CDB Abstracts

1 Maria Bahramzi

Dr. Dwayne Stupack

Role of integrin alpha-4 in Neuroblastoma

This research focused on determining whether integrin alpha-4 (a4) expression promotes neuroblastoma malignancy. The aim was to develop and optimize protocols for immunofluorescence and immunohistochemical staining of human neuroblastoma tissue for integrin a4 expression. Once this was achieved, we determined the a4 expression on the primary tumor samples from Rady Children's Hospital. We can use the results to define an association of integrin a4 expression with primary tumor stage and site of metastasis.

2 Kyle Begovich

Dr. Steve Briggs

Role of Ubiquitination and the Proteasome in Arabadopsis Innate Immunity

Examining plant resistance and signaling is of great importance to preserve the plants and crops being grown today. The main pathway that we are interested in focuses on the effector proteins that pathogen secrete to bypass the basal defense system and increase virulence. These effectors are recognized by resistance (R) proteins, which triggers the resistance pathway. There is not much known about the events that occur downstream of activation of R proteins. In order to help determine the events that follow R protein activation, we used a proteomics methodology to quantify changes in protein expression and specifically phoshorylation levels during resistance signaling. We used an inducible system that would trigger RPM-1 activation via the production of the bacterial effector protein, avrRpm1. Following quantitative LC/MS, it was found that numerous protein levels changed during immune signaling and about 1000 proteins were found to have post-translational modification, specifically in their phosphorylation levels. Here, we focus on two proteins of which phosphorylation levels increased dramatically; an Ubiquitin-like (UBI) and Ubiquitin-Interaction protein (UBINT). Ubiquitination and protein degradation are important during R-protein signaling, as literature shows that RPM-1 is degraded with coincident with the hypersensitive response (HR), and the proteasome is essential for resistance responses. We are currently conducting functional analysis of these two proteins using knockout mutants and overexpression of phospho-mimetic and phospho-dead proteins. Preliminary assays show increased resistance of ubi-knockouts to bacteria expressing avrRpm1, suggesting UBI could be a negative regulator of RPM1 function. Using the phosphorylation-mutants, we will test the function of UBI and UBINT phosphorylation sites.

3 Laura Bliss

Dr. Lars Bode

Galacto-Oligosaccharides reduce Entamoeba Histolytica cytotoxicity in vitro with and without the presence of Lactose.

Human Milk Oligosaccharides (HMO), complex sugars found in human milk, and Galacto-Oligosaccharides (GOS), sugars utilized in formula milk, protect the body from infection by inhibiting the adhesion of several microbial pathogens to human intestinal epithelial cells. One such pathogen is Entamoeba histolytica, the parasite that causes Amebiasis. It is suspected that GOS and HMO protect host cells by blocking binding of E. histolytica to host cells through interaction with the parasite's Gal/GalNAc-lectin, a major virulent protein that mediates adhesion and cytotoxicity. Of the two types of sugars, GOS protects most strongly. The objective of this project is to determine if GOS's effectiveness relies on its lactose content. Lactose, the smallest galacto-oligosaccharide, is highly protective in vitro but would be ineffective in vivo because it is cleaved by lactase before reaching the colon. To determine if the larger, indigestible galacto-oligosaccharides can protect against E. Hystolytica, without lactose present, a solution of GOS was separated by size on an FPLC and the fractions containing more than 4% lactose were found using HPLC analysis and excluded. Co-culture experiments with HT-29 cell monolayers showed that the lactose-free GOS was effective. Although these results need to be confirmed in vivo, GOS' effectiveness sans lactose raises hope for a GOS-based drug to help prevent amebiasis.

4 Kimberly Chia

Dr. Steve Briggs

Plant Immune Signaling through NDR1

The plant immune system is bipartite, consisting of PTI (PAMP-triggered immunity) and ETI (effector-triggered immunity). PTI involves the recognition of characteristic molecular motifs (e.g. LPS and flagellin) that are integral to pathogen structure or viability, and leads to reactions such as thickening of the cell wall and production of reactive oxygen species. ETI, meanwhile, employs highly specific resistance (R) proteins to directly or indirectly recognize pathogen effector molecules, triggering localized programmed cell death known as the hypersensitive response (HR). NDR1 is an Arabidopsis thaliana protein that has been shown to have an important role in PTI and ETI. It has also been suggested that NDR1 is involved in signal transduction, implicating it in key protein interactions. Building off NDR1 co-immunoprecipitation data, interactions between NDR1 and several proteins of interest were investigated via bimolecular fluorescence complementation (BiFC), a technique that allows in vivo visualization of protein interactions. NDR1 was observed to interact strongly in planta with NTMC (a membrane-associated protein), as well as WHY3 (an ssDNA-binding transcription factor). Protein expression was confirmed via Western blot. Preliminary bacterial growth assays with Pseudomonas syringae revealed that NTMC may also play a role in the defense response. Additional bioassays on other NDR1 interactor mutants, using P. syringae strains carrying different effectors, are currently under way.

5 Keval Desai

Dr. Susan Golden

Using cyanobacteria to drive down cost of Algal biofuel production

One of the challenges of Algal biofuels is that it can become expensive to provide nutrients for large biofuel farms. This could delay the commercial application of the biofuels research that is currently being done by many labs. We want to engineer another photosynthetic organism to produce the required nutrients to nourish algal biofuel strains. Cyanobacteria, also known as blue-green algae, are photosynthetic organisms that are often used for biotechnology and are a suitable base for a prototype. We are creating a strain of cyanobacterium Anabeana, a nitrogen fixing species, to produce and export a nitrogen rich molecule. To prove the system works we will co-culture the strain with S. elongatus, a non-nitrogen fixing species, and show that it can survive in a nitrogen-limiting environment using the nitrogen exported by the feeder. Octopine is a nitrogen rich molecule that can be used as a specific nutrient source. The system will be selective since only those organisms capable of metabolizing octopine will be able to benefit. Cyanobacteria are not known to synthesize or metabolize octopine, but through biotechnology we will engineer them to do so. Octopine synthesis and metabolism genes can be found in the T-DNA of Agrobacterium tumefaciens. Primarily, the ocs gene and the occ operon are of interest. The ocs gene is known to promote the synthesis of octopine from argenine and pyruvate, and we will clone this into anabaena to induce octopine production. The occ operon codes for octopine uptake and utilization proteins. I am going to use the occ and other suitable operons to create an octopine recipient strain from S. elongatus. I hypothesize that by cloning the occ operon into S. Elongatus, it can uptake octopine and use it as a nitrogen source under nitrogen limiting conditions. Methods: So far I have isolated the entire occ operon, 3000 bps long, through PCR amplification and cloned it into an expression vector. First I conducted a PCR using the T-DNA of Agrobacterium Tumefaciens and amplified the all the genes in the operon (occQ, M, P, and J). These were purified and cloned into an entry vector using the TOPO cloning technology. Using the LR gateway reaction from invitrogen I created an expression vector that can now be transferred to S. Elongatus. The expression plasmid will allow my genes to be inserted into the chromosome of S. elongatus. The genes will be under the control of the IPTG-inducible lacIq promoter. There is also a c-myc tag placed before the occQ gene. The c-myc tag will transcribe a sequence of amino acids that can bind with a special antibody and be isolated to measure the amount of protein expression. Future work: I will perform viability tests to make sure that the mutants are able to survive. To make sure that the proteins are being expressed I will perform western blot analyses using anti-cmyc antibodies. Then I will check if the mutants are able to import Octopine. To do this we will culture it in media supplemented with commercially purchased octopine. In addition I will also clone in ooxAB genes. These genes have been annotated to code for enzymes that facilitate the break down of octopine. Finally co-culture experiments with octopine producing Anabeana in nitrogen limiting environments will be used to show that it is possible to use a photosynthetic biological feeder to provide an economical nutrient source for other strains.

6 Leila Haghighat

Dr. Patricia Ann Thistlethwaite, M.D, Ph.D.

APJ directs venous differentiation of the coronary endothelium and is regulated by ERG

Recent data suggests that arterial-venous identity in the very early embryo is established before the onset of circulation and that the acquisition of arterial or venous phenotype is governed in endothelial

cells by the expression of specific genes. While much research has focused on the development and pathobiology of coronary arteries, little work has been done to understand the signals responsible for the identification and development of coronary veins. Previously, our lab has shown that APJ, a 7-transmembrane domain G protein-coupled receptor, is a lineage-specific marker for the endothelium of the developing coronary venous system and the earliest genetic marker of coronary venous phenotype. In mice, venous endothelial cells have been found to derive from a committed cell population that migrates to the right atrial epicardium on day E9.5, and the right atrium is, in fact, the first site of venous network formation at day E10.5. Apelin, the endogenous ligand for APJ, has polarized expression within the right atrium as well. These results implicate an important role for APJ in the early identity and network development of coronary veins and give rise to the need for studying its transcriptional regulation in vivo. We propose a molecular mechanism for venous endothelial determination that involves regulation of APJ expression by the transcription factor ERG. We show that the expression of ERG is temporally and spatially associated with the development of APJ-expressing endothelial cells in the developing heart. Overexpression of ERG via AAV-transduction in coronary endothelial cells transcriptionally upregulates APJ, and APJ -/- mice exhibit a compensatory increase in ERG expression, as assessed by Western blotting. These findings suggest that ERG controls the transcription of APJ, and, as such, is important in venous differentiation of coronary endothelium.

7 Michelle Hoang

Dr. Susan Golden

Identification of CikA Localization Partners in Synechococcus elongatus

Michelle Hoang, Juliana Bordowitz, and Susan Golden Synechococcus elongatus PCC 7942 is the simplest unicellular organism, with a genome of ~2.7 Mb (1), in which an endogenous biological circadian oscillator has been documented. The cyanobacterium has a robust circadian clock that consists of the KaiABC complex that interacts with other proteins to sense environmental stimuli. S. elongatus does not appear to use photoreceptors to set the clock; instead, a key input protein, CikA (circadian input kinase), uses redox to sense light indirectly based on photosynthetic activity and relays information regarding the external time to the oscillator (2). Bioinformatics have shown that CikA contains three domains necessary for proper functioning: a histidine protein kinase (HPK), a GAF, and a pseudo-receiver (PsR) (6). The PsR domain is also predicted to function as the domain that interacts with peripheral membrane proteins to position CikA at its specific pole position (6). Mutation of CikA's PsR domain results in delocalization that can be visualized using a green fluorescent reporter ZsGreen (ZsG) from a Zoanthus sp. coral (6). A similar pattern of mislocalization in the CikA-∆PsR mutant is expected in cells lacking the necessary factors that interact with PsR. To identify CikA's localization partners, I performed a screen of single-gene knockout mutants from the S. elongatus library (UGS 1-26). An E. coli plasmid containing the ZsG-CikA fluorescent construct was incorporated into Neutral Site I (NS I) of the S. elongatus chromosome by reciprocal homologous recombination to allow for visualization of delocalized CikA. Of the approximately 2426 library mutants representing about 88% of all genes in the S. elongatus genome, 158 mutants have shown the delocalized phenotype.

Bioinformatics analyses are being conducted on these candidates in order to identify whether they could be transmembrane proteins required for localization of CikA at the cell pole.

8 Soheil Karbassi

Dr. Milto Saier

Transport Proteins Encoded within the Genome of Myxococcus xanthus

Transport Proteins Encoded within the Genome of Myxococcus xanthus Abstract Myxococcus xanthus is an unusual bacterium with the capability of undergoing two mechanistically distinct types of gliding motility and forming fruiting bodies containing differentiated environmentally stable spores. The entire genome has been sequenced. It is one of the largest prokaryotic genomes yet to be sequenced. It is 9,139,763 nucleotides long, and consists of a single circular chromosome. This genome is predicted to encode 7,316 proteins. We have analyzed the proteome of M. xanthus and identified 436 potential transport proteins. These have been analyzed to determine (a) the matching hit in the Transporter Classification Database (TCDB), (b) the regions of the two proteins that show homology, (c) the positions of the transmembrane segments, if any, that align with each other, (d) soluble protein domains present within the subject and hit sequences, and (e) the blast score (e-value) relating these two proteins. Some of the matches proved to be in hydrophilic domains which are not indicative of transport function. Those subject proteins that exhibit significant similarities in the transmembrane domains were retained for further study. The TMS's that aligned were analyzed for evolutionary significance, and when necessary, the evolutionary pathway taken for the appearance of these proteins was determined. Repeat elements were identified, and the possibility that the presence of repeats is a characteristic feature of members of this family was evaluated. The results revealed the distribution of the transporters of various classes and subclasses as well as the range of substrates transporters. Additionally, the query proteins in each family within each class was evaluated for the presence of numbers of TMS's and topological types. These studies serve to define the transporters encoded within the genome of M. xanthus.

9 Zixuan Shao

Dr. Yunde Zhao

Genetic Screening for Arabidopsis Mutants that Suppress the Phenotypes of npy1 cuc1 Double Mutant

Auxin is an essential regulator of plant organogenesis. Biosynthesis, transport and signaling pathways of auxin have previously been studied using the model organism Arabidopsis. Yet, the exact mechanisms of these pathways are still not well understood. Here, we conducted a novel genetic screen for enhancers in the npy1 cuc1 double-mutant background, which are defective in auxin signaling. The npy1 cuc1 mutants display defects in embryogenesis, seedling development, and flower development. Several mutants have been found that develop pin-like inflorescence in the nyp1 cuc1 background, but not in wild type background. Our results suggest that the new mutants are informative in dissecting the mechanisms for flower organogenesis. Each of the enhancer mutants shows a different level of

inflorescence deformity indicating there are a variety of genes that could possibly be involved with auxin related flower initiation. Future work will be done to genetically map the gene associated with each of the enhancers.

10 John Tat

Dr. Seth J. Field, M.D., Ph.D.

Characteristics of Golgi Morphology Under the Condition of DNA Damage

GOLPH3 binds to phosphatidylinositol-4-phosphate and myosin-18A. This complex links the Golgi membrane to the actin cytoskeleton, which creates a tensile force that functions to pull vesicles and tubules off the Golgi. An addition consequence of this tensile force is the stretching of the Golgi ribbon around the nucleus. Golgi morphology is not static, however, and can change under different cellular conditions. In particular, in vitro studies of HeLa cells reveal that when DNA damage is induced with anti-cancer agents, Golgi morphology changes from an organized ribbon around the nucleus into tiny vesicles dispersed throughout the cell. An important question to answer then: where does the Golgi go after DNA damage causes it to disperse? These studies also discovered that DNA damage does not cause the Golgi to colocalize with the transitional endoplasmic reticulum.

11 Allison Van Vooren

Dr. Ryan Philippe

Investigating the Metabolic Evolution of Chalcone Isomerase (CHI) with high-throughput protein-ligand screens

In vascular plants, Chalcone Isomerase (CHI) catalyzes the committed step in flavonoid biosynthesis, converting chalcones to s-flavanones. This unique enzyme exhibits impeccable stereospecificity with a rate that approaches the diffusion limit of efficiency. The evolutionary lineage of CHI is still unclear, but research of the Arabidopsis thaliana genome has revealed the presence of five CHI homologs. These basal members of the CHI-like family are non-catalytic, fatty acid (FA) binding proteins, suggesting the appearance of catalytic activity in the evolution from primary to secondary metabolism. This study aims to identify interactions between CHI-family proteins (AtCHI, AtCHIL, AtFABa1, and AtFABb) and a 960 chemical compound library using high-throughput thermofluor assays. Relative shifts in fluorescence temperature indicate protein interaction, and these findings potentially have great significance in understanding the catalytic evolution of CHI. Three positive interactions with CHI have been identified thus far, and further investigation into the nature of these protein-compound interactions remains do be deciphered. Nevertheless, these findings will aid in the understanding of CHI ancestry, a vital component in the study of protein mechanisms and metabolic evolution in plants.

12 Alexander Vu

Dr. Benjamin Yu

Understanding Stem Cell Differentiation through Adenovirus E1A Oncoprotein

The early steps in differentiation by embryonic stem (ES) cells are just becoming understood. In classic embryological models, the E1A oncoprotein has been used to study the early events of neuroectoderm and mesoderm patterning. The Yu laboratory has applied a similar approach to mouse ES cells using E1A to investigate the transcriptional and cell cycle changes that take place during the transition of pluripotency toward more differentiated states. We found that expression of E1A induces a cardiac mesoderm fate over an approximately six-day period. During this period, mouse ES cells transition from early primitive streak mesoderm (Brachyury, Goosecoid-positive) to cardiac markers (Nkx2.5).

13 Robert Yuan

Dr. Dwayne Stupack

Characterizing a novel isoform of caspase-8

Caspase-8 is a cytosolic protein that functions as an initiator of apoptosis, or programmed cell death. Procaspase-8 is comprised of a prodomain region, consisting of two adaptor domains, as well as a catalytic domain, which effects the proteolytic activity of caspase-8. In the presence of an appropriate apoptotic cue, the adaptor domains function to recruit caspase-8 to a death-receptor activation complex. However, a number of caspase-8 isoforms lack proteolytic activity and encode only the adaptor domains, suggesting that these domains may serve cellular functions aside from facilitating apoptosis through the death-receptor pathway. In fact, it has been proposed that some of these isoforms might function as inhibitors of apoptosis, competing with other forms of caspase-8 for recruitment to the activation complex. Here, I characterize a previously hypothesized adaptor-only isoform of caspase-8, designated caspase-8 isoform 6. I identify and isolate Isoform 6 from differentiated monocytes and perform functional studies utilizing caspase-8-null cells reconstituted with this novel isoform. Isoform 6 expression does not influence cellular proliferation and is associated with the formation of filamentous structures within the cell. Additionally, Isoform 6 can induce cell death in the absence of other death signals. Thus, Isoform 6 serves to promote caspase-mediated apoptosis rather than inhibit it.