



*GGB Symposium
September 19, 2017*

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Schedule

9:00 – 10:00 Breakfast and Registration

9:30 – 10:00 New Student Orientation

10:15 Opening remarks

Session I

10:30 The Grape expectations: a microbial journey through the vine by Ella Deyett

10:50 High-resolution haplotype construction by single gamete cell sequencing by Ruidong Li

11:10 A Neuroendocrine Network Connecting Gustatory Signals to Developmental Timing in Drosophila by Mikkal Blick

11:30 - 11:40 Tea/Coffee Break

Session II

11:40 Population Variation in post harvest rot *Rhizopus stolonifer* by Sawyer Masonjones

12:00 The Roles of An RNA Polyphosphatase PIR-1 in RNA interference by Lichao Li

12:20 - 1:20 Lunch

Session III

1:20 The Effects of Aluminum on Genome Stability in Arabidopsis by Stephen Bolaris

1:40 Interactions between the RNA-binding proteins Pumilio and Argonaute, and their effects on gene expression by Erin Sternburg

2:00 - 2:15 Refreshments

2:15 - 3:00 Keynote

Gene expression and the molecular acrobatics of its control by translation presented by Dr. Seán O'Leary

3:00 - 4:00 Poster Session and Reception

4:00 Awards

Abstracts

Session I

Identifying Grapevine Microbial Endophytes for Control of Pierce's Disease

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Host-pathogen interactions are highly dynamic and not always well understood, but targeted metagenomics has revealed a great deal about the diversity within microbial communities and their biological functions. Bioinformatic tools can be used to identify and correlate microbes that act antagonistically or beneficially to a pathogen. Specifically, this research looks at the microbial composition of grapevines under pressure from a devastating plant pathogen, *Xylella fastidiosa* (*Xf*), the causative agent of Pierce's Disease (PD). The overarching goal of our project is to identify both prophylactic and curative measures for PD by utilizing the microbial communities associated with grapevine. We hypothesized that the differences in the vines' microbiome influence disease outcome. The goals of our project are to (i) study microbial community temporal dynamics in PD-symptomatic and non-symptomatic grapevines, (ii) evaluate biological control agents approach for preventative PD management and (iii) identify anti-*Xylella* bioactive natural molecules of microbial origin as curative control strategies for PD. Metagenomic tools and culture-independent methods were utilized to reveal potential beneficial organisms that mitigated PD. *In vitro* and *in planta* assays were developed to evaluate these beneficial organisms for both their anti-*Xf* properties and for their plant growth promoting attributes. The implementation of natural microbes to combat pathogens could be an alternative approach to limit the risks of pesticide resistance, increase crop productivity and create sustainable agriculture.

High-resolution haplotype construction by single gamete cell sequencing

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A haplotype is a set of DNA variants (or alleles) that tend to inherit together from a single parent. Evidences have shown that phenotypic variations can be better explained if haplotype data are available. Many specific haplotypes have been identified to be associated with a particular disease susceptibility or drug response. Unrevealing the haplotype structure is also critical for understanding allele-specific events such as methylation, and for identifying the parental origins of de novo mutations.

Although deep-sequencing of individual genome has made it easy to determine the genotypes for millions of single nucleotide polymorphisms (SNPs), the genotype data usually take unphased format. It remains challenging to phase these molecular variants into specific haplotype for species with heterozygous genomes like human. Many computational methods have been proposed for haplotype inference of heterozygous individuals. However, these methods either require genotypes of both parents of the individual which sometimes are not available, or require a large sample from the individual's progeny (>100) which are costly and time-consuming. Genotyping of single haploid cells (e.g., gametes) from the heterozygous individuals provides opportunities for reconstructing haplotype efficiently due to the substantial reduction of the complexity of mixtures of paternally and maternally inherited DNA in these cells.

We have developed an advanced and powerful algorithm allowing missing genotypes and genotyping errors from sequencing a small number of haploid cells at low coverage to construct high-resolution haplotypes. Both simulation and real gamete sequencing datasets show that our algorithm outperforms those proposed by other research groups. Further studies will be performed to seek the potential biological/clinical applications of the new program.

A Neuroendocrine Network Connecting Gustatory Signals to Developmental Timing in *Drosophila*

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Drosophila melanogaster has multiple life stages, with the release of the steroid hormone ecdysone being responsible for developmental transitions from one life stage to the next. There are multiple controls for ecdysone release, including signaling by prothoracicotropic hormone (PTTH). PTTH, released by two pairs of bilateral neurons in the protocerebrum, plays a role in the timing of ecdysone release from the prothoracic gland, with a delay in development from third instar to pupariation caused by the ablation of the PTTH secreting neurons. Determining what factors signal for PTTH secretion would show what plays a role in the timing of development of *Drosophila* larvae to pupariation. Here, we show that the neurons producing the neuropeptide *hugin* in the subesophageal zone (SEZ) send signals to the PTTH neurons and contribute to the control of developmental timing in *Drosophila*. GFP Reconstitution across Synaptic Partners (GRASP) analysis suggests that the *hugin* neurons likely form synapses with the PTTH neurons. Additionally, null mutations of both *hugin* and the *hugin* receptor-encoding genes cause a delay in developmental timing similar to that caused by PTTH neuron ablation, and the receptor mutation phenotype can be rescued through expression of the *hugin* receptor driven by PTTH promoters. The SEZ is known to be innervated by gustatory receptor neurons (GRNs), which sense taste signals and have been shown to be involved in food preference. GRN signaling of the *hugin* neurons would potentially link external stimuli to developmental timing. Using gustatory receptor Gal4 lines, we have been able to show that multiple GRNs have a likely synaptic connection with the *hugin* neurons through GRASP analysis. Additionally, both the silencing and activation of some GRNs can alter the timing to pupariation. Taken together, our results suggest that PTTH control of developmental timing can be influenced by taste signals from the environment through a neuronal circuit that goes from GRNs to PTTH signaling via *hugin* neurons.

Session II

Population variation in post-harvest rot *Rhizopus stolonifer*

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Globally postharvest diseases contribute to one third of food losses. *Rhizopus stolonifer*, a Mucoromycota fungus, causes postharvest soft rot in fruits and vegetables. Control is typically focused on quick harvesting, packing at cool temperatures combined with treatment of fungicides. However, many fungicides are not approved for direct use on fruits and understanding mechanisms of resistance development is limited. We have phenotyped 130 geographically and substrate diverse *R. stolonifer* strains and sequenced the genome of half of these. Strains have been cultured from California and Florida strawberries, from almond hulls from California orchards, and obtained from culture stocks from the USDA-NRRL collection.

Fungicide resistance varies among isolates with reduced sensitivity to Fludioxonil in some strains. Linear growth rates in race tubes at 12C, 23C, and 30C show significant differences among strains that originate from varied climates or isolation substrates. Whole genome sequencing is being performed on 60 strains from this collection and analysis of patterns of genetic variation was performed to test for evidence of population structure and demography. Preliminary data from a geographically diverse subset of 12 samples identified 40,000 SNPs across the 38 Mb genome. Variants were analyzed to scan for highly diverse gene loci, evidence of directional selection, or insertion/deletions of transposable elements. The strains group into 4 main clades, but no phylogeographic pattern has emerged yet. Further sequencing will test robustness of these potential sub-populations, examine genotype and phenotype correlations, and identify genetic loci with signatures of rapid evolutionary change.

The Roles of An RNA Polyphosphatase PIR-1 in RNA interference

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RNA interference (RNAi) is a highly conserved process in eukaryotes that responsible for transposon silencing, gene regulation and antiviral response. During RNAi process, 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 an RNA polyphosphatase PIR-1 interacting with Dicer, who may participate in RNAi but the mechanism is unrevealed. The project is to demonstrate the functions and mechanism of PIR-1 in RNAi pathways.

We firstly elucidated that the in vitro activity of *C. elegans* PIR-1 is to remove β and γ phosphates from the 5' end of triphosphorylated RNA molecule. Secondly we investigate the role of PIR-1 in antiviral RNAi. Orsay virus propagation enrichment in infected *pir-1* null mutant suggests that PIR-1 is significant for antiviral RNAi response to Orsay virus. dsRNA extraction and strand-specific qPCR results show that viral dsRNAs are depleted in *pir-1* null mutant but not in other RNAi deficient mutants such as *rde-1*, *rde-3* or *dcr-1*, which indicated that PIR-1 is involved in dsRNAs processing. We are constructing *pir-1;dcr-1* double mutant and preparing *pir-1* catalytic site mutant using CRISPR/Cas9 to understand the specific roles of PIR-1 in dsRNA stability and cleavage.

Thirdly, previous study revealed that PIR-1 is required for synthesis of endogenous siRNAs which targets self RNAs. We designed cloning method for endogenous siRNA precursors to investigate the roles of PIR-1 in endogenous RNAi pathways.

Session III

The Effects of Aluminum on Genome Stability in Arabidopsis

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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 plant's reaction to the toxicity with the goal of applying this knowledge to agriculturally relevant plant species.

Interactions between the RNA-binding proteins Pumilio and Argonaute, and their effects on gene expression

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Post-transcriptional regulation has revealed itself as a key mechanism in quickly and effectively regulating cellular activity. RNA binding proteins (RBPs) play a major role in post-transcriptional regulation by binding to specific sites in the mRNAs, typically in the 3' untranslated region (UTR), and acting to increase or decrease the stability of the mRNA. These mRNA-RBP interactions are essential to proper cellular function, and many pathologies are associated with their misregulation. Although the activity of many single RBPs has been studied, what remains largely unexamined transcriptome-wide is how extensively multiple RBPs interact with one another when bound to an mRNA, and how these interactions affect its protein expression. In conducting this study, a global approach was taken to assess the extent of interaction between two well characterized and highly conserved RBPs, Argonaute (Ago) and Pumilio (Pum). Crosslinking followed by immunoprecipitation (CLIP) experiments were performed, followed by high throughput sequencing, to determine the transcriptome-wide binding profiles of each protein. Candidate 3' UTR interaction sites where Ago and Pum are colocalized were selected for functional validation using luciferase reporter assays. Of the 26 sites tested, 3 sites in 3 different transcripts (VLDLR, RRAGD, and FNIP2) were shown to host antagonistic interactions between Pum and Ago, where Pum acts to decrease Ago binding. Interestingly, the binding sites for the two proteins are too far for potential antagonism due to steric hindrance, and suggests some alternate mechanism. The importance of this research lies in its elucidation of complex interactions between RBPs, and how extensive these interactions are transcriptome-wide. Our evidence indicates that PUM, a protein characterized as mostly repressive, can change its behavior in a context dependent manner. Many diseases society faces today are due to gene misregulation. A more complete understanding of the relationships that RBPs can have with one another can provide insight into gene expression regulation, how misregulation leads to disease, and may further lead to more effective treatments against disease.

Poster Session

Identification of a Novel Endogenous Small RNA Pathway Specifically Targeting the 3' UTRs of mRNAs

James W.J. Randolph, Lichao Li, Weifeng Gu

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We are reporting a novel WAGO small RNA pathway which specifically targets the 3' UTR of hundreds of functionally important genes. In *C. elegans*, endogenous small RNAs, 22G-RNAs, bind Argonautes to regulate almost all germline genes. There are two major 22G-RNA-mediated pathways in *C. elegans* germline cells: One is mediated by Argonaute CSR-1 and plays important roles in chromosome segregation and embryonic development; the other is mediated by multiple Argonautes, WAGOs, and play critical roles in silencing transposons, pseudogenes, viruses, and some functional genes. In all these small RNA pathways, 22G-RNAs are generated by RNA-dependent RNA polymerases (RdRPs) using mRNAs and other RNAs as templates. Usually these 22G-RNAs are generated from both coding regions and UTRs of RNAs. Here we are reporting a novel small RNA pathway which specifically targets the 3' UTRs of hundreds of genes, many of which have been well studied and play important roles in germline and embryonic development. Our preliminary results indicated that these genes are targeted both by CSR-1 and WAGO Argonautes. However, CSR-1 majorly targets the 5' UTR and coding regions, while the WAGO Argonautes only target the 3' UTRs. Interestingly, the WAGO-22Gs are not dependent on *rde-3*, which is usually required for generating 22Gs in other WAGO-dependent pathways including exogenous RNAi pathways. Our RNA-seq results suggest that these 22G-RNAs may be involved in silencing the target RNAs. We are currently using genetics, high-throughput sequencing and ribosome profiling to investigate why these small RNAs are only generated from the 3' UTR regions and if these small RNAs are involved in translation regulation. We are also analyzing if these 22G-RNAs affects miRNA-mediated gene regulation at the 3'UTR of RNAs. In all, we are reporting a novel WAGO-mediated 22G pathway which specifically targeting the 3'UTR of hundreds of functional genes and this pathway is different from the canonical WAGO pathway since RDE-3 is not required for the 22G biogenesis.

Assembly of Eleven Pseudomolecules Representing the Cowpea Genome Sequence

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Cowpea (*Vigna unguiculata* L. Walp.) is a legume crop that is resilient to hot and drought-prone climates, and a primary source of protein in sub-Saharan Africa and other parts of the world. Despite its relevance to food security, actual yields of cowpea are much lower than the known yield potential and cowpea genome resources have lagged behind other major crop plants.

Here we report the first high-quality sequence of the cowpea genome (~620Mb estimated size), from IT97K-499-35. Using Single Molecule Real-Time Sequencing (Pacific Biosciences) ~6M reads were generated for a total of 56.8Gbp (~91x genome equivalent), with a read N50 of ~14.5Kbp. We obtained an assembly of 505Mbp using CANU, with contig N50 of ~5.3Mbp, contig L50 of 27 and the longest contig of ~18.4Mbp. We polished the draft assembly using Pacific Biosciences' Quiver pipeline in SMRT Portal, then evaluated the quality and completeness using several independent metrics, including Illumina reads. We generated super-scaffolds by mapping the polished assembly onto a BioNano genome (BNG) optical map. The BNG map consisted of 508 contigs for a total length of ~622Mbp, and a N50 of ~1.6Mbp. The super-scaffold N50 was ~12Mbp, with the longest scaffold of ~30Mbps. Finally, we produced pseudomolecules by anchoring and orienting super-scaffolds to five genetic maps including

37,372 unique SNPs. We obtained eleven pseudomolecules for a total of 474Mbp. Cowpea pseudomolecule sequences will be available from <http://www.int-cowpea.org/>. Project was supported by the NSF BREAD program.

Transcriptome-wide identification and validation of interactions between the miRNA machinery and HuR on mRNA targets

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The 3' UTR of mRNAs is the primary regulatory region that mediates post-transcriptional control by microRNAs and RNA-binding proteins (RBPs) in the cytoplasm. Aside from individual sequence-specific binding and regulation, examples of interaction between these factors at particular 3' UTR sites have emerged. However, the whole picture of such higher-order regulatory modules across the transcriptome is lacking. Here, we investigate the interactions between HuR, a ubiquitous RBP, and Ago2, a core effector of the miRNA pathway, at the transcriptome-wide level. Using HITS-CLIP, we map HuR and miRNA binding sites on human 3' UTRs and assess their co-occurrence. Additionally, we demonstrate global effects of HuR knockdown on Ago2 occupancy, suggesting a co-regulatory relationship. Focusing on sites of Ago2-HuR overlap, 13 candidates were screened in luciferase reporter assays. Eleven sites showed miRNA-dependent repression, as confirmed in Dicer-null cells. To test for HuR's role in co-regulation, we measured the reporters in HuR KO cells. Three of the miRNA sites demonstrated altered activities, indicating that HuR has an effect on miRNA repression at those sites. Our study presents an efficient search and validation system for studying miRNA-HuR interactions, which expands our understanding of the combinatorial post-transcriptional control of gene expression at the 3' UTR.

ER stress inhibits nonsense mediated decay

Zhelin Jeff Li, John Vuong, Min Zhang, Cheryl Stork, and Sika Zheng

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Nonsense-mediated RNA decay (NMD) selectively degrades mutated and aberrantly processed transcripts that contain premature termination codons (PTC). Cellular NMD activity is typically assessed using exogenous PTC-containing reporters. Guided by that NMD directly targets some endogenous alternatively spliced transcripts, we developed a broadly applicable strategy to reliably and conveniently monitor changes in cellular NMD activity after overcoming inherently problematic aspects of assaying endogenous NMD targets. Our new method was validated genetically for distinguishing NMD regulation from transcriptional control and alternative splicing regulation. Applying this robust method for screening, we identified NMD-inhibiting stressors and also found that NMD inactivation was not universal to 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 of calcium signaling mediating thapsigargin-induced NMD inhibition as previously reported. Instead, we discovered that of the three unfolded protein response (UPR) pathways activated by thapsigargin, mainly protein kinase RNA-like endoplasmic reticulum kinase (PERK) was required for NMD inhibition. Finally, we reported that ER stress compounded TDP-43 depletion in the upregulation of cryptic NMD isoforms that have been implicated in the pathogenic mechanisms of amyotrophic lateral sclerosis and frontotemporal dementia.

The dynamic and varied genome of the oomycete pathogen *Phytophthora infestans*

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As the causal agent behind the late blight disease of tomato and potato, the oomycete *Phytophthora infestans* causes considerable crop losses around the world. Controlling epidemics via host-mediated resistance is hampered by the remarkable adaptability of the *P. infestans* genome, since new R gene introductions into commercial varieties are quickly overcome. To investigate mechanisms driving genome plasticity, we used whole genome Illumina sequences of progeny from two sexual crosses and >50 diverse global individuals to identify copy number variation (CNV), structural variation (SV), and loss of heterozygosity (LOH) events. We determined through examining SNP allele ratios that the four parental isolates behind our two sexual crosses were mostly 2N, but that about 10% of progeny were mostly 3N. Local CNVs larger than 5kb comprised around 4.5% of genomic regions which could be uniquely mapped, and affected as many as 400 individual genes depending on the strain. Structural variants in the form of inversions and deletions were also identified among nine examined diverse global isolates using Pindel and Breakdancer. Finally, we observed considerable tracks of LOH among our progeny using the YMAP toolset. To investigate the functional consequence of LOH, we sequenced four progeny which spontaneously produced fast-growing sectors in the presence of the fungicide metalaxyl. These exhibited numerous sequence differences which may reveal the basis of resistance to the fungicide.

Upcoming GGB events

GGB Ice Cream Social and October meeting

Wednesday, October 4 at 4:30 PM

Meet in Genomics lobby



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GGB Graduate Students



<https://www.facebook.com/groups/300820133677669/>

ggbgsa.slack.com



Faculty seeking students

Alan Brelsford, I study population genomics of non-model animals. Areas of interest include genetic basis of coloration, sex determination, social behavior, and reproductive barriers between species. Major study systems are ants and birds.

Anand Ray, Use the olfactory system as a model to understand behavior, gene regulation, development etc in *Drosophila*, mosquito, mammalian cell culture

Caroline Roper, host-microbe interactions (plant and insect-bacterial), plant microbiome

Dan Koenig, Plant evolutionary genetics

Joel Sachs, evolutionary genetics of bacterial symbionts

Meng Chen, My lab uses light signaling in *Arabidopsis* as a genetic model to understand how cell signaling controls developmental programs through spatial reorganization of the nuclear architecture.

Rong Hai, Understanding pathogenicity of emerging pathogen

Sarjeet Gill, Mechanism of Bacterial toxin action

Seán O'Leary, Single-molecule biophysics of the mechanism of eukaryotic translation initiation

Sika Zheng, Brain-specific RNA regulation in health and disorders

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