Biology (and Biology related collaborations) student abstracts 2012
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The Effects of Aquatic Acidification on *Desmognathus fuscus fuscus* Behavior and Vulnerability

Ashley Ann Alex and Andrew Gannon

Since the 1980's, scientists world-wide have become increasingly concerned by alarming declines in amphibian populations. Acid mine drainage and the subsequent acidification of streams and other aquatic environments are leading causes of anthropogenic habitat degradation. The Northern Dusky salamander (*Desmognathus fuscus fuscus*) is considered an indication of biodiversity and environmental health and thus, is widely recognized as an ecologically important species among eastern American ecosystems. We observed the behavioral response and physiological vulnerability of Northern Dusky salamanders to various aquatic acidities meant to represent degrees of acid mine drainage. Thirteen salamanders were exposed to four pH treatments: 6.9(control, equivalent to natural streams), 4.9, 3.9, and 2.9. For each treatment, the time each salamander spent voluntarily immersed in water, partially immersed and out of water was measured, as was the change in mass. Change of mass was used to determine desiccation. Preliminary results indicate that salamanders exposed to more acidic aquatic conditions spend less time immersed and suffer the greatest amount of desiccation. This study is important in better quantifying consequences of acid mine drainage on Northern Dusky populations and its potential effect on amphibian declines.

Characterization of the Effects of *sec72* Deletion on Endocytosis and Vacuolar Morphology in *Schizosaccharomyces pombe*

Justin Bailey and Melanie Styers

Microcephaly is a brain disorder in which defects in the migration of early cerebral neurons results in irregularities in cranial growth. This disorder affects the ability of immature brain tissue to grow at a normal rate, often leading to slower cognitive development and mental retardation. Previous studies have shown that mutations in the human *ArfGef2* gene, which encodes the protein BIG2, result in a disorder characterized by periventricular heterotopia with microcephaly. Mutations in BIG2 have shown to cause deficiencies in the function and morphology of recycling endosomes, but further study is needed to fully understand the connection between BIG2 and cerebral growth defects. The goal of this study was to characterize sec72p, a homolog of BIG2 in *Schizosaccharomyces pombe***,** as a model to better understand the function of this family of proteins. Fluorescence microscopy was used characterize endocytosis and morphology of the vacuole in the wildtype and Δ*sec72* strains. FM4-64 was used to visualize movement through the endocytic pathway and, ultimately, uptake into the vacuole. MDY-64 was used to analyze vacuolar size and morphology. Lysosensor© was used to determine whether the function of the vacuole was affected by the loss of *sec72.* Our results showed that Δ*sec72* yeast displayed no significant differences in these assays compared to wildtype cells. These observations suggest that *sec72* deletion alone does affect the endocytic pathway or vacuolar morphology. Future studies will characterize whether double deletion of the related homologs *sec72* and *sec71* will result in defects in these pathways.

Characterization of the Effects of *PDR5* Deletion on Pma1-RFP Localization in *Saccharomyces cerevisiae*

Agee Baldwin, III and Pamela Hanson

Hexadecylphosphocholine, or HePC, is a lipid analog that has shown promising results in combating breast cancer metastases and cutaneous lymphomas. HePC acts at cell membranes to interfere with turnover and formation of natural phospholipids and to disrupt membrane-signaling networks. Research suggests, lipid rafts and drug efflux pumps function as major regulators of HePC resistance. It is proposed this resistance is related to an alteration in lipid raft structure causing improper inhibition of phosphatidylcholine synthesis. Using *S. cerevisiae*, a widely accepted model for studying the behavior of cancer cells, this research investigates the mechanism that confers HePC resistance in yeast cells lacking pleiotropic drug response gene Pdr5, a member of the ATP-binding cassette family of membrane transporters. In the present paper we characterize the effects of HePC and of *PDR5* deletion on the localization of intrinsic lipid raft proteins in yeast cells. Analysis of captured fluorescent microscope images suggests altered lipid raft localization may not be a large contributing factor to HePC resistance.

Folate Levels Affect *S. cerevisiae* Growth Inhibition by the Anticancer Ruthenium Complex KP1019

Amy Banks and Pamela Hanson

For years, platinum complexes have been used successfully in cancer treatment, but they also cause harsh side effects. These side effects have led to a search for effective alternatives. One such alternative is the ruthenium complex indazolium *trans*-[tetrachlorobis(1*H*-indazole)ruthenate(III), also known as KP1019. Since KP1019's mechanism of action is poorly understood, we previously conducted a phenomic screen to identify *S. cerevisiae* genes that cause KP1019 resistance when deleted. Initial results of this screen suggested that deletion of *ABZ1, ABZ2, and ADE3* – three genes involved in folate metabolism – increases resistance to KP1019. Here we report findings regarding the impact of extracellular folate and reduced expression of the essential folate metabolism genes *FOL1* and *FOL3* on KP1019 tolerance. Specifically, we studied strains with reduced levels of *FOL1* and *FOL3* expression due to decreased abundance by mRNA perturbation (DAmP). Under all conditions studied, the *FOL3* DAmP strain was hypersensitive to KP1019, a phenotype that may result from reduced fitness caused by accumulation of dihydropteroate, a toxic metabolic intermediate of folate biosynthesis. In a liquid-based assay using media lacking folate, the *FOL1* DAmP strain was significantly more sensitive to KP1019 than the wild-type control. Interestingly, in spot assays, the *FOL1* DAmP strain was slightly resistant to KP1019, a phenotype that could be reversed by incubating plates under anaerobic conditions. Future studies will examine whether some of these complex interactions between folate metabolism, oxygen levels, and drug tolerance might result from KP1019 impacting folate metabolism, a phenomenon that has been reported for cisplatin.

Characterization of the Role of *lvs1* on Vacuolar Morphology and Function in *Schizosaccharomyces pombe*

Matthew Barnes and Melanie Styers

Chediak-Higashi syndrome (CHS) is a human disorder characterized by partial albinism, bleeding disorders, and persistent infections. CHS has been shown to be caused by alterations in the *LYST* gene in humans resulting in enlarged lysosomes, suggesting a defect in endo-lysosomal fusion. However, the molecular mechanisms underlying this defect remain uncharacterized. In *Schizosaccharomyces pombe*, sequence analysis has identified the *lvs1* gene as a homolog of the human *LYST* gene. The goal of this study was to determine whether the *S. pombe lvs1* gene is indeed a functional homolog of *LYST*. Staining of vacuoles with MDY-64 revealed that *lvs1*Δ cells contain enlarged vacuoles, while strains overexpressing *lvs1* had smaller vacuoles in comparison to the wild-type. LysoSensor™ Blue DND-167 was used to assess whether defects in vacuolar size were associated with changes in vacuolar acidification, revealing a decrease in vacuolar acidity in the *lvs1*Δ strain. Fluorescence microscopy revealed that endocytosed FM4-64 reached the vacuole at an earlier time point in the *lvs1Δ* strain compared to the wild-type strain and that FM4-64 staining was decreased at 45 min. This decrease in FM4-64 fluorescence was confirmed by flow cytometry. Together, these results suggest that *S. pombe lvs1* is a functional homolog of *LYST* and reveal novel changes in the endocytic pathway in *lvs1*Δ cells. Future studies will further characterize the endocytic defect in *lvs1Δ* cells and will utilize *S. pombe* as a model for investigating the detailed molecular mechanisms that underlie the characteristic cellular defects of CHS.

Late Rectal Toxicity Predictors in Patients Treated with Radiation Therapy for Prostate Cancer

Justin Bishop, Wayne Shew, Andrew McDonald, and John Fiveash

Purpose: To conduct a retrospective study to find possible predictors of late rectal toxicity in prostate cancer patients treated with radiation therapy.
Materials/Methods: 89 patients treated at UAB Radiation Oncology Department between 2005 and 2009 were sampled for this study. The dose-volume histograms (DVH) of patients exhibiting grade II or higher late rectal toxicity were compared to patients exhibiting no rectal toxicity. All patients were treated on a dose schedule of 70Gy over 28 fractions. Tomotherapy and Eclipse software were used for dose calculation and data collection from the plans of all patients. Unpaired t-tests were conducted to determine any significant difference in the data points taken from the DVHs.
Results: Of the patients sampled, the radiation plans of 7 patients with late rectal toxicity and the plans of 26 patients exhibiting no toxicity were available for collection at the time of this study. Both the planned percent rectal volume and absolute rectal volume receiving 70Gy (V_{70}) showed a significant difference between patients with toxicity and without toxicity ($p= 0.007$, 0.005 repectively). The planned maximum amount of dose to any part of the rectal was on average 2 Gy higher for patients with toxicity. This difference was not significant but was close ($p=.065$).
Conclusion: The planned V_{70} seemed to be a strong predictor of late rectal toxicity. While a more complete data set is needed, this study suggests a dose response for rectal toxicity and will aid treatment planners in determining radiation plan quality.

Infection Rates by *Batrachochytrium dendrobatidis* of Tadpoles at Ruffner Mountain, AL

Ginny Boehme and Andy Gannon

Batrachochytrium dendrobatidis (Bd) is a virulent fungal pathogen that attacks keratinized epidermal cells of amphibians. Bd causes chytridiomycosis, a disease that rarely exhibits visible deformities (such as lesions) in adults, but is known to cause visible defects in the keratinized mouthparts of amphibian larvae. Although Bd has been implicated in the decline of amphibian species worldwide, the mechanism behind its lethality is not yet known. Interspecies susceptibility is highly variable, with some species being extremely vulnerable to the disease and others being resistant. Resistant species are an ecological issue, as they can be carriers of the pathogen and can spread it to areas previously free of Bd. In 2009, the first known mortality event by Bd in Alabama was found at the Red Lakes in Ruffner Mountain Nature Preserve in Irondale. Since then, Bd has been found in the Ruffner Mountain Wetlands. An ongoing study at Birmingham-Southern College has examined the distribution of Bd in the Birmingham area and its infection of both adult and larval amphibians. This study has found positive results (using PCR analysis) of Bd infection in none of the adults tested, only in the larvae. As part of this ongoing study, we examined interspecies infection rates in tadpoles collected from the Ruffner Mountain Wetlands, and also examined the keratinized mouthparts for signs of chytridiomycosis. By doing this, we hope to identify possible carriers of the Bd pathogen.

Influence of Habitat Isolation on Moth (Geometridae: Lepidoptera) Populations in Central Alabama

Aisha Bonds and Peter Van Zandt

Due to the amount of environmental changes caused by humans, once-contiguous habitats can become fragmented, leading to isolated populations of species. In this study, two habitat sites were compared: the small, isolated forest patch of Birmingham-Southern College (BSC) located beside the Ecoscape and the large, contiguous Cahaba River National Wildlife Refuge (CRNWR) which is located in Bibb County. In order to analyze the effects of isolation on populations of insect species, moths from the family Geometridae were studied. Moths were collected from May to October, 2011 via black light traps. The Geometridae found in each site were sorted to species if possible, and from that the relative richness of Geometridae species in each habitat was determined. Furthermore, the numbers of individuals of Geometridae that occurred in both habitat sites were counted as an index of relative population size. Because the BSC campus is an isolated habitat, I expected to find A) more species of Geometridae in the CRNWR than the BSC forest and B) greater relative population sizes of the Geometridae species in the CRNWR than in the BSC forest. While final data results are pending, at this time there have been 24 species of Geometridae identified from the BSC site, and there have been 37 species of Geometridae identified from the CRNWR site. Of these species, 14 occurred in both habitat sites.

Analyzing Effects of Trapping and Removal on the Population Dynamics of an Invasive Crayfish, *Orconectes virilis*

Courtney Campbell and Megan Gibbons

The removal of a dam in 2008 in Roebuck Springs, Alabama, caused the water level in a spring pool to lower rapidly and resulted in the death of thousands of individuals of the endangered watercress darter, Etheostoma nuchale. Further threats to the darter may exist from predation by *Orconectes virilis*, an invasive crayfish. In addition, *O. virilis* may also be outcompeting at least one native crayfish species, *Cambarus striatus*. Intensive trapping of the invasive crayfish species *O.virilis* in Roebuck Springs, Alabama has taken place since 2008 after the dam removal. The purpose of this study was to research the impact that our intensive trapping has had on the *Orconectes virilis* population size (using catch rates), sex ratio, and body size. For each *O. virilis* crayfish removed, we recorded the location from which it was removed, its sex, total body length measurement, carapace length, and carapace width. Individuals of *C. striatus* were measured (in the same fashion as *O. virilis*), tagged using a standard Alpha IV single-shot coded wire tag injector and returned to the spring. We compared sex ratios and catch rates between years since 2008, and investigated the change in body size of captured individuals since trapping began. Understanding the effects of trapping and removal of *Orconectes virilis* on their population dynamics may help with conservation efforts to restore the *Etheostoma nuchale* and *Cambarus striatus* populations in Roebuck, Springs, Alabama.

Carnivore Influence in Fossil Accumulation at Sterkfontein, South Africa

Antonio Slavco Castanon III and Jason Heaton

Sterkfontein, a fossil hominid site in South Africa, has one of the largest collections of fossil humans anywhere in the world. Our research on the fossil collection attempted to determine if carnivores played a role in the accumulation of the fossil primates, specifically the Cercopithecoids, or Old World monkeys. To assess the possible roles of carnivores, skeletal part counts were obtained and subsequently organized into side and element (cranial, lower/upper jaw, postcranial) for over 3,000 Cercopithecoid elements in the Sterkfontein collection. From this, the percent of minimal animal units (%MAU) were calculated and compared to other archaeological assemblages of known carnivore origin (i.e. John Nash Nature Reserve, Mapungubwe National Park and an experimental collection). Our research found that the collection is dominated by crania and mandibles with other bones such as femora, humeri, and ulnae contributing to the collection at a lesser extent. The greater occurrence percent of crania and mandible fossils in the collection supports previous research revealing that the collection is biased by analyst selectivity. Due to the strong artificial collection bias, we are unable to securely determine if carnivores played a role in the accumulation of the Sterkfontein Cercopithecoids.

Regulating Fate Commitment of Mesenchymal Cells by Runx2 Gene Reconstitution

Chris Clarke, Megan Gibbons, and Amjad Javed

Runt-related transcription factor 2 (Runx2) is essential for skeletal development. Runx2 plays a critical role in the differentiation of mesenchymal stem cells (MSC) to osteoblast, chondrocyte and odontoblast lineages. Previous results from our lab have shown that Runx2 deficient mice lack bone formation, however cells isolated from the calvaria of these mice show a preference to differentiate towards adipocytes. We hypothesize that the Runx2 gene is a molecular switch for the control of MSC lineage differentiation. To understand if Runx2 is responsible for this altered lineage commitment, we used two model system. Mesenchymal progenitors C3H10T1/2 cells express extremely low levels of endogenous Runx2 gene. These cells were used to over-express Runx2 during adipogenic differentiation. The second reconstitution model was Runx2 null cell. Both cell types were infected with Runx2 expressing lenti-virus. Parallel cultures of these cells were infected with GFP expressing lenti-virus as control. Levels of GFP expressing cells were used to compare infection/reconstitution efficiency. Our results revealed ~ 40% infection efficiency in Runx2 null and ~80% in C3H10T1/2 cells. The number of adipocytes in C3H10T1/2 cells gradually increases from 3, 5, and 7-days only in GFP infected or untreated control cells. In sharp contrast Runx2 over-expressing cells show dramatically reduced adipogenesis and very limited change from day-3 to days-7. Furthermore, adipocyte in control and GFP expressing cells exhibited larger uni-locular fat vacuoles, while Runx2 expressing cells showed small multi-locular fat vacuoles. Reversal in adipogenic activity of reconstituted cells was monitored by RT-PCR analysis of gene expression. Similar pattern of inhibited adipogenesis was noted in reconstituted Runx2 null cells. Together, these results suggest that transcriptional control of adipogenesis by Runx2 normally entails a negative feedback.

Aggressive Behavior of Ovigerous and Non-ovigerous Female Northern Crayfish, *Orconectes virilis,* and the Effect of Trapping and Removal on their Overall Population Characteristics

Caroline Crawford and Megan Gibbons

Invasive species can create problematic consequences in their invaded habitat, causing a threat to biodiversity and economic debts. In aquatic ecosystems, invasive crayfish have been shown to reduce the biomass of macroinvertebrates, hydrophytes, periphyton, and microalgae. A large population of the invasive northern crayfish species, *Orconectes virilis,* poses a threat to the native hay crayfish, *Cambarus striatus,* and the endangered watercress darter, *Etheostoma nuchale,* in Roebuck Springs of Birmingham, Alabama, creating a need for conservation efforts. Continuing the trapping and removal of *O. virilis* aids in these conservation efforts by decreasing the size of the invasive population. The success of the trapping and removal of *O. virilis* was determined by examining the catch rate and total body length over the trapping period. The results of this study followed the results of the previous research, showing significant decreases in catch rate and body size of *O. virilis* since October 2008. This paper also studied aggressive behavior between ovigerous and non-ovigerous *O. virilis* females, and found a trend indicating that ovigerous females demonstrate higher intensity and more aggressive behavior than non-ovigerous females.

Secretion of the Eng1p Glucanase Is Impaired in *gea1+/- Schizosaccharomyces pombe*

Alan Eckler and Melanie Styers

Guanine nucleotide exchange factors (GEFs) are proteins that drive the formation of vesicles used for transport of proteins and lipids between organelles. Viruses have recently been shown to manipulate this essential process for their own replication. Viruses such as poliovirus, ebolavirus, and severe acute respiratory syndrome (SARS) coronavirus require the activity of a specific GEF, called Golgi-specific Brefeldin-A resistance guanine nucleotide exchange factor 1 (GBF1), for viral replication. GBF1 regulates membrane trafficking in the early secretory pathway between the endoplasmic reticulum (ER) and the Golgi apparatus. The gea1p protein has been identified as the fission yeast ortholog of GBF1. Analysis of this yeast protein can, therefore, provide insight into the role of GBF1 in human cells. Characterization of the *gea1+/-* mutant strain, which lacks one copy of the *gea1* gene, revealed that these cells exhibit defects in septum degradation, a process required for the cytokinesis phase of the cell cycle. To determine the underlying cause of this phenotype, we conducted a 1,3-β-glucanase activity assay, which measured the internal and secreted amounts of Eng1p, an enzyme responsible for degrading the septum. We also attempted to endogenously tag the *eng1* gene with green fluorescent protein (GFP) to localize this protein within the cells. Our results showed that Eng1 was not secreted at normal levels in the mutant cells compared to the wild-type cells, suggesting that defects in septation in *gea1*+/- cells are due to altered secretion of the enzymes that degrade the septum.

The Role of *REV1* in Cell Tolerance of KP1019 in Yeast and Human Cells

Amanda Farrell and Pamela Hanson

The anticancer drug KP1019 is in early clinical trials and seems especially promising due to its effectiveness in inducing apoptosis in cancer cells while showing relatively low toxicity to patients. Because it is fairly new, relatively little is known about its mechanism of action. However, studies suggest that DNA damage is a major contributor to the induction of apoptosis by KP1019. To understand the mechanisms at work, we previously carried out a genome-wide screen in *S. cerevisiae*, which showed certain DNA repair mutants to be especially hypersensitive to the drug. For example, KP1019 sensitivity was observed for yeast lacking *REV1,* a gene encoding a translesion synthesis polymerase. To determine the role this gene plays in cell survival following KP1019 treatment, we exposed a wild type strain of *S. cerevisiae* and a Δ*rev1* mutant to various concentrations of the drug and found significantly lower survival in cells lacking *REV1*. We are currently determining the role of the analogous gene in human cells by using siRNA to knock down *REV1* in HeLa cervical cancer cells and exposing both the transfected and control HeLa cells to the KP1019. We will compare induction of apoptosis in these samples by measuring caspase activity. These results will help us better understand the mechanism KP1019 uses to induce cell death and will also help us determine whether *S. cerevisiae* is a good model for predicting KP1019 resistance and sensitivity in mammalian cancer cells.

The Effect of *Gea1* Haploinsufficiency on Sac1p Function in *Schizosaccharomyces pombe*

Veronica Ferris and Melanie Styers

Single-stranded RNA viruses can cause severe symptoms, including poor liver function and respiratory and intestinal infections. GBF1, golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1, is a guanine nucleotide exchange factor (GEF) that is required for plus-strand RNA virus replication. GBF1 activates ADP Ribosylation Factor 1 (ARF1), which, in turn, recruits coatomer I (COPI), a coat protein that functions in transport of vesicles between the endoplasmic reticulum (ER) and the Golgi. The goal of this study was to use *Schizosaccharomyces pombe* as a model system to characterize *gea1*, a homolog of human GBF1. We have previously shown that *gea1* haploinsufficiency results in defects in septation. Similar defects have been observed in *sac1Δ* budding yeast. Sac1p is a lipid phosphatase that localizes predominately to the ER. However, sac1p is constantly recycled from the Golgi back to the ER by COPI, suggesting that gea1p may be involved in its localization. We found that deletion of one copy of *gea1* resulted in changes in the localization of sac1p, specifically, accumulation in the Golgi. The function of sac1p was also measured by quantifying the amount of PtdIns(4)P present in the cells. We expected the mutant *gea1* cells to contain less PtdIns(4)P phosphate due to impaired *sac1* function; however, no significant difference was found between the wild-type and mutant cells. These results suggest that *gea1* haploinsufficiency leads to accumulation of sac1p in the Golgi, likely due to impaired COPI-dependent recycling back to the ER, but no global changes in PtdIns(4)P levels.

Potential Correlations between 2^{nd}:4^{th} Digit Ratios and Autism

Rana Festok, Jason L. Heaton

Recently, high levels of prenatal testosterone have been shown to be linked with autism, as it stops growth in areas of the brain related to communication. It's been proposed that the ratio of the 2^{nd} to 4^{th} digits is influenced by exposure to testosterone in utero so these ratios may be used as an essential measure. The purpose of this study is to elaborate on previous studies and provide more data in order to establish solid results. Data was collected for male and female children, autistic or not, ages 4 and up. A questionnaire was given to the participants or guardians to fill out. Then photocopies of the participants' right and left hands were made using a scanner. A ruler was used to measure the 2^{nd} and 4^{th} digits. The total number of participants was 31, 17 females and 14 males, with 1 female and 1 male diagnosed with Asperger's syndrome The results show that the average ratios for the left and right hand were 0.978 and 0.98, respectively. The average ratios for males and females for the left hand were, 0.965 and 0.931, correspondingly, and for the right hand they were, 0.898 and 0.992, respectively. The ratios of the left and right hands do not differ greatly, however, the average ratios for male and female varied for both hands, with the Asperger's male having a lower ratio for the left hand and higher ratio for the right, while the Asperger's female had a higher ratio for both hands.

Interaction of the MRL Proteins MIG-10, RIAM, and Lamellipodin with Acetylcholinesterase from *Ciona instestinalis*

Caitlin Glover and Leo Pezzementi

Acetylcholinesterase (AChE) is a key enzyme in the termination of neuronal signaling via the hydrolysis of acetylcholine. AChE has three homomeric molecular subunit forms that result from alternative splicing: (G_1), dimers (G_2), and tetramers (G_4). The tetramers are also capable associating with the proline rich attachment domains (PRADs) of the proline-rich membrane anchor (PRiMA) on cellular membraned or triple helical collagen Q (ColQ), which organizes groups of three tetramers in the extracellular matrix, via their WAT (tryptophan amphiphilic tetramerization) domains at their C-termini. A PRAD from the cytoskeletal protein lamellipodin (LpD), a cytoplasmic cytoskeletal protein and member of the MRL (MIG10, LpD, RIAM) protein family, has recently been shown to be associated with soluble tetrameric plasma butyrylcholinesterase (BuChE), a enzyme similar to AChE. The AChE of the invertebrate *Ciona intestinalis* has recently been found to be capable of assembling into tetramers and associating with ColQ and PRiMA. We are using *in vitro* expression in conjunction with velocity sedimentation on sucrose gradients to characterize the binding capabilities of the MRL family of proteins to *C. intestinalis* AChE in order to investigate whether PRADs are a key requirement for tetramerization and whether the PRADs in these proteins may play a role in organizing tetramers of AChE and BChE.

Characterization of BIG2 Function in Vesicular Transport Using the Fission Yeast *Schizosaccharomyces pombe*

Bryan Benjamin Grissett and Melanie Styers

The BIG2 protein is a large Arf-GEF that is thought to have a role in maintenance of endosomal morphology. Interestingly, mutations in the gene that encodes BIG2, *ARFGEF2,* have been linked to a developmental disorder characterized by microcephaly, a condition of reduced cranial size, and periventricular heterotopia, a fetal neurological migration disorder. This study aims to characterize the role of sec71p, a homolog of BIG2, in the fission yeast *Schizosaccharomyces pombe* by analyzing the effects of *sec71* deletion on endocytic membrane traffic. Based on the localization of human BIG2 and preliminary data, it was hypothesized that *sec71* deletion would result in impaired endocytic function. Filipin staining was used to observe endogenous 3-β hydroxysterol localization and ascertain whether endocytosis was impaired. Endocytosis of FM4-64 dye at various timepoints was also monitored in *sec71* null (Δ*sec71)* cells to determine whether an endosomal block was present in the endocytic pathway. Based on our preliminary results and previous studies of the human protein, it was predicted that endocytosis would be delayed in the recycling endosomes of Δ*sec71* cells, resulting in lateral sterol accumulation along the Δ*sec71* cell surface and accumulation of FM4-64 in the recycling endosomes. The results of the study were contradictory as Δ*sec71* cells exhibited no block in FM4-64 endocytosis, but increased lateral filipin staining was present. This research, paired with subsequent study, may provide insight into Arf-GEF function at the cellular level and contributes to our understanding of the role of BIG2 in human brain development.

Characterization of a Native American Site at Turkey Creek Preserve, Jefferson County, Alabama.

David Hall and Jeannette Runquist

Numerous surveys and excavations have characterized the Turkey Creek Nature Preserve as a site of significant historical and cultural interest. However, little research has been conducted with a direct focus on Native American habitation. Artifact collections of local residents contain projectile points, flint tools and chips of Native American origin. A rock shelter located in the preserve could have served as a seasonal occupation site and the decision was made to test the site for evidence of Native American utilization. The site was parameterized using surveying equipment and three test pits were placed in the interior and exterior of the shelter. The exterior deposit includes a collection of approximately 45 flint fragments and pieces of modified stone. The interior pits indicate a similar collection with approximately 40 stone and flint artifacts, but also includes a grit-tempered plainware potsherd (possibly dating back to the Woodland period). Additionally, substantial deposits of approximately 80 snail shells of a single species were found in a soil layer along with remains of burnt wood and charred rock. Although species identification is pending, the presence of these snails could provide insight into the lifestyle patterns of migratory Native Americans. The presence of charring within the shelter, the artifacts recovered, and the availability of flint deposits in the preserve suggest that the site was used as a temporary campsite and tool manufacturing location. This study could provide grounds for continued cultural resource management and protection of archaeological sites within the Turkey Creek Preserve.

Role of the Pentose Phosphate Pathway in *Saccharomyces cerevisiae* Resistance to KP1019

Ashley Hudson and Pamela Hanson

Chemotherapy is a widely used form of cancer treatment, but it is common for cancer cells to develop resistance to these drugs, thus decreasing their effectiveness. Mechanisms of drug resistance can vary greatly and are poorly understood. Resistance to the ruthenium complex KP1019, which is used to treat colorectal tumor cells and damages DNA through oxidative stress, has been seen in the genetic model organism *Saccharomyces cerevisiae*. Previous research has shown that the pentose phosphate pathway (PPP) is upregulated in response to oxidative stress and DNA damage, suggesting that the PPP could be involved in resistance to KP1019. Deletion of the phosphate-encoding gene *PHO13* has previously been shown to upregulate the PPP in yeast. Here we show that deletion of *PHO13*causes increased resistance to KP1019. To further examine the role of the PPP in KP1019 tolerance, we overexpressed *ZWF1*, which is involved in the NADPH production step of the oxidative branch of the PPP. The effect of *ZWF1* overexpression on KP1019 resistance was measured by percent survival and analysis of cell growth through spot assays. Our hypothesized increase in resistance to KP1019 was not observed, suggesting that other portions of the PPP may be more important for yeast tolerance of KP1019. A better understanding of the pathway to resistance may suggest a way to combat this problem, leading to better and more effective long-term treatment for cancer.

The Yeast HOG Pathway May Be Involved in Cellular Response to KP1019

Will Jackson and Pamela Hanson

KP1019 is a ruthenium-based chemotherapy that has entered phase II clinical trials for the treatment of solid-sate tumors; however the mechanism of cellular response has not been fully elucidated. A potential mode of induction involves the mammalian p38 MAP kinase pathway. The p38 pathway is important for cellular response to osmotic stress, temperature stress, and hypoxia as well as regulation of the G2/M checkpoint of the cell cycle via modulation of the tumor suppressors *p53* and *p73*. The homologous *Saccharomyces cerevisiae* High Osmolarity Glycerol (HOG) pathway is structurally less complex with highly conserved function and provides a model for studying the importance of p38 in the induction of cellular response to KP1019. Growth assays with strains lacking HOG pathway components *HOG1*, *PBS2*, *SSK1*, and *STE11*showed that each of these genes are necessary for normal cell growth on KP1019-containing media. Surprisingly, KP1019-induced localization of Hog1-GFP to the nucleus was not observed as would be anticipated if the pathway was activated. These conflicting results demonstrate the need for further research on the role of p38 and orthologous pathways in cellular response to KP1019.

Estimating Wildlife Use of Riparian and Interior Habitats in Urban Nature Preserves Using Wildlife Camera Traps

Claire Johnston and R. Scot Duncan

Urban nature preserves can be crucial habitats to wildlife in a highly developed landscape. However, little is known about movement patterns and habitat use within urban preserves. In this study we examined whether wildlife preferentially used riparian or forest interior habitats within 10 preserves in the Birmingham metropolitan area. Baited wildlife camera traps were deployed in each treatment along routes of movement, such as game trails, for two weeks at a time. We detected 12 species and a minimum of 74 individual animals. The three most common species captured were *Procyon lotor* (Common Raccoon)*, Odocoileus virginianus* (White-tailed deer)*,* and *Sciurus carolinensis* (Eastern gray squirrel). Measured by species richness or capture frequency, wildlife used riparian and interior habitats equally. One interpretation is that both habitat types may be important for urban wildlife. It is also possible that our use of baited traps (vs. unbaited) influenced wildlife movement patterns. More research with additional techniques is needed to examine habitat use by wildlife in urban preserves.

Pollen Presence on Moth Proboscides and Other Body Parts as Evidence of Nocturnal Moth Visitation to the Flowering Plants of the Ketona Dolomite Glades of Bibb County, AL

Kathryn Ann LeCroy, Wayne Shew, and Peter Van Zandt

There is a significant lack of knowledge concerning nocturnal moth pollination ecology, especially in studies answering community level questions about pollination or visitation of nocturnal moths to plants. Furthermore, the gap between ecological modeling and pollination ecology leaves us with many unanswered questions about pollination and visitation networks between animals and plants. We wanted to know if evidence of visitation by nocturnal moths to flowering plants could reveal any potential network structure of nocturnal moth-to-flower visitation at the Ketona Dolomite glades of Bibb County, Alabama. These glades provide a habitat for many rare as well as endemic plant species. It has the highest biodiversity in the state of Alabama, but no studies on pollination biology at the glades have been conducted. During May 2011 to October 2011, students from the BSC Biology Department conducted a nocturnal moth survey of The Nature Conservancy's Bibb County Kathy Stiles Freeland Preserve. Moths were trapped, killed, mounted, and identified for collection. Approximately 270 moth specimens of four understudied moth families (Erebidae, Geometridae, Noctuidae, and Pyralidae) were examined for pollen grains on moth body parts by removing grains and using light microscopy to record grain size, quantity, and morphology. Grains from moth specimens were then crosschecked with a pollen reference collection constructed from the flora of the glades and matched to the lowest plant taxon possible. Our analysis may reveal undiscovered associations between the nocturnal moth fauna and flowering vascular flora of the Bibb County Glades.

The Relationship Between Weaponry Signaling, Chela Force, and Dominance in Males of the Invasive Northern Crayfish *Orconectes virilis*

Lauren Metzger and Megan Gibbons

When it comes to competition, many species resolve their disputes before they escalate into physical bouts. Physical encounters are costly, thus natural selection should favor the ability for competitors to asses each other's resource holding potential (RHP) before engaging in a fight. Assessment typically involves displays of weaponry or other traits that express RHP, which then allows the competitors to determine if one of them is more likely to win a fight; if competitors appear equally matched, a physical encounter is more likely. It has been predicted that weapon signaling should be a reliable indicator of an organism's RHP, either because the weapons are "indices," or because they represent a "handicap." There is evidence that dishonest signaling in weaponry display is more common than once believed, especially in crustaceans, possibly due to the exoskeleton surrounding the muscle, disabling direct assessment of muscle size. A dishonest display of a weapon that suggests a high RHP may allow an individual with a low RHP to win agonistic interactions, as long as they do not escalate into fights. We will use behavioral, physiological, and morphological measurements to determine if males of an invasive species of crayfish (*Orconectes virilis*) use dishonest signaling in agonistic encounters. We predict that males with larger chela size will win interactions that do not escalate beyond displays, but that males with greater chela force will win fights.

Second to Fourth (2D:4D) Ratios and Female Athletic Ability

Eric Nordgren and Jason Heaton

The 2D (2^{nd} digit, index finger): 4D (4^{th} digit, ring finger) ratio has been shown to be a sexual dimorphic trait influenced by prenatal levels of testosterone and estrogen. Research has shown that males have lower ratios than females resulting from higher developmental levels of testosterone. Additionally, the 2D:4D ratio has been shown to be correlated to conditions and/or behaviors, such as autism, breast cancer, diabetes, and athleticism, just to name a few. In previous research, it has been shown that male athletes exhibit lower ratios than non-athletes (i.e. larger 4D-to-2D). Correspondingly, there is a potential link between athletic ability and digit ratios of females. In our study, the 2D:4D ratio of females, athletes and non-athletes, was examined further. Photocopies were made of each of the participants left and right hands, and the digit lengths were measured using Vernier calipers (to nearest 0.01 mm). In our sample (athletes and non-athletes), there is no significant difference in the ratio of an individual's left and right hands. It was also found that with a mean ratio of 0.966, athletes show a significantly ($p < 0.001$) lower ratio than non-athletes (mean = 0.990). Furthermore, the female athletes exhibited a slightly higher mean ratio (0.966) than the male athletes found in the literature (0.95). As a result, we conclude that the 2D:4D ratio is also correlated to athleticism in females, but ratios are not as extreme, as males.

Identification and Characterization of Novel Components of Unfolded Protein Response(UPR) in Human Glioma Cancer Using Comparative Genomics

Faris Pacha, Jason Heaton, and Shahid Mukhtar

The goal of this project is to identify the unfolded protein response (UPR) genes in cancer cells through the use of comparative genomics. We used recently published data by Jonikas *et al.*, 2009 that displayed genes whose deletions causes both up and down regulation of UPR in *S. cerevisiae*. Bioinformatic techniques such as Database for Annotation, Visualization, and Integrated Discovery (DAVID) were used to classify a large gene list into functionally related gene groups. Next we intended to search for an overrepresentation of *cis*-regulatory elements in their promoters. We obtained 1000 bp upstream regulatory regions of these genes and subjected to a set of databases such as Multiple Em for Motif Elicitation (MEME and POBO). This bioinformatic strategy allowed us to identify statistically significant motifs. We performed experiments to identify transcription factor(s) that can regulate these large sets of UPR genes. We selected twenty candidate genes for further study of their expression patterns in six different glioma cancer cell lines under normal (21% O_2), hypoxic (5% O_2) and UPR (21% O_2 treated with 2mM DTT) conditions. We found differential transcription of these genes in the cancer cell lines. Future experiments will examine the functions of these genes in glioma cancers using cell biology. The significance of this research will allow for further innovation in cancer treatment and the eventual help of identification of harmful targets.

Habitat Use of Invasive Northern Crayfish (*Orconectes virilis*): A Threat to the Endangered Watercress Darter (*Etheostoma nuchale*)

Josh Rabbit and Scot Duncan

The watercress darter (*Etheostoma nuchale*) is a species that is endangered by urbanization and introduction of an invasive crayfish. *E. nuchale* are found exclusively in five springs in Jefferson County, Alabama. In September 2008 a dam at Roebuck Spring was removed, killing an estimated 11,760 darters. Although *E. nuchale* are found in five springs, the Roebuck Spring population is genetically distinct from all others. After the dam removal, biologists observed surviving darters being eaten by the invasive northern crayfish (*Orconectes virilis*). While previous studies had focused on the spring's pool, after dam removal, a series of studies on the spring run was initiated to assess the size and condition of darter population. One of these studies described the habitat of the spring run and habitat use by *E. nuchale*. The present study examined habitat use patterns of *O. virilis* through trapping in the five most abundant habitats found in the spring run: *Alternanthera*, *Ceratophyllum*, *Nasturtium*, silt, and *Sparganium*. *O. virilis* were found in moderate densities in all five habitats and the average number trapped was 2.8 (±2.5 SD) per trap per 24 hour period; however, *O. virilis* capture frequencies were similar among all habitats. A negative correlation was found between habitat biomass and capture frequencies. Using data from a parallel study, we found no correlation between *O. virilis* and *E. nuchale* for capture frequencies when matched by habitat type. Due to the limited time frame of this study, further investigation is needed to improve our knowledge of habitat use patterns of *O. virilis*.

Nonsense Mutation Suppression Potential of RTC#13 and RTC#14 in a Hurler Mouse Model

Patrick Rowan, Pamela Hanson, David Bedwell, and Kim Keeling

Hurler syndrome is a lysosomal storage disease characterized by the accumulation of the glycosaminoglycans (GAG) dermatan sulfate and heparan sulfate. The condition is the result of nonsense mutations in the *IDUA* gene that normally encodes for α-L-iduronidase, an enzyme vital for GAG degradation. Suppression of these nonsense mutations by aminoglycosides has previously shown to reduce GAG accumulation, yet the strong cytotoxic effects of the drugs limit them as a viable treatment option. In the present study, we measure the nonsense mutation read-through ability of two novel drugs RTC#13 and RTC#14, using mouse *IDUA-392W* and *IDUA-W392X* plasmid constructs. In addition, cell viability tests are conducted to assess the cytotoxicity of these two compounds.

Role of Poly(L-Proline) and MRL Proteins in hBChE Assembly and Enzymatic Activity

Megan Sandlin and Leo Pezzementi

Butyrylcholinesterase (BChE) is a serine hydrolase found in most vertebrate tissues. It has been suggested that proline-rich attachment domains (PRADs) are essential to the assembly of ChEs into tetramers by binding with the C-terminus tryptophan (W) amphiphilic tetramerization (WAT) domain of ChEs. Thus, we would like to determine the WAT-PRAD binding interactions involved with BChE that cause recruitment of BChE subunits into tetramers. PRiMA (proline-rich membrane anchor), a protein found in the brain, and ColQ, a specific collagen found in neuromuscular junctions, both contain PRADs that have been found to associate with BChE. It has recently been found that hBChE is assembled into tetramers by association with a PRAD derived from Lamellipodin (Lpd), a cytoplasmic cytoskeletal protein. However, the exact mechanism by which this interaction and interactions with other MRL proteins, MIG-10 and RIAM, has not yet been extensively studied. To study these binding interactions we determined the molecular forms of hBChE by velocity sedimentation along a sucrose gradient and enzymatic activity through the Ellman colorimetric assay when BChE is expressed alone or co-expressed with an associate protein. Data was analyzed to determine the relative enzymatic activity to each of the molecular forms of BChE produced, monomers, dimers, or tetramers. We found that Lpd and RIAM were capable of recruiting hBChE into tetramers. However, it was found that Lpd down regulates enzymatic activity in a dose-dependent manner while RIAM upregulates enzymatic secretion. Our results show that MIG-10 A did not have an effect on the oligomerization or enzymatic activity of hBChE.

Birth weight and associated Infant Mortality related to Maternal Obesity in Alabama

Katherine Screven and Jason Heaton

Since 2002, there has been a prominent trend in infant mortality rates related to birth weight in the United States. Changes in birth weight may result from factors such as maternal weight gain, but the specific biological mechanism linking birth weight to infant mortality is unknown. Increased maternal weight gain has been linked to the development of gestational diabetes (in mothers) and higher birth weight (in infants). Previous research has shown that mothers with increased weight gain during pregnancy give birth to higher weight infants. Using data available from the CDC VitalStats and the Alabama Department of Public Health, we analyzed the relationship between maternal weight gain and infant mortality. Additionally, we assessed the correlation between infant mortality and county specific obesity rates in the state of Alabama. Our analysis shows that there is an increase in infant mortality with increases in maternal weight gain. Furthermore, we found a significant difference ($p<0.01$) in county specific obesity percentages and infant mortality rates within the state. There was a positive correlation ($r=0.647$, $n = 20$) between the two variables of increased maternal weight gain during pregnancy and increased infant death. Finally, we assessed the relationship of increased maternal weight gain/infant mortality when compared to urban and rural designations. Our results may serve to improve maternal prenatal education about obesity aiming towards decreasing maternal obesity rates and, in turn, decreasing infant mortality rates within the state, and possibly the country.

Role of the Mitotic Kinesin – KifC1 – in Tumor Growth and Invasion

Amy Sessions, Melanie Styers, and Gretchen Repasky

Metastasis, which requires tumor cell migration and invasion, is the primary cause of breast and ovarian cancer-related deaths. Cancers with increased cell motility, a contributor to invasiveness, have been shown to be genetically unstable. One such form of genetic instability, centrosome amplification (CA), is a common feature of invasive cells that is exhibited by 80% of invasive breast tumors. The minus-end directed mitotic kinesin, KifC1, plays a critical role in proliferation of cancers with a high level of CA. KifC1 promotes stable cell division in cancer cells exhibiting CA, by tethering supernumerary centrosomes to form a pseudo-bipolar mitotic spindle. This mitotic kinesin has been shown to be essential in division of cancer cells with CA, but is not essential for proper cell division in normal cells. This study investigates the role of KifC1 in tumor cell invasion and survival through the overexpression and inhibition of KifC1. Knockdown of KifC1 by siRNA transfection caused a decrease in cell viability of some breast and ovarian cancer cell lines, while overexpression of KifC1 and motor domain truncation of KifC1 had no significant effect on cell viability or migration. The DNA damaging agents, idorubicin and camptothecin, caused a dose-dependent change in KifC1 expression. A better understanding of the role of KifC1 in cell growth and invasiveness is beneficial, as this protein may represent a potential chemotherapeutic target. This study contributes on a larger level to the understanding of the mechanisms that underlie chromosome instability, resolution of aberrant mitosis as well as tumor cell invasion.

Pentose Phosphate Pathway Upregulation Increases Yeast Resistance to the Anticancer Ruthenium Complex KP1019

Sarah Sharman and Pamela Hanson

Indazolium *trans*-[tetrachlorobis(1*H*-indazole)ruthenate(III), KP1019, is a promising chemotherapeutic due to its efficacy against drug resistant cell-lines *in vitro* and its relative lack of toxicity to patients in early clinical trials. Previous *in vitro* studies showed that KP1019 induces oxidative stress and DNA damage in colorectal cancer cells. Interestingly, both human and yeast cells have been shown to react to oxidative stress by shifting from the use of glycolysis to the pentose phosphate pathway (PPP), presumably as a means to adjust levels of intracellular reductants such as NADPH. Furthermore, in some cancer cell lines, resistance to anticancer drugs correlates with elevated PPP activity. In *S. cerevisiae*, deletion of the phosphatase-encoding gene *PHO13* has been shown to upregulate the PPP, in part by increasing expression of the PPP transaldolase Tal1. Here we report that the deletion of *PHO13* causes resistance to KP1019. Moreover, growing yeast on xylose and arabinose, two substrates of the PPP, induced resistance to KP1019 in a wild-type strain. To assess Tal1's role in this drug resistance, we examined the expression and localization of this enzyme in response to treatment with xylose and KP1019. Fluorescence microscopy and western blotting revealed no changes in expression or localization of Tal1-GFP, suggesting that other enzymes of the PPP may mediate KP1019 resistance.

A Comparative Survey of Moth Community Composition in the Bibb County Glades Preserve and the Cahaba River National Wildlife Refuge, Bibb Co., AL

John-Paul Tortorich and Peter Van Zandt

Regional diversity of moth species remains understudied and unexplored in much of the southeastern United States despite their important contributions to local biological communities as pollinators, detrivores, herbivores, and food sources. The Bibb County Glades (BCG) and Cahaba River National Wildlife Refuge (CRNWR), both located in Bibb County, account for a large portion of the fauna which characterize Alabama as the fifth most biodiverse state in the U.S. In this study, we examined the moth species and family diversity of these two unique and understudied habitats by compiling a site-specific species inventory. We sampled the BCG and CRNWR from May 7th - October 27th, 2011 on 10 nights using black light traps. While final results are still pending, current collected data suggest that inter-site moth species/family composition (taxonomic identity) and richness (total number) differ between the BCG and CRNWR. Only 47 out of 219 total species are shared between the sites despite sample locations only being 3.7 km apart; yet, 61 species are unique to the BCG and 44 only occur in the CRNWR, which implicates the strong influence of habitat distinctiveness on moth species distribution within the unique sampling sites. These data highlight the effects of proximity and habitat uniqueness on moth community composition, and further analyses will explore these important trends. This study represents one of the first assessments of moth biodiversity in the BCG and CRNWR, and we hope that our results will inform future environmental conservation efforts in Bibb County.

Molecular Forms of Mouse (*Mus musculus*) Acetylcholinesterase in the Presence of the MRL Proteins, Mig-10, RIAM, and Lpd

Janelle West and Leo Pezzementi

Acetylcholinesterase (AChE) breaks down acetylcholine in the neuromuscular junction, terminating synaptic transmission. An evolutionarily related enzyme, butyrylcholinesterase (BChE), protects AChE from inhibition, thus preserving synaptic transmission, by binding and inactivating nerve agents in synapses. AChE has a greater half-life when it is in its tetrameric form, though it also exists in less stable forms as monomers and dimers. The C-termini of cholinesterase subunits have a WAT (tryptophan amphiphilic tetramerization) domain which associates with the PRAD (proline-rich attachment domain) of either ColQ (collagen Q) or PRiMA (proline-rich membrane anchor). This WAT-PRAD interaction induces tetramerization of cholinesterase subunits, anchoring the enzymes to cell membranes in synapses. Recently, it was found that BChE associates with a polyproline sequence derived from lamellipodin (Lpd), a cytosolic cytoskeleton protein. Lpd, RIAM (Rap1-GTP-interacting adaptor molecule), and Mig-10 (migration protein) are homologues and make up the MRL proteins. To determine if vertebrate AChE interacts with the MRL proteins, the molecular forms of mouse (*Mus musculus*) AChE were determined in the presence of each protein. AChE enzyme activity was measured using the Ellman's assay. The distribution of molecular forms was analyzed by velocity sedimentation. It was found that the MRL proteins produced no significant change in the levels of AChE tetramers when compared to the polyproline control. The results suggest that there must be important differences in the WAT domains of AChE and BChE of various species to allow for interactions with the MRL proteins. Implications regarding the effect of the MRL proteins on trafficking and degradation of AChE are also evident.

A Role for Neuronal Cilia in Learning and Memory Behavior

Siamak Mohammad Zaki Rismanchi Yazdi, Megan Gibbons, and Bradley Yoder

Primary cilia are small microtubule-based cellular appendages found on the surface of nearly all eukaryotic cells that serve complex sensory and signaling roles in diverse tissues. Significant roles for cilia have been demonstrated for vision, olfaction, and mammalian development. Strikingly, little is known about the roles of cilia in most adult tissues, such as the brain. For more than fifty years, it has been recognized that neurons throughout the brain each possess a solitary cilium, but knowledge regarding their functions remains modest. The goal of this research was to determine if neuronal cilia have a role in learning and memory. Specifically, we utilized conditional mutant mice in which cilia formation is disrupted in the cortex and hippocampus, resulting in loss of cortical and hippocampal cilia. Polymerase chain reaction, immunoblotting, β-galactosidase staining, and immunofluorescence confirmed cilia loss. Learning and memory in wild-type and mutant mice were assessed through Morris Water Maze (MWM) to assess spatial learning and memory and fear conditioning to assess contextual and cued aversive learning and memory. While MWM data indicate no phenotype, fear conditioning assays suggest a deficient contextual and cued memory phenotype in mutants, thus further implicating cilia in neuron function and a potential role in neuronal signaling pathways. This study uncovered novel functions for neuronal cilia, and follow-up studies will allow for improved understanding of cognitive deficits associated with cilia dysfunction.

Deletion of *MMS22* Increases Sensitivity to the Anticancer Ruthenium Complex KP1019

Brian Zhao and Pamela Hanson

Indazolium trans-[tetrachlorobis(1*H*-indazole) ruthenate (III)], also known as KP1019, is a ruthenium-based anti-cancer drug that is thought to kill cancer cells *in vitro* by targeting their DNA. In our study, we used the yeast model *Saccharomyces cerevisiae* to study the effect of KP1019 on *MMS22*-null yeast cells. *MMS22*, a gene implicated in resolution of blocked replication forks, has been shown to be involved in accurate cell replication and DNA-damage repair. To determine the impact of *MMS22* on cellular tolerance of KP1019, wild-type and *MMS22* deletion strains were treated with KP1019 and allowed to recover for different time periods. We found that KP1019 treatment increased the percentage of large-budded cells in both wild-type and mutant strains. Furthermore, in the absence of drug, a disproportionately large number of *mms22*Δ cells had an elongated nucleus in the bud neck. The percentage of *mms22*Δ cells with this and other morphological defects increased upon KP1019 treatment. Evidence from recent studies suggested that *MMS22* works synergistically with another yeast gene, *CTF4*, in preserving the structural integrity of the DNA replisome during DNA replication. My ongoing work attempts to explore and validate this possible synergistic relationship between the two genes.

Calcium Levels in the Digestive System of *Macrobrachium rosenbergii* During the Molt Cycle

Matt Zieman and Andy Gannon

In order for crustaceans to grow, they shed and reform their exoskeleton in a process called ecdysis. Significant amounts of calcium, a major constituent of the exoskeleton, are lost to the environment in this process. Due to low Ca levels in freshwater environments, crustaceans face major problems with maintaining Ca homeostasis throughout the molt cycle. Some crustaceans have evolved mechanisms to make up for the Ca lost in the shed exuvium after molting. For example, crayfish store calcium carbonate gastroliths in their stomachs prior to molting and eat their exuvium after molting to recover lost Ca. The giant freshwater prawn, *Macrobrachium rosenbergii*, has shown neither of the previously mentioned adaptations for conserving Ca. To determine Ca allocation through the molt cycle, we used acid digestion and atomic absorption spectroscopy to measure Ca concentrations in the digestive system tissues (stomach and hepatopancreas) of *Macrobrachium rosenbergii* just prior to molting and compared those results to Ca levels in other tissues as a proportion of the total Ca budget throughout the molt cycle. Our preliminary results indicate that exoskeleton calcium levels substantially decrease as the calcium concentrations in the digestive gland increase from <1% to about 4% in the premolt stages. By studying crustaceans, we can gain insight into calcium-dependent physiological processes, like bone growth and muscle contraction, due to the similar Ca transport mechanisms common to all eukaryotic cells.