16th Annual Research Symposium
Genetics, Genomics, and Bioinformatics
September 13th, 2016
University of California, Riverside

GGB Student Symposium 2016

Genomics Auditorium

8:00-9:00AM New Student Orientation

8:30-9:30AM Breakfast and Registration
(Genomics Lobby)

9:30-10:45AM Session I

Chair: Maggie Lu

9:30AM **Dr. Hailing Jin, Julio Sosa, and Michael Matson**, Welcome & Opening Remarks

9:45AM **Stephen Bolaris,** Larsen Lab
Effects of aluminum toxicity on the stability of plant genomes

10:00AM **Jonathon Deans,** Sladek Lab
A differential role of HNF4α isoforms in liver metabolic and circadian processes.

10:15AM **Zhelin Li,** Zheng Lab
A robust method for monitoring changes in cellular nonsense-mediated RNA decay activity

10:30AM **Suhas Sureshchandra,** Messaoudi Lab
Maternal obesity-induced epigenetic changes contribute to a dampened inflammatory transcriptional program in neonatal monocytes

10:45-11:00AM Refreshments (Genomics Lobby)

11:00-12:00PM Session II

Chair: Sawyer Masonjones

11:00AM **Thanin Chantarachot,** Bailey-Serres Lab
Characterization of an RNA helicase family that functions in mRNA turnover in *Arabidopsis*

11:15AM **Lichao Li,** Gu Lab
The roles of RNA polyphosphatase PIR-1 in RNA interference

11:30AM **Yahui Li,** Karginov Lab
Transcriptome-wide analysis of regulatory interactions between microRNAs and the RNA binding protein HuR

11:45AM **Erin Sternburg,** Karginov Lab
Investigation of interactions between miRNA machinery and the RNA binding protein, Pumilio.

12:00-1:30PM Lunch (Genomics Lobby)

1:30-2:30PM Keynote Address:

Chair: Michael Matson

Dr Jaimie Van Norman, Department of Botany & Plant Sciences

Identification of a receptor-like kinase with polar localization in the radial axis and roles in tissue patterning

2:30-2:45PM Refreshments (Genomics Lobby)

2:45-3:45PM Session IV

Chair: Thanin Chantarachot

2:45PM **Eric Smith,** White Lab
Recombination rate mapping in African malaria mosquitoes

3:00PM **Maggie Lu,** Le Roch Lab
Nascent RNA sequencing reveals transcriptional regulation mechanisms in the human malaria parasite life cycle

3:15PM **Michael Matson**, Judelson Lab
High-throughput mapping of loci linked to sporulation, pathogenicity, and fungicide sensitivity in the plant pathogen *Phytophthora infestans*

3:30PM **Patrick Schreiner,** Atkinson Lab
piClusterBusteR: Software for automated classification and characterization of piRNA cluster loci

3:45-4:30PM Poster Session and Reception
(Genomics Lobby)

Albert Do, Reddy Lab
Testing the significance of homodimerization of a stem c inducing transcription factor in stem cell gene expressior

Mikkal Blick, Yamanaka Lab
The analysis of chemosensory neurons that affect developmental timing in *Drosophila melanogaster*

Sawyer Masonjones, Stajich Lab
Population genomics of *Rhizopus stolonifer*

Jui-Yu Liao, Zidovetzki Lab
Investigating the role of endothelial-to-mesenchymal transition in the human primary brain tumor, glioblastoma multiform

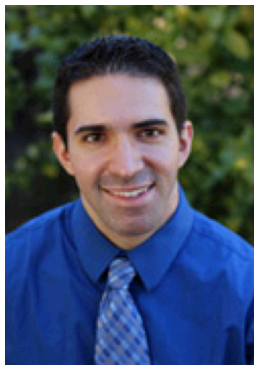
4:30PM Awards and Closing Remarks

Oral Presentations From GGB Students
Session I

Effects of aluminum toxicity on the stability of plant genomes

Stephen C. Bolaris, and Paul B. Larsen
Department of Biochemistry

Stephen Bolaris



9:45-10:00 AM

Aluminum (Al) is one of the most abundant elements in the earth's crust; in acidic soil the normally inert Al speciates to its trivalent form which can lead to a toxic environment for most plants to grow. Al toxicity is a global problem affecting more than 30% of the world's arable land (von Uexkull and Mutert, 1995). Previous research has shown that exposure to these toxic conditions lead to a stoppage of root growth in an ATAXIA AND TELANGIECTASIA AND RAD3 RELATED (ATR) dependent manner (Rounds et al, 2008). ATR, a kinase activated as part of the cell's response to DNA damage and detects persistent single stranded DNA as well as replication fork stalls. Additional DNA damage response factors such as SUPPRESSOR OF GAMMA RESPONSE1 (SOG1) were also discovered to part of the plants response to the toxicity. These findings have directed the research towards understanding the genomic impact of exposure while trying to extrapolate the larger picture of what other biological factors are involved in cells DNA damage response after exposure to Al. In order to accomplish this, both RNA sequencing and whole genome sequencing will be applied to this biological question, with results helping to realize of the full impact of Al toxicity and identify putative Al tolerance genes in plants. As a means to accomplish this, the well-established Arabidopsis model will be utilized to better understand the plants reaction to the toxicity with the goal of applying this knowledge to agriculturally relevant plant species.

A differential role of HNF4α isoforms in liver metabolic and circadian processes.

Jonathan Deans, L Vuong, N Titova, Frances M Sladek
Department of Cell Biology & Neuroscience

Jonathan Deans

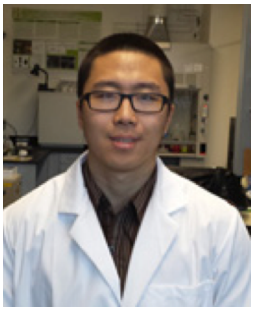
10:00-10:15 AM

Hepatocyte nuclear factor 4 alpha (HNF4α) is a liver-enriched transcription factor and a member of the nuclear receptor (NR) superfamily, a family of nutrient-sensitive transcription factors that lie at the intersection of metabolic and circadian pathways. HNF4α is one of the most abundant transcription factors expressed in the liver where it is best known as a master regulator of liver-specific gene expression and is essential for adult and fetal liver function. The *HNF4A* gene can be expressed via alternative promoters, P1 and P2, driving alternative first exons resulting in an altered A/B domain and the loss of activation function 1 (AF-1) domain in P2 isoforms. Cytochrome P450s (CYPs) are heme-containing enzymes that are responsible for oxidative metabolism of various endogenous steroids, hormones, bile acids, and fatty acids, as well as xenobiotic compounds. HNF4α is known to play an important role in CYP gene expression and more than half of all NRs and many CYP genes display rhythmic patterns of expression in multiple metabolic tissues. It is thought that fatty acids, sterols, and other hormones may be able to communicate information about nutrient and energy status to the circadian clock via their cognate receptors. Here we investigate the role of HNF4α alternative promoter isoforms in the control of circadian and metabolic signaling in the liver. We performed RNA-seq and rapid immunoprecipitation mass spectrometry of endogenous proteins (RIME) in the mouse liver at several time-points to identify differentially expressed genes and identify factors that interact uniquely with HNF4α isoforms. Analysis of ChIP-seq data HNF4α and other transcription factors provides additional insight into mechanism. The results reveal a unique role for HNF4α isoforms at the crossroads of drug and ligand metabolism and regulation of circadian clock components.

A robust method for monitoring changes in cellular nonsense-mediated RNA decay activity

Zhelin Li, John Vuong, Min Zhang, Cheryl Stork, and Sika Zheng
Division of Biomedical Sciences, School of Medicine

Zhelin (Jeff) Li



10:15-10:30 AM

Nonsense-mediated RNA decay (NMD) selectively degrades mutated and aberrantly processed transcripts harboring premature termination codons (PTC). Cellular NMD activity is typically assessed using exogenous PTC-containing reporters. Guided by the fact that NMD directly controls the levels of a suite of endogenous alternatively spliced transcripts, we developed a broadly applicable strategy to reliably and conveniently monitor changes in cellular NMD activity after overcoming several inherently problematic aspects of assaying endogenous NMD targets. Our new method was validated genetically in distinguishing NMD regulation from alternative splicing regulation. With this robust method, we tested a panel of widely used chemical inhibitors for their impacts on NMD and identified NMD-inhibiting stressors consistent with previous reports but also found that NMD inhibition was not a universal response to various cellular stresses. The high sensitivity and broad dynamic range of our method revealed a strong correlation between NMD inhibition, endoplasmic reticulum (ER) stress and polysome disassembly upon thapsigargin treatment in a temporal and dose-dependent manner. We found little evidence for the involvement of calcium signaling, which was previously reported as the mechanism underlying thapsigargin-induced NMD inhibition. Instead, consistent with studies reporting NMD inhibition via eIF2α phosphorylation, we found that of the three unfolded protein response (UPR) pathways activated by thapsigargin, only protein kinase RNA-like endoplasmic reticulum kinase (PERK) was required for NMD inhibition. Finally, we discovered that ER stress compounded TDP-43 depletion in the upregulation of TDP-43-repressed cryptic isoforms that have been implicated in the pathogenic mechanisms of amyotrophic lateral sclerosis and frontotemporal dementia

Maternal obesity-induced epigenetic changes contribute to a dampened inflammatory transcriptional program in neonatal monocytes

Suhas Sureshchandra[1,5], Maham Rais[5], Randall Wilson[2,5], Jonathan Purnell[3], Kent Thornberg[3], Nicole Marshall[4], Ilhem Messaoudi[1,2,5]
[1]Graduate Program in Genetics, Genomics, and Bioinformatics, University of California, Riverside CA, [2]Graduate Program in Cell, Molecular, and Developmental Biology, University of California, Riverside CA, [3]The Knight Cardiovascular Institute, Department of Medicine, Oregon Health & Science University, Portland OR, [4]Maternal-Fetal Medicine, Department of Medicine, Oregon Health & Science University, Portland OR, [5]Division of Biomedical Sciences, School of Medicine, University of California, Riverside CA.

Suhas Sureshchandra

10:30-10:45 AM

Obesity during pregnancy is associated with adverse health outcomes for the offspring, particularly increased early incidence of bacterial sepsis and necrotizing enterocolitis. Furthermore, babies born to obese mothers are more likely to develop chronic inflammatory diseases such as type 2 diabetes, cardiovascular diseases, asthma, and cancer culminating in an increased risk of all-cause mortality, suggestive of *in utero* reprogramming of neonatal immunity. We have previously shown that monocytes obtained from umbilical cord blood cells of babies born to obese mothers (obese group) had reduced cytokine production in response to TLR (Toll-like receptor) ligands compared to those from babies born to lean mothers (lean group), indicative of an overall dysfunction in monocytes. These observations are clinically important because monocytes play a critical role in chronic diseases that occur more frequently in offspring of obese mothers. However the mechanisms that regulate this dysfunction are less understood. To address this critical knowledge gap, we proposed to test the mechanisms underlying one critical aspect of monocyte function - the ability to respond to microbial antigens. Here, we show that that the dampened cytokine responses in neonatal monocytes from obese group are mediated by transcriptional changes resulting from *in utero* epigenetic reprogramming. While resting monocytes from both groups had similar

transcriptional profiles, *ex vivo* stimulation with lipopolysaccharide (LPS) failed to trigger robust inflammatory gene expression changes in the obese group. DNA cytosine methylation has emerged as a possible regulatory mechanism that mediates allostatic inflammatory processes in immune cells. Therefore, we hypothesize that the transcriptional block in monocytes from obese group is mediated via obesity-induced DNA methylation changes. Using targeted bisulfite sequencing, we demonstrate global hypomethylation in monocytes from obese group, with changes occurring on promoter regions of key defense response and innate immune genes. We found limited association between methylome and transcriptome of resting cells. However, the methylation status strongly correlated to the *ex vivo* inflammatory response of monocytes to LPS stimulation, indicating a critical role of obesity-induced DNA methylation in influencing inflammatory responses in innate immune cells.

Session II

Characterization of an RNA helicase family that functions in mRNA turnover in *Arabidopsis*

Thanin Chantarachot[1], Reed Sorenson[2], Maureen Hummel[1], Lauren Dedow[1], Alek Kettenburg[1]
Leslie Sieburth[2] and Julia Bailey-Serres[1]
[1] Department of Botany & Plant Sciences, Center for Plant Cell Biology, UCR
[2] Department of Biology, University of Utah, Salt Lake City, UT

Thanin (George) Chantarachot

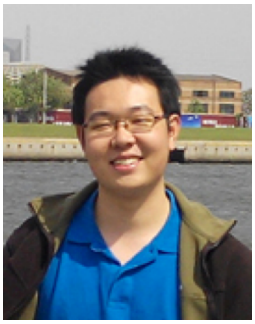
3:00-3:15 PM

RNA helicases are motor proteins that unwind nucleotide duplexes in an ATP-dependent manner. Their roles have been implicated in every step of RNA metabolism, including decay of cytoplasmic mRNAs. In yeast and animals, the DEAD-box helicase DHH1/Rck/p54 orthologs function in translational repression and activation mRNA decapping during the 5' to 3' mRNA decay. We identified three closely related helicases, *RNA HELICASE*6, *8* and *12* (*RH6*, *RH8* and *RH12*), as encoding the plant counterparts of the DHH1/Rck/p54 family. To assess the roles of these RHs, we obtained loss-of-function T-DNA insertion alleles for each gene. Neither single nor double mutants of *rh6*, *rh8* or *rh12* show obvious developmental abnormalities. However, the triple homozygote *rh6 rh8 rh12* displays pleiotropic phenotypes including delayed germination and seedling growth, altered vein patterning, diminutive organs and severe maturation defects similar to those of mRNA decapping mutants (i.e. *VARICOSE* and *DCP2*). Confocal microscopy imaging resolved all three RHs in cytoplasmic complexes resemble mRNA processing bodies. These structures enlarge under mild hypoxia, as the bulk of cytoplasmic mRNAs become arrested in translation. Additionally, RH6 colocalizes with the decapping enzyme DCP2 and Oligouridylate Binding Protein (UBP)1C, which binds mRNAs that are sequestered or degraded during hypoxia. To identify the mRNAs regulated by RH6/8/12, a global analysis of RNA decay was performed after cordycepin treatment followed by RNA-seq. Steady-state mRNA accumulation was also monitored in the mutant versus wildtype (Col-0) Decay rates of 4,247 mRNAs were significantly attenuated in the triple *rh6 rh8 rh12* mutant. Interestingly, these are enriched for abiotic stress response function genes, including mRNAs dynamically regulated by hypoxia stress. This systems-level exploration of RH6/8/12 provides new insight into the relevance of mRNA turnover in plants.

The Roles of RNA Polyphosphatase PIR-1 in RNA interference

Lichao Li, Weifung Gu
Department of Cell Biology & Neuroscience

Lichao Li



11:15-11:30 AM

RNA interference is a highly conserved process in eukaryotes that is responsible for transposon silencing, gene regulation and anti-viral response. During RNAi processing in C. elegans, double-stranded RNAs (dsRNAs) are cleaved by Dicer into 20-26 nucleotides long primary short interfering RNAs (siRNAs). Then these primary siRNAs guide specific RNA binding proteins, Argonautes, to target mRNAs and recruit RNA-dependent RNA polymerases (RdRPs) to produce secondary siRNAs, for direct silencing of target mRNAs. Previous Dicer immunoprecipitation discovered that the RNA polyphosphatase PIR-1 interacting with Dicer may participate in RNAi but the mechanism is unrevealed. This project is to demonstrate the functions and mechanism of PIR-1 in RNAi pathways.

Firstly, We elucidated that the in vitro activity of C. elegans PIR-1 is to remove β and γ phosphates from the 5' ends of triphosphorylated RNA molecules. Secondly, an antiviral RNAi deficiency phenotype in pir-1 null mutant suggests that PIR-1 is important for exogenous RNAi. Our preliminary results support a model that PIR-1 may serve as a specific sensor for viral dsRNAs with a 5' triphosphate group, and is required for the proper loading of primary siRNAs into Argonautes. Thirdly, depletion of endogenous siRNAs targeting self RNAs in pir-1 null mutant suggest that PIR-1 is required for synthesis of endogenous siRNAs. Thus, we designed cloning method for endogenous siRNA precursors to investigate the role of PIR-1 in endogenous RNAi pathway.

Transcriptome-wide analysis of regulatory interactions between microRNAs and the RNA binding protein HuR

Yahui Li, Ted Karginov
Department of Cell Biology & Neuroscience

Yahui Li

10:30-11:45 AM

MicroRNAs (miRNAs) and the RNA-binding protein HuR are both important trans-acting factors in post-transcriptional regulation. Recent studies have shown that these two regulators can act on shared target mRNAs in an interactive way, either cooperatively or competitively. However, these previous studies have focused on the regulation of individual transcripts, and a comprehensive understanding of functional interactions between miRNAs and HuR across all transcripts is lacking. We used transcriptome-wide technique CLIP-Seq to look for the sites bearing such interactions, and the activities of the sites were tested by luciferase assays. 28 candidate sites have been screened. Although many of them were shown to be miRNA regulated sites, few were regulated by HuR. HuR was found to be abundant in nucleus but undetectable in cytoplasm. However, post-transcriptional regulation of HuR only occurs in the cytoplasm. Accordingly, we are trying to induce cytoplasmic HuR by stresses such as UV treatment and actinomycin treatment. If any condition is found, we will redo the site activity testing to check the interactions.

Investigation of interactions between miRNA machinery and the RNA binding protein, Pumilio.

Erin Sternburg, Ted Karginov
Department of Cell Biology & Neuroscience

Erin Sternburg

11:45-12:00 PM

RNA binding proteins (RBPs) play a major role in post-transcriptional regulation, acting on messenger RNAs to affect both their stability and translation efficiency. One of the major contributors to mRNA regulation is Argonaute, along with a small RNA, termed microRNA (miRNA). The miRNA serves as a sequence specific guide strand which, when associated with Argonaute, binds to the mRNA strand, preferably in the 3'UTR, causing repression. It has been shown through bioinformatics analysis that ~60% of genes have miRNA target sites. The presence of other RBPs may also play a major role in modulating Argonautes activity, leading to more specific regulatory mechanisms. One candidate RBP, Pumilio, has been suggested to play a role in affecting Argonaute behavior. Pumilio, also a conserved RBP, is part of a larger group of PUF proteins which are known to regulate many genes involved in cell division, differentiation, and development. CLIP-seq experiments show that Argonaute and Pumilio binding overlaps, and that knockdown of one protein can influence the binding behavior of the other. My project is to investigate the mechanism of interaction between Pumilio and Argonaute, and how this translates into effects on gene expression.

Session III

Keynote Address

Identification of a receptor-like kinase with polar localization in the radial axis and roles in tissue patterning

Jaimie M. Van Norman, Roya Campos, and Jason Goff
Department of Botany & Plant Sciences

Dr. Jaimie Van Norman

1:30-2:30 PM

Development often relies on extrinsic (non-cell-autonomous) signals as positional cues and determinants of cell fate. During plant development, intercellular communication serves a critical role in coordination of asymmetric cell divisions and differential cell fate specification that are essential to establish and maintain tissue patterning. While polarly localized factors involved in apical-basal (root-shoot) patterning have received considerable attention, how polarity is involved in tissue patterning in the radial axis remains largely unknown. Using high-resolution spatial and temporal gene expression data, an in silico screen was conducted for genes predicted to function in radial tissue patterning. One of the genes we identified, designated here as POLARLY LOCALIZED KINASE 1 (PLK1), encodes a transmembrane receptor-like kinase (RLK) that is polarly localized to lateral domains in embryonic and root tissues. Unexpectedly, PLK1 polarity varies with cell type and is maintained under a variety of conditions that disrupt the localization of other transmembrane proteins. plk1 mutants exhibit altered cell morphology that appears to arise from abnormal positioning of cell division planes. Our results suggest that laterally polarized, RLK-mediated signaling domains function in the perception of directional cues in developmental patterning. Furthermore, we propose that a distinct paradigm in cell polarity operates to establish these polarized signaling domains.

Oral Presentations from GGB Students
Session IV

Recombination rate mapping in African malaria mosquitoes

Eric Smith, David Turissini, Bradley White
Department of Entomology

Eric Smith

2:45-3:00 PM

Despite progress in reducing malaria transmission with insecticide-based vector control, the disease continues to claim over 500,000 lives per year, most of which are African children. A significant impediment to control in Africa is the presence of *Anopheles gambiae* – a uniquely efficient mosquito vector endemic to the continent. While great advancements have been made in the molecular biology and genetics of *An. gambiae* over the past decade, inadequate data on recombination rate in this species prevents novel and traditional vector control strategies from being deployed with maximum effectiveness. Recombination is a fundamental biological process with profound evolutionary implications. In mosquitoes and other sexual eukaryotes, recombination between homologous chromosomes is required for both the proper formation of haploid gametes from diploid germ cells and the production of new combinations of alleles. However, the rate at which recombination occurs varies with genomic position, sex, and the presence of chromosomal inversions. Such variation in recombination rate influences a myriad of evolutionary processes including the efficacy of natural selection, levels of standing diversity, and the elimination of deleterious mutations. Using a backcrossing strategy, we have developed high-resolution recombination rate maps for both male and female *An. gambiae* constructed using data from thousands of mosquitoes, each sequenced at thousands of loci. These maps indicate that there is a significant reduction in recombination in chromosomal inversions and that there are significant differences in recombination rate between male and female mosquitoes. The maps developed in this study give us insight into some of the basic evolutionary processes that make *An. gambiae* such an efficient and adaptable vector. These maps can be used to aid in the development of novel vector control strategies, as well as to help predict of the efficacy of these vector control strategies.

Nascent RNA sequencing reveals transcriptional regulation mechanisms in the human malaria parasite life cycle.

Xueqing Lu, Gayani Batugedara, Evelien Bunnik, Jacques Prudhomme, and Karine G. Le Roch
Department of Cell Biology & Neuroscience

Xueqing (Maggie) Lu

11:00-11:15 AM

Plasmodium falciparum, the deadliest malaria-causing parasite, has a complex life cycle involving multiple hosts and morphological stages. Progression through the parasite life cycle is finely tuned by coordinated changes in gene expression. However, the machinery regulating transcription in the parasite remains poorly understood. In particular, relatively few sequence-specific transcription factors and regulator elements have been identified in the Plasmodium genome. In this study, we have explored mechanisms regulating transcription in P. falciparum by profiling newly transcribed RNA (or nascent RNA) using global run-on sequencing (GRO-seq). By generating nascent RNA coverage profiles for a total of eight erythrocytic stages, we were able to accurately monitor transcriptional activity during asexual and sexual parasite development. In addition, we analyzed the interplay between mechanisms regulating transcription at the initiation and post-transcriptional levels by a comparison between GRO-seq, RNA Pol II Chip-Seq, and steady-state mRNA at the various life cycle stages. Results from this study not only offer key insights into the parasite's gene expression regulatory system but also provide a foundation for future research on the identification of novel anti-malarial strategies.

High-throughput mapping of loci linked to sporulation, pathogenicity, and fungicide sensitivity in the plant pathogen *Phytophthora infestans*

Michael EH Matson, Howard S. Judelson
Department of Plant Pathology & Microbiology

Michael Matson

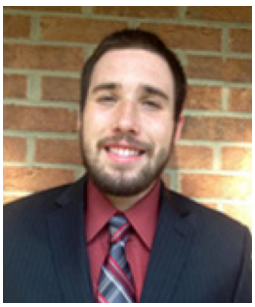
3:15-3:30 PM

The late blight disease of potato and tomato, caused by the filamentous oomycete *Phytophthora infestans,* is one of the most destructive plant pathogens in modern-day agriculture. The basic biology of the pathogen is poorly understood outside of the interactions between *P. infestans* and its host species. Understanding traits of the pathogen such as those important to asexual dispersal, its adaptation to existing fungicides, or aggressiveness, could inform the development of future means of chemical control. In order to identify genetic loci underlying these traits, we generated a cross of *P. infestans* showing phenotypes that appear to roughly segregate in a 1:1 ratio, facilitating genetic mapping. Seventy-seven progeny from this cross were Illumina-sequenced and the resulting reads mapped to a new draft reference genome generated from one of the parental isolates using Pacific Biosciences sequence data. These progeny were separated into phenotypic groups of high and low resistance to the fungicide metalaxyl, sporulation proficiency, radial growth rate, and potato tuber infection ability. Following SNP identification using the GATK suite, we identified genomic regions linked to resistance to metalaxyl and infection proficiency; once the new reference genome is annotated, candidate genes will be identified. We are also identifying regions contributing to these phenotypes by measuring copy number variation, differential gene expression, and loss of heterozygosity. A combination of linkage mapping and non-mapping methods will significantly narrow down the list of candidate genes contributing to the phenotypes of interest.

piClusterBusteR: Software for automated classification and characterization of piRNA cluster loci

Patrick Schreiner, Peter Atkinson
Department of Entomology

Patrick Schreiner



3:30-3:45 PM

Piwi-interacting RNAs (piRNAs) are sRNAs that have a distinct biogenesis and molecular function from siRNAs and miRNAs. piRNAs are well-conserved and shown to play an important role in the regulatory capacity of germline cells in Metazoans. A significant subset of piRNAs is generated from discrete genomic loci, referred to as piRNA clusters, that are composed mostly of transposable element remnants. Given that the contents of piRNA clusters dictate the target specificity of primary piRNAs, and therefore the generation of secondary piRNAs, they are of great significance when considering transcriptional and post-transcriptional regulation on a genomic scale.

We have developed a general tool, piClusterBusteR, that accurately, automatically, and efficiently performs nested annotation to ensure high-quality characterization of piRNA cluster contents, provides a quantitative representation of piRNA cluster composition by annotated feature, and makes available both annotated and unannotated piRNA cluster sequence that can be utilized for downstream analysis. The data necessary to run piClusterBusteR and the skills necessary to execute this software on any species of interest are not overly burdensome for biological researchers.

piClusterBusteR has been utilized to compare the composition of top piRNA generating loci amongst Metazoan species. Characterization and quantification of cluster composition allows for comparison within piRNA clusters of the same species and between piRNA clusters of different species. The results from piClusterBusteR has provided an in-depth description and comparison of the architecture of top piRNA cluster within and between species, as well as a description of annotated and unannotated sequences from top piRNA cluster loci in Metazoans.

Poster Presentations from GGB Students

Testing the significance of homodimerization of a stem cell inducing transcription factor in stem cell gene expression

Albert Do, Kevin Rodriguez, Stephen Snipes, Alex Plong, G. Venugopala Reddy
Department of Botany & Plant Sciences

Albert Do

Above ground growth of plants is mediated by the shoot apical meristem (SAM); a collection of stem cells at the tips of shoots. The biology of the meristem is controlled by a complex network of genes and regulators maintaining a delicate equilibrium between the preservation of tissue in an undifferentiated state and differentiation into more mature specialized structures. One of the most critical components of the SAM regulation network is WUSCHEL (WUS); a homeobox transcription factor which serves as a cornerstone to maintaining the balance in the meristem cells by altering expression of several other key genes. The activity of the WUS protein has been shown to be bifunctional with monomers tending to serve to activate targets while dimers tend to repress them. However, pinning down the effects of the dimeric or monomeric forms in isolation from each other is difficult as the protein can transition between the two forms stoichiometrically and the DNA binding homeodomain also participates in homodimerization. To understand the functional significance of homodimerization and more accurately study the downstream activities of the WUS dimeric form, a gene encoding two translationally fused WUS proteins was generated and transformed into *Arabidopsis thaliana* carrying a heterozygous knockout of the wildtype WUS. Phenotypic analysis showed that plants carrying the construct appeared to have overall stunted growth with abnormal traits such as shortened and altered silique patterns, abnormal flowers, and terminated meristems. The plants that showed termination of SAMs indicated that the dimeric form might have acquired a new function or the monomeric form is required for activating a regulator that induces SAM growth. Confocal imagery of meristems of transformed plants showed signs of a possibly altered gradient of expression of the WUS target *CLV3* by extension indicating altered WUS activity. The current results may give further weight to the role of WUS as a repressive agent and are the first step to separating out the possible effects of the WUS dimeric form from its other functionality. Further investigations will continue to examine the details of WUS spatial expression and the influence of the different WUS forms on cells of the SAM and overall growth.

The analysis of chemosensory neurons that affect developmental timing in *Drosophila melanogaster*

Mikkal Blick, Naoki Yamanaka
Department of Entomology

Mikkal Blick

In insects, the developmental transition from one life stage to the next is initiated by the release of the steroid hormone ecdysone from the prothoracic gland (PG). This is strongly influenced by prothoracicotropic hormone (PTTH), which signals for ecdysone production to begin. In Drosophila melanogaster, PTTH is produced by a bilateral pair of neurons that innervate the PG. Using Drosophila, it was found that ablating PTTH producing neurons caused a delay in the amount of time it took for larvae to pupariate. The hugin neurons, found in the subesophageal zone (SEZ) of the brain, produce the neuropeptide hugin, which is received by the PTTH neurons. Hugin mutant flies display a similar delay in development compared to the one caused by the ablation of PTTH neurons. Determining the factors that interact with the hugin neurons will lead to a better understanding of what influences developmental timing in Drosophila larvae. It is known that the gustatory receptor neurons (GRNs), which receive taste input in the larvae, project towards the SEZ. Activating or silencing some these GRNs have resulted in altered developmental times to pupariation. Additionally, a GFP reconstitution across synaptic partners (GRASP) screening has shown that many of the GRNs potentially synapse with the hugin neurons, lending support to the idea that GRNs can influence the release of PTTH and therefore developmental timing through the hugin neurons.

Population genomics of *Rhizopus stolonifer*

Sawyer R Masonjones, Jason E Stajich
Department of Plant Pathology & Microbiology

Sawyer Masonjones

Worldwide, about one third of food goes uneaten due to postharvest losses and waste. One culprit of this loss is Rhizopus stolonifer, a Mucoromycota fungus that causes soft rot in many fruits and vegetables globally. The recommended control strategy is quick harvesting, packing, etc at cool temperatures combined with treatment of fungicides. However, not all are approved for human contact limiting their use in strawberry. In addition, fungicide resistance is expected to evolve in populations with frequent application. The organic farming sector's limited use of fungicides with mixed efficacy, could impact or establish population structures of R. stolonifer. We present preliminary work in phenotyping in 40 geographically and substrate diverse strains and genomic diversity from 8 geographically diverse R. stolonifer samples. Strains are being cultured from strawberries grown in organic and conventional farms found in California and from culture stocks at the NRRL. Fungicide resistance is being assayed in a collection of strains from global distribution and isolates from California and Florida strawberry fields. Initial growth rate experiments of 30 samples vary from 0.5 cm/day to 3.4 cm/day with a mean of 1.98 and a standard deviation of .54, and ANOVA demonstrates wide variation between samples ($p<<0.05$) for the current collection. Further growth experiments will measure growth rate under various abiotic stressors. Genomic resequencing of 8 strains obtained from NRRL were analyzed for patterns of variation in the genomes to establish if there is a strong signal of population structure and demography. The identified variants were further analyzed to scan for gene loci with high accumulation of differences and evidence for transposable element activity. Future work with expanded collections of strains will scan for selection and variation.

Investigating the role of endothelial-to-mesenchymal transition in the human primary brain tumor, glioblastoma multiform

Jui-Yu Liao[1], Don Armstrong[2], Florence M. Hofman[3,4], Raphael Zidovetzki[1]
[1]Department of Cell Biology & Neuroscience, University of California-Riverside
[2]University of Illinois at Urbana-Champaign, Champaign, IL
[3]Department of Pathology, Keck School of Medicine, University of Southern California, Los Angeles, CA
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Jui-Yu Liao

Glioblastoma multiforme (GBM) is the most aggressive type of human malignant brain cancer. The median survival time of the patient is only 12-15 months despite the earlier diagnosis and therapy. In the brain tumor microenvironment, the tightest specialized endothelium, which formed the critical selective part in the blood-brain barrier (BBB), is quickly controlled for the primary tumor progression. The properties of the brain endothelial cells (BECs) in the tumor-surrounded vessel were found to be quite different. Isolated tumor-associated brain endothelial cells (TuBECs) have large, flat, and veil-like appearance and exhibited high activity by using the mesenchymal markers. Based on the observations in cell morphology, we hypothesize that the stem cell-liked cellular mechanism, endothelial-to-mesenchymal transition (EndMT), may occur in the blood-brain barrier. There were total 72 genes presented the significant changes. In order to verify the potential molecular mechanism of the specialized BECs in GBM, we conducted the approaches of gene set analysis among three different types of BECs. Five of significant hallmark gene sets from the GSA analysis indicated their roles in EndMT. Currently, we have found that twelve candidates have the connection with EndMT. We will establish the small RNA-induced silencing system and conduct the migration/invasion assay to further verify their roles in GBM cell. Our aim of this research is to first clarify the molecular mechanism of the specialized BEC transition in GBM and further identify an approach to terminate or reverse the mechanism in primary brain tumors.

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