Project Descriptions

1**. Neuropeptide Modulation of Vertebrate Behaviors.**
Project Advisor: Sunny Boyd
Introduction: The long-term objective of this research program is to identify the interactions among chemical messengers that control behaviors. Neuropeptides and steroid hormones alter a variety of vertebrate behaviors, including parental, aggressive, and reproductive behaviors. The mechanisms of action of these compounds and the site in the brain where they act on specific behaviors are poorly understood. We currently focus on the neurohypophysical peptides which modulate the display of vocalizations in vertebrates. Vocal behavior is a critical component in social interactions of many species, including humans.
Research Projects: Student projects will be designed to provide experience at both the whole-animal behavior level and also at the cellular and molecular level of investigation. Students will thus (1) analyze the effects of peptides and steroids on animal behavior, (2) localize peptides, steroids and their receptors in the brain, and/or (3) sequence genes involved in the synthesis of these factors or their receptors.
What the Student will Learn: Students will learn a variety of basic techniques in behavioral neuroendocrinology, including: observation of animal behavior, survival surgeries, brain sectioning, neuroanatomy, immunocytochemistry, confocal microscopy, receptor binding techniques, PCR, Northerns, and *in situ* hybridization. Through development of their own research projects, students will also learn literature analysis, experimental design, data analysis and data presentation.

2. **Subzero Temperature Adaptations Antifreeze Proteins**
Project Advisor: John G. Duman
Introduction: My research concerns the physiological and biochemical adaptations of poikilothermic organisms to subzero temperature. Most studies are with insects and plants, but other organisms (including spiders and other terrestrial invertebrates, fungi and bacteria) are also under investigation. These organisms adapt to subzero winter temperatures by either becoming freeze tolerant (able to survive extracellular freezing) or freeze avoiding. Freeze avoiding species generally produce antifreezes, such as polyols (glycerol, etc) and/or antifreeze proteins. We have been particularly concerned with the antifreeze proteins and our studies have ranged from investigations of the function of the proteins, to hormonal and environmental cues controlling their production, to protein chemistry and molecular biology designed to determine the structure - antifreeze function relationships of the proteins. Studies with freeze tolerant organisms have concentrated on ice nucleating proteins which function to induce ice formation in the extracellular fluid at high subzero temperature, and also on antifreeze proteins. In addition to functioning as antifreezes in freeze avoiding organisms, antifreeze proteins appear to function in certain freeze tolerant organisms as cryoprotectants to inhibit the damage resulting from freezing of body water. The mechanism of this process is under investigation.
Research Projects: Current ongoing research includes: (1) structure/function relationships of insect, plant and bacterial antifreeze proteins; (2) the cloning and expression of antifreeze protein genes; (3) potential cryoprotection mechanisms of

antifreeze proteins; (4) applied studies on the potential uses of antifreeze proteins in agriculture (i.e., transgenic plants which produce insect antifreeze proteins) and for the cryopreservation of biological materials; and (5) studies of antifreeze proteins in Alaskan insects. However, within the broad theme of low temperature adaptations a wide variety of student projects may be accommodated. These may range from physiological ecology to protein biochemistry.
What the Student will Learn: Depending on the project students will learn protein purification and characterization, including protein/protein interactions; low temperature physiology and biochemistry; cryoprotective techniques; gene cloning, sequencing, microarray, and other molecular techniques.

3. **Baculovirus recombinants capable of simultaneous expression of multiple gene products. Project Advisor: Malcolm Fraser**
Introduction: The insect pathogenic Baculoviruses have been adapted for high efficiency recombinant protein production in infected insect cells and larvae. Multi-gene proein complexes such as antibodies or human pathogenic virus structures can be produced by co-infecting multiple Baculovirus recombinants, but increased efficiency of multi-gene products could be attained if all genes were expressed from a single baculovirus recombinant. We are constructing a multi-gene Baculovirus expression vectors that could be used for efficient expression of multi-gene products by adapting several non-essential regions of the virus to recombinant protein expression.
Research Projects: The student will begin by constructing and expanding novel Baculovirus shuttle vectors containing the gene of interest. These vectors will be used for transfection of insect cells in culture, along with Baculovirus DNAs, to generate recombinant Baculoviruses. Recombinants will be identified using flourescent protein markers and cloned. Southern and PCR analyses will confirm the structuer of the desired gene in the recombinant virus. Recombinant-infected cells will be analyzed for the expressed protein products.
What the Student will Learn: The student will gain experience in cloning and characterization of recombinant DNA molecules, working with animal viruses in cell culture, and the genetic and molecular characterization of DNA viruses. Subsequent analyses of protein products produced by the generated recombinants will complete the training. This will provide a useful survey of recombinant DNA and protein technologies that are standard practice in molecular genetics laboratories.

4. **Protein Trafficking Across Plastid Membranes.**
Project Advisor: Kristin Hager

Introduction: Our lab utilizes state of the art techniques such as time lapse video microscopy and advanced genetic analysis to study interactions between the parasite and host cell in order to answer this question. We believe that the protozoan parasite, *Toxoplasma gondii,* is spectacularly successful due to its ability to secrete proteins that allow it to interact with virtually *any* nucleated host cell during invasion and intracellular survival. A key step in protein secretion is the organisms' ability to

synthesize and properly target these invasion/maintenance proteins to their respective organelles. Our laboratory is interested in dissecting the central steps involved in these phenomena and in general are interested in intracellular trafficking of proteins in protozoan parasites. Our current model system is *T. gondii.* It is an obligate intracellular parasite normally controlled by an active immune system. Unfortunately, there has been an alarming rise in the number of immunosupressed individuals such as HIV patients. Congenital toxoplasmosis is a major source of neurological birth defects. The early secretory pathway has several compelling features. Our laboratory is interested in events that occur between the endoplasmic reticulum (ER) and Golgi. In *T. gondii* , the apical end of the nuclear envelope appears to be the sole site of intense vesicle trafficking and recycling of proteins between the Golgi and ER (see Figure 1 and 1b) (Hager et al. 1999). It is likely that this region represents the organisms 'Achilles heel'; a bottle-neck through which all secretory proteins must process. The long-term goal of my lab is to determine what genes are expressed at this site, and characterize their role in regulating the trafficking of the specialized 'invasion/maintenance' proteins.

Research Project: Projects currently in progress are investigation of: the molecular and biochemical mechanisms of ER-Golgi recycling proteins using the receptor, *Tg* ERD2, investigation of the nature of these protein interactions with other components of the secretory apparatus in *T. gondii* by identifying specific vesicular coat proteins (COPI) such as *Tg* b COP, and determining the essential nature of the recycling genes for parasite viability. To achieve these ends, a variety of cell biological, molecular and genetic approaches will be used to characterize the recycling-specialized protein interactions.

What the student will learn: The student will be provided an excellent opportunity to participate on a research project which will teach them skills in a 'wet lab' setting such as molecular biology skills and 'dry lab' setting such as computational approaches. Our computational approaches for understanding protein function will be based on two search methods – weighted profile Hidden Markov Models (HMMs) and the FAST_PAN tool for integrated multiple query similarity searching. Both methods utilize information from multiple query sequences to attain high levels of sensitivity and discrimination in detecting novel and remote homologs within gene superfamilies. Students will acquire molecular biology skills with cloning on of a fluorescent tag, followed by cell biology skills of using a state of the art motorized microscope with video camera and fluorescent imaging capabilities. Lastly, students will gain a basic understanding of how proteins move within a cell and why this type of 'fundamental concept' is ultimately important for understanding life within plant cells and cells possessing plastid-like organelles.

5. **Genetic expression in butterflies under climate change.**
Project Advisor: Jessica Hellmann

*Introduction:*The geographic range limits of many species are strongly affected by climate and are expected to change under global warming. For species that are able to track changing climate over broad geographic areas, we expect to see shifts in species' distributions toward the poles and away from the equator. A number of ecological and evolutionary factors, however, could restrict this shifting process. These factors include

habitat loss and fragmentation, dispersal limitation, and our particular interest, local adaptation. We study whether populations at the northern edge of a species' range are locally adapted to the climates that occur there or if they prefer climates characteristic of the range core. These two scenarios could lead to very different outcomes under climate change with possible declines in the former and likely range shifts in the later. We pursue our research using two butterfly species that reach their northern range limit on Vancouver Island, British Columbia.

Research Projects: The student will play a key role in rearing caterpillars of our two focal butterfly species in growth chambers. These individuals are collected in the field and then exposed to a variety of conditions at Notre Dame. We will sample caterpillars at a variety of stages of development and extract their mRNA to determine which genes are expressed during larval development. These expressed proteins then will be sequenced, ultimately to generate microarrays for use in subsequent climate experiments.

What students will learn: The student will gain an appreciation for organismal biology and genetic techniques. The student will learn how to operate environmental chambers and care for living organisms. The student also will interact with graduate students, providing an opportunity to see she/he wishes to pursue graduate study.

6. **The Genetic and Developmental Basis of Divergence in *Drosophila***
Project Advisor: Hope Hollocher

Introduction: My lab studies all aspects of speciation, everything from the evolution of reproductive incompatibilities to morphological differentiation. We are particularly interested in determining the genetic and developmental changes responsible for differences we observe between species to reveal general patterns underlying speciation processes. Are certain traits more prone to change during speciation than others? If so, why? Do differences between species mostly arise from changes in regulatory sequences? These are a few of the many questions we aim to answer in our research.

Research Projects: We have two ongoing research projects in the lab. The first focuses on the evolution of hybrid incompatibilities in African *Drosophila* (specifically, *Drosophila melanogaster* and its sister species). Using the arsenal of genetic tools available in *D. melanogaster*, we are working to identify which genes are involved in disrupting germ line development in hybrids. In addition, we have started to use microarrays to assay gene expression profiles of pure species and hybrids to investigate how the genetic cascades affecting germ line development are altered during speciation. The second project focuses on the evolution of abdominal pigmentation between different species of the *Drosophila cardini* group, which inhabit the Caribbean Islands and nearby mainland of Central and South America. Here, we are analyzing patterns of sequence variation from multiple genes involved in pigmentation to determine how these patterns correspond to changes in the developmental control of melanin synthesis and deposition in the different species.

What the Student Will Learn: Students will have the opportunity to learn a variety of molecular techniques (e.g. DNA extraction, DNA amplification, cloning, and sequencing) as well as population genetic analyses used to interpret these molecular data. In addition, students will be introduced to Drosophila identification, dissection, tissue preparation,

antibody staining and microscopy used in developmental research. Students are also instructed on how to keep a laboratory notebook, troubleshoot, and design experiments to test specific hypotheses. All students are encouraged to read and discuss the primary literature associated with their research and are given the flexibility to pursue their own lines of inquiry directed toward the overall research goals of the laboratory.

7. **Molecular Genetic Analysis of Zebrafish Eye Development and Retinal Regeneration. Project Advisor: David R. Hyde**

Introduction: My lab studies the mechanisms involved in eye development (both retina and lens) in zebrafish. Eye development proceeds very quickly, with a functional eye (based on behavior and electrophysiology) present within 72 hours after fertilization of the egg. Because the embryo develops external to the mother and the embryo is translucent, it is relatively straightforward to follow many of the developmental events using non-invasive microscopy. The advanced state of the zebrafish genome project, the ability to reduce the expression of embryonic genes using morpholinos and RNAi, and the panel of antibodies that my lab has generated, it is relatively straightforward to observe and perturb in precise ways the development of the functional eye. Using microarray experiments, we have identified a number of interesting genes that are candidates for being essential in lens and retinal development. We have also developed methods to kill specific neuronal populations in the retina and observe their regeneration. Microarray experiments have identified that a number of the genes differentially expressed in neuronal regeneration correspond to genes that are likely to be important in early retinal development.

Research Project: Regardless of the project that the student selects, the student and I will devise experiments that incorporate molecular and genetic techniques to examine one or two genes. This analysis may include cloning a candidate gene or promoter, DNA sequencing, in situ hybridization to examine the expression pattern of the candidate gene and morpholinos to knock-down the expression of the candidate protein. The student will then use a combination of histology, immunohistochemistry, and in situ hybridization to examine the effects that result from the loss of the candidate protein. We will then examine the potential role of the candidate protein in a biological process (such as eye development or retinal regeneration) by studying the process in wild-type and morpholino-injected embryos, which will again require histology, immunohistochemistry, and in situ hybridization. Thus, the student will gain an appreciation of the relationship between gene-protein-function-role in a biological process.

What the Student Will Learn: The student will be exposed to modern techniques in molecular genetics, including the generation and analysis of transgenic zebrafish, generation and analysis of transient mutants using RNAi and morpholinos, cloning of genes and analysis of their promoters, immunohistochemistry to examine cell-specific expression of proteins. They will be exposed to experimental design and the use of stringent controls, interpretation of data, and assembling the data into a presentation format (manuscripts or meeting posters). The students will also have the opportunity to see how their molecular genetic analysis relates to the physiological and cell biological studies that are being performed by graduate students and postdocs in the lab.

8. **Ovarian Follicle Selection and Differentiation; Ovarian Granulosa Cell Tumors. Project Advisor: Alan Johnson**

Introduction: This laboratory focuses upon two distinct, but related, lines of research: 1) viability and differentiation of ovarian granulosa cells during normal follicle growth and development; and 2) the etiology of granulosa cell tumors. Emphasis is placed upon comparing processes that regulate normal differentiation and cell viability *versus* cell death (via apoptosis) in primary cultured granulosa cells, to those processes that characterize transformed cells derived from a tumor origin. Our long-term goals are directed towards defining differences and similarities in granulosa cell function between these two model systems as a means to expand our understanding follicle development and ovarian function in amniotes.

Research Projects: Projects are developed according to the student's interests, and may incorporate the use of primary cells and/or continuous cell lines. Each project is hypothesis-based and emphasizes experimental design and execution, followed by in-depth analysis and summarization of research data.

What students will learn: Students will develop hands-on expertise with a variety of cellular and molecular techniques tailored to address each unique research question. We routinely utilize techniques that aid in identifying genes (polymerase chain reaction, rapid amplification of cDNA ends, nucleic acid sequencing), quantifying RNA (northern blots), characterizing protein expression (Western blots, confocal microscopy), and monitoring activity of cell signaling pathways involved in cell viability (apoptotic pathways) and cell differentiation (hormone assays) in cultures of primary and continuous cell lines. Students are ultimately encouraged to integrate information derived at a cellular level as a means to better understand ovarian physiology.

9. **Modulatory Effect of Intrinsic and Environmental Cues on Circadian Rhythms of Visual Sensitivity. Project Advisor: Lei Li**

Introduction: One of our research projects is to study intrinsic and environmental cues that play a role in the regulation of circadian rhythms of visual system functions. Like humans, the bahavioral visual sensitivity of zebrafish (our study model) fluctuates between day and night. They are most sensitivity in the late afternoon and least sensitive in the early morning. A number of factors, such as daily light-dark cycles, vitreal dopamine and the input from olfactory bulbs may affect the circadian rhythms of visual sensitivity. We are interested in understanding how that happens.

Research Project: To investigate mechanisms underlying multiphasic opsin gene expression in rod photoreceptor cells in transgenic zebrafish.

What the Student Will Learn: You will be exposed to modern bio-techniques, such as time lapse imaging, immunochemistry and molecular biology. You will learn how to design and perform experiments, how to interpret the data, and how to assemble the data into a presentation format, i.e., research articles or meeting posters.

10. **Utilization and Metabolism of Vitamin A in Insects**
Project Advisor: Joseph E. O'Tousa

Introduction: Most animals cannot synthesize Vitamin A and so must obtain it directly or indirectly from plant sources. In insects, vitamin A is used only in vision where it serves the role of the chromophore for all the visual pigments. However, different chemical

forms of retinal are used by different insects and there are dramatic differences in the ability of different species to store these compounds. This project will define the chemical composition of the retinal stored in four representative insects from different orders by quantitative HPLC, and use genetic and genome information to determine how specific genes are involved in vitamin A utilization.
Research Projects: The student will execute one or more of the following projects: 1. Extraction and HPLC analysis of retinoid and carotenoid compounds from different tissues of the following insects: house fly, honey bee, beetle, moth. 2. Test the ability of specific Vitamin A derivatives to act in formation of visual pigments in the *Drosophila* model. 3. Identify the *Drosophila* transporters and retinoid binding proteins responsible for movement of various forms of vitamin A to the retina. 4. Characterize the relationships the vitamin A transporters from other insects using genomic sequence information.
What the Student will Learn: The student will produce novel information on the process by which vitamin compounds are extracted from diet and used productively by different animals. They will gain an appreciation of the benefits associated with the use of *Drosophila melanogaster* genetic and molecular analyses in addressing current questions in basic insect biology. Finally they will see the rationale for, and insights to be gained from, comparative analysis of genomic information.

11. Mycobacteria
Project Advisor: Jeffrey Schorey

Mycobacteria have a long history as pathogenic organisms and are the etiological agents of such well known diseases as tuberculosis and leprosy. Tuberculosis is a particularly deadly disease accounting for over 2 million deaths annually and is the 2nd leading cause of death due to an infectious organism. A further concern in recent years has been the dramatic increase in the number of individuals infected with multi-drug resistant strains of Mycobacterium tuberculosis. Other pathogenic mycobacteria include M. avium, one of the most common opportunistic pathogen in AIDS patients within the United States and M. Leprae, the causative agent of leprosy.
My lab focuses on the interaction between mycobacteria and its' host cell the macrophage. As intracellular pathogens, mycobacteria require invasion of macrophages for their survival. However, macrophages, which function as part of the innate immune system, also serve an essential role in controlling a mycobacterial infection. Interestingly, macrophages infected with pathogenic, relative to non-pathogenic mycobacteria, show limited production of inflammatory mediators (i.e. cytokines, chemokines, nitric oxide, etc.) which are required to control bacterial growth. However, the molecular mechanisms responsible for this difference in macrophage response is not well defined. Our studies have identified a number of macrophage-signaling pathways activated upon mycobacterial invasion including the mitogen activated protein kinases and have shown that production of inflammatory mediators are dependent on the activation of these pathways. Moreover, we have determined that macrophages infected with pathogenic M. avium and M. tuberculosis strains show only limited activation of these signaling systems. Studies are ongoing to further characterize the macrophage signaling molecules activated upon mycobacterial infection, how these responses differ upon infection with pathogenic and non-pathogenic mycobacteria and to characterize the

mycobacterial components which initiate or inhibit these macrophage signals. We are particularly interested in studying the importance of glycopeptidolipids (a major surface component of M. avium) in modulating macrophage-signaling responses and in mycobacterial pathogenesis.

12. **Genetic Models in Drosophila for Human Disease**
Project Advisor: Robert Schulz

My research involves using the fruit fly *Drosophila melanogaster* to generate and study genetic models for human disease, especially as they relate to abnormalities in heart, blood cell, and muscle development. *Drosophila* is a superb model organism for these investigations because of its short generation time, the numerous experimental approaches available, the known DNA sequence of its genome, and the wealth of genetic and information resources on hand and emerging. Studying problems in *Drosophila* development has clear ramifications for our understanding of human development and disease due to the substantial conservation of genes between the two species. That is, greater than 60% of all *Drosophila* genes have homologues in humans, including many genes known to be causal of or associated with specific diseases. Research projects ongoing in my lab include identification and analysis of (1) genes required for heart formation, relevant to the study of congenital heart disease in humans, (2) genes controlling blood cell development, with relevance to our understanding of human leukemias, and (3) signaling pathways controlling indirect flight muscle formation and function, relevant to the understanding of certain human muscular dystrophies. Genetic, developmental, and cell biological approaches are used in these analyses.

13. **Regulation of Microtubule-Based Membrane Transport**
Project Advisor: Kevin T. Vaughan
Introduction: The movement of membranes, chromosomes and organelles in the cell is deliberate and highly orchestrated. Most of this movement is mediated by a novel class of "molecular motor" proteins which bind to these cargo types and carry them to their destination along cytoskeletal filaments. At the end of transport, the motors release the cargo and reset for a new round of transport. The precise delivery of membranes and chromosomes to the correct destination at the correct time is tightly regulated. The interests of the laboratory are centered on how motors identify the correct cargo, and how motor function is regulated by phosphorylation. The motor protein we are focused on currently is the microtubule-based motor cytoplasmic dynein. This motor is responsible for many aspects of membrane transport and chromosome segregation during mitosis. We are working currently on several cytoplasmic dynein and dynactin subunits whose functions are regulated by phosphorylation. We are also working on how the dynactin complex functions as a dynein receptor on chromosomes and organelles. Recently, we delineated the chain of events which lead to motor protein loading and the onset of motility. Our lab integrates the use of conventional cell biology approaches (advanced quantitative imaging, biochemistry, DNA cloning and mutant analysis and live cell analysis) with advanced proteomic methods (mass spectrometry), functional genomics (intelligent design chimeras) and kinomics (signal transduction complexity). These cutting edge approaches provide students with training in multi-dimensional dissection of

complex and highly integrated systems.
Research Project: Students can pursue one of several projects which include: 1) analysis of protein kinase function by drug treatment and analysis of proteins with mutated phosphoryation sites, 2) live-cell imaging analysis of cells lines expressing GFP-tagged cell surface adhesion molecules.
What the student will learn: Each project will incorporate a mixture of advanced molecular biology (RT-PCT, cloning, etc.), immunocytochemistry and immunofluorescence microscopy, live-cell imaging, biochemistry of culture cell lines, and sophisticated software analysis. The particular project will be tailored to the interests and goals of the student.

14**. Comparative Analysis of Photoreceptor Cell Types in the Compound Eyes of Insects. Project Advisor: Michelle Whaley**
Introduction: Analysis of the genome content of many different organisms now allows comparative studies into the expression of genes in particular tissues and cell types. Members of the rhodopsin family of visual pigments are expressed in subsets of photoreceptor cells to tune the photoreceptor to colors of light. Analysis of this gene family in many insects, including ants, butterflies, fruit flies and mosquitoes has shown a wide scope of the type and number of these genes in different genomes. In this project we seek to understand the cellular architecture of the mosquito compound eye relative to the expression of these individual genes.
Research Projects: The student will play a key role in one or more of the following projects: 1. Gene cloning work to prepare gene expression constructs to allow visual pigments found in mosquitoes to be expressed and characterized in *Drosophila melanogaster*, 2. *Drosophila* genetic crosses and mating schemes to characterize transgenic strains and place transgenes in required genetic backgrounds, 3. histological examination of the retinal organization in mosquitoes, providing description of the cellular anatomy and the cells expressing particular visual pigments.
What the Student will Learn: The student will gain an appreciation of the similarities and differences in genome structures of different insect species, and how these are manifested in differences in the development and organization of the insect retina. The student will be exposed to hypotheses on how these adaptations benefit the behavior and life cycle strategies of various species. The student will also learn genetic and molecular analysis in the widely used model organism *Drosophila melanogaster.*

15. Genetics and Genomics of Drug Resistance and Virulence in the Malaria Parasite. Project Advisor: Michael Ferdig
Malaria is flourishing in the form of drug-resistant parasites and insecticide-resistant mosquitoes. The complexity of Plasmodium parasites' life cycle and biology renders them elusive to drugs and vaccines. Currently, 40% of the global population is at risk for the disease. The nearly completed P. falciparum genome sequence, along with integrated, analytical tools, offers fresh hope for gene discovery and identification of novel control strategies. My lab is using methods to overlay critical biological processes on whole-genome data to bridge the gap between critical phenotypes, like drug resistance and virulence, and their underlying gene mutations, with the long-range goal of elucidating

new avenues of malaria intervention. We are focused on identifying genes that confer complex P. falciparum traits, specifically, susceptibility to antimalarial compounds and parasite proliferation in red blood cells (RBC). To do this, we study inheritance patterns of precisely measured phenotypes and high-resolution microsatellite markers to identify the genetic regions carrying genes that direct these traits' expressions. Such quantitative trait loci (QTL) profiles act as "biological filters" of massive sequencing and transcriptional databases emerging from the genome project. In this way a biological framework can be imposed on the data to narrow the search window and to pinpoint specific genes, gene interactions, pathways and transcriptional networks that drive drug responses and parasite growth.

16. **The molecular basis of the mammalian circadian clock**
Project Advisor: Giles Duffield

Circadian ('about a day') rhythms are an integral component of biochemistry, physiology and behavior. Circadian clock biology is relevant to human health. Dysfunction of the circadian clock underlies several disease states, including Seasonal Affective Disorder, and sleep disorders. My lab is interested in elucidating the molecular basis of the circadian clock in mammals using a range of traditional and state of the art molecular, cellular and behavioral approaches.

The molecular circadian clock consists of an autoregulatory transcriptional-translational feedback loop composed of positive and negative regulators. Work over the last 8 years has identified 9 such components, but additional genes and modifiers are being identified. In addition, most tissues of the body harbor cell-autonomous circadian clocks. One such additional gene, identified in my laboratory using a cDNA microarray screen, is the transcriptional inhibitor Inhibitor of DNA-binding 2. It is rhythmically expressed in the master clock structure in the hypothalamic brain known as the suprachiasmatic nucleus (SCN), and throughout the body in various peripheral tissues (e.g. heart and liver). Current studies are to evaluate the role of genes such as Id2 in the organization of the central oscillator, and to identify novel molecules relevant to cellular clock function. We are also interested in understanding how light resets the molecular clock (input), and how the clock regulates down-stream clock-controlled genes (i.e. output, hands of the clock).

My lab is using continuous activity monitoring to identify behavioral phenotypes in transgenic mouse models (e.g. *Id2* knockout mice) that are maintained under a variety of photocycle conditions, and exposed to artificial time-zone changes and acute light/pharmacologic/behavioral treatments. Results thus far have revealed that in the absence of the *Id2* gene, mice adapt to large time-zone changes (e.g. mimicking a flight from Berlin to Los Angeles) more rapidly than wildtype individuals. We are also using real-time monitoring of clock gene expression in tissues and cells derived from transgenic mice that express Firefly luciferase in a rhythmic manner. We are using DNA microarray and real-time quantitative RT-PCR analyses to identify and characterize clock regulated genes, tissue culture of immortalized fibroblasts that exhibit circadian oscillations in gene expression as a model of the *in vivo* rodent circadian clock, and traditional neuroanatomical techniques (e.g. *in situ* hybridization, immunohistochemistry, neuronal track tracing) to characterize clock gene function in the brain. These studies have been supported by the Royal Society, the Wellcome Trust and the NIMH.

17. **Membrane Trafficking, Cytoskeletal Remodeling and Signal Integration**
Project Advisor: Crislyn D'Souza Schorey

Well-defined changes in cell morphology accompany an array of cellular activities such as epithelial to mesenchymal transitions, cell migration and invasion, neuronal growth cone remodeling, phagocytosis and cell cycle progression. All of the above entail coordinated changes in membrane and cytoskeletal architecture. Signaling pathways that govern these processes can influence the cell's ability to undergo morphological changes in response to intracellular and extracellular signals. Proteins of the ARF and RHO families of the RAS superfamily of GTPases, via directed effects on membrane traffic and actin remodeling, can impinge on a variety of cellular responses that involve rapid and progressive changes in cell shape and motility. Research in our laboratory aims at investigating how signal transduction mediated by the ARF and RHO GTPases regulate the following cellular processes.

Cell adhesion and migration: A major focus of our laboratory is to investigate how extracellular and oncogenic signals through coordinated changes in membrane traffic and the actin cytoskeleton govern cell-cell adhesion and cell motility. Our efforts are directed at defining the signaling pathways and molecular mechanisms that underlie the acquisition of a migratory / invasive phenotype in normal and tumor cells.

Cell cycle progression: We are investigating the regulation of morphological changes which occur as cells progress through the cell cycle. Presently we are exploring the role of ARF6 and the regulation of membrane remodeling processes during cytokinesis.

Protein aggregation: We are interested in understanding the cellular processes that lead to protein aggregation in neurodegenerative disorders, such as Huntington's disease (HD). Our current goals are to elucidate the cellular machinery requisite for mutant huntingtin aggregation as well as cytoplasmic alterations that may lead to neurodegeneration in HD.