Descriptions of the Research Conducted by the Students:

2008 Final Abstracts presented at the Tri-University Summer Symposium in Science and Engineering at the University of Michigan. The schools involved are: Hope College, U of Michigan, and Notre Dame.

LOCATING THE GENE RESPONSIBLE FOR THE "BIG HEART" PHENOTYPE IN *DROSOPHILA* EMBRYOS

Kelly Biedron, Saint Mary's College
Douglas Shoue, Dr. Robert Schulz*, University of Notre Dame

Drosophila melanogaster is a model organism to study heart development because the earliest stages of vertebrate cardiogenesis parallels the conserved mechanisms of heart formation found in the fly. To determine the location of the gene that produces a dorsal vessel with an excess number of cardioblast cells, referred to as "big heart", deficiency mapping was utilized. *Drosophila* containing numerous chromosomal deletions were crossed to flies heterozygous for the "big heart" phenoytpe to obtain a quarter of hemizygous offspring with newly formed dorsal vessels, observed with ultra-violet in-vivo microscopy. Additional *Drosophila* strains will be used to further subdivide the specific deletion areas into smaller sections in which fewer genes are located to map the gene of interest. One third of the chromosomal deletions have been investigated thus far, none of which showed the "big heart" phenotype. Future experiments will involve crossing the remaining chromosomal deletions to determine the location of the altered phenotype to a specific gene involved in cardiogenesis.

The Biochemical Structure of Tektin2 in Mammalian Somatic Cells

Christopher D. Higgins, Elizabeth S. Halpin and Edward H. Hinchcliffe, University of Notre Dame

The tektin proteins have recently been discovered to play an essential role in cytokinesis, the division of the cell membrane after mitosis, yet their biochemical structure remains unknown in mammalian somatic cells. This study seeks to characterize the structure of the tektins with the goal of delineating a mechanism by which they participate in cytokinesis. Sucrose gradient centrufugation and size exclusion chromatography followed by immunoblotting identified tektin2 in three separate fractions which corresponded to monomeric, dimeric, and higher oligomeric molecular weights. Furthermore, coimmunopreciptation pull down identified α-tubulin, Mitotic Kinesin-like Protein 1, Aurora B kinase, and Protein Regulating Cytokinesis 1 as *in vitro* binding partners of tektin2 in mammalian cells. Tektin2 siRNA knockdown caused mislocalization of tektin1 and tektin3 and disorganization of microtubules in the midbody. Thus, tektin2 has multiple quaternary structures, interacts with several important cytokinetic proteins, and is required for proper localization of tektin 1 and tektin3.

Expressing Rhodopsin visual proteins in the mosquito, *Anopheles gambiae*

Joshua Lelemia Irvine, University of Hawai‘i at Mānoa, Joseph O’Tousa*, University of Notre Dame, Michelle Whaley*, University of Notre Dame, Xiabong Hu*, University of Notre Dame

Vision contributes in mosquito behavior such as resting, mating, and host-feeding. Rhodopsin, a visual protein in the mosquito, initiates the transduction of a light stimulus to a neural signal. This study is designed to characterize rhodopsin proteins 1, 7, 8, and 9 in *Anopheles gambiae*, the principal tropical vector for human malaria. The C-terminal sequences of these four rhodopsin genes were cloned into the expression vector PET-32a(+) and then were expressed in *E.coli*. The purified proteins were successfully prepared for injection into mice to produce polyclonal antibodies. These antibodies will then be used for immunolocalization experiments to visualize the pattern of rhodopsin expression in the mosquito.

Targeting of Cytoplasmic Dynein to Distinct Mitotic Structures by Phosphorylation of the Intermediate Chain Subunit

Caitlin Lazar, Jason Bader, Dr. Vaughan*, University of Notre Dame

Errors in mitosis have been implemented in aneuploidy and cancer, but knowledge of the mechanisms in cell division is incomplete. Dynein is a microtubule motor protein that functions at the cell cortex, spindle pol, and kinetochores. The process for targeting of dynein to the distinct locations within the cell is unclear. Previous research has suggested that phosphorylation specifies the location of a specific population of dynein. In this study, we compare two phosphorylation sites on the dynein intermediate chain and the impact of phosphorylation on the localization of dynein during mitosis. Phosphorylation at the first site (PT89) directs dynein to the kinetochore where it plays a crucial role in the spindle assembly checkpoint (SAC). Phosphorylation at the second site (PY130) locates this population of dynein to the spindle pole, but the function of PY130 is not known. To test if PY130 is the same population of kinetochore dynein that is cycling in the SAC, or if Y130 is a distinct population that functions at the spindle pole, we inhibited kinetochore dynein regulation or perturbed the spindle pole and assayed for PY130 dynein localization. Disruption of kinetochore dynein with taxol or calyculin A resulted in retention of PT89 at the kinetochore but did not have an impact on the localization of PY130 at the spindle poles. Based on colocalization of PY130 with NuMa, an essential spindle pole protein, we used a mutant NuMa construct to disrupt the spindle pole. This assay showed a disrupted spindle and the absence of PY130 dynein at the spindle pole. The results suggest site-specific phosphorylation of dynein intermediate chains in targeting of dynein to different loci in the cell. They also suggest that phospho-regulation is crucial in successful cell division.

Investigation of the role of LIF in activation of the regeneration pathway in the zebrafish (*Danio rerio)* retina

Lauren R. Lilley, Travis J. Bailey, and David R. Hyde*
University of Notre Dame, Notre Dame, IN 46656

Light-induced photoreceptor apoptosis and regeneration has been characterized in the adult zebrafish retina (Vihtelic and Hyde 2000). However, little is known about the signals that initiate the proliferation of Müller glia, the source of neuronal progenitors. This study investigated the ability of LIF to induce proliferation of Müller glia. Three injections of LIF at .25 ug/uL, .5 ug/uL, and 1 ug/uL were made over 72 hours. Eyes were harvested at 24 and 72 hours post injection. PCNA immunofluroescence indicates that LIF is sufficient to elicit INL proliferation at 72 hours post injection. Cell counts suggest that 1 ug/uL LIF and CNTF (previously characterized; Kassen *et al.,* unpublished) produce similar amounts of PCNA-positive cells in the INL (ANOVA, α=0.05) at 72 hours post injection. These results implicate LIF as a putative initiator of regeneration and merit further investigation into the role of LIF and its gp130 signaling in the regeneration pathway.

THE ROLE OF AURORA B IN DYNEIN RECRUITMENT TO THE KINETECHORE

Meghan Morrissey, Jamie Kasuboski, Dr Kevin Vaughan*, University of Notre Dame

Accurate chromosome segregation, regulated by the Spindle Assembly Checkpoint (SAC) is an essential function of cell division and defects in the process have tragic consequences, including birth defects and cancer. Proper recruitment of kinetochore dynein is important both to resist anaphase onset during prometaphase and for silencing the SAC once the chromosomes are properly aligned. The transition in dynein function is controlled by dynein dephosphorylation by the phosphatase PP1g. Because PP1g often dephosphorylates substrates of Aurora B, Aurora B may be the kinase responsible for recruiting dynein to the kinetochore. To test this hypothesis, we used the Aurora B inhibitor ZM447439 or kinase-dead Aurora B constructs and monitored the levels of dynein at the kinetochore. Although Aurora B inhibition did not reduce the total amount of phosphorylated dynein, it did reduce the ability of the kinetochores to recruit phosphorylated dynein. Further assays revealed that the entire scaffold of proteins believed to recruit dynein to the kinetochore, as well as the checkpoint protein BubR1, were absent from the kinetochore after Aurora B inhibition. Consistent with this effect, treated cells displayed lagging chromosomes and premature anaphase onset. These findings suggest that the Aurora B kinase is essential for proper functioning of the SAC, acting on dynein indirectly through other kinetochore proteins, and is also important for the assembly of the kinetochore-microtubule interface during mitosis.

Neuronal Targeting of Cytoplasmic Dynein Through Isoform-Specific Phosphorylation.

Brian O'Rourke, University of Notre Dame, Kevin T. Vaughan* University of Notre Dame

Dynein is a large multi-subunit motor protein composed of catalytic heavy chains (HCs) and a series of intermediate chains (ICs), light intermediate chains (LICs) and light chains (LCs) that are thought to mediate cargo-binding. Uniquely in neurons, the IC subunits undergo alternative splicing of neuron-specific (IC1) and ubiquitously expressed (IC2) gene products giving rise to six protein isoforms that differ only by the alternative splicing of two coding exons. MS/MS mapping of neuronal phosphorylation sites reveals differential phosphorylation of each isoform in the cargo-binding domains of the protein. We tested the hypothesis that neuronal IC phosphorylation mediates binding to neuron-specific cargos involved in axonal transport. Consistent with previous work, optimized blot overlay assays we revealed dephosphorylated ICs bound to p150 in total neuronal extracts, identifying dynactin as the dominant binding partner for dephosphorylated dynein. To generate phosphorylated ICs, we performed in vitro phosphorylation reactions with recombinant ICs and purified PKCu. Although phosphorylation activity was evident in these reactions, we were unable to stimulate sufficient levels of phosphorylation in vitro for biochemical analysis. Because the second site of interest fits the consensus for p38MAPK, we performed in vitro phosphorylation reactions with four isoforms of p38MAPK. Blot overlay assays with the samples phosphorylated by p38MAPK displayed an intriguing difference in binding to neuronal samples. Phosphorylated ICs displayed reduced binding to the p150Glued protein but enhanced binding to a new protein with a Mw of ~65kD. Together, these results suggest that phosphorylation of the neuron-specific pair of residues might not be mediated by PKCu. However, p38MAPK-based phosphorylation of the ICs could mediate binding of the dynein ICs to a novel binding partner in neurons. This would support the hypothesis that phosphorylation drives isoform-specific binding of dynein to cargo in neurons.

Characterization of *olig1*, *ngn1,* and *pax6a* in adult zebrafish retinal regeneration.

Robert Plasschaert, University of Notre Dame, Sean Kassen, David Hyde*, University of Notre Dame

The persistent cell proliferation in the zebrafish (*Danio rerio)* retina makes it a useful model to study retinal regeneration. Our recent microarray analysis has implicated several transcription factors that might participate in the regenerational pathway. I tested several polyclonal antisera for their potential to characterize the Olig1, Ngn1, and Pax6a proteins during regeneration of the light-damaged adult retina. Full length *olig1*, *ngn1*, and *pax6a* sequence were individually cloned into the pET-32a expression vector to generate S-tag fusion proteins which were subsequently used to purify the corresponding polyclonal anti-sera. Antiserum specificity was determined through injecting anti-*olig1*, *ngn1*, or *pax6a* morpholinos into early stage zebrafish embryos and using immunoblotting techniques to determine successful protein knockdown. The antisera were used to determine the spatial and temporal expression patterns of Olig1, Ngn1, and Pax6a during retinal regeneration. This data further suggests these transcription factors regulate critical molecular processes during zebrafish retinal regeneration.

Determining the inhibitor of phagocytosis in the retinal regeneration pathway of *Danio rerio.*
Rachel Slaughter, Pensacola Christian College, Dr. David Hyde*, Dr. Travis Bailey, University of Notre Dame

Retinal cells of the zebrafish die following light damage, but are regenerated by Müller glial cells, differentiated cells that can be triggered to act as stem cells. The portions of the regeneration pathway observed suggest that apoptosis and phagocytosis precede regeneration of photoreceptors. Inhibiting apoptosis or phagocytosis limits regeneration of photoreceptors. The signal for apoptosis is supposedly phosphatidylserine (PtdSer); PtdSer is exposed on the surface of the apoptotic cell. This exposure signals Müller glial cells to phagocytose the apoptotic cells and divide to produce two neuronal progenitor cells. One progenitor cell migrates to the ONL to replace the phagocytosed cell while the other progenitor cell remains in the INL to form a Müller glial cell. To determine the mechanism of the signaling, the retinal cells of the zebrafish were light-damaged after injecting the vitreous humor with various inhibitors of the phagocytosis to analyze their effects on regeneration.

THE RECRUITMENT AND LOCALIZATION OF TYPE 1 PI 3-KINASE GAMMA FOLLOWING PHAGOCYTOSIS OF *M. BOVI'*S BCG
Stephanie Vivanco, Loyola Marymount University, Dr. Jeffrey Schorey*, Chris Lemaire, University of Notre Dame

M. tuberculosis is an intracellular pathogen which resides primarily inside macrophages within an infected host and can modulate the cell signaling processes that occur in the macrophage upon mycobacterial infections. Mechanisms used to suppress macrophage activations include limiting production of immune effector molecules such as cytokines, chemokines and reactive nitrogen intermediates. The PI 3-kinase plays a significant role in the macrophage because it consists of signaling molecules that are involved in regulating cellular proliferation and survival especially when dealing with an invading microorganism. Previous studies in the Schorey laboratory have indicated that the PI-3 kinase is activated in macrophages upon mycobacterial infection but to differing extent with significantly less activation following infection with *M. tuberculosis*. The main objective of this work is to determine the recruitment and localization of the signaling molecule, Type 1 PI 3-kinase γ following phagocytosis of *M. bovis* BCG (a close relative of *M. tuberculosis* but less pathogenic). Results are pending but will be presented at the Michigan Symposium.

HedgehogF4 expression in Drosophila lymph gland development and haematopoietic precursors

Alisha Wainwright, University of Florida
Dr. Schulz* Jessica Stoller, The University of Notre Dame

The blood cells of Drosophila melanogaster are a prime model for research of hematopoiesis and microenvironment niche development. Hematopoiesis of blood cell precursors occur within the posterior signaling center (PSC) in the lymph glands where the hedgehog molecule plays a significant role in niche development evidenced by its presence early in the PSC's formation. Hedgehog is a transcriptional enhancer that aids in the management and development of the niche, and further exploration of the gene will give incite to blood stem cell development and maintenance. The hedgehog gene section hhF4 expressed green florescent protein within the PSC, so that region of the gene is significant. Hedgehog was sectioned into six segments to find the location along the gene responsible for transcribing and managing the blood precursor niche. Functional constructs were developed and injected into Drosophila white flies. Such research is pivotal for blood stem cell research.

The role of microtubule binding of p150^{Glued} during mitosis in mammalian cells

Michael Werner, Stetson University, Maury Raycroft, University of Notre Dame, Kevin T. Vaughan,* University of Notre Dame

Dynactin is a large multi-subunit protein complex that binds to both the motor protein dynein and MTs through the same p150^{Glued} subunit. In this study, we tested if MT binding was required for MT-kinetochore interactions during mitosis. We utilized a shRNA/rescue scheme to deplete cells of endogenous p150^{Glued} coupled with GFP-tagged rescue constructs of either wild-type (WT), a G59S mutation that lacks MT binding, or a deletion mutation that displays impaired MT binding. The WT construct displayed complete rescue. In contrast, although both mutants rescued initial MT-kinetochore contact and chromosome alignment, most failed to form a metaphase plate and never reached anaphase onset. Further, the small percent that completed mitosis displayed delayed alignment and drifting away of chromosomes upon anaphase onset. Together these results suggest a role for MT binding of p150^{Glued} in dynein related chromosome function, MT anchoring at the kinetochore, and the spindle assembly checkpoint.