II ANNUAL CELL & MOLECULAR BIOLOGY SYMPOSIUM

CMB seniors invite you to a two-day conference event featuring presentations of their undergraduate research projects. Research interests will cover a wide range of topics, and will be divided into 3 sessions.

Register by email to:
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When emailing to register, provide your name, year, and major, need for a parking pass, and lunch attendance

When:
Session 1
Fri. March 24, 4-7p
Sessions 2-3
Sat. March 25
8:30-11:30a & 12:30-3:30p
Lunch & conversation in between sessions

Sessions to be held in KHS 1101
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Anna E. Barry, Eric Moore, Ashley K. DeWitt
Mentor: Dawn M. Clifford Hart
Grand Valley State University, Department of Cell and Molecular Biology, Allendale, MI, USA.

*Schizosaccharomyces pombe* (*S. pombe*) is a family of fission yeast that divide by medial fission and are an appropriate model for the study of human cell division. In *S. pombe*, the scaffolding protein Mid1 is required for proper placement of the actomyosin ring, which determines where the cell will divide. Mid1 localization to the cell cortex is regulated through phosphorylation and the kinases involved have been identified. However, a phosphatase that reverses phosphorylation has not yet been reported. Previous experiments demonstrated that Mid1 is a substrate of the serine/threonine phosphatase Dis2. Here, we investigated the relationship between Dis2 and Mid1 in *vivo*. We generated a catalytically inactive version of Dis2, by introducing a mutation (H124A) at a conserved position in Dis2 to observe the consequences of Mid1 regulation. Comparison of wild type Dis2 to the mutated version shows altered Mid-GFP localization. When Dis2 is present, Mid1 localizes to the nucleus, cortical nodes and division site, as expected. The catalytically inactive version of Dis2 shows cytoplasmic dispersed Mid1-GFP. Our results suggest that Mid1 localization during interphase and mitosis is specifically regulated by Dis2 phosphatase activity. Further experiments on the localization of Dis2 will be helpful in cancer research to study mechanisms involved with how and where a cell divides. Mutating Dis2 to inhibit its catalytic activity will provide further insight to contractile ring and Mid1 regulation.

This research is funded by the National Science Foundation RUI Award# 1157997.
Dis2-PP1 Phosphatase Dephosphorylates Mid1 during Cytokinesis in *Schizosaccharomyces pombe*

Audrey Arbogast\(^1\), Anna Barry\(^1\), Eric Moore\(^1\)
Mentor: Dawn M. Clifford Hart\(^1\)
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*Schizosaccharomyces pombe* is an organism used to study defects in general cell division, which are often the underlying causes of many human diseases, including cancer. *S. pombe* expresses a scaffolding protein called Mid1 that is necessary for the establishment of the actomyosin ring, as it recruits proteins to form the contractile ring by providing spatial cues and acting like a “docking port”. During metaphase, Mid1 becomes hyperphosphorylated and recruits several key proteins for ring formation. In late anaphase, Mid1 disassociates from the established ring and becomes hypophosphorylated. Dis2 is a type 1 protein phosphatase, thus it catalytically removes phosphate groups from other proteins through a hydrolysis reaction. Previous work revealed that Dis2 undergoes an *in vivo* interaction with Mid1 during mitosis. The aims of this research include determining if Dis2 directly dephosphorylates Mid1 as well as characterizing the effect it has on Mid1 localization. Confocal microscopy was used to visualize the stages of the cell cycle in Dis2-deficient *S. pombe* to determine how Mid1 localization varies. The data shows that while Mid1 is normally restricted to the medial region of the cell, Mid1 is irregularly dispersed in phosphatase-dead Dis2 and *dis2Δ* cells. Thus, the phosphatase activity of Dis2 is necessary in accurately localizing Mid1, developing a proper contractile ring, and dividing the contents of the cell equally to ensure normal growth patterns. This discovery can be applied to human cancers derived from malfunctions in the localization of Anillin, the human analog of Mid1. There is also a substantial amount of evidence that defective PP1 phosphatases play a role in human diseases.

This research is funded by the National Science Foundation RUI Award #1157997.
Regulation of chromatin structure is essential for several cellular processes including DNA replication and repair in eukaryotes. Heterochromatin refers to parts of the genome that are highly compacted throughout all or most of the cell cycle and thus generally transcriptionally inactive. The Δdim-5 mutation in the model filamentous fungus *Neurospora crassa* disrupts heterochromatin formation and causes multiple phenotypes including hypersensitivity to the DNA damaging agent methyl methanesulfonate (MMS) and increased production of small RNAs from heterochromatic regions. To help characterize the mutant, the effect of the Δdim-5 mutation on small RNA production and nuclear division in *Neurospora crassa* were analyzed. Small RNAs can arise from the degradation of full length RNA Polymerase II transcripts or could be generated by the specialized RNA-dependent polymerase, QDE-1. In this study, the hypothesis that QDE-1 is responsible for small RNA production from heterochromatin regions in the Δdim-5 strain was analyzed as qde-1 knock-in constructs with Human influenza hemagglutinin (HA), FLAG, and green fluorescent protein (GFP) tags were created using overlapping PCR. qde-1 knock-in strains were crossed with a Δdim-5 mutant for future analysis using ChIP-seq. The hypothesis that MMS-hypersensitivity of the Δdim-5 strains reflects a defect during the S phase of DNA replication was also examined. Δdim-5 and wild type strains containing both mcm-2-rfp and h2a.z-gfp were compared using fluorescent microscopy. Δdim-5 mutants appeared to have higher frequencies of mcm-2-rfp nuclear foci, suggesting replication defects occur.
Nucleoside versus non-nucleoside reverse transcriptase inhibitor treatment for endometrial cancer

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Endometrial carcinoma is the most common gynecological malignancy in women in the US. With variable risk factors ranging from age to obesity, prevention methods are limited; however, treatment options are being explored. Telomerase, a ribonucleoprotein enzyme maintaining the telomeric repeats at the end of eukaryotic chromosomes, is reactivated in most malignancies, making it a novel treatment target. Previous studies with anti-telomerases in our laboratory showed a reduction in proliferation of breast cancer cells. Therefore, in this study, we compared the effects of a non-nucleoside reverse transcriptase inhibitor (BIBR1532) to that of a nucleoside reverse transcriptase inhibitor (Azidothymidine, AZT) on endometrial cancer cells (HEC 1A). Culture flasks were seeded with approximately 5x10⁵ HEC 1A cells and exposed to either BIBR1532, or AZT, or non-supplemented media (T-25, n=4) for 7, 14 and 21 days. Viability of cells was assessed by use of the Trypan Blue exclusion test. BIBR1532 showed a marked decrease on proliferation at day 7 (P <0.05) compared to AZT, but no difference was observed at days 14 and 21. Additionally, the number of dead cells did not change significantly between the two treatments during the period of observation. Studies to quantify steady-state levels of select genes are currently in progress, and will aid in determining the direct effects of BIBR1532 and AZT on HEC 1A cell proliferation.
RNAi and Transgenesis of *Drosophila melanogaster* Embryos: A Reverse Genetics Approach to Investigating the Function of Cyclin Y

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Cyclin Y is a member of the cyclin superfamily of proteins, which play key roles in transcription and cell cycle regulation. Cyclin Y is highly conserved across species, and is overexpressed in human colorectal cancer cells. However, it is also one of the least studied cyclins, and its function is not well-known. In this study, we investigate its function in the *Drosophila melanogaster* model. The cyclin Y gene was knocked out via RNAi using shRNA (short hairpin RNA) and dsRNA (double stranded RNA) to create two distinct phenotypes in the posterior wing. A cyclin Y transgene was then injected into the posterior of the resulting embryos, and in the case of successful transgenesis, integrated into the germ line. The F1 flies were then evaluated for transgenesis using the presence of the w⁺ marker, which results in orange eyes. The majority of injected embryos did not survive to adulthood, and the F1 resulting from the mating of the surviving flies did not express the w⁺ marker, which indicates that they were not transgenic. Even the embryos which were not injected had a poor survival rate, suggesting that the lethality was due to manipulation of the embryos rather than the transgene. Further investigation will be necessary to explain these results, and hopefully provide a better understanding of cyclin Y. The ultimate goal is that this will lead to the development of possible treatments for colorectal cancer and other cell cycle anomalies in humans.
Telomere as Biologic Currency: Why Those with Older Fathers May Have a Greater Inheritance

Jon Richards

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Heterochromatin is densely structured DNA localized in the centromeric and telomeric regions of chromosomes. Telomeric heterochromatin inheritance as well as telomere length (TL) are of interest due to their respective effects exhibited on nearby gene expression and putative impact on longevity. Telomeric heterochromatin-induced silencing of gene expression, known as Telomere Position Effect (TPE), and maintenance of telomere length have been studied in *Drosophila melanogaster*, though mechanisms of their heritability are not well understood. We sought to determine whether telomere maintenance, occurring via the male germline, influences TPE and offspring longevity. In the context of the Hayflick limit theory, we proposed that the offspring of older males would exhibit enhanced longevity due to the ongoing process of telomere maintenance over time. Potential paternal-age effects were studied by grouping cohorts of flies by paternal age and enrolling them in a longevity assay. Our data shows inheritance of long-lived phenotypes, due to TL, is likely related to paternal age but confounded by potential genetic variations within a population. Heritability of chromatin states at the telomere as revealed by TPE was investigated by utilizing a UAS/GAL4 ectopic expression system with a telomeric reporter. We found definite changes in TPE phenotype among flies whose father expresses the GAL4 in the male germline and carries a UAS insert within the TART and TAHS regions of a *Drosophila* telomere. Our work has established a system in which to study the factors affecting the heritability of telomere chromatin state as well as the role played by paternal age and telomere length in affecting offspring longevity.
Structure-function analysis of boronic acid transition state inhibitors of *Acinetobacter*-derived cephalosporinase (ADC-7)

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Resistance to β-lactam antibiotics in the nosocomial pathogen, *Acinetobacter baumannii*, is an enormous challenge in antimicrobial chemotherapy today. The expression of class C β-lactamases, such as *Acinetobacter*-derived cephalosporinases (ADCs), provides the pathogenic bacteria with a mechanism to inactivate β-lactams. Boronic acid transition state inhibitors (BATSIs) offer a potentially effective way to inhibit ADCs; however, it is essential to characterize the structure and function of these compounds within the active site of an ADC enzyme. Here, the X-ray crystal structures of four BATSIs in complex with ADC-7 were solved to explore key amino acid side chain interactions within the active site, and Kᵢ values were determined via steady state kinetics. These BATSIs bind with high affinity to ADC-7, as demonstrated by Kᵢ values in the low nM range. Each BATS contained the R1 moiety found in the β-lactam antibiotic cephalothin yet possessed a unique R2 group that varied in size and flexibility. The X-ray crystal structures of the BATS complexes (approximately 2.0 Å resolution) revealed several canonical binding modes as well as unique interactions with the side chain of R340, a residue that distinguishes ADC-7 from related class C enzymes. These ADC-7/BATSI complexes provide insight into the recognition of non-β-lactam inhibitors by ADC enzymes and provide the basis for optimization of a novel series of inhibitors against a clinically relevant resistance target.
Analysis of Hydrogen Bonding Networks in OXA-66 Wild-Type and P130Q

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For antibiotic resistant bacteria, the carbapenem class of β-lactam antibiotics are often the antibiotics of last resort. Thus emerging Class-D β-lactamases that can hydrolyze carbapenems are an ever increasing threat. A P130Q mutation in the β-lactamase, OXA-66, significantly increases activity against carbapenems. This research seeks to identify structural differences in the hydrogen bonding networks stabilizing the tertiary structures between the wild-type and P130Q enzymes with the hope of explaining the mechanism of enhanced carbapenemase activity. A 200 ns long molecular dynamics simulation trajectory of fully hydrated OXA-66 for both wild type and P130Q were analyzed in order to determine the most prevalent hydrogen bond tertiary contacts in both proteins. Comparison of both networks revealed that four pairs of bonds: D105-R109, T81-E178, D134-R138, and E100-R138 are affected by the mutation. Three of them are significantly weakened in the mutant. Three out of the four hydrogen bonded pairs are located in the area of the omega loop, which contributes to the enzyme’s binding pocket and helps to explain the improved binding of doripenem. Identifying shifts in hydrogen bonding interactions helps elucidate the mutation effects in this protein family and provides targets for innovations in the design of new antibiotics and inhibitors.
Molecular Dynamics Simulations Help Elucidate the Mechanism of Improved Doripenem Binding in Clinical Mutants of OXA-51 and OXA-66

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Antibiotic resistance mediated by $\beta$-lactamases poses a serious threat to public health. The class D (OXA) $\beta$-lactamases are rapidly evolving to provide resistance to modern antibiotics including carbapenems and 3rd generation cephalosporins. Several single amino acid mutations have emerged recently in the OXA-51/66 subfamily which have been shown to increase carbapenem binding. The mechanism of this gain of function is not clear. Here, we investigate the structure and dynamics of these mutant enzymes to better understand their interactions with the carbapenem, doripenem. In order to study the impact of mutations on protein dynamics, 250 ns Molecular Dynamics simulations were carried out on fully solvated models of OXA 51, OXA 66, and the I129L, P130Q, and L167V mutants. Trajectory analysis revealed the impact of these mutations on side chain rotations of several residues forming the binding pocket. I129 and W222 play particularly important roles in doripenem binding and the mutations impact their rotational freedom through direct and allosteric interactions. The rotational freedom of I129 is affected directly either through mutation to leucine or indirectly by mutation of nearby residues - an adjacent proline residue or leucine residue on the omega loop. The increased rotational freedom of W222 is connected to a breakdown of the hydrophobic bridge over the entrance to the binding pocket. While all mutations promoted carbapenem binding by increasing the rotational freedom of I129 and W222, P130Q had the greatest effect. These results help us to understand the evolutionary path of antibiotic resistance in OXA $\beta$-lactamases.

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Avian malaria (majority caused by *Plasmodium relictum*) is present globally and can have detrimental effects on avian health, mostly reproduction. The presence/absence of infection patterns vary depending on multiple factors: geographical location, genetic variation and ecological conditions such as: plant species available, predators and weather patterns, can influence the resistance of the host to the infection. This study focused on the effects of ecological conditions and geographical location to determine the prevalence of infection. The study was conducted observing prevalence of *Plasmodium re.* in the blood of 40 mating pairs of *Tachycineta bicolor* in Allendale, Michigan. PCR primers were designed to bind to conserved regions of *Plasmodium spp.* and ran on an agarose gel to screen the birds for infection. Amplified *Plasmodium spp.* DNA was extracted from agarose gel, cloned in a plasmid vector, and will be sequenced to determine the species. We found that 15/80 of birds were infected in Michigan, compared to the 0/8 in Tennessee and 43/89 in New York. This research will strengthen the relationship that different environmental factors such as temperature, ecological environment and geographical location influence the health of *Tachycineta bicolor*. Future studies will determine the importance of the relationship between the negative effects of chronic infection to physiological detriments, such as life span and reproduction. This would be done by infecting birds with avian malaria and comparing their health versus that of non-infected birds.
Phage and Susceptible Bacillus Host Isolated from Soil

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Colony Collapsing Disorder (CCD) is a disease prevalent in beehives which causes worker bees to evacuate their hives indefinitely. A definitive cause of CCD has not been found but contributory factors have been identified. *Paenibacillus larvae* is a honeybee pathogen tied to a disease affecting honeybee larvae and a contributor to CCD. Treating beehives for these infections has been a struggle to agriculturalists and scientists. To address this issue we developed a phage therapy model with the use of *G. mellonella* larvae, also pests to beehives. Phage therapy is a microbiological technique using bacteriophage to infect and kill bacteria. In this experiment, spore-forming bacillus and bacteriophage were isolated from the rhizosphere. Bacteriophage were isolated through incubation of the soil in growth media, centrifugation, and filtration of the supernatant. The filtrate was incubated with the bacterium isolated from the same soil sample. From this, a complete bacterial lawn was formed, accompanied by the formation of plaques—holes in the bacterial lawn—which indicated the lysis of the bacterium. A lethal dose 50% test was performed on *G. mellonella* in order to quantify the amount of bacteria necessary to kill half of the larvae. Upon completion of the LD-50, it was determined that the bacillus we had isolated was not lethal. Future testing would involve the use of a bacterium, lethal to *G. mellonella*, to carry out the phage therapy experiment.
Plant growth and development is controlled by the different wavelengths in the electromagnetic spectrum. Phytochrome B (phyB), a red and far-red light photoreceptor, plays an integral role in shade avoidance, flowering time, seed germination, and de-etiolation. Proper degradation of this photoreceptor is crucial to the function of these processes. Degradation of phyB occurs via the ubiquitin-proteasome system. The light regulating Bric-a-Brac/Tramtrack/Broad Complex (LRB) protein, in conjunction with CUL3, forms an E3 ligase complex which facilitates the attachment of ubiquitin to phyB. Preliminary research has revealed a highly conserved region of the LRB protein near its N-terminus which may function in controlling rubylation. In order to determine if the LRBs play a role in rubylation, a truncated version of the LRB gene missing this region was inserted into a wild type and a lrb1 lrb2 double mutant Arabidopsis thaliana. Phenotypic and immunoblot analysis of homozygous lines featuring the truncated LRBs suggest that the N-terminal region may play a role in their function. Future analysis of these lines will provide insight into the role of the N-terminal region of the LRB’s in phytochrome degradation and CUL3 rubylation.
Chronic inflammation in endothelial cells is a characteristic of atherosclerosis, a very serious disease affecting over 3 million people every year. Atherosclerosis is the hardening and narrowing of arteries, reducing blood flow to vital organs such as the heart. Fatty acids present in the bloodstream, usually at elevated amounts due to dietary factors, can lead to pro or anti-inflammatory responses depending on the binding receptor. In our study, expression of Connexin 43 (Cx43), a potential marker of arterial inflammation, was examined in bEnd.3 cells in the presences of 18-carbon fatty acids oleic acid and alpha-linolenic acid. Protein concentration was determined through a BCA protein assay and western blot analysis was used to confirm protein expression. Our research findings show that cis-unsaturated oleic acid decreased endothelial cell Connexin 43 expression, whereas n-3-cis polyunsaturated alpha-Linolenic acid increased it. Further studies with using linoleic acid (n-3 cis-polyunsaturated) and elaidic acid (trans-polysaturated) can be performed in order to help expand on our findings. Potential research can show that cis fatty acids have anti-inflammatory properties and could help prevent cardiovascular disease. Polyunsaturated fatty acids are shown to increase arterial inflammation and should removed or limited in one’s diet.
**Stick It to the Man: The Effect of Nrgp1 Repression on Two Unnamed Adhesion Genes**

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*Candida albicans* is an opportunistic pathogen and the causative agent of candidiasis. *C. albicans* exhibits high virulence when in its hyphal form, and the BRG1 gene is crucial to hyphal growth. Also crucial to virulence is colony adhesion; it is currently hypothesized that candidiasis begins through colonization of objects such as catheters and IV drip tubes. BRG1 is repressed by Nrgp1, a protein which prevents expression of hyphal growth by binding the operon of BRG1. However, the effects of this repressor protein on other genetic targets – particularly other virulence-related genes – is poorly understood. Evidence from previous studies suggests that two currently unidentified cell adhesion target genes, loci orf19.2302 and orf19.6705, may also be repressed by Nrgp1. Orf19.2302 codes for an ER-related ion transport protein, deletion of which causes total loss of adhesion. Orf19.6705 is a cell receptor coding gene which, uniquely, causes differential levels of cell adhesion dependent on the media the colony is grown in. To test this, colonies of knockout, heterozygous compliment, and wildtype *C. albicans* of each strain were grown in 96-well plates and assayed for thickness of biofilm. It was determined that adhesion was affected by gene knockout, and may be media-dependent. This indicates that cell adhesion may be affected in a similar manner to Nrgp1-mediated repression. There exists a possibility of clinical applications of NRG1 Nrgp1 as a method to control *C. albicans* infections by decreasing cell adhesion, however research is currently ongoing.
Cerebral Palsy (CP) is a term that describes a group of complex neurological disorders which causes non-progressive, life-long motor impairments. Besides motor dysfunctions, CP also causes severe comorbidities including visual, speech, cognitive, and learning disabilities. The standard clinical diagnostic of CP is primarily based on subjective observation of delayed motor development or muscle reflexes at age ≥ 2; this prevents opportunity to obtain therapies—physical, language, speech and occupational—at an early age. Thus, it is important to develop objective biomarkers to early detect CP. Our long-term research goal is to identify molecular biomarkers that can diagnose CP infants immediately after birth. Previously, we used microarray technology to examine gene expression of CP children and healthy control (HC) from neonatal blood spot samples (NBS). Here, we applied quantitative real-time PCR (qRT-PCR) to further evaluate gene expression of the two most differentially-expressed genes: S100 calcium binding protein A9 (S100A9) and ectonuclease triphosphate diphosphohydrolase-1 (ENTPD1). qRT-PCR results showed up-regulation of S100A9, but not ENTPD1, in all CP samples when compared to HC. Our preliminary results showed that S100A9 in NBS may serve as an early detection biomarker for CP to allow early intervention.
Investigation of \textit{TNFRSF1A} (tumor necrosis factor receptor super family member 1A) gene expression in children with typhoid fever

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Typhoid fever is an enteric bacterial infection caused by Salmonella enterica serovar Typhi (S. Typhi), a human-specific pathogen, a health problem worldwide, especially in developing nations. In Africa, typhoid fever can be a major cause of morbidity in infected children. The onset of this illness is insidious and clinical diagnosis is often unreliable due to a variety of unspecific symptoms. In poor-resourced clinical settings, diagnostic services are limited, often invasive, and expensive. Thus, developing a rapid, sensitive, and affordable diagnostic test is essential. The study and understanding of such mechanisms provides a comprehensive genomic component of immunological responses and allow new insights for possible diagnosis and treatment of typhoid fever. We will focus on evaluating the gene expression of TNFRSF1A (tumor necrosis factor receptor super family member 1A) in the blood of children with typhoid fever during phases of infection using quantitative real-time PCR. TNFRSF1A codes for tumor necrosis factor receptor 1 protein (TNFR1) which is an outer membrane protein that triggers an immune response, the caspase cascade. Studying the expression of TNFRSF1A in S. Typhi-infected patients will provide better understanding of host immune response that may lead to better diagnostics. Our current research shows no significant relation of TNFRSF1A to Typhoid Fever identification but our experimental method may be used in typecasting other genes as biomarkers.
A known indication of Parkinson’s disease is the abnormal accumulation of \( \alpha \)-synuclein protein aggregates in the dopaminergic neurons of the \textit{substantia nigra} and later degeneration of the affected neurons. Although \( \alpha \)-synuclein has been extensively studied, its normal function, and its role in toxicity are unclear. This experiment aims to determine if toxicity is associated with nuclear localization of \( \alpha \)-synuclein in dopaminergic neurons. We hypothesize that targeting of \( \alpha \)-synuclein to the nuclear compartment in dopaminergic neurons promotes cell death. Lenti-virus is used to deliver human \( \alpha \)-synuclein protein to overexpress it in human SHS5Y5 cells. Constructs of \( \alpha \)-synuclein tagged with a nuclear localization signal or nuclear export signal are used to confirm specificity of the observed effects. Cell death is quantified by cell counts and protein expression is quantified by western blotting and immunostaining. These results will help determine if \( \alpha \)-synuclein is a key contributor to neuron death, thus leading to development of therapeutic approaches regarding Parkinson’s disease, as well as other neurodegenerative diseases.