have shown that the FZD7-Fc fusion protein interferes with signaling of WNT3A. These fusion protein receptors are critical reagents to investigate WNT-receptor interactions and to specifically modulate WNT signaling during the process of hPSC proliferation and differentiation.

59 Linh Truong

Dr. Paul Price

The Calcification of Staphylococcus aureus Bacteria: A Potential Defense Mechanism Against Bacterial Infections

The emergence of antibiotic resistant bacteria has become a worldwide concern. Our goal was to develop a new strategy to treat antibiotic resistant bacterial infections. We investigated whether bacteria are killed by the Mineralization by Inhibitor Exclusion (MIE) mechanism. This mechanism exploits the size exclusion characteristics of the bacterial cell wall, and therefore has no impact on mammalian cells. Our studies demonstrate that live Staphylococcus aureus are calcified by the MIE mechanism, and that calcification kills bacteria. The MIE procedure kills bacteria within hours at room temperature and physiological pH, and requires only calcium, phosphate, and a macromolecular calcification inhibitor.

60 Andrew Yatteau

Dr. Alain Dabdoub

Loss of R-spondin2 leads to additional hair cell development in the mammalian cochlea

Recent studies have demonstrated the importance of R-spondins, a novel family of secreted proteins, in canonical Wnt signaling and Fgf signaling, two pathways that play significant roles in the development of the cochlea, the hearing organ. Cochlear development requires several events including growth, proliferation and cell fate specification. Furthermore, normal cellular patterning in the cochlea, the organ of Corti, is crucial for auditory function as any patterning defects result in hearing deficit. R-spondins (Rspo) consist of four members in mammals, encoded by separate genes. To begin to investigate a possible role in cochlear development, the expression of all Rspo members was assayed at the time of mechanosensory hair cell differentiation. Rspo2 and Rspo3 were detected by PCR but not Rspo1 or Rspo4. The role of Rspo2 in the development of the mouse cochlea was determined in this study by examining the Rspo2-/- mice. Within the organ of Corti, a single row of inner hair cells and three rows of outer hair cells extend along the basal-to-apical axis of the cochlea. Every sensory hair cell is separated from the next by an intervening non-sensory supporting cell resulting in an invariant mosaic. In the Rspo2 mutant cochleae, there was an extra row of outer hair cells along with an extra row of support cells in the apical half of the cochlea. This data suggests that R-spondins are involved in cell fate determination in the mammalian cochlea.

61 Tabi Zara

Dr. Robert Schmidt

Positional Cloning and Characterization of the Rotten Ear (Rte)/Truncated Inflorescence Development (Tid) Gene

Maize (Zea Mays), in addition to being one of the most widely grown crops in the United States, is a widely studied plant model organism. Our group is particularly interested in identifying genes important in maize floral development. We identified a novel recessive mutant, rotten ear(rte)/truncated inflorescence development1(tid1), that is impaired in the growth of male and female floral organs and fruit. The Rte/Tid1 gene was successfully isolated through a map based cloning approach, with initial sequence analysis suggesting that Rte/Tid1 encodes a putative boron transporter. We are interested in characterizing and further investigating the role of Rte/Tid1 during the development of maize inflorescences.

62 Weihao Zheng

Dr. Milton Saier

Evolutionary Relationships of ATP-Binding Cassette Uptake Porters

The purpose of this study was to understand how ABC uptake transporters with different number of transmembrane segments (TMSs) evolved. All of the 34 families of ABC uptake proteins except for one (family 21) were shown to be homologous using the BLAST search tool applied to NCBI or TCDB as well as the IC, SSearch and GAP programs. Topological analyses revealed that these porters contain numbers of TMSs ranging from four to twenty. Phylogenetic analyses revealed which families share most recent ancestries. Intragenic duplication events occurred multiple times during the evolution of several of these uptake porters. They originated from a simple primordial protein containing 3-TMSs, which duplicated to 6 TMSs, and then produced porters of the various topologies via insertions, deletions and duplications. They all proved to be related to members of the previously classified ABC2 family (Wang et al., 2009).