Genetics, Cellular, and Molecular Sciences
Training Grant Symposium

Indiana University

May 21, 2015
Simon Hall 001
Wednesday May 20th
6:30pm  Group Dinner with Faculty (meet at IMU lobby)
     Nick Sokol, Pat Foster, Steve Bell, and Craig Pikaard

Thursday May 21st
8:30 - 9:10  Registration and Breakfast
            Speakers will be escorted from IMU to Simon Hall by GCMS-TG trainees
9:10 - 9:15  Opening Remarks by GCMS-TG Director
9:15 - 10:15  Dr. Lucas Argueso (Colorado State University)
              Genomic context of mitotic copy number variation in yeast diploids
10:15 - 10:45  Christopher Puccia (Soni Lacefield lab)
               Polo Kinase CDC5 Governs Meiotic Commitment in \textit{Saccharomyces cerevisiae}
10:45 - 11:00  Coffee Break
11:00 - 11:30  James Pease (Matthew Hahn lab)
               Phylogenomics of two closely related species groups
11:30 - 12:00  Katie Hummels (Dan Kearns lab)
               A conserved translation elongation factor, EF-P, regulates motility in \textit{Bacillus subtilis}
12:00 - 12:30  Courtney Ellison (Yves Brun lab)
               Elucidating the Initial Steps of Bacterial Adhesion
12:30 - 1:30  Lunch
1:30 - 2:00  Ryan Over (Scott Michaels lab)
            Using FRAP in \textit{Arabidopsis} roots to investigate novel questions about linker histone dynamics
2:00 - 2:30  Benny Walker (Jared Cochran lab)
            \textit{Mechanism of force generation by kinesin 10s}
2:30 - 3:30  Dr. Michael Bustin (National Cancer Institute)
            Epigenetic Regulation by Chromatin Architectural Proteins
3:30 - 4:30  GCMS-TG meeting between speakers and trainees (current and former)
4:30 – 6:00  Poster Session (Jordan Hall Atrium)
6:00 Dinner with GCMS-TG trainees
Dr. Lucas Argueso w/ Katie Hummels, Christopher Puccia, and James Pease
Dr. Michael Bustin w/ Rachel Samson, Ryan Over, and Jered Wendte
Lucas Argueso, Ph.D.
Colorado State University
Symposium Invited Speaker

Title: Genomic context of mitotic copy number variation in yeast diploids

Abstract: Population-based surveys of the human genome have conclusively established that copy number variations (CNVs) are a substantial source of genetic diversity. Despite their abundance and association with disease processes, our understanding of the underlying mechanisms leading to CNV formation is still incomplete. We have optimized an assay for CNV detection in diploid yeast cells that takes advantage of two genes, SFA1 and CUP1, that confer gene dosage-dependent tolerance to formaldehyde (FA) and copper (Cu), respectively. Cells carrying a single chromosomal insertion of the SFA1-V208I-CUP1 gene-dosage reporter are plated on media containing high levels of FA and Cu, and only the rare individuals carrying a genome rearrangement resulting in two or more copies of the reporter are able grow. The assay is quite versatile since it can, in principle, detect chromosomal rearrangements initiated by DNA lesions occurring anywhere in the genome, as long as the final outcome of repair involves amplification of the region where the reporter was inserted. We investigated the role of genomic context on CNV formation by analyzing a panel of tester strains with insertions of the reporter at sites of interest on chromosomes (Chr) 4, 5, and 15. Differences in genomic context of the reporter resulted in a 15 fold range in CNV rates (0.16 to 2.45 x 10^{-6}); and exposure to sub-toxic doses of Hydroxyurea (HU), Camptothecin, Methyl Methanesulfonate, and ionizing radiation stimulated CNV rates by 3 to 17 fold. The selected formaldehyde-copper resistant yeast clones were then analyzed by PFGE karyotyping and array-CGH to characterize the spectra of CNV-associated genome rearrangements. The majority of the CNVs were formed by non-allelic homologous recombination (NAHR) between dispersed repetitive DNA sequences, primarily Ty retrotransposon insertions. Non-homologous and microhomology mediated events were also detected, albeit at lower frequencies. The qualitative spectra of CNV events were heavily influenced by the chromosomal context of the reporter. The primary mechanisms of gene amplification on Chr5 were whole chromosome aneuploidy and segmental duplication, whereas on Chr4 and Chr15 most CNVs resulted from unbalanced translocations involving loss of a terminal segment of one chromosome and gain of an extra copy of the chromosome arm containing the reporter. Our results demonstrated the broad diversity of genome rearrangements that can be detected by the SFA1-CUP1 reporter and the key role that genomic context plays in CNV formation.

Unexpectedly, our analysis also identified a robust bias for terminal deletions of the right arm of Chr7 associated with unbalanced translocations. This bias was observed at approximately constant strength across all three reporter insertion sites, as well as for all four DNA damage conditions tested. We investigated the possibility of the presence of a fragile site on Chr7 by comparing the rate copy-neutral loss-of-heterozygosity (LOH) for the right arms of Chr7, Chr13 and Chr4. The LOH rates were very similar between all three regions regardless of the absence or presence of DNA replication stress (HU exposure), and LOH breakpoints on Chr7 were not clustered at a discrete locus. These results disfavored the fragile site model, and indicated that the Chr7 bias applies to non-allelic rearrangements, but not to allelic interhomolog recombination. Finally, to independently validate the existence of a CNV bias at Chr7, we developed a genome rearrangement competition assay that does not rely on selection for FA and Cu resistance. In this case, a single homologous recombination donor substrate on Chr5 (ura3D3') can interact with any one of seven simultaneously available insertions of a recipient sequence (ura3D5') dispersed across the genome of a diploid strain. NAHR between the donor and any
one of the recipients generates a functional *URA3* gene at the breakpoint of a chromosomal translocation. Spontaneous independent clones containing such rearrangements were selected in uracil drop-out media, and using a custom multiplex PCR assay we determined that the *ura3D5'* recipient inserted at the right arm of Chr7 was the most frequent substrate used in the competition. DNA replication stress quantitatively increased the overall NAHR competition rate, yet it did not change the Chr7 NAHR qualitative bias. Taken together, our results suggested that even though this specific region of the genome does not appear to be more prone to DNA breakage than other regions, once a DNA lesion is formed there, it has a high propensity to undergo inappropriate repair leading to a chromosomal rearrangement. This observation may provide a suitable model system to investigate the cellular mechanisms associated with the formation of recurrent chromosomal rearrangements in mitotic cells.

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**Michael Bustin, Ph.D.**  
Center for Cancer Research, National Cancer Institute  
National Institutes of Health  
Invited Symposium Speaker  

**Title:** Epigenetic Regulation by Chromatin Architectural Proteins  
**Abstract:** Chromatin structure plays a central role in the orderly progression of transcription, replication and repair, and in the epigenetic regulation of gene expression. Aberrations in chromatin structure leading to alterations in epigenetic regulatory processes have been linked to birth defects, aging, and various diseases. All vertebrate nuclei contain structural proteins such as linker histone H1 and high mobility group (HMG) proteins, which bind to nucleosomes without specificity for DNA sequence and alter the local and global architecture of the chromatin fiber. I will present recent findings which suggest that a dynamic interplay between H1 and members of the HMGN protein family maintains the landscape of chromatin regulatory sites, modulates the fidelity of the cellular transcription profile, and impacts the cellular phenotype.
Christopher Puccia - Soni Lacefield lab
GCMS-TG current trainee: speaker
Title: Polo Kinase CDC5 Governs Meiotic Commitment in *Saccharomyces cerevisiae*
Abstract: Maintaining commitment to a particular cell cycle is key for maintaining genome stability. For example, if a cell enters but fails to maintain meiosis and returns to mitosis, genome instability can result. We are studying meiotic commitment in budding yeast by a response to nutrients. Yeast cells will enter meiosis when starved of nutrients, but are able to return to mitosis if nutrient-rich medium is added prior to reaching commitment. However if cells have progressed past the meiotic commitment point of Prometaphase I, they will finish meiosis even when subjected to nutritive growth conditions. Importantly, cells that do not stay properly committed to meiosis, and return to mitosis at meiotic stages beyond the normal commitment point increase their genome content and nuclei number. Termed inappropriately uncommitted to meiosis, this abhorrent phenotype is caused by reduced levels of Ndt80, a major meiotic transcription factor. Because phosphorylation is a major meiotic regulatory mechanism, I have assessed several meiotic kinases downstream of Ndt80 and active during the meiotic commitment point. Utilizing a microfluidics assay coupled to time-lapse fluorescence microscopy, I have found polo kinase Cdc5 responsible for properly maintaining meiotic commitment. A meiotic null allele of Cdc5 causes cells beyond the commitment point to inappropriately bud and return to mitosis. Similar to reduced Ndt80 levels, this absence of Cdc5 during meiosis results in genome instability through accumulation of many nuclei, as well as a deformed bud phenotype. Future work aims to identify relevant downstream targets of Cdc5 in the meiotic commitment pathway.

James Pease - Matthew Hahn lab
GCMS-TG current trainee: speaker
Title: Phylogenomics of two closely related species groups
Abstract: When closely related clades are formed by a rapid succession of speciation events, phylogenies inferred from different genes and genomic regions often conflict due to a variety of evolutionary factors. I present evidence that massive genome-wide introgression all but completely obscured the phylogeny of the Anopheles gambiae species complex. Additionally, I describe a rapid radiation of wild tomato species (Solanum), which shows evidence of ancestral allele sorting, introgression, and both lineage- and environment-specific patterns of sequence evolution. These findings and new methods demonstrate how phylogenomic analysis can enrich and expand our broader understanding of speciation histories.

Katherine Hummels - Dan Kearns lab
GCMS-TG current trainee: speaker
Title: A conserved translation elongation factor, EF-P, regulates motility in *Bacillus subtilis*
Abstract: Translation Elongation Factor P (EF-P) is a tRNA mimic conserved in all domains of life. In *Escherichia coli* and *Saccharomyces cerevisiae*, EF-P aids in the translation of poly-proline stretches which are particularly difficult for the ribosome to translate. Here, we investigate the role of EF-P in a Gram-positive bacterium, *Bacillus subtilis*. We show that, contrary to what is observed in *E. coli* and *S. cerevisiae*, EF-P is not required for cell growth and is instead essential for motility. Further, we determine that EF-P regulates swarming motility at the level of the flagellar hook, with an efp mutant producing fewer hooks and therefore fewer mature flagella. Finally, we show that the need for EF-P can be bypassed by overexpression of Yeel, a highly conserved gene of unknown function. We provide data indicating that Yeel acts in a pathway parallel to EF-P, suggesting a conserved partnership of these two proteins.
Courtney K. Ellison - Yves Brun lab
GCMS-TG current trainee: speaker
Title: Elucidating the Initial Steps of Bacterial Adhesion
Abstract: *Caulobacter crescentus* is a freshwater alphaproteobacterium that undergoes a dimorphic life cycle. It begins this life cycle as a motile, non-reproductive swarmer cell that eventually differentiates into a sessile, reproductive stalked cell. The transition from motile to nonmotile phases is accompanied by the secretion of an adhesive material called the holdfast that allows the cell to permanently adhere to surfaces. Although it is known that pili and the single, polar flagellum of the motile cell assist in temporary adhesion to a surface, how these structures contribute to adhesion and the secretion of the holdfast remains to be elucidated. In order to clarify the mechanisms behind the transition from reversible to irreversible adhesion including how surface sensing occurs, I am using single-cell tracking to observe cell behavior upon surface contact. I am able to image holdfast production upon surface contact by selectively labeling holdfast with a fluorescent conjugated lectin. By using cell tracking software MicrobeJ, I can track the production of holdfast in response to surface contact in mutants to directly test the roles of different genes in the surface sensing process.

Ryan Over - Scott Michaels lab
GCMS-TG current trainee: speaker
Title: Using FRAP in *Arabidopsis* roots to investigate novel questions about linker histone dynamics
Abstract: The structure and characteristics of chromatin play a large role in controlling gene expression patterns. Linker histones (H1) bind DNA between nucleosomes to further compact chromatin. In contrast to nucleosomes, linker histones bind chromatin dynamically, and their relative levels and primary variants change during development. This suggests they contribute greatly to overall chromatin characteristics. Fluorescence recovery after photobleaching (FRAP) has been used effectively in mammalian cell culture to explore factors affecting H1 dynamics. The Arabidopsis root provides an excellent system for studying H1 dynamics in an organism, which allows us to address previously unanswerable questions including dynamics in different cell types and stages of differentiation. This system will also shed light on plant specific questions such as the role of the divergent histone H1.3. Both monocots and dicots have this variant of unknown function, which is expressed late in old tissue and under drought conditions. An additional intriguing factor to consider is the role of the histone chaperone NAP1, which recently was shown to affect linker histone loading and dynamics in Xenopus egg extracts.

Benny Walker - Jared Cochran lab
GCMS-TG current trainee: speaker
Title: Mechanisms of force generation by kinesin 10s
Abstract: Force generation by molecular motors and microtubule dynamics are essential for faithful cell division and misregulation can lead to genetic instability and cancer. My main research interest is the force generation pathways of the molecular mitotic motor family called kinesin-10, specifically the human and *Drosophila* homologue KID and NOD, respectively. Kinesin-10s bind chromosome arms during mitosis and via an unknown mechanism exert the polar ejection force (PEF) as they interact with spindle microtubules. Kinesins share a structurally similar motor domain which converts the chemical energy from nucleotide hydrolysis into mechanical energy (e.g. the PEF). Our goal is to characterize the kinetic nucleotide hydrolysis mechanisms of NOD’s and KID’s motor domains as they interact with tubulin and polymerized microtubules to build biophysical models of force generation. In conjunction we want to investigate whether NOD and KID produce force at the microtubule lattice or at the dynamic microtubule plus ends by utilizing fluorescent microscopy. Combining the biophysical and microscopy data we hope to build a robust model of the force generation mechanism for NOD and KID.
Brittany A. Niccum – Pat Foster lab
GCMS-TG former trainee: poster presenter
**Title:** Determinants of Wave Pattern of Base Pair Substitutions Across the Escherichia coli Genome
**Abstract:** The mutation rate and mutational profile of an organism as determined by whole-genome analysis act as molecular markers for internal and external influences on genomic stability. Past studies of genomic stability have relied on analyzing mutation rates and mutational profiles using either mutations of specific reporter loci or base substitutions that accumulate at neutral DNA sites. These studies were limited to specific areas of the genome. By using a mutation accumulation protocol followed by whole genome sequencing, we are able to analyze a mutational density pattern that spans the genome of Escherichia coli and resembles a sine wave. These results suggest that mutation rates change across the genome, which could indicate that different areas of genome could evolve at different rates. The wave-like pattern was shown to correlate with the binding sites of two nucleoid-associated proteins (NAPs), HU and Fis. Different strains of E. coli carrying deletions of mutL as well as either fis, hupA, recA, matP, or rnhA were used in a mutation accumulation protocol followed by whole genome sequencing. We were able to observe a change in the wave like pattern when using these different strain backgrounds. Our results suggest that recombination and chromosomal structure are major causes of the pattern. Chromosomal structure and recombination play a part in how the genome evolves at different sites.

Briana Whitaker - Keith Clay lab
GCMS-TG current trainee: poster presenter
**Title:** Ecotype by Environment interactions drive microbiome community assembly in Panicum virgatum
**Abstract:** Foliar fungal endophytes are a ubiquitous and asymptomatic microbiome of plants. However, a core question in the field of host-associated microbiomes, such as for fungal endophytes, is whether microbes preferentially colonize certain host ecotypes or genotypes, reflecting some degree of co-adaptation in the symbioses, or whether community assembly is simply a byproduct of microbial source type and availability within the local environment. In order to explore this question, replicate plant hosts from three locally-adapted plant populations (i.e. ecotypes) of the native grass Panicum virgatum (Switchgrass) were first propagated under controlled greenhouse conditions and then transplanted at three field sites across the Midwest in a reciprocal transplant experimental design. We found that the fungal endophyte microbiome varied as a function of both host ecotype and environment. Specifically, the environment of the host plants had the greatest effect on total fungal colonization frequency and community diversity. Community structural analyses showed that the endophyte microbiome across host plants was driven by a host ecotype by environment interaction. NMDS ordination and visualization shows that environmental site of the host plants most strongly drives these community structure changes. However, the three ecotypes hosted structurally distinct microbial communities, particularly at the tallgrass prairie and riparian sites. These results indicate that community assembly in the fungal endophyte microbiome of Switchgrass is driven by both environmental and genetic controls.

Rachel Samson - Steve Bell lab
GCMS-TG current trainee: poster presenter
**Title:** Recruitment of the MCM Helicase to Origins of Replication
**Abstract:** Origins of replication in Eukarya and Archaea are defined by initiator proteins belonging to the AAA+ superfamily of ATPases. In eukaryotes, it is well-established that the combined action of the Origin Recognition Complex (ORC) and Cdc6 facilitates the recruitment of the replicative helicase MCM(2-7) to replication origins. However, the molecular basis of this recruitment has remained elusive. In our current work, we reveal that a single ancestral Orc1/Cdc6 protein performs both the ORC-like function of binding to replication origins and the Cdc6-like function of directing MCM recruitment to replication origins in Archaea. ATP binding by the *Sulfolobus* Orc1-1 initiator transduces structural movements within the protein that appropriately position a specific module responsible for the
recruitment of the MCM hexamer. Subsequent ATP hydrolysis renders the Orc1-1 protein incapable of recruiting additional MCM complexes to origins, ensuring fidelity within the process of replication.

Michael Washburn - Heather Hundley lab
GCMS-TG former trainee: poster presenter

Title: Role of the dsRBP ADR-1 in regulating RNA Editing in C. elegans.

Abstract: With over 100 million predicted editing sites in the human transcriptome, the hydrolytic deamination of adenosine to inosine, or A-to-I editing is one of the most prevalent types of RNA editing. A-to-I editing is catalyzed by a family of proteins called adenosine deaminases that act on RNA, or ADARs, that target regions of double-stranded RNA (dsRNA) for deamination. ADARs are present across metazoa and their expression is required for proper development. Importantly, A-to-I editing levels are altered in several cancers and neurological diseases; therefore, understanding how RNA editing by ADARs is regulated is important for human health.

An interesting area of A-to-I editing regulation is the influence of the RNA binding protein (RBP) landscape in regulating editing levels. The model organism C. elegans provides an excellent system to study the regulation of A-to-I editing by RBPs due to the viability of ADAR mutants. In C. elegans, two ADARs are present in the genome, ADR-1 and ADR-2; however, only ADR-2 is capable of catalyzing A-to-I editing. Recently, we have demonstrated that ADR-1 acts as a global regulator of A-to-I editing levels by promoting editing at the majority of affected adenosines. While the catalytic domain of ADR-1 harbors mutations rendering it catalytically inactive, ADR-1 is able to utilize its dsRNA binding domains (dsRBDs) to bind to the editing targets of ADR-2 in the absence of A-to-I editing. Furthermore, ADR-1 binding to the editing targets of ADR-2 is required for its ability to regulate editing levels in those substrates. However, it is unknown whether all RNAs bound by ADR-1 are targets of editing regulation.

While our initial approach found that ADR-1 editing regulation required dsRNA binding by ADR-1, this raised the questions of whether ADR-1 only binds to targets of RNA editing and if ADR-1 regulates the editing in all of its dsRNA binding targets. To test this hypothesis, we are in the process of coupling our previously utilized RNA-immunoprecipitation (RIP) assay to deep-sequencing analysis to identify the binding targets of ADR-1 across the transcriptome. In addition, as our initial analysis of ADR-1 editing regulation examined RNA from whole worms which could mask subtle tissue-specific events, we are also examining if ADR-1 regulates specific editing events solely in the context of C. elegans neurons.

Jeff Adrion - Matthew Hahn lab
GCMS-TG current trainee: poster presenter

Title: Transposable element insertion bias contributes to genome-wide TE landscape in Drosophila melanogaster

Abstract: Transposable elements (TEs) make up a significant portion of most multicellular eukaryotic genomes and their movement represents a major influx of genetic variation. Knowing the rates at which TEs insert and delete is critical for understanding their role in genome evolution. TEs are enriched in regions of low recombination and gene density. This bias has been explained by both the reduced efficacy of selection to remove deleterious TEs and the attenuation of deleterious fitness effects caused by insertions in these regions. However, this pattern could also be driven by an insertion bias. Here we take a mutation-accumulation (MA) approach to estimate the rates of TE insertions and deletions genome-wide and explore the spatial landscape of TEs in D. melanogaster. Our genome-wide survey of rates provides critical empirical estimates for understanding how the dynamics of TE movements influence long-term evolutionary patterns of TEs. Our data show that spatial patterns of insertions and deletions dramatically differ along chromosomes. Importantly, new insertions are not only more likely to persist in regions of low recombination and gene density (based on the distribution of extant copies), they are more likely to occur in such regions in the first place.
**Title:** Pterin-Dependent Surface Attachment Control in *Agrobacterium tumefaciens*

**Abstract:** One of the primary factors mediating surface attachment in *Agrobacterium tumefaciens* is an adhesin called the unipolar polysaccharide (UPP). Mutants disrupted in a gene called *dcpA* encoding a dual diguanylate cyclase (DGC)/phosphodiesterase (PDE) protein were found to exhibit elevated UPP production. DcpA has discrete DGC and PDE domains that catalyze synthesis and degradation, respectively, of the second messenger cyclic-diguanylate-monophosphate (c-di-GMP). This intracellular signal molecule plays a prominent role in regulating the motile-to-sessile transition in bacteria. Studies in *A. tumefaciens* and *E. coli* revealed that DcpA has both DGC and PDE activities, with the PDE activity dominating in *A. tumefaciens*. However, the DGC activity is revealed in *A. tumefaciens* mutated for an unlinked gene called *pruA* (*pteridine reductase A*). Mutation of a predicted PruA catalytic site residue indicated that it is required to maintain the PDE-dominant activity of DcpA. PruA catalyzes the synthesis of the novel pterin, 2'-O-methylmonapterin in *A. tumefaciens*. The gene immediately upstream and transcriptionally linked to *dcpA* encodes a putative pterin-binding protein (*pruR*, pterin responsive UPP Regulator) that is required to impart the pterin–dependent regulation of DcpA. PruR is annotated as a YedY-type molybdopterin binding protein, but is missing the key cysteine residue considered crucial for coordinating the molybdenum. The protein also has a predicted N-terminal secretion signal. Together, the PruA protein, through production of a monapterin product, and requiring the PruR protein, biases DcpA strongly towards its PDE activity, and this bias is critical for maintaining UPP production and other attachment processes in the off state.

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**Title:** Exploring the role of internal and external constructive processes in the development of *Onthophagus* beetles

**Abstract:** Traditional models of trait evolution consider development to be a unidirectional and linear translation of genotype into phenotype. However, many developmental processes are complex, nonlinear, and constructive, in the sense that they constantly create developmental environments that allow subsequent steps of phenotype construction to take place. In addition, a growing body of work illustrates that phenotype construction extends beyond the boundaries of the organism itself through *niche construction*, the process by which organisms alter the environmental conditions they experience during development, ranging from e.g. choice of diets to the construction of shelters or pupal cases. Here we explore the role of internal and external constructive processes in the development of horned beetles of the genus *Onthophagus*. *Onthophagus* beetles feed exclusively on nutrient-poor dung, and develop inside a subterranean brood ball made of dung which contains all of the nutrition available for larval development. Depending on the dung quantity and quality, male beetle larvae plastically develop into either small, hornless or large, horned morphs, which differ in gene expression profiles, responsiveness to hormone manipulations, and reproductive tactics. In one project, we report on the role of the neurohormone serotonin in mediating developmental transitions and morph specification in *O. taurus*. In a second project, we manipulate the degree to which larvae themselves can modify (i.e. niche construct) the brood ball environment and assess the effect of this manipulation on growth, rate of development, and larval mortality of *O. gazella*. Here we present our most recent results and discuss their implications within the context of developmental plasticity and evolution.

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**Title:** Using the Alphavirus 6K Protein to Understand the Role of Small Membrane Proteins in Enveloped Virus Assembly and Exit from Host Cells

**Abstract:** To maximize successful assembly and transmission, viruses co-opt resources from their host in clever ways. Enveloped viruses acquire their membrane from infected host cells during exit in a
process called budding. For alphaviruses, enveloped RNA viruses, budding is the final step to generate the new stable infectious virus particles. Alphavirus budding is known to occur independent of canonical host machinery and other factors that might facilitate alphavirus budding are unknown. Though the budding mechanism is not fully defined, all sequenced alphavirus species encode the small membrane protein isoforms 6K and TF that positively affect budding. I hypothesize 6K promotes the optimal environment for budding through interactions with host components. While not essential for virus production in a tissue culture system, recombinant viruses lacking 6K or TF negatively impact infectious and total virus output from infected host cells compared to wild-type. Furthermore, virus particles released from host cells when 6K or TF are absent are less stable in the extracellular environment and display morphological abnormalities upon purification. Additional assays indicate the major defect in the absence of 6K and TF occurs at budding. Despite their influence on the number, infectivity, and morphology of released virus particles, the 6K protein localizes mainly inside the host cell. Therefore, 6K interactions with host proteins and membrane components are being assayed inside the host cell where budding is initiated. Results from this study will help us understand how the 6K protein actively promotes assembly and exit from host cells at the molecular level.

Jamie L. Kostyun - Leonie Moyle lab
GCMS-TG former trainee: poster presenter
Title: Examining Evolutionary Developmental Genetics of Floral Diversity in Jaltomata (Solanaceae)
Abstract: The evolution of phenotypic diversity and lineage diversification, and their interaction, are key processes contributing to biodiversity. Although we have many data to inform these processes, we still lack a detailed understanding of their mechanistic basis especially among closely related species. My research focuses on floral diversity, by examining the genetic and developmental basis of observed floral trait differences among 12 closely related species within the genus Jaltomata (Solanaceae). Flowers of these species span multiple axes of variation, including petal and nectar color, overall size, and aspects of morphology – providing an excellent opportunity to dissect numerous traits contributing to floral disparity. Here, I show my work to develop genetic and genomic resources for this non-model system, and two approaches (QTL mapping and comparative transcriptomics) to identify gene regions controlling floral variation in this new and highly diverse system.

Matt Neubauer - Roger Innes lab
GCMS-TG current trainee: poster presenter
Title: Exploring the Role of a GNAT in EDR1 Stress Signaling in Arabidopsis
Abstract: Enhanced Disease Resistance 1 (EDR1) is a protein kinase involved in regulating cell signaling in response to biotic and abiotic stress. EDR1 regulates Keep on Going (KEG), an E3 ubiquitin ligase involved in abscisic acid signaling as well as endomembrane trafficking. Mutations in KEG suppress all edr1 mutant phenotypes, implying that KEG is a downstream component of EDR1 signaling. To identify other members involved in EDR1 and KEG signaling, multiple yeast two-hybrid screens were performed. Interestingly, an uncharacterized GCN5-related n-acetyltransferase (GNAT) was found to interact with both EDR1 and KEG. Through a number of molecular assays, I have verified that GNAT physically interacts with both EDR1 and KEG. I have also uncovered a number of interesting stress-related phenotypes displayed by gnat mutant plants. gnat mutants, like edr1 mutants, exhibit enhanced resistance to powdery mildew infection, as well as heightened levels of dark-induced senescence. Interestingly, unlike edr1 mutants, gnat mutants display insensitivity to abscisic acid treatment. My preliminary results suggest that EDR1 may directly phosphorylate GNAT. This suggests that GNAT may be a downstream elicitor of EDR1 signaling. Furthermore, early work investigating the functional interaction between GNAT and KEG suggests that GNAT may inhibit KEG ubiquitination activity. I propose that GNAT may function as a regulator of KEG, and, therefore, abscisic acid signaling. Future experiments will be focused on uncovering the role GNAT plays in regulating plant stress responses, and the nature of its interactions with EDR1 and KEG.
Luke Baker - Justin P. Kumar lab
GCMS-TG former trainee: poster presenter
Title: Eyeless Participates in the Establishment of Dorsal-Ventral Patterning Within the Developing Eye
Abstract: Eye development in all seeing animals is controlled by the Pax6 transcription factors. In Drosophila, there are two Pax6 genes known as twin of eyeless (toy) and eyeless (ey). These genes are part of an evolutionarily conserved retinal determination (RD) network. Ectopic expression of these genes is sufficient to induce ectopic eye development. ey mutants are characterized by a reduction in the size of the adult eye. It is currently thought that eye development in ey mutants is compensated for by the presence of toy. The ey² mutant carries a transposable element insertion into an eye-specific enhancer, reducing the amount of ey transcript in the eye. It has been assumed that retinal development in these mutants was largely normal except for the overall reduction in size. However, recent studies have shown this to be untrue. Analysis of developing retinal tissue and adult retinal tissue in the ey² mutant show that ventral tissue is preferentially lost. In some cases, ey² eyes are composed of exclusively dorsally fated tissue. This suggests that ey participates in either establishing or maintain dorsal-ventral compartments in the eye. Examination of other RD genes, including sine oculis (so) and eyes absent (eya) suggest that this phenotype may be exclusive to ey.

Jered Wendte - Craig Pikaard lab
GCMS-TG former trainee: poster presenter
Title: Arabidopsis RNA polymerase V transcript processing and function in RNA directed DNA methylation
Abstract: Plants possess a specialized multi-subunit RNA polymerase, called Pol V, which functions to transcribe long non-coding RNAs that mediate RNA directed DNA methylation (RdDM). Pol V is evolutionary derived from duplications of several of the genes that encode for the subunits of canonical Pol II, which is the essential enzyme found in all eukaryotes that functions to transcribe messenger RNAs (mRNAs). At the amino acid sequence level, Pol V has diverged from Pol II in many important functional domains, including the carboxy-terminal domain (CTD) of the largest subunit. The Pol II CTD is involved in many protein-protein interactions required for mRNA biogenesis, including the recruitment of proteins that process the 5’ and 3’ ends of nascent transcripts to produce the 5’ 7-methyl guanosine cap and 3’ poly-adenosine tail that characterize mature mRNAs and are required for their function. The implications of sequence divergence at the Pol V CTD for Pol V transcript end processing are yet to be discovered since full length Pol V transcripts and their end processing steps, if any, have not been described in detail. Here, I will discuss my efforts to identify full length Pol V transcripts and characterize their 5’ and 3’ ends and provide evidence that Pol V transcripts possess unique features that distinguish them from Pol II-derived mRNAs. I will also provide evidence that the divergent Pol V CTD has an analogous role to the Pol II CTD in recruiting proteins involved in transcript processing.

Alex Porambo – Scott Michaels lab
GCMS-TG current trainee: poster presenter
Title: Understanding Large Scale Chromatin Remodeling in Arabidopsis thaliana
Abstract: DNA molecule wraps ~149bp around the nucleosome, consisting of histone proteins, to form the core particle of the nucleus organizational tool, chromatin. In the cell there are multiple ways to modify and effect the packaging of chromatin by various demethylases/methyltransferases that alter heterochromatic environments. These changes can occur on a gene by gene bias, or they can be more large scale (i.e. condensation to form chromosomes). However, one such event in Arabidopsis thaliana found in ATXR5/6 mutants show a link between large scale chromatin rearrangements and response to DNA damage. The formation of these RACs (re-replication associated centers) in the mutant are highlighted by their localization of DNA repair mechanism specific proteins such as γ-H2Ax, RAD51, and pH1. Currently, specific promotors driving GFP constructs are being constructed to understand the relationship of gene and epigenetic regulation work towards this shift towards the observed phenotype. The screen formulated by the promoter driven GFP constructs will search for RAC regulators, it is only
by identifying the direct effectors of RAC formation that we will learn the genes responsible for such
dramatic shifts in chromatin dynamics. By marking genes specific for ATXR5/6 mutant regulation, but
apart from that of defined DNA damage response mechanisms with the GFP to show mutations that are
affecting the creation of RACs.

Michelle Marasco - Craig Pikaard lab
GCMS-TG former trainee: poster presenter
Title: Dramatic active site divergence in multisubunit RNA polymerases IV and V: does it make them
error-prone?
Abstract: RNA Polymerase IV and RNA Polymerase V are central components of the transcriptional
silencing pathway in plants known as RNA-directed DNA Methylation (RdDM). These polymerases
evolved as specialized forms of Pol II. More than half of the twelve subunits of Pols II, IV and V are
encoded by the same genes. However, Pol IV and Pol V have diverged from Pol II at many amino acid
positions that interact with the DNA template, incoming nucleotide triphosphate, or nascent RNA
transcript, including the bridge helix and trigger loop. Given this divergence of Pols IV and V in regions
thought to be critical to RNA polymerase function, we are investigating what effects these changes may
have on Pol IV and V activity. One aspect of Pol IV and Pol V activity that we are particularly interested in is fidelity. Using an in vitro transcription assay, I have demonstrated that, in regards to selectivity of
the nucleotide base, Pol IV is more error prone than Pol II, while Pol V appears to be more
accurate. We are in the process of trying to determine whether these changes in base accuracy are
due to altered selectivity for the correct nucleotide or differences in error correction through
backtracking. In regards to discrimination of the nucleotide sugar, we have found that both Pol IV and
Pol V have higher levels of misincorporation of deoxyribonucleotides into their transcripts compared to
Pol II. We are very interested in determining whether these differences in fidelity also occur in vivo.

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Title: Tandem master regulators synergistically control blue-green chromatic acclimation in marine
Synechococcus
Abstract: Cyanobacteria of the genus Synechococcus are ubiquitous in oceans and are responsible for
a significant portion of the primary productivity and oxygen production on Earth. Synechococcus
contain light harvesting structures called phycobilisomes, which increase surface area for photon
capture. Synechococcus undergo blue-green chromatic acclimation, adjusting the composition of their
phycobilisomes to further optimize photon capture in blue and green light.

Using a reverse genetic approach, I have identified two genes, fciA and fciB, that encode master
regulators of chromatic acclimation. These genes are completely conserved in all strains capable of
chromatic acclimation and are absent in most strains incapable of chromatic acclimation. The
interruption of either of these genes leads to a complete loss of chromatic acclimation, as judged by
changes in phycobilisome composition. RNA sequencing has uncovered that FciA and FciB control a
very small regulon, consisting of only two genes, which appear in the same genomic island as FciA and
FciB.

Interestingly, fciA and fciB mutants are locked in opposite phenotypic states, suggesting that FciA and
FciB have opposite effects on chromatic acclimation. In addition, overexpression of FciA or FciB is
sufficient to alter the organism’s ability to undergo chromatic acclimation. However, overexpression of
both FciA and FciB has no effect, suggesting that FciA and FciB work synergistically to control
chromatic acclimation.