Biology (and Biology related collaborations) student abstracts 2009
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Determining the Effect of T-peptide Mutations on the Tetramerization of *Ciona intestinalis* Acetylcholinesterase and its Ability to Associate with the Proline-rich Membrane Anchor (PRiMA)

Claire Anderson and Leo Pezzementi

Acetylcholinseterase hydrolyzes the neurotransmitter acetylcholine at the cholinergic synapse in the brain and at the neuromuscular junction. The study of AChE has revealed that the functional enzyme in vertebrates is found in a tetrameric form associated with a structural protein that specifies its location. AChE exists as four splice variants $AChE_S$, $AChE_R$, $AChE_H$, and $AChE_T$. The $AChE_T$ variant contains a tryptophan (W) amphiphilic tetramerization (WAT) domain that allows it to associate with the proline-rich attachment domain (PRAD) of structural proteins such as the collagenous tail ColQ and the proline-rich membrane anchor (PRiMA). The WAT domain of AChE contains seven aromatic residues and is conserved in all vertebrates. *Ciona intestinalis* is the invertebrate most closely related to the vertebrates and has a mutation of the fourth aromatic residue, Tyr20, to Ser20. *C. intestinalis* AChE has been found to form tetramers, and to associate with the PRAD of ColQ and PRiMA. In a previous study, mutations were made in the t-peptide of *vertebrate* AChE; when the Tyr20 residue was mutated to proline and alanine residues tetramer formation and association with the PRAD of ColQ was completely suppressed, indicating the importance of the chemical properties of amino acid side chains of the WAT domain residues to its interaction with the PRAD of structural proteins. Clearly, The Y20S substitution does not affect tetramer formation and association with PRADs in *C. intestinalis.* We made S20N and S20R mutations to further investigate the chemical properties of the amino acids at position 20. Data and results are still being collected and analyzed at this time.

Effect of NAMI-A on *K-Ras*-mediated Oncogenic Transformation and Signal Transduction

Natalie L. Ausborn, Laura K. Stultz, and Gretchen A. Repasky

The ruthenium-based drug imidazolium *trans*-imidazoledimethlysulfoxide-tetrachlororuthenate (NAMI-A) has previously demonstrated anti-proliferative and anti-metastatic effects, in addition to limited toxicity. The goal of this study was to elucidate potential molecular mechanisms for the anti-cancer effects of NAMI-A on rat intestinal epithelial (RIE-1) cells transformed by *K-Ras 12V,* a genetic mutation that causes the Ras GTPase to be chronically activated, leading to unregulated cell cycle progression. RIE-1 cells were treated with NAMI-A, and morphological changes and effects on cell signaling pathways downstream of Ras were investigated. Exposure of control or *Ras*-transformed cells to 100 μM or 200 μM NAMI-A for up to twenty-four hours did not appear to alter or revert the morphological appearance of RIE-1 cells. Western blot analysis was used to examine active and total levels of both ERK and Akt after twenty-four hours of treatment with 100 μM NAMI-A. NAMI-A treatment caused an upregulation of ERK 1/2 and Akt activation in non-transformed cells and caused a slight downregulation of Akt activation in *Ras*-transformed cells. Given that the anti-proliferative and anti-metastatic effects of NAMI-A on RIE-1 cells have been previously shown to be selective towards the *Ras*-transformed cells, this study proposed an interesting shift to the potential effects of NAMI-A on molecular pathways downstream of Ras in nontransformed cells. This study indicates that NAMI-A upregulates ERK and Akt activation in nontransformed cells and provides novel

evidence of the effects of NAMI-A on Akt, suggesting that NAMI-A could target other proteins that lie upstream of both ERK and Akt signaling.

Determination of Coral Reef Diversity Using Indicator Fish Species as a Representation of Biodiversity

Tyler Brown and Andrew Gannon

Coral reef ecosystems are the most complex systems known in the marine environment worldwide. Because of their high levels of productivity and genetic diversity, they support vast numbers of marine organisms, many of which are fishes. Since many anthropogenic and natural impacts are leading to degradation of the reefs, marine protected reserve sites have been created in many places to conserve biodiversity. In the Sandy Bay-West End Marine Reserve in Roatán, Honduras, we surveyed 32 indicator fish species to calculate density and percent sighting frequency at nine dive sites to determine if the diversity of the reef has changed over three years. There was no significant difference comparing the percent sighting frequency between any of the years 2009, 2008, and 2007 (2 tailed t-test; p= 0.385, 0.285, and 0.370) and the density index from 2009 to 2008 (p= 0.133). However, there was a significant difference in the density index from 2008 to 2007 (p= 0.044, mean= 1.53, S.D.= 0.815). Species that showed the most difference were the Peacock Flounder, Trumpetfish, and Green Moray Eel, which are now less common on the reef. This suggests that the diversity on the reefs at Roatán, Honduras have not significantly changed over recent years due to the present phase shift from hard coral dominance to algal dominance. Data from our study will be compared to a long-term study provided by REEF to examine the effectiveness of the reserve.

Trapping and Removal of Invasive Northern Crayfish, *Orconectes virilis*, from Roebuck Springs, Alabama: An Effort in Protecting the Endangered Watercress Darter.

Kathryn Carroll and Megan Gibbons

The endangered watercress darter (*Etheostoma nuchale*) populates an area (Roebuck Springs near Birmingham, Alabama) that has recently been altered due to dam removal. Although loss of habitat has caused a drastic decline of *E. nuchale* abundance, the invasive, Northern crayfish (*Orconectes virilis*) is still found at high densities in the spring and has been observed preying on the watercress darter. This predation could exacerbate the problems initiated by the dam removal, and lead to the demise of this darter population. In addition to preying on the endangered *E. nuchale*, *O. virilis* out-competes native crayfish in Roebuck Springs. During October 2008 to April 2009, I began an effort to trap and remove the *O. virilis* from Roebuck Springs in order to reestablish the watercress darter population, reinstate the native crayfish, and restore ecological stability to the disturbed ecosystem. I was able to determine *O. virilis* catch rates based on the average amount of crayfish caught each day in each trap. Since I began removing *O. virilis*, body size of trapped individuals has significantly decreased and the sex ratio has become significantly less male-biased. The results allow for an analysis of the potential change in *O. virilis* population and any native crayfish that may repopulate the area. This project will be one of the many steps toward restoration of an endangered fish

population in a disturbed ecosystem, and it will also provide insight concerning the effectiveness of using trapping to manage invasive crayfish populations.

Temperature Effects on Osmoregulation and Salinity Preference in *Uca pugilator*

Vince Carpri and Andrew Gannon

Osmoregulation was studied in *Uca pugilator* to determine how different ambient temperatures affect ability to regulate hemolymph (= blood) concentrations and salinity preference among a range of six different salinities ranging from freshwater (50 mOsm/kg) to full strength seawater (1100 mOsm/kg). It was hypothesized that *Uca pugilator* would be a strong euryhaline osmoregulator (maintain a constant hemolymph osmolarity) in a full temperature range. It was also hypothesized that *Uca pugilator* would prefer higher salinities in cooler temperatures and lower salinities in higher temperatures. Individual crabs were maintained at 5^{o}, 15^{o}, 25^{o}, 35^{o} and allowed access to water at the 6 different salinities. After 24 hours of acclimation to the ambient temperature, hemolymph osmolarity was measured with a Vapor Pressure Osmometer. Hemolymph samples were taken immediately before and after each trial. Behavioral observations were collected by using a video camcorder to examine number of visits to each water concentration as well as time spent at each water dish. We found that at 35^{o} *Uca pugilator* is unable to regulate hemolymph concentrations (initial = 831 mOsm/kg, final = 718 mOsm/kg). However, at 25^{o} *Uca pugilator* is a good euryhaline osmoregulator (initial = 735 mOsm/kg, final = 716 mOsm/kg). No salinity preference was seen at any temperature settings.

Analysis of Exogenous Estrogen Compounds in the Upper Cahaba River Watershed

Katie Clingan, R. Scot Duncan, and H. Wayne Shew

The Upper Cahaba River Watershed (UCRW) encompasses the first 100 miles of the Cahaba River running from Trussville, AL through Centreville, AL. The UCRW receives discharge from 30 municipal wastewater treatment plants (WWTPs). Both excessive nutrients and non-point runoff have resulted in decreased biodiversity and habitat degradation in the Cahaba River. Effluents from WWTPs generally carry large nutrient loads, in addition to organic and pharmaceutical compounds, including estrogenic compounds. Exogenously introduced estrogens are referred to as endocrine-disrupting chemicals. These chemicals act as hormone signals that elicit adverse effects on natural endogenous estrogens by mimicking, antagonizing, or altering their metabolism and synthesis. Natural hormones include: estrone (E1), 17β-estradiol (E2), and estriol (E3). Research has shown that exogenous estrogenic compounds are capable of inducing feminization in male wild fish species at nanogram per liter concentrations. The present study aims to develop a site map plotting the variation in estrogen concentrations across the UCRW based on a coupled solid-phase extraction – enzyme-linked immunosorbent assay (SPE-ELISA) method. Estrogen concentrations are known to fluctuate depending on seasonal variability in temperature and flow rate. The present study is the first study focusing on the presence of estrogenic compounds in the Cahaba River.

Human Cytomegalovirus Strain Variation in Congenitally Infected Babies

Vishwanath Danthuluri, Shannon Ross, Zdenek Novak, Jennifer Blumenthal, Nitin Arora, Karen Fowler, William Britt, Suresh Boppana and Jeannette Runquist

Human cytomegalovirus (HCMV) is a common congenital infection affecting approximately 40,000 infants per year in the United States, and is a leading cause for sensorineural hearing loss (SNHL) in children all over the world. The aims of this study are to determine if multiple HCMV strains occur in congenital infection and to define the distribution of gN genotypes in two different compartments, urine and saliva, in congenitally infected infants. Infants were screened for congenital HCMV infection by rapid culture of saliva by a standard direct early antigen detection of fluorescent foci (DEAFF). Urine and saliva samples obtained to confirm congenital infection were utilized for this study. The gN genotyping was carried out by cloning of the gN gene followed by nucleotide sequencing of the plasmid DNA. Urine and saliva samples from 13 infants with congenital infection were examined and all four gN genotypes were detected. Five infants were found to have multiple gN genotypes in saliva and/or urine. Three infants had different genotypes found in different compartments: two subjects with gN genotype 4a in the urine and type 1 in the saliva, and one subject with gN genotype 4c in the urine and 4b in the saliva. Thus congenital infection with multiple HCMV strains was observed in over 1/3 of our patients studied. To our knowledge, this is the first report to demonstrate compartmentalization of gN genotypes in congenitally infected infants and raises the possibility that certain HCMV types are associated with specific clinical outcomes, such as SNHL.

Site Directed Mutagenesis of the Tryptophan Amphiphilic Tetramerization Domain in *Ciona intestinalis* Acetylcholinesterase

Brian Davis and Leo Pezzementi

The enzyme acetylcholinesterase (AChE), which hydrolyzes the neurotransmitter acetylcholine, has been highly conserved throughout evolution. The AChE gene possesses several exons that result in different domains at the c-terminus. Vertebrates produce $AChE_T$ that has a tryptophan (W) amphiphilic tetramerization domain (WAT) capable of forming tetramers (G_4). Vertebrates also have genes that transcribe anchoring proteins, which possess a proline-rich attachment domain (PRAD) that interacts with four WATs, leading to the formation of tetramers. Collagen Q (ColQ), expressed in muscle, attaches AChE to the basal membrane. Proline-rich membrane anchor (PRiMA), expressed in brain, attaches AChE to the cellular membrane. Recently, it was demonstrated that AChE of an invertebrate, *Ciona intestinalis*, assembles into asymmetric forms when expressed with ColQ *in vitro*. We have shown that AChE of *Ciona intestinalis* also forms tetramers with and without PRiMA. The WAT domain of *Ciona intestinalis* has six out of the seven conserved aromatic residues found in vertebrates. At the twentieth position, *C. intestinalis* has serine (S) instead of tyrosine (Y), the amino acid conserved in vertebrates. We used site directed mutagenesis to investigate the possible role of this position in tetramer formation. The serine was substituted with glycine (S20G) and threonine (S20T). Velocity sedimentation on sucrose gradients demonstrated that S20G and S20T formed tetramers with and without PRiMA. We propose that the interactions of the WAT domain's conserved aromatic residues and the prolines of the PRAD are sufficient in the formation of tetramers. Hydrophobic and polar interactions play a secondary role in stabilizing the WAT-PRAD interactions.

Toxicity of Triclopyr and Clopyralid to the Red Swamp Crayfish, *Procambarus clarkii*

David Frantz and Andrew Gannon

Triclopyr and clopyralid are pyridine herbicides in common use both individually and in combination. While both herbicides are water soluble and can migrate to water supplies, little research has been done to determine their individual or combined toxicity to aquatic life. The limited research that has been done indicates that both clopyralid and triclopyr may be toxic to aquatic species. The red swamp crayfish *Procambarus clarkii* has been proposed for use as a bioindicator species, to monitor the health of ecosystem; therefore, investigation of the toxic effect of these herbicides upon *Procambarus clarkii* is useful in determining both the extent of usefulness of *Procambarus clarkii* as a bioindicator and in establishing a baseline for the toxicity of triclopyr and clopyralid to crustaceans. Using several different concentrations, the red swamp crayfish was exposed to the herbicides triclopyr and clopyralid to find the 96 hour 50% lethal concentration (LC50.) *Procambarus clarkii* was also exposed to triclopyr and clopyralid in the herbicide Confront® 1 : 2.73 clopyralid : triclopyr mixture to find the 96 hour LC50, determining if a synergistic toxicity effect occurs during concurrent exposure. Future research in this area might examine the LC50 for other herbicides on *Procambarus clarkii* or might focus on the effects of clopyralid and triclopyr upon other more sensitive aquatic species.

The Anti-Cancer Ruthenium Complex KP1019 Induces Rad52-GFP Focus Formation

Sarah Gammons and Pamela Hanson

The ruthenium-based chemotherapy drug trans-[tetrachlorobis (1H-indazole) ruthenate (III)], otherwise known as KP1019, is currently in clinical trials on human patients. However, a lot is still unknown about the specific mechanisms by which KP1019 induces DNA damage and how cells respond to such damage. Our research indicates that *RAD52*, a gene required for recombination repair, is important in the mechanism by which cells cope with KP1019-induced DNA damage. Specifically, a *rad52Δ* strain was found to be ten times more sensitive to KP1019 than the wild type control. Published research has shown that Rad52p aggregates around DNA lesions, forming centers of DNA repair and that Rad52-GFP can be used to visualize these foci. Here we show that KP1019 induces formation of Rad52-GFP foci in a concentration dependent manner. Furthermore, we have found that treatment with KP1019 induces Rad52-GFP focus formation primarily in cells with small buds. These results are consistent with published research showing that focus formation occurs predominantly in S phase. To further explore the effect of the cell cycle on the cellular response to KP1019 induced DNA damage, we isolated quiescent and nonquiescent cells from a stationary phase culture and studied the effect of KP1019 on cell viability of these populations. These results give us a deeper insight into the mechanism by which cells respond to DNA damage induced by KP1019.

Investigating Rho Proteins: Relationship with Intracellular Trafficking and Effects on Invasive Cell Growth

M. Patricia George, Elizabeth Sztul, Daniel Welch, Melanie Styers, and Gretchen Repasky

Rho proteins are molecular switches that regulate various downstream effectors and play a central role in mediating cell shape, structure, adhesion, and motility through the reorganization of the actin cytoskeleton. Such characteristics are essential to facilitate motility and invasion of cells when cancerous. Rho proteins share an inactivating regulatory protein called ARAP with ADP-Ribosylation Factor (ARF), a coat protein complex I (COP-I) recruiter. Both ARF and Rho proteins make up families within the Ras superfamily of GTPases, which act as molecular switches cycling through an active GTP-bound and an inactive GDP-bound form. Through the recruitment of COP-I, ARF1 has been shown to mediate both retrograde and anterograde transport of proteins, between the Golgi and ER. COP-I consists of seven subunits that can be divided into two subcomplexes: α-, β'-, and ε-subunits and γ-, ζ-, δ-, β-subunits. We have found that siRNA-mediated depletion of the β-COP subunit results in an increase of actin stress fibers and focal adhesions; similar to the phenotype observed in cells that overexpress activating mutations in Rho proteins. We propose that β-COP depletion results in accumulation of its recruiter ARF1, and this accumulation may then saturate ARAP leading to prolonged Rho activation. In addition, we fabricated human breast epithelial cell lines that stably overexpress Rho isoforms and examined the effect on invasion. This study may provide new insights into cancer progression, as overexpression and/or hyperactivation of Rho isoforms has been found in a variety of high-grade human carcinomas including breast and prostate.

Examining KP1019: The Effects on *K-Ra*s Transformed Cells and *pso2Δ S. Cerevisiae*

Joshua D. Hooper, Laura K. Stultz, Gretchen A. Repasky, and Pamela K. Hanson

Ruthenium-based chemotherapeutic drugs have received attention due to their effectiveness against an array of cancers including cell lines showing resistance to previous platinum-centered drugs. The compound KP1019 (indazolium *trans*-[tetrachlorobis(1H-indazole)ruthenate(III)]), has gained considerable interest due to its success through early stages of clinical trials. Further investigation into the hydrolysis mechanism of this compound was obtained through kinetic studies giving a better view into the rate of ligand exchange for the complex. *K-Ras* mutated oncogenes, a mutation causing a perpetually active Ras protein that results in unregulated cell proliferation, are present in over 90% of pancreatic and 40% of colorectal cancers. Through a morphology study of the RIE-1 cell line the results in this study indicated that KP1019 does not induce a reversion of oncogenic phenotypes in the *K-Ras* mutated cells. Through the use of alamarBlue assay, KP1019 was determined to be much more cytotoxic than discussed in previous literature. There was no sign of significant specificity of attack by KP1019 towards transformed *K-Ras12V* cells over vector cells. This study also uncovered that KP1019 causes DNA damage via interstrand cross-links in a mutated strain of *S. Cerevisiae* conataining a deletion of the *PSO2* gene.

Analysis of the Importance of *ELM1* in Activation of the Pdr Network by Hexadecylphosphocholine

Kaleigh Hussey-Tomich and Pamela Hanson

Drug resistance is an escalating problem when treating many different types of diseases. Cells become resistant through various mechanisms, including drug efflux. In *Saccharomyces cerevisiae* the pdr network is an integral part of efflux-mediated drug resistance. The pdr network is composed of transcription factors Pdr1 and Pdr3 which control expression of efflux pumps including the ABC transporter Pdr5 and the putative long chain base transporter Rsb1. However, the pdr network has also been shown to be activated in response to membrane perturbation. Previous published research indicates that expression of *PDR5* increases when cells are exposed to membrane perturbing compounds, such as amphipathic drugs and detergents. Using a *PDR5-lacZ* reporter construct, we have determined that hexadecylphosphocholine (HePC) activates *PDR5* gene expression. An *RSB1-lacZ* reporter construct was used to examine the activation of *RSB1* by HePC and it was determined that *RSB1* is not significantly activated by HePC. Although we know HePC increases expression of *PDR5*, the molecular mechanism by which this occurs is unknown. Elm1, a protein kinase involved in signal transduction, was a potential candidate. One published study reports that Elm1 functions upstream of *PDR5*, indicating a potential connection between the two. To determine the its importance, *ELM1* deletion strains were transformed with a *PDR5-lacZ* reporter construct, and β-Galactosidase activity was measured. It was determined that ELM1 does not have significant importance in activation of the pdr network. This research assists in determining the pathway that begins at membrane perturbation and leads to increased expression of transporters involved in drug resistance.

Site Directed Mutagenesis of Ser20 of the T-peptide of AChE from *Ciona intestinalis*: Effect on Tetramer Formation without and with the Proline-rich Membrane Anchor

Julie Johnson and Leo Pezzementi

To better understand the evolution of cholinesterases (ChEs), the molecular forms of acetylcholineserase (AChE) were studied in the invertebrate *Ciona intestinalis*. In the vertebrates, the $AChE_T$ subunit assembles into tetramers that may associate with the collagen tail subunit (ColQ) and with the proline-rich membrane anchor (PRiMA) protein. Previously, it was thought that invertebrates were only capable of forming the soluble globular forms (monomers, dimers or tetramers) of the enzyme, but not tailed forms. The urochordate, *C. intestinalis,* is one of a few invertebrates known to possess the $AChE_T$ subunit. Although *C. intestinalis* possesses the $AChE_T$ subunit, it has been shown to express only globular forms *in vivo*. *In vitro*, however, *C. intestinalis* is capable of forming tailed forms of AChE in association with PRiMA or ColQ. Although the AChE of *C. intestinalis* closely resembles vertebrate AChE, the tryptophan (W) amphipathic tetramization (WAT) domain of the t-peptide, which associates with PRiMA or ColQ of *C. intestinalis* differs from the vertebrate WAT domain: Tyr20 of the WAT domain is a replaced by Ser20. In the vertebrates, mutations of Tyr20 prevent formation of PRiMA or ColQ associated tetramers. Clearly, the S20Y substitution does not prevent this association in *C. intestinalis*. In the present paper, we created the S20I and S20E mutations in the t-peptide of *C. intestinalis* AChE to determine the properties of amino acids at this position that promote the formation of tetramers in the absence and presence of PRiMA.

T-Cell Trafficking, Activation, and Proliferation in a Murine Model of Allergic Asthma

Benjamin Jones, Kari Dugger, Lisa Schwiebert, and Andrew Gannon

Asthma symptoms have been reported both clinically and experimentally to be attenuated through the implementation of a regimen of moderate-intensity aerobic exercise. The mode of this attenuation is currently unknown, though hypothesized to result from changes in the activation and proliferation of T-cells. Bioluminescent CD4+ T-cells harvested from transgenic donor mice were injected into female BALB/cj mice. Subjects underwent a course of OVA sensitization and challenge. Half of the subjects were exercised regularly, three times per week for 45 minutes. Bioluminescent imaging was then used to track the trafficking, activation, and proliferation of T-cells *in vivo* throughout the course of the protocol, allowing a comparison between exercised and non-exercised mice.

The Effects of Decreased Hemolymph pH on Ghost Crabs, *Ocypode quadrata*, on Ventilation and Heart Rate

Sarah Juliana and Andrew Gannon

Terrestrial vertebrates have a pH-sensitive ventilatory drive that responds to CO_2 as a respiratory acid in the blood. Terrestrial crabs' ventilation can be extremely CO_2-sensitive but may respond to CO_2 as a gas and not as a respiratory acid. Therefore, evolving a pH-sensitive ventilatory drive may not be a necessity for breathing air. We examined the cardioventilatory response of an intertidal species of crab, *Ocypode quadrata,* to decreased hemolymph pH to determine if they have a pH-sensitive ventilatory drive when breathing air. Metabolic acidosis (decreased hemolymph pH) was induced via HCl injections into the pericardial cavity, alternating with saline injections. Heart and ventilatory rates were then measured for thirty minutes. Despite acid injections, hemolymph pH remained constant, indicating pH regulation by non-ventilatory means. Following acid injections, both heart and ventilation rates remained constant. Therefore, these crabs have not demonstrated the pH sensitive ventilatory response found in terrestrial vertebrates, indicating that they regulate hemolymph pH through non-ventilatory means.

A Comparison of Outcomes in Obese Women with Endometrial Cancer Undergoing Open versus Minimally Invasive Surgery: A Rationale for Robotic Surgery as the Standard of Care

Kristen Kerr, Warner Huh, and Andrew Gannon

Because obesity is a primary risk factor for developing endometrial cancer, it is important to determine the best treatment option for this patient population. The traditional treatment, laparotomy, is associated with both intraoperative and postoperative complications for the morbidly obese, such as an increased estimated blood loss (EBL), wound infection, and wound dehiscence. The purpose of this study was to retrospectively evaluate the surgical outcomes of morbidly obese women with endometrial cancer undergoing laparoscopy, compared to the newer, minimally invasive robotic surgery. We hypothesized that many of the advantages offered by robotic technology would prove beneficial for this particular patient population. The study was conducted by examining patient characteristics and surgical outcomes for all robotic and laparotomy cases performed on severely obese women with endometrial cancer at the

University of Alabama at Birmingham from 2006-2008. Patient characteristics for both surgery groups including body mass index (BMI), EBL, surgical time, length of postsurgical hospital stay, and postoperative complications were statistically compared. Both length of postoperative hospital stay and EBL were significantly greater in morbidly obese patients treated laparoscopically (ttest; $p<0.05$). Although postoperative complications were not statistically significant between the two groups, it is important to note that patients who underwent robotics surgery did not experience any notable postoperative complications. Consequently, because of the great number of morbidly obese women diagnosed with endometrial cancer, and due to the surgical complications associated with the current treatment, the results of this study could be used as an initial step in providing better patient care.

The Contribution of Ischemic Insults to the Pathogenesis of Alzheimer's Disease

Anne Lawrence, Inga Kadish, and Leo Pezzementi

Alzheimer's disease (AD) is a devastating neurodegenerative disease characterized by the progressive loss of cognitive function, finally leading to the inability to perform daily activities. Alzheimer's disease is associated with the two pathological hallmarks 1) intracellular neurofibrillary tangles (NFTs) and 2) accumulation of amyloid beta (Aβ) in neuropil plaques and intracellular sites as well as substantial Aβ deposition in the cerebral vasculature. Although alterations in the cerebral vasculature are clearly not the sole cause of AD, mounting evidence indicates that the accumulation of microinfarcts does contribute to the cognitive impairments associated with AD. Despite intense study of AD and the increasingly widespread use of transgenic models of Aβ deposition in AD research, few studies have analyzed the possible relationship between cortical infarcts and cognitive dysfunction in AD. In this study, we examined the relationship between cortical infarcts and the development of cognitive dysfunction in an AD model mice. Intraperitoneal injection of a photosensitive dye (Rose Bengal) followed by laser illumination of the specific area of the brain were utilized to induce focal ischemic lesions unilaterally in the cortex of transgenic AD model mice. Two months later mice were behaviorally tested for cognitve deficits in the Barnes maze and the Water maze. The results of this study showed 1) that ischemic AD mice performed significantly worse on behavioral tests to compare with control AD mice, 2) that small ischemic infarcts considerably increased the amyloid beta pathology and glial upregulation in the brain of APP-DI mice.

Mutations of Serine 20 in the WAT Domain of Acetylcholinesterase: Effects on the Ability to form Tetramers and Associate with PRiMA

Jeff Lucas and Leo Pezzementi

Acetylcholinesterase (AChE) hydrolyzes acetylcholine into its components, acetate and choline, rapidly at cholinergic synapse to terminate its action. $AChE_T$, an alternatively spliced form of AChE, contains a tryptophan (W) amphiphilic tetramerization domain (WAT) at its C-terminus and can form tetramers alone or when associated with a collagen-like tail (ColQ) or with the proline-rich membrane attachment protein (PRiMA). The PRAD is the proline-rich attachment domain of ColQ and PRiMA, and contains a stretch of

prolines where AChE attaches via its WAT domain. The WAT domain is highly conserved in vertebrates. However, in the invertebrate *Ciona intestinalis* Tyr20 is substituted by Ser20. Site-directed mutagenesis was used to determine the effect of this substitution on tetramer formation and association with PRiMA. Ser20 was mutated to the amino acids glutamine (Q) and histidine (H). The enzyme was expressed with and without PRiMA *in vitro,* extracted, and sucrose gradients were run to determine the relative abundance of monomers, dimers, and tetramers of AChE. We found that AChE does form tetramers and associates with PRiMA upon the insertion of glutamine or histidine in the place of the serine at the 20th position, indicating this amino acid is not crucial to the function of the WAT domain.

The Effects of the S20K and S20W Mutations on the Tetramerization of *Ciona intestinalis* Acetylcholinesterase Without and With the Proline-Rich Membrane Anchor, PRiMA

Elizabeth Molony and Leo Pezzementi

Acetylcholinesterase (AChE) hydrolyzes the neurotransmitter acetylcholine to facilitate the end of signal transduction in cholinergic synapses and neuromuscular junctions. One variant of acetylcholinesterase, the tailed variant ($AChE_T$), contains a t-peptide at the carboxy terminus that gives the enzyme the ability to form monomers, dimers, and tetramers. Within the t-peptide is the tryptophan (W) amphiphilic tetramerization (WAT) domain that allows tetramers to associate with a proline-rich attachment domain (PRAD) present, for example, in PRiMA, the proline-rich membrane anchor. The WAT domain of vertebrates contains a Tyr20, but the acetylcholinesterase of the invertebrate *Ciona intestinalis* contains a Ser20 while maintaining the same tetramer formation and association properties. C*iona* does not produce its own PRADs *in vivo*, but will associate with them *in vitro*. This association led to our interest in the properties of the amino acid at position 20 that allow the enzyme to assemble into globular forms and associate with PRADs. We used site-directed mutagenesis to create S20K and S20W mutants in the *Ciona* acetylcholinesterase t-peptide, and transfected COS-7 cells without and with PRiMA to produce enzyme *in vitro.* The molecular forms were determined by velocity sedimentation on sucrose gradients. The data are still being collected and analyzed, but should show the effects of the mutations on the formation of oligomers, and the importance of size, shape, charge, hydrophobicity, and polarity of the amino acid at the 20th position of the t-peptide in its involvement in tetramer formation and the WAT-PRAD interaction.

Establishment of A Doxycycline-Responsive Dominant-Negative IκBα Construct and Determining its Effect On Medulloblastoma Cell Proliferation and Tumor Progression

William Moore, Naomi Logsdon, Sue Spiller, and Jeannette Runquist

Over 400 children every year in the U.S. are diagnosed with medulloblastoma, a highly malignant brain tumor. Survivors of this disease often suffer from detrimental side-effects due to treatment-induced damage of surrounding neural tissue. Better understanding of the unique features of medulloblastoma cells will aid in developing treatments targeted directly toward medulloblastoma cells. The purpose of this study was to

understand the role of NFκB, a signaling pathway involved in the survival of medulloblastoma. Preliminary data indicate NFκB is aberrantly active in medulloblastoma; our goal was to create a genetic method to specifically control the pathway. Two preliminary steps were taken to establish a double-stable cell line containing a doxycycline-inducible mutated form of IκBα, the inhibitor of NFκB. First, a plasmid containing a doxycycline-controlled transcription factor was transfected into the medulloblastoma cell line D425. Second, the gene for a dominant-negative mutant of IκBα, dnIκBα, was amplified from the pCMV/IκBαM plasmid and inserted into a Tet-responsive plasmid. Assays using a luciferase reporter gene downstream of a Tet-response element showed four clones with greater than 20-fold induction upon treatment with doxycycline, indicating that they may be used for establishment of a double-stable cell line expressing the dnIκBα gene in an inducible fashion. Future *in vitro* and *in vivo* studies using this line will demonstrate the effect of the dnIκBα construct on medulloblastoma cell proliferation and tumor progression. Expression of the dnIκBα construct is expected to prevent NFκB nuclear translocation, thus shutting down the pathway and possibly leading to cell and tumor death.

Characterization of Efficacy and Mechanism of Function of Ruthenium Complex Cholinesterase Inhibitors

Brian Perrin, Leo Pezzementi, and Laura K. Stultz

Previous research has demonstrated acetylcholinesterase (AChE) inhibition by complexes of ruthenium and polycyclic aromatic ligands, such as phenanthroline (Phen) and diphenylphenanthroline (Dpp); however, a thorough quantitative analysis of their efficacy and characterization of their mechanism of action has not been done. We synthesized a library of these compounds and characterized their inhibition with electric eel AChE and horse serum butyrylcholinesterase (BChE) to determine the type of inhibition and the efficacy of different ligands/ligand combinations. Current data indicate mixed inhibition, indicating that the complexes can bind in the absence and presence of substrate, and suggest that, of the complexes studied, the greatest inhibition results from either the Dpp ligand or a synergistic effect resulting from the Dpp-Phen ligand combination. The bulky nature of the ruthenium complexes make it seem likely that the competitive inhibition component of inhibition is related to π-π stacking of the aromatic moieties of the ligand and the aromatic residues of the enzyme's catalytic gorge; this notion is supported by molecular modeling simulations suggesting face-to-face and face-to-edge stacking of the inhibitors with aromatic residues in the enzyme.

Using Water Quality Testing and Habitat Assessments to Determine the Presence and Effects of Acid Mine Drainage in Clear Creek and Mulberry Fork, Alabama

Rebekah Pine, Peter Van Zandt, and R. Scot Duncan

Surface mining for coal has several environmental consequences including loss of riparian habitat and the release of metals into surrounding waterways. One of the most harmful aspects of mining is acid mine drainage that enters the creeks and rivers surrounding the mine. Acid mine drainage (AMD) is caused by the oxidation of pyrite and other minerals found in coal and associated rocks. AMD causes an increase in

acidity, an increase in conductivity and an increase in concentration of metals such as iron and manganese. These changes in water chemistry can cause certain intolerant species of macroinvertebrates, fish and bryophytes to become extirpated and replaced by more tolerant species. Six mines in the Mulberry Fork subwatershed and four mines in the Clear Creek subwatershed, both in Winston and Walker County, Alabama are the focus of this study. In addition, ten reference tributaries with no known mining damage were chosen for reference tributaries to serve as a control. At these 20 sites, water quality testing and habitat assessments will be conducted. Water quality parameters include pH, alkalinity, hardness, conductivity and concentrations of iron and manganese. The habitat assessment in this study includes ten measurements of stream health including bank stability and sediment deposition. It is expected that tributaries affected by mining will have lower pH, higher conductivity, lower alkalinity and hardness, and higher concentrations of iron and manganese in comparison to reference tributaries. Reference tributaries are expected to have better habitat assessment rankings than the tributaries adjacent to the mines. This project gives current research for this understudied area of Alabama with a focus on the effects of the surface coal mining.

Outcomes of Total Hip Arthroplasty Performed by a Single Surgeon in Brazil: a Comparison between Private and Public Hospitals

Thiago Queiroz, Diego Lima, and Megan Gibbons

The demands for primary total hip arthroplasty (THA) increases with a boost in population longevity in developed countries. The success rate of this surgery is very high in most developed countries, but less is known about the success rates in moderately developed countries. Data were acquired using patients' records from a surgeon in Brazil who has conducted several years of total hip replacements at both public and private hospitals. Diagnosis, age, gender, brand of prosthetic material, and fixation methods were compared between patients at public and private hospitals, using Multivariate Regression analyses. Our results showed a significantly higher rate of patients diagnosed with femur fracture for public hospital while for private hospital, coxarthrosis was the preeminent diagnosis. Because our study used data from a single surgeon, this eliminated biases associated with particular skills and techniques used by different surgeons. Furthermore, this is the first study to investigate the outcomes of total hip replacement between patients at public and private institutions in a moderately-developed country. This research could provide useful information concerning the quality of public health care in Brazil.

Effects of Site Directed Mutagenesis of *Ciona intestinalis* AChE T-subunit Ser20 Residue on Tetramerization and Association with PRiMA *in vitro*

Ashley Russell and Leo Pezzementi

AChE is a highly conserved enzyme and is found in both invertebrates and vertebrates. The t-splice variant of AChE ($AChE_T$), which is of interest in this study, may exist as soluble monomers (G_1), dimers (G_2) and tetramers (G_4). It may also associate with proline-rich membrane anchoring protein, PRiMA, and the collagen-tailed membrane anchoring subunit, Col-Q. The $AChE_T$ splice variant is primarily found in the vertebrates, but prior studies have identified the $AChE_T$ variant in the deuterostome invertebrate, *Ciona intestinalis.* The t-peptide contains a region of seven aromatic residues, known as the tryptophan (W) amphiphathic tetramerization (WAT) domain, which interacts with the proline rich attachment domain

(PRAD) found in Col-Q and PRiMA. In the vertebrates, all seven aromatic residues of the t-peptide are conserved. However, in *C. intestinalis* AChE, only six of the seven are conserved: the tyrosine located at the 20^{th} position is replaced by a serine residue. Site directed mutagenesis was used to create S20M and S2oL point mutations of the t-peptide in *Ciona intestinalis* AChE. The cDNA was tranfected into COS-7 monkey cells and the molecular forms of the enzyme in the presence and absence were analyzed via velocity sedimentation on sucrose gradients. Both the S20M and S20L mutants are capable of forming tetramers in the absence and presence of PRiMA, though to varying degrees.

Exploring the Binding Mode of $Ru(phen)_3^{2+}$ to Apocytochrome C

Lane Schlitz and Clyde Stanton

The intercalative binding of tris(1,10-phenanthroline)ruthenium(II) ($Ru(phen)_3^{2+}$) to DNA occurs when one of the phenanthroline ligands is stacked between nucleotide base pairs. These base pairs form a hydrophobic pocket which shields $Ru(phen)_3^{2+}$ from quenching by water; this results in both a lengthening of the emission lifetime and a decrease in the quenching constant (Kq) of the ruthenium. The binding constant (K_b) of the phenanthroline to the DNA can be determined using a Scatchard plot. The purpose of this project is to see if $Ru(phen)_3^{2+}$ binds to apocytochrome c, a protein containing a large hydrophobic region, in this same way. Heme-free apocytochrome c was synthesized from horse heart cytochrome c, and the emission lifetimes and Kq values were determined from laser induced studies on mixtures of $Ru(phen)_3^{2+}$ with various concentrations of the protein. If binding occurs intercalatively, then apocytochrome c should show effects similar to those of DNA on the emission lifetimes and the Kq values of $Ru(phen)_3^{2+}$. Also, if shielding is shown, a Scatchard plot will be used to extract the binding constant.

Effects of Nitrogen-Fixing Rhizobial Bacteria on Growth Rates and Alkaloid Chemical Defenses of *Baptisia alba* and *Baptisia australis*

Scott Shashy and Peter Van Zandt

Plants have a variety of physical and chemical adaptations that defend against herbivory. Chemical defenses may be either constitutive or induced. In constitutive defenses, the concentration of defense chemical remains constant. In induced chemical defense, the plant synthesizes toxic chemicals depending on the amount of plant tissue damage. Plants may vary in inducibility, caused by factors such as nutrient availability and water stress. Legumes have a highly coevolved endosymbiotic relationship with bacteria. In this symbiosis, nitrogen-fixing bacteria are able to convert ambient nitrogen into ammonia, which is then available for their host plant. Legumes benefit from this symbiosis in many different ways including increased growth rate, nutrient content, and possibly herbivore resistance. In my study, I will investigate the effects of the nitrogen-fixing rhizobial bacteria on the growth rates and the inducibility chemical defenses of two legume species, *Baptisia alba* and *Baptisia australis*. I will grow *B. alba* and *B. australis* in a greenhouse and apply rhizobial bacteria to the roots of half of the plants of each species. I will use *Trichoplusia ni* caterpillars to induce 0 % – 50 % damage on each of the plants. I will measure their height

and calculate the relative growth rate for each plant species. I will analyze plants for alkaloid content by gas chromatography. Because rhizobia increase the amount of available nitrogen and *Baptisia*'s chemical defenses are nitrogen-based, I hypothesize that rhizobia will increase the concentration of alkaloid defense chemicals in both *B. alba* and *B. australis*.

Deletion of the Inositol Phosphotransferase Gene *IPT1* Increases Expression of the Multidrug Transporter Pdr5p

Lisa Speake and Pamela Hanson

Pleiotropic drug resistance in pathogenic fungi leads to difficulty in adequately treating patients with infections. In yeast, the genes *PDR1* and *PDR3* encode Cys6-Zn (II) transcription factors that bind to pleiotropic drug response elements (PDREs) in the promoters of genes such as *PDR5* and *IPT1,* which encode an ABC transporter and inositol phosphotransferase, respectively. With respect to drug resistance, the role of Pdr5p in pumping cytotoxic agents out of the cell is well characterized, whereas little is known about the role of Ipt1p, an enzyme that catalyzes the last step in sphingolipid biosynthesis. Deletion of *IPT1* increases resistance to the Pdr5p substrates cycloheximide, rhodamine 6G and fluconazole. This resistance is greater at 37°C compared to 30°C and correlates with increased expression of Pdr5p. However, *pdr5Δipt1Δ* yeast are more resistant to cycloheximide than *pdr5Δ* yeast, demonstrating that there is also a Pdr5p-independent mechanism of resistance in *ipt1Δ* cells. The goal of this study was to further elucidate the causes of cycloheximide resistance in *ipt1Δ* strains. Filipin staining was used to uncover potential differences in membrane sterol composition between wild-type and *ipt1Δ* cells. While no major differences were seen in sterol staining between mutant and wild-type cells, it was noticed that mutant cells grown at 37°C are larger and display an abnormal shape.

Determination of the Mechanism of Ras-mediated PEDF Downregulation and the Role of PEDF in Oncogenesis

Jannese Stallworth and Gretchen Repasky

Oncogenic mutations in Ras GTPase are found in approximately 95% of all pancreatic ductal cancers. The multifunctional protein, Pigment Epithelium-derived Factor (PEDF), has been suggested to have anti-cancer properties. Preliminary studies show that oncogenic Ras downregulates, PEDF. Additional preliminary studies have shown that the extracellular signal-regulated kinase (ERK) pathway, but not the phosphoinositide 3-kinase (PI3K) pathway, leads to PEDF downregulation. However the role of the RalGEF-Ral signaling pathway in PEDF downregulation is still unknown. This pathway is important because it plays a role in metastasis and in the formation of invasive tumors. Furthermore, it has been shown that when PEDF is removed from Ras-transformed pancreatic intraepithelial neoplasia (PanIN) cells, the progression of the disease is greatly increased. But, it is unknown whether addition of exogenous PEDF can cause Ras mutated cells to revert to the normal phenotype. Therefore, to determine whether the Ral-GEF pathway leads to PEDF downregulation, western analysis was done using a constitutively activated Ral-GDS expressing cell line. It was found that the PEDF expression was decreased in these cells. In order to determine PEDF would cause Ras transformed cells to revert to the normal phenotype, a morphological assessment and a low serum growth assay were done. It was found that stable reintroduction of

PEDF did not cause a reversion to the normal phenotype. These studies allowed us to come one step closer towards a fuller understanding of the relationship between Ras and PEDF.

Rapid Geomorphic and Habitat Assessments of Urbanization's Impacts on the Upper Cahaba Watershed

Virginia Sturgis and R. Scot Duncan

Urbanization leads to stream degradation by altering natural sediment and water flow. In 2002 the United States Environmental Protection Agency (EPA) determined the Cahaba River was 29th most imperiled river in North America as a result of urbanization. The unstable and hazardous environments created by urbanization affect the unique species that dwell in streams. *Notropis cahaba*e, Cahaba Shiner, is a sensitive and endemic species in Cahaba Watershed, which shows signs of urbanization. In 2003, the Alabama Department of Environmental Management (ADEM) assessed the Upper Cahaba Watershed, most notably by performing a rapid geomorphic assessment (RGA). The current study followed the protocol described by ADEM's report at 17 of the 29 sites, to compare current and previous conditions of each site. The condition of the watershed was determined when results from ADEM's study were compared to the results collected from this study. The second assessment, designed by the EPA, rapid habitat assessment (RHA) was used for sites to study several more parameters to rate the condition of the Upper Cahaba Watershed. Both assessments rate the effects of urbanization (e.g. scouring, bank erosion and excess sediments), they have different objectives. The ADEM assessment focuses on channel morphology, which is important in determining the evolutionary stage, channel shape, of the stream. The RHA of streams, designed by the EPA focuses on the overall health of a stream which is conscious of the aquatic species future. The overall health of the Cahaba Watershed was assessed by using the two different protocols.

Associations between Glial Cells Involved with Aging, Cognitive Decline, and White Matter Degradation in Mice

Corey Thomas, Inga Kadish, and Peter Van Zandt

In the field of neuroscience, a resurgence of interest in the roles of glial cells has occurred. Glial cells serve to support neurotransmission by maintaining homeostasis in the brain and providing nutrients and physical support to neurons. While a great deal of knowledge has been acquired about the individual roles of the different types of glia, little is known about the communication and interactions between them. Atrophy in the number of glial cells and their functional efficiency is often associated with aging and cognitive decline. We attempt to develop a deeper understanding of interactions between glia on a molecular level and to investigate changes in these interactions associated with aging. Immunohistochemical analysis was performed in order to evaluate the responses of astrocytes, oligodendrocytes, and microglia to white matter damage induced by ischemia. We also investigate disturbances in the communication between astrocytes and oligodendrocytes in aging mice. Decreased communication between astrocytes and oligodendrocytes, as well as the upregulation of astrocytes and microglia, have been tied to inflammation in previous studies. A series

of behavioral tests were carried out in order to analyze the effects of non-steroidal anti-inflammatory drugs (NSAIDs) on the glia of aging mice. By investigating communication between glial cells and their roles in aging, we can better understand how the nervous system functions and deteriorates with age.

Analyzing Fluorescence Lifetime Characteristics of Apomyoglobin on Ruthenium(II) tris Phenanthroline ($Ru(phen)_3^{2+}$)

Jacob Vaughn and Clyde Stanton

This experiment attempts to expound on the photochemical studies of Ruthenium (II) tris phenanthroline ($Ru(phen)_3^{2+}$) in the presence of biological molecules. Specifically, this experiment analyzed the effects *equine* apomyglobin (*eq*-apoMb) had on $Ru(phen)_3^{2+}$. Quenching and fluorescence lifetime lengthening via intercalation were the characteristics of interest in this study. The average fluorescence lifetime for $Ru(phen)_3^{2+}$ with no *eq*-apoMb present was 982 $\pm$ 45 nanoseconds (ns), while the derived quenching rate constant (k_q) was $3.95E^9$ M^{-1}sec. The data showed no evidence of quenching or fluorescence lifetime lengthening occurring as *eq*-apoMb was introduced to $Ru(phen)_3^{2+}$. The absence of the expected fluorescence lifetime lengthening trend via intercalation could be a result of the smaller concentration of *eq*-apoMb with respect to the concentration of $Ru(phen)_3^{2+}$.

Physical Investigative Behavior and Shell Selection of the Land Hermit Crab *Coenobita clypeatus* in Response to Shell Damage

Terza Weston and Andrew Gannon

In Roatan, Honduras, *Coenobita clypeatus*, a common land hermit crab, was used to determine a pattern of shell investigative behavior and shell selection. As they grow, hermit crabs must select and move to larger shells. The ability to select higher quality shells will greatly impact their survival. Hermit crabs were given the choice between a shell with one hole and a shell with three holes. It was hypothesized that the shell with one hole would be selected more frequently than the shell with three holes. The pattern of behavior during shell investigation was observed for thirty minutes. A Kinematic diagram was constructed and most the frequent sequence of behaviors was: approach shell, touch outer shell with antennae, chelae, then walking legs, climb onto shell, turn shell/reach into aperture with chelae, and enter the shell. Novel behaviors such as a shell roll were also observed. The shell selected for most frequently after eight hours was a shell with only one hole. However, a chi-square test value of 2.25 shows this difference was not significant. This study provides the first Kinematic diagram depicting hermit crab shell investigative behaviors. It also suggests that land hermit crabs may select shells based more heavily upon factors other than drilling damage such as the tactile, gustatory, or chemosensory quality of potential shells.

Analysis of Growth Rates, Induced Defenses, and Constitutive Defenses in Two *Baptisia* Species (*B. alba* and *B. lactea*)

Olivia Woodard and Peter Van Zandt

We characterized plant response to herbivory for two *Baptisia* species, *Baptisia alba* (white wild indigo) and *Baptisia lactea* (white false indigo). The Growth Rate (GR) hypothesis was used to predict constitutive defenses (toxins that are always present) and induced defenses (additional toxins produced after herbivory) for each species. The maximum growth rates of each *Baptisia* species framed our predictions about the differences in defenses according to the GR hypothesis, which predicts that faster growing plants will exhibit greater induced defenses and lower constitutive defenses than slower growing plants. We used the generalist herbivore *Trichoplusia ni* (cabbage looper moth) to trigger induced resistance by damaging each *Baptisia* plant. These caterpillars were later used for a bioassay comparing caterpillar growth (μg/day). It is expected that there would be differences in *B. alba* and *B. lactea* induced defenses and constitutive defenses only if there was is a significant difference in their maximum growth rates; however, this result is still to be determined. Future studies could test additional plant species using the Growth Rate hypothesis, allowing further insight into how plants respond to herbivory.