18 Aditi Abraham

Dr. Patricia Finn

Microbiome: Relevance for the Understanding of Lung Transplant Rejection

Introduction: Chronic Obstructive Pulmonary Disease(COPD) and cystic fibrosis have become major public health problem over the last 40 years. COPD is the fourth leading cause of death in America and cystic fibrosis afflicts 30,000 Americans and 1,000 new cases are diagnosed every year. Currently the only viable treatment for these end stage diseases is lung transplantation. Unfortunately, rejection occurs in 35-50% of lung transplants. The term microbiome refers to the microbes that inhabit the human body. It has been suggested that disturbances in the microbiome may cause or exacerbate disease states. We hypothesize that disturbances in the lung microbiota increases the risk of lung transplant rejection Methods: Bronchoalveolar lavage (BAL) was collected from transplant subjects via bronchoscopy in compliance with the Institution review board. Transplant subjects are from the Center for Lung Transplantation at the UCSD Medical Center. BAL samples were collected from lung transplant (n=5) and COPD (n=46) patients. Bacteria were collected using centrifugation at 14,000rpm. DNA was isolated from the bacterial pellet using the Qiagen DNeasy kit. DNA concentration and purity was verified using Nanodrop spectrophotometry. Extracted bacterial DNA was amplified by PCR 16s expression. Samples will be sequenced by Illumina 1G analyzer and identified by BLAST and other bioinformatics software for taxonomy assignment. Results: Lung transplant samples collected 24 hours after transplantation showed a very high expression for 16S. About 1/2 of COPD samples showed a moderate 16S expression level. Interestingly, for those COPD samples that did not have 16S expression at 35 cycles, expression was significantly increased to a moderate level when the number of PCR cycles was increased to 70. Relative expression is higher in lung transplant samples than in COPD. Conclusion: There is a significant difference in the amount of bacteria present in lung transplant versus COPD samples. It remains to be elucidated whether this increase corresponds to a completely different microbial profile or an increase in normally commensal bacteria that causes pathogenesis. To answer these questions we are working toward collecting samples at set time points during the transplantation process as well as collecting normal samples to act as a control for comparison.

19 Ishita Desai

Dr. Suresh Subramani

The Role of Doa4 in Peroxisome Degradation in Saccharomyces cerevisiae

Doa4 is a deubiquitinating enzyme that plays a major role in the ubiquitin-proteasome system in S.cerevisiae. Past research has indicated the protease plays no role in the cell's general autophagic machinery. Our research conducted with GFP-tagged thiolase as a marker in DOA4 deletion mutants indicates that such a role may exist in the peroxisome degradation pathway known as pexophagy. Strains were grown in a glucose- and nitrogen-rich medium prior to transfer to an oleate-containing medium in order to induce peroxisome biogenesis. The subsequent transfer to a glucose-containing, nitrogen-deficient medium results in pexophagy in the WT strain. Western blot results indicate that the

DOA4 deletion mutant demonstrates a near complete lack of pexophagy. Various other ubiquitin-proteasome system components were also tested for involvement in the process. Hrd2-1 and Δpdr5, complemented with MG-132, are deficient in proteasome function. Utilizing thiolase as a marker for pexophagy, results indicate both mutants are unaffected in the process. UFD2, UFD3, UFD4, UFD5 and OTU1 deletion mutants, deficient in stages of the ubiquitin-proteasome system, also do not indicate a deficiency in pexophagy. The results imply that the function of Doa4 in pexophagy appears to be unrelated to its role in the ubiquitin-proteasome system. Microscopy analysis of the results, however, indicates that the DOA4 deletion mutant is deficient thiolase import. Thus, additional research will serve to confirm Doa4's unique role in pexophagy via GFP-tagging of peroxisomal membrane proteins rather than matrix proteins similar to thiolase. Further research will be conducted in order to determine the role Doa4 plays in organelle-specific autophagy machinery as well as its localization under pexophagy conditions.

20 Randal Du

Dr. Dong-Er Zhang

The Transcription Factor RUNX1 and its Role in Hematopoiesis

The transcription factor RUNX1, also known as AML1, CBFA2, or PEBP2alpha, has been shown to play a critical role in hematopoiesis. Runx1 KO mice have been shown to die early in embryonic development. Conditional knockout mice exhibit imperfections in megakaryocytic maturation, along with imperfections in lymphocyte differentiation. The translocation t(8;21) can also lead to the RUNX1/ETO fusion protein, which has been identified in about 12% of acute myeloid leukemia patients. Here, we examine various possible targets of Runx1 regulation using promoter luciferase assays and site directed-mutagenesis techniques. Ultimately, the goal of finding these targets is to identify other factors that are important for hematopoiesis.

21 Aleksandr Gorin

Dr. Amy Pasquinelli

Interaction of Argonaute with tRNAs in Caenorhabditis elegans

MicroRNAs (miRNAs) are a family of small non-coding RNAs that post- transcriptionally regulate gene expression. miRNAs are crucial for the proper developmental timing in animals and their misregulation has been associated with several forms of human cancer. miRNAs function by guiding the Argonaute proteins to 3' untranslated terminal region (UTR) of the target mRNA by imperfectly base-pairing to target sequences. The miRNA/Argonaute complex then induces the translational repression and/or degradation of the target mRNA. Here, we identify that transfer RNAs (tRNAs), a class of non-coding RNA molecules not previously known to interact with Argonaute or the miRNA machinery in Caenorhabditis elegans, associated with Argonaute. Previous studies in our lab identified RNA molecules that interact with Argonaute and created a map of these interactions on the C. elegans transcriptome. Using the data from this experiment, I identified tRNA molecules that appeared to interact with

Argonaute in this assay. Bioinformatic analysis revealed that the initiator methionine tRNA interacted with the Argonaute complex at a reproducibly higher level than other tRNAs. To further investigate this interaction, levels of tRNAs in Argonaute mutant C. elegans worms were compared to those of wild type worms using PCR and northern blot analysis. Our findings suggest a novel role for Argonaute in which this protein may play a role in the biogenesis/maturation of tRNA molecules.

22 Danielle Hagtrom

Dr. Suresh Subramani

Requirement of Cysteine Residues for the Function of the Peroxisomal Targeting Signal Receptor, Pex5

Import of most matrix proteins into peroxisomes requires the binding of cargo proteins containing a peroxisomal targeting signal 1 (PTS1) to the receptor protein Pex5, so that the receptor can aid translocation of the cargo across the peroxisomal membrane. Recent evidence from this laboratory suggests that the Pex5 homodimer contains an intermolecular disulfide bond which possibly affects the binding and release of cargo. In a joint project with my post-doc mentor, Dr. Ma, we investigated the three conserved cysteines that could be involved in Pex5 intermolecular disulfide bond formation for their functional significance. We found that cysteine 10 was critical for the function of Pex5 as mutation of this residue caused complete loss of disulfide bond formation and loss of function of Pex5. In contrast, cysteines 338 and 444, when mutated, could still form disulfide bonds but had less efficient function as a receptor. We also found that the binding of cargo to, and release from, Pex5 is subject to redox regulation. This work was supported by a Biological Sciences Eureka! scholarship to DH and NIH grant DK41737 to SS.

23 Aleksandar Jamborcic, Garrett Brooks, Alexandra Chen, Vicky Hwang, Sher Khan, John Magarian

Dr. Joseph and Kit Pogliano

Structural Protein Identification and Analysis: A Parts List of a Novel Mycobacteriophage

Bacteriophage exist at the nanometer scale as tiny packages of protein and DNA, yet they contain all the structural components needed to carry out an infection. Our study focuses on mycobacteriophage, a diverse group of bacterial viruses that infect bacteria primarily in the Mycobacterium genus. We isolated and characterized the novel mycobacteriophage, Fionnbharth, from an environmental sample. Beginning with genome sequence data, we used computational approaches (BLAST, Phamerator) to predict many of the structural proteins belonging to the phage particle. We confirmed the expression and presence of these proteins in the phage particle using tandem mass spectrometry. We identified the following major components necessary to build the bacteriophage including: tail, capsid, portal, tape measure, and assembly proteins. Here we will discuss how these phage components compare to other phage, and the insights we gained from confirming genomic data with proteomic data. Our results provide insight into the evolution of our bacteriophage with respect to other phage.

24 Yujung Lee

MB Abstracts

Dr. Steven Briggs

Role of Thioredoxins in Plant Immunity

Title: Role of Oxidoreductases Thioredoxin 3, 4, and 5 in Plant Immunity Plants are regularly exposed to stresses that negatively affect their development and nutritional content. One of the most severe stresses that plants are exposed to is pathogen infection, caused by plant pathogens such as Pseudomonas syringae. Plants have two main types of defense against pathogens the PAMP triggered immunity (PTI) and effector triggered immunity (ETI) which recognizes and defends the plants against pathogen associated molecular patterns (PAMPs) and effector molecules. When a plant is infected by pathogens there is an in salicylic acid (SA) which causes an important reduction in Nonexpresser of Pathogenesis Related genes-1 (NPR1) by the oxidoreductase Thioredoxin 5 (TRX5). This reduction in NPR1 allows its monomerized form to become nuclear localization and it leads to transcription of defense related genes. In order to determine what role of redox signaling in plant defense against pathogen infection we are studying plant TRX3, TRX4, and TRX5 and their role in reducing the disulfide bonds of various proteins that are involved in plant defense against pathogen, such as NPR1. Our research involves creating double and triple mutants of trx3, trx4 and trx5 and subjecting these mutants to pathogen infection to determine their defense phenotype. We are also interested in using in vivo TRX pull down to determine the interacting partners of these oxidoreductases and how these interactions change when the plant is treated with different defense elicitors. This study will help us better understand the plant immunity.

25 Shiqian Li

Dr. Maho Niwa

The recognition of substrate by Ire1 is determined by a variable loop site in the ribonuclease domain

The unfolded protein response (UPR) is activated due to increased amounts of misfolded proteins inside the lumen of the endoplasmic reticulum (ER). Ire1, an ER transmembrane kinase and ribonuclease protein, will sense the levels of misfolded proteins in the ER lumen and start to activate the UPR. After the luminal domain of Ire1 senses the misfolded proteins, Ire1 will oligomerize in the plain of the membrane and the kinase domain will start to auto phosphorylate other juxtaposing Ire1 proteins. The phosphorylation of the kinase domain activates the ribonuclease domain of Ire1, which will start to cleave Hac1 mRNA substrates in yeast or Xbp1 mRNA substrates in higher metazoan species. The resulting mRNA substrates will be expressed into chaperone proteins that will assist the ER in regulating the amounts of misfolded proteins back to homeostasis. Ire1 is a conserved protein from yeast to higher metazoan eukaryotes. The protein crystal structure has been resolved for in both Saccharomyces cerevisiae yeast and Homo sapiens. Here we show that the structure of Ire1 will determine what substrate the protein will be more likely to recognize. By using bioinformatics structural predictions, we have located an important residue in the ribonuclease domain that greatly differentiates the structures between yeast and human. In yeast, a long cleft can be seen that stretches into a previously predicted nuclease site. This cleft is blocked by a single amino acid residue difference in the human Ire1 structure. The hydrophobicity of the area is also affected by the different amino acid residue change, resulting in a

more hydrophobic area in humans than yeast. We show that by changing this amino acid in yeast to the same one in humans through a quick change mutation, the yeast Ire1 will start to recognize and splice the human Ire1 substrate more efficiently than the original yeast Ire1 substrate. These findings provide a basis for the understanding of the exact location of where substrate splicing occurs in Ire1, as well as a better understanding of ribonuclease functionality.

26 Jeffrey Maloy

Dr. Jens Lykke-Andersen

Elucidation of the Mechanism of hDcp2 Stabilization by Hedls

The regulation of mRNA turnover in the cell plays an important role in modulating gene expression, and has been linked to many diseases including cancer. When mRNA is produced in eukaryotes, it contains a 5' cap and a 3' polyA tail to protect it from degradation. The removal of the cap is a key step in silencing gene expression by causing translational inhibition and 5'-3' mRNA decay. The decapping enzyme hDcp2, which is unstable alone, exists in a complex with the decapping enhancer Hedls, and may be stabilized by this interaction. In this study, the stabilization of hDcp2 by Hedls was explored further. hDcp2 was found to contain a 5 amino acid region that was required for its degradation. Additionally, an attempt was made to discover the amino acid sequence in hDcp2 that is required for its interaction with Hedls. A comparative analysis of the sequences required for hDcp2 stabilization and Hedls binding of hDcp2 could provide further insight into the mechanism of hDcp2 stabilization by Hedls.

27 Timothy Mok

Dr. Milton Saier, Ph.D.

Bioinformatic Analysis of Bacterial Mercury Ion Transporters

Currently, there are five known types of mercury transporters in bacteria: MerC, MerE, MerF, MerH, and MerT. Their general function is to mediate ion uptake across the bacterial membrane into the cell. Although they are present in prokaryotes of different phyla, and although they lay within five distinct families, I have utilized bioinformatics and the Superfamily Principle to show that they are related by common descent. After using programs such as GAP and SSearch to establish homology, I aligned and analyzed their amino acid sequences to find conserved motifs. This will allowed me to support the conclusion of homology, thus establishing that the proteins belong to a single superfamily. This allows me to extrapolate information about structures, function and mechanisms from one protein to all superfamily members.

28 George Murano

Dr. Ellen C. Breen

Lung overexpression of TNFalpha impairs locomotor skeletal muscle function

Transgenic mice that over express the inflammatory cytokine TNfalpha under the control of a lung-specific promoter exhibit emphysema and pulmonary hypertension. In this study, we hypothesized high circulating TNFalpha levels in these mice could also alter skeletal muscle function and contribute to an impairment in overall exercise capacity. We found that (1) endurance capacity in a treadmill running test is reduced by 46% in male and 29% in female SP-C/TNFalpha transgenic mice. (2)Overall body weight is reduced by 18% in male, but not female, mice after 6 months of age. (3) Gastrocnemius complex in situ fatigue time is also decreased in both male (33%) and female (44%) SP-C/TNFalpha mice compared to WT mice. Our resuts suggest that high circulating TNFalpha levels lead to muscle dysfunction that could contribute to exercise limitation in addition to the primary lung pathology.

29 Tonhu (Tiffany) Nguyen

Dr. Paul A. Price

Apatite Mineral Formation in Humans

Our goal is to understand the biochemical mechanisms responsible for the normal mineralization of bones and teeth and the abnormal mineralization of atherosclerotic plaques. Human serum is supersaturated with respect to the apatite mineral found in biological calcifications, but crystals will not form in serum unless a catalyst of mineral formation is present. We have investigated possible catalysts of crystal formation using an in vitro assay that closely resembles the calcium, phosphate, and pH of human serum. The rate of crystal formation was monitored by visual inspection and by measuring the amount of calcium and phosphate incorporated into mineral. Scanning Electron Microscopy was used to investigate the structure of the mineral and powder X-ray diffraction was used to identify the calcium phosphate mineral phase. The results of our studies provide an insight into the biochemical defects responsible for osteoporosis (low bone mineral density) and atherosclerosis (abnormal deposition of mineral in the artery intima).

30 Chiara Ricci-Tam, Eileen Shi, Joanna Coker, Yasaman Pirahanchi, Rebecca Mazahreh, Hedieh Matinrad, Sean Jones

Dr. Kit Pogliano and Dr. Joe Pogliano

Application of Mass Spectrometry to Bacteriophage Genome Annotations

This project seeks to annotate the genome of novel M. smegmatis bacteriophage Fionnbharth, isolated in 2010 from La Jolla, CA. The genome was originally annotated using auto-annotation computer programs, a map of predicted coding potential, and NCBI BLAST database comparisons. To confirm these annotations and predicted start sites, tandem mass spectrometry was conducted on both crude and purified phage samples. By screening the peptide sequences detected by mass spectrometry against the genes predicted, the annotated start sites of ten of the genes were confirmed or changed. Some genes were only found from the crude phage stock, suggesting that their products are related to phage structure and assembly. In addition, insight was gained into possible post-translational modifications,

including methionine cleavage. The use of this new method adds a greater degree of certainty to the predicted gene calls for the Fionnbharth genome by supplementing the auto-annotation data with experimental evidence for the tangible gene products.

31 Ying Sun

Dr. Colleen Doherty

Exploring Transcription Co- activator overexpression effects on the Circadian Clock

The Earth's rotation provides predictable environmental changes that oscillate in approximately 24 hour periods, known as Circadian Rhythms. These rhythms are endogenous and driven by an internal Circadian Clock. Many organisms from cyanobacteria to plants to animals have gained the ability to regulate their biology to coordinate with this cycle, allowing the rhythm of the clock to regulate their biological processes. By conserving clock components, organisms are better fit in both biomass accumulation and reproductive performance. Plants especially utilize the Circadian Clock to coordinate their biology with the temporal environment. Due to this consistency, plants have gained an internal ability to anticipate dawn and dusk. This clock- regulated ability results in increased chlorophyll content, enhanced photosynthetic activity, and optimization of nightly starch use. Certain clock components could also influence response to biotic stimuli that gives disease and pathogen resistance. In unraveling the mechanism behind the molecular organization of the plant Circadian Clock, we can then utilize this knowledge to better adapt plants, enhancing agriculture and ecological growth. The Circadian Clock has a complex transcriptional regulation mechanism of multiple interlocked loops, which control gene expression. Through the regulation of transcription, the clock will affect flowering, leaf movement, growth, germination, enzyme activity, photosynthetic activity, and fragrance emission. This project aims to investigate a certain family of transcriptional co- activators that facilitate the binding of a transcriptional activator to the TATA box binding protein (TBP). This specific co-activator gene is conserved and evolutionarily ancient, with a strong cycling phenotype. It exists in many organisms and serves as a highly important mechanism in regulating active transcription. Due to its notably strong cycling, it can be hypothesized that this gene will contribute to the production of clock genes. Therefore, to determine if this family of co- activators will affect clock- regulated output and clock- regulated genes, it is important to analyze differences in clock- regulated outputs, and also the effects of the co-activator on gene transcription. The model organism for plant research, Arabidopsis Thaliana will be used to conduct this research. However, the study of these co- activator genes becomes more complicated in Arabidopsis since Arabidopsis is prone to redundancy and has an ortholog (family) of three transcriptional co- activator genes: 1A, 1B, and 1C. It has since been determined that 1C shows greatest expression in the morning, while 1B peaks in the evening, and 1A doesn't cycle at all. However, to determine the link between this transcriptional co- activator and clock output, all three lines need to be tested.

32 Tin-Yun Tang

Dr. Randall Johnson

Hif-1a's Effects on Metastatic Success

Adaption to hypoxia is a key event in carcinogenesis and has been correlated with poorer prognosis. Micrometastases also encounter a hypoxic environment following the extravasation processes associated with metastatic spread. The constitutively expressed transcription factor Hypoxia Inducible Factor 1 (HIF-1) is the master regulator of homeostatic responses to O2 levels. Downstream targets of HIF-1a include a number of key effectors involved in angiogenesis such as Vascular Endothelial Growth Factor (VEGF). HIF-1a is rapidly marked for degradation by prolyl hydroxylases (PHD) under normoxia and is upregulated during hypoxia. Increased expression of Hif-1a has been demonstrated to have profound effects on the tumor microenvironment and has been strongly connected with more aggressive phenotypes in primary tumor growths, which have increased proliferation and invasiveness. Despite the usage of pharmacological angiogenesis inhibitors in treatment regimens, Hif-1a's role and its viability as a treatment target during the formation and progression of metastatic nodes is poorly understood. Here, we examine Hif-1a's role in metastatic spread with an in vivo model. A murine model of metastatic spread was created by giving injections of Lewis Lung Carcinoma cells (LLC) directly into the lateral tail vein. This model was used in conjunction with daily administered doses of pharmacological inhibitors of Hif-1a or PHD. Metastatic success was then measured by quantifying the size and number of metastatic nodes in the lungs. We show the differential effects of tampering with Hif-1a expression on metastasis.

33 Geraldine Tran

Dr. Roger Y. Tsien

Seeing Through Skin: Evolving a Near Infrared Fluorescent Protein for Live Animal Imaging

In molecular biology, fluorescent proteins (FPs) are indispensible tools to image protein expression and localization. FPs are extensively used in all fields of biology, including labeling individual neurons, locating tumor cells, and small molecule sensing. The discovery of FPs and optimization won the Nobel Prize in 2008. Mutations to the original green and red FPs have created a rainbow across the visible spectrum, but few FPs emit fluorescence in the near infrared region. FPs that emit in the far red have been derived from coral and bacteria. Our research shows that a phycobilisome protein from Trichodesium erythaeum, a cyanobacteria, can be engineered into a near infrared FP with excitation and emission maximum of 646 and 672 nm, respectively. This near infrared phycobilisome FP (NIRPhFP) has a quantum yield of 0.15 and fluorescent properties make NIRPhFP ideal for FRET pairing with other FPs and fluorescent imaging in living animals.

34 Kit Wu, Carolyn Zhang, Jeremy Chou, Adriee Wong, Daphne Chen, Dieter Lam, James Zhang

Dr. Kit Pogliano and Dr. Joe Pogliano

Genomic Organization and Comparisons of Cluster K Phage, Fionnbharth

Bacteriophage are one of the most ubiquitous and numerous biological entities that exist on the planet. With their genomic diversity and potential role as an effective treatment against multi-drug resistant

bacteria, being able to compare and group different bacteriophages into clusters of relatedness becomes important in organizing the vast amounts of genetic information. After sequencing the genome of cluster K bacteriophage, Fionnbharth, we noticed some of its genes were related to other clusters. Our analysis is set up to determine the likely subcluster of the K-cluster phage, Fionnbharth, and identify genes that are more commonly found on unrelated clusters of phage. Using DNA-DNA comparison programs and diagonal dot plot analysis, we determine the likelihood of Fionnbharth belonging to one of the existing K subclusters, or whether it is the first phage discovered belonging to a new K-subcluster.

35 Tony Yu

Dr. Alexander Hoffmann

Faithful Expression of Canonical NFkB Inhibitor IkBa and Single Cell Level Analysis of the NFkB/RelB Activation Kinetics

NFkB describes a family of transcription factors that is implicated in many different pathways: immune response, senescence, and apoptosis. NFkB misregulation can lead to abnormal immune response and certain types of cancer. While the canonical NFkB pathway, involving RelA dimers, deals with rapid immune response the noncanonical NFkB pathway deals with cellular development. RelB is a part of the non-canonical NFkB pathway. IkBs operate within the canonical NFkB pathway and are proteins that bind the free form of NFkB/RelA dimers in the cytoplasm to sequester it there and keep it inactive. Active NFkB/RelA dimers translocate into the nucleus and bind kB sites, upregulating the transcription of many genes including its own inhibitor IkBa. This negative feedback mechanism ensures the proper regulation of NFkB. A 5xkB IkBa promoter overexpressed IkBa compared to the endogenous wildtype promoter. The first project aims to produce more faithful expression of IkBa by reducing the promoter to 3xkB sites through site directed mutagenesis. Faithful expression of IkBa will allow for a more complete understanding of NFkB regulation. Results show that the 4xkB mutants had no effect on expression levels and 3xkB mutants express IkBa more faithfully but still produce slightly more IkBa. The behavior of a biological system is a sum of its parts. Recently, evidence has emerged that individual cell behavior may be heterogeneous in terms of NFkB dynamics. This discrepancy has been observed between population-level assays such as EMSAs and cell-level studies of RelA activation kinetics. Stimulus dependent activation and kinetics of RelB has been studied with bulk assays at the cell population level. Single cell level stimulus dependent kinetics of RelB, on the other hand, is not well elucidated. Varying concentrations and frequencies of stimulus such as LPS and TNF will be applied to examine the underlying stimulus dependent kinetics of RelB activation in knockout MEFs at the single-cell level. NFkB/RelB dimers will be visualized in the cell via a mCherry and GFP amino-terminus fusion of RelB in a fluorescent microscope. NFkB in its inactive state is sequestered in the cytoplasm. Nuclear localization indicates that NFkB is active. The RelB transgene RelB -/- MEFs will show the stimulus dependent kinetics of RelB in the non-canonical NFkB pathway. The RelB transgene RelB -/- NFkB2 -/- MEFs are deficient in p100/p52, the non-canonical RelB dimer partners. Lacking these, RelB can only dimerize with p50 which shunts it towards the canonical pathway. RelB -/- NFkB2 -/- MEFs with a

transgenic RelB reporter fusion will allow for the stimulus dependent kinetics of the NFkB/RelB:p50 to be studied under canonical regulation.

36 Nathan Zemke

Dr. Beverly Emerson

c-MYC Specific Defects of TGFβ1/Smad Signaling in Human Cancer Cells

TGFβ1 signaling is a ligand-induced pathway that regulates cell growth at the level of gene expression after TGFβ1 receptor activated DNA binding complexes formed by Smad proteins translocate to the nucleus. Upon TGFβ1 stimulation phosphorylated Smad-2/3 heterodimerize with Smad-4 and migrate to the nucleus where they regulate transcription of several genes. One of the major TGFβ1 responsive genes involved in the growth arrest induced by TGFβ1 is the proto-oncogene c-MYC. Upon TGFβ1 stimulation, c-MYC expression is down-regulated in normal epithelial cells but this repression is missing in many cancer cells. Interestingly the mechanism explaining this defect is unclear. Our data shows that TGFβ1 represses c-MYC expression in MCF10A and HaCaT epithelial cell lines while the highly metastatic cancer cell line MDA-MB-231 has no changes in c-MYC expression upon TGFβ1 induction. This observed difference in the c-MYC expression response to TGFβ1 between normal epithelial cells and many cancer cell lines suggests that cancer cells experience a transformation in the TGFβ1 pathway. Also previous findings suggest that the lack of response of c-MYC in cancer cells upon TGFβ1 stimulation is due to a defect of the Smads translocation to the nucleus. However, our preliminary data shows that transcription of the PAI-1 gene, a well known target of TGFβ1/Smad signaling does respond to TGFβ1 treatment in all the cell types tested while c-MYC repression is specifically missing in MDA-MB-231 cells. This argues against the first hypothesis and rather suggests that the defect in the TGFβ1 pathway is specific for c-MYC while other genes such as PAI-1 still respond to TGFβ1 stimulation. We are currently investigating possible specific defects in transcription factors binding to the c-MYC promoter upon TGFβ1 induction by Chromatin Immunoprecipitation as an approach to elucidate the mechanism underlying the observed differences in c-MYC gene expression in the different cell types.

70 Varun Cidambi, Ben Rich, Ander Beckham, Rose Hill, Colee Evangelista, Jim Mcburney-Lin, Christina Dimopoulos

Dr. Kit and Joe Pogliano

Bacteriophage and Bacteria exchange genes between their respective genomes, increasing their gene diversity. We isolated a phage, which we named Fionnbharth, and annotated its sequenced genome. We used NCBI protein BLAST to identify amino acid sequences with similarities to our predicted open reading frames. Because phage often acquire genes from their host, identifying bacterial genes within a phage genome can provide information about the phage's native bacterial hosts in the environment as well as mechanisms of gene exchange. We found 15 proteins within the Fionnbharth genome that had significant matches to proteins encoded within the genome of several different bacterial species. We examined possible similarities between the bacteria containing Fionnbharth related genes. We explored at the bacterial gene product’s function within the bacteria to look for clues of how these genes were

transferred between Fionnbharth and the bacteria. We used mass spectrometry to determine if Fionnbharth was expressing the genes of interest. These genes may be important in understanding the phage genome evolution as they may play an important role in phage replication, host infection, or environmental survival.