Compensatory Responses to Dopamine Loss

Dopamine (DA) is an important neurotransmitter/neuromodulator throughout the animal kingdom. In humans, dysfunction of the DA system leads to motor, cognitive, and affective disorders. Detailed studies feasible in the genetic model system Drosophila melanogaster permit circuit level definition of pathways responsible for specific behaviors, allow for detailed temporal/spatial control of DA synthesis, and have the potential to uncover novel neural mechanisms relevant to human diseases of DA dysfunction. Our model is particularly relevant to early stage Parkinson’s disease, where a poorly described pathway can partially compensate for decreased DA levels. This proposal focuses on the mechanisms of this compensation.

1) **Determine transcriptional responses to loss of brain dopamine and in a subline that has bypassed the need for dopamine.**

We will characterize transcriptome changes in response to models of both short- and long-term loss of brain dopamine, encompassing partial or total DA loss, using both pharmacologic and genetic approaches. Parallel studies will be performed in mouse brain to aid in detecting similarly regulated transcripts. In an effort to discover genes and pathways involved in compensation for loss of DA, transcripts will then be analyzed in flies showing partial suppression of the dopamine loss phenotypes. Further studies will look at transcriptome changes during the early stages of DA depletion to better model the process occurring during Parkinson’s disease.

2) **Determine the genetic basis of the DA bypass phenotype by bulk segregant analysis.**

Bulk segregant analyses, followed by whole genome sequencing, will be used to identify the quantitative trait loci (QTL’s) and the genetic lesions responsible for the suppression phenotypes. This technique, borrowed from population biology, is the most cost-effective means of identifying the responsible genes. Candidate genes identified by this technique will then be tested using classical (forward) and reverse genetic tools to identify those responsible for the suppression.

3) **Determine the neural basis of the DA bypass phenotypes.**

We will test whether other DA-dependent behaviors are suppressed in the locomotor DA bypass flies. We will determine whether aminergic neurons are required for DA bypass, and whether DA post-synaptic regions are required. In either case, follow up studies would further dissect the pathways using genetic tools directed at the relevant neurons. Candidate genes and pathways, as identified in the previous aims, will be checked for roles in suppression via RNAi based expression constructs, and using classical genetics when available. Similarly, genes identified from previous studies as modulating locomotor activity will be tested for complementation to determine whether they are critical for our model.

Dr. Hirsch previously supervised Karol Cichewicz, who has since enrolled in the PhD program in Biology at UVA and continues his research in the Hirsch laboratory.
The CRISPR/Cas9 system is a bacterial immune response system that has been adapted as an RNA-guided genome-editing tool. Due to its simple design and high efficiency, application areas for this exciting technology are ever expanding. Currently, the wild-type active Cas9 is being used in a wide range of organisms for targeted gene editing or for whole-genome genetic knockout screenings. In this project, we will use CRISPR technology to identify small molecule targetable essential genes and drugs targets deriving in vivo progression of Pancreatic ductal adenocarcinoma (PDAC). PDAC is associated with the shortest survival duration of any solid malignancy. Central to this poor outcome is the lack of effective chemotherapy. It is clear that the genetic and epigenetic diversity of PDACs have confounded the development and delivery of effective therapies. Furthermore, the progression of tumors to therapy resistance (often in a short period of time) has complicated the design of strategies to treat established disease. The focus of the project is to use CRISPR/Cas9 based targeted functional genetic knockout screening to identify druggable novel targets that govern the progression of human PDAC in vivo. In our screening strategy, will specifically target three groups of proteins; transcription factors, epigenetic modifiers and kinases to identify targets that will synergize with inhibitors of MEK signaling. The proposed idea of in vivo screening is highly novel and is based on complementary and transdisciplinary expertise of our team members. Dr. Adli has extensive experience in genomic and epigenomic technology development and their applications. He previously developed the Nano-ChIP-Seq technology (Nature Methods, 2010) and has recently established CRISPR/Cas9 technology in his lab at UVA (Nature Biotechnology, 2014). Dr. Adli has teamed up with Drs. Todd W. Bauer and Tom Parson who have developed and validated a unique patient derived xenograft (PDX) model of PDAC and identified several novel treatment strategies using this model. This proposal is applying the novel CRISPR/Cas9 technology to a unique in vivo human tumor model to identify genes with essential regulatory functions of pancreatic tumor progression and identify novel drug targets.

**Project 1** Use CRISPR/Cas9 gene knock-out technology to screen for human kinases, transcription factors and epigenetic regulators that are functionally antagonistic to MEK inhibition in patient-derived xenograft model of pancreatic cancer. We will use lentivirus to deliver Cas9 and individual sgRNAs that target the genes of interest. Human tumors will be infected and

**Figure 1:** CRISPR based genetic knock-out screening. Human tumors will be infected with pool of barcoded sgRNAs. The barcodes will be quantified in control vs xenograft tumors by deep sequencing.
sgRNA expressing cells will be transplanted into the pancreas of mice. Since sgRNAs are barcoded, we will then sequence genomic DNA from cells prior to transplantation and after PDX tumor formation to identify genes essential for in vivo tumor outgrowth (i.e. sgRNAs present after infection but missing after PDX formation). We will PCR amplify barcoded sgRNAs from the genomic DNA and perform high throughput sequencing to quantify enriched and depleted sgRNAs in the population relative to control group (Figure 1).

**Timeline:**
- Months 0-2: Finish lentivirus multiplicity of infection (MOI) optimizations.
- Months 2-8: Perform in vivo screening, DNA sequencing.
- Months 8-12: Computational data analysis and integration of results with existing genomic and epigenomic data.

**Additional Related projects:** In addition to PDX model, we have several other CRISPR screening projects currently going on in the lab. We are performing screening in Ovarian cancer, Breast cancer and glioblastoma cancers. These projects include both screenings as well as genomic and epigenomic profiling. Therefore, Although expertise with genomic and epigenomic technologies are not absolutely required, having these skills are highly recommended. In particular, Students who are interested and have computational skills with whole-genome data analysis tools are particularly encouraged to apply.

BACKGROUND:

The central dogma of molecular biology says that genes (DNA) are copied to RNA molecules that then give rise to proteins. RNA was considered only as the mold (template) for producing proteins. However, we now know that there are RNA molecules that have biological functions without coding for proteins. In the lab we study these types of RNA molecules, which are functional as RNA: microRNAs, tRFs deriving from tRNAs and highly heterogeneous group of long non coding RNAs (lncRNAs). We are pursuing various projects to show that these noncoding RNAs are important in normal development and cancer.

PROJECT:

Differentiation of muscle-specific stem cells (myoblasts) to myotubes occurs during vertebrate development, growth and after muscle injury. There are known transcriptional factors which play a role during the process, mainly muscle regulatory factors (MRFs): MyoD, myogenin, MRF4, and Myf5. Their expression levels change very specifically during the differentiation process, so each step of myogenesis is controlled appropriately. There are still many gaps in our understanding of regulatory mechanisms controlling MRFs expression during differentiation. Patients with chronic muscle diseases like myotonic dystrophy would benefit if we can alter the kinetics of proliferation or differentiation of the myoblasts in their skeletal muscle. Elderly persons with sarcopenia could be benefited similarly. Finally, malignancies of the skeletal muscle, rhabdomyosarcomas, are notorious for the failure of the malignant cells to differentiate, and so that molecules that can force differentiation of the cancer cells will be of immense interest. Noncoding RNAs have emerged as critical regulators of differentiation and the study of myoblast to myotube differentiation is important not only for understanding how stem cells differentiate, but also for medical reasons.

We have identified a group of long noncoding RNAs (lncRNAs) that are induced during differentiation of myoblasts. We want fully characterize these transcripts, establish their importance during myogenesis, and identify their mechanisms of action.

SPECIFIC AIMS:

Student will work with one of the candidate lncRNAs that are induced >10-fold during differentiation.

Aim 1: To characterize the transcript: length, number of exons, number of transcripts deriving from this locus, coding potential of the transcript (PCR-based methods, computational analysis). To test whether knock-down/overexpression of the lncRNA affects differentiation (work with muscle cell lines, deregulation IncRNA level by siRNA transfection, or by transfection and overexpression of exogenous IncRNA; isolation of RNA from affected cells and running RT-qPCR analysis; Western blotting and immunofluorescence for changes in myogenic proteins).

Aim 2: To find out the molecular mechanism of how the lncRNA works. To identify the interaction partners for the IncRNA whether they are proteins, other RNAs or genomic DNA sequences (using pull down methods: Chromatin immunoprecipitation, RNA immunoprecipitation).

REQUIRED SKILLS/KNOWLEDGE:

Student is expected to have strong understanding of the basics of molecular biology. Laboratory experience in culturing cell-lines will be desirable.

Dr. Anindya Dutta is supervising a current PhD student in the lab, Magda Cichewicz, who is working on the project (and is an alumna of this program).
Papers published from the laboratory on the topic are:


3) Sarkar S, Dey BK and Dutta A. MiR-322/424 and 503 are induced during muscle differentiation and promote cell-cycle quiescence and differentiation by downregulation of Cdc25A. Mol. Biol. Cell. 2010, 21:2138-49. PMC2893979


Jaideep Kapur, MD PhD Eugene Meyer III Professor of Neuroscience, & Professor of Neurology

Project: Progesterone regulation of excitatory transmission in

A. Specific Aims

Problem: Women constitute a majority of the patients with epilepsy, and many of them experience a cyclical exacerbation of seizures related to periodic changes in serum progesterone and estrogen levels during the menstrual cycle (catamenial epilepsy) (Herzog et al. 1997; Herzog et al. 2004; Quigg et al. 2008). Currently, there are no scientifically tested effective treatments for catamenial exacerbation. Notably, a multi-center placebo controlled trial of progesterone therapy, 200 mg taken three times a day for 14 days failed to show efficacy (Herzog et al. 2012). The proposed studies seek to understand the effect of prolonged progesterone exposure on excitatory and inhibitory synaptic transmission and on seizure frequency and intensity.

Progesterone is secreted by the corpus luteum following ovulation and is maintained at a high level for 7-8 days before declining at the end of the menstrual cycle, when seizure exacerbation occurs (Herzog, Klein, & Ransil 1997). Progesterone suppresses pentylenetetrazol-induced and maximal electroshock induced seizures or electrically kindled seizures in animals (Gangisetty and Reddy 2010; Maguire et al. 2005; Reddy et al. 2001; Smith et al. 1998). However, effects of chronic progesterone treatment on recurrent spontaneous seizures have not been studied. Furthermore, most studies investigated progesterone induced plasticity of GABA-A receptors (GABARs) (Maguire, Stell, Rafizadeh, & Mody 2005; Reddy 2009; Smith, Gong, Hsu, Markowitz, ffrench, JM, & Li 1998) because progesterone is converted to neurosteroids in the brain, which activate GABARs. However, the activation of GABARs inhibits the neuronal firing in networks, which could trigger feedback mechanisms to restore firing rates. In addition to actions through neurosteroids, progesterone activates progesterone nuclear receptors, which are widely expressed on neurons including principal neurons of the hippocampus. These receptors regulate gene and protein expression and could alter synapse strength. Progesterone receptor knockout mice are slow to develop kindling and do not maintain kindled state as well as control mice (Reddy and Mohan 2011).

Preliminary data: 1) We developed a model of chronic temporal lobe epilepsy (TLE) in female rats; treatment with progesterone caused initial anticonvulsant action followed by rapid return of baseline seizure frequency and even worsening of seizure frequency. Termination of progesterone injections caused seizure exacerbation and inhibition of conversion of progesterone to neurosteroids had similar effects. 2) We measured the effects of progesterone treatment on synaptic transmission in the principal neurons of the hippocampus. The amplitude of spontaneous excitatory postsynaptic currents (sEPSCS) recorded from CA1 pyramidal neurons (CA1 PN) of female epileptic rats treated with progesterone was larger than that recorded from CA1 PN of untreated epileptic females. 3) These changes were accompanied by the increased expression of the AMPA receptor (AMPAR) subunits GluA1 and GluA2 in hippocampal cell membranes.

Hypothesis: We propose that chronic elevation of progesterone in female epileptic animals enhances AMPA receptor-mediated glutamatergic synaptic transmission and suppresses GABAR-mediated synaptic transmission. The changes are associated with the reduced anticonvulsant action of progesterone. The following proposed aims test these predictions. Project) To characterize the impact of progesterone treatment on glutamatergic synaptic transmission on CA1 PN and DGCs of naive and epileptic female mice and Progesterone receptor knockout mice using a combination of patch clamp electrophysiology and analysis of the expression of the AMPAR subunits via both biochemical and immunohistochemical techniques.

Main technique Patch clamp electrophysiology.

Significance: These studies will provide novel insights into the mechanisms of catamenial epilepsy. They will demonstrate for the first time that chronic progesterone treatment enhances glutamatergic synaptic transmission. A recent phase III multicenter trial of progesterone treatment of catamenial and non-catamenial epilepsy demonstrated that progesterone treatment did not improve seizures. These studies could explain the mechanism of failure and potentially identify novel therapeutic targets for the treatment of catamenial exacerbation of seizures.

Timeline:

• Months 0-4: EPSC recording from CA1 pyramidal neurons control mice during estrous cycle.
**Months 5-8:** ESPSC recording from CA1 pyramidal neurons of brain-specific PR receptor knockout mice.

**Months 8-12:** ESPSC recording from CA1 pyramidal neurons of brain specific PR receptor knockout epileptic mice treated with progesterone or not.

Dr. Kapur is a new mentor in the Program.

**References**


Smith, S.S., Gong, Q.H., Hsu, F.C., Markowitz, R.S., ffrench, M., JM, & Li, X. 1998. GABA(A) receptor alpha4 subunit suppression prevents withdrawal properties of an endogenous steroid [see comments]. *Nature*, 392, (6679) 926-930
David Wotton, PhD
Associate Professor of Biochemistry and Molecular Genetics
The overall interest of our lab is understanding how Transforming Growth Factor beta (TGFβ) signaling controls gene expression, normal mammalian development, and cancer progression.

Understanding the role of TGIF1 and MYT1L in glioblastoma.

Glioblastoma (GBM) is a highly aggressive cancer with poor prognosis and limited treatment options. We have found that the expression levels of the two transcription factors, MYT1L and TGIF1, are highly predictive for survival in human GBM patients. MYT1L is a DNA-binding transcriptional activator, whereas TGIF1 is a transcriptional repressor that can be recruited to DNA via interactions with other DNA binding proteins. Our preliminary in vitro analyses demonstrate that TGIF1 and MYT1L interact and suggest that TGIF1 may limit activation of gene expression by MYT1L. We suggest a model in which these two factors control expression of a common set of target genes. These genes will be activated by MYT1L and repressed by TGIF1, and we predict that de-regulation of this gene set drives GBM (Fig 1).

![Figure 1. Left: Our proposed model for the role of TGIF1 and MYT1L in GBM. Right: Kaplan-Meier analysis of TGIF1 and MYT1L in GBM.](image)

We plan to test the model that increased TGIF1 expression and decreased expression of MYT1L drives GBM, by expressing MYT1L, or knocking down TGIF1, in GBM cells to examine effects on proliferation, invasion and tumorigenicity using mouse xenograft models and in vitro cell culture. Additionally we will perform global gene expression analysis to identify TGIF1 and MYT1L target genes in GBM cells.

Students joining this project will: Analyze gene expression using real-time RT-PCR, and will be involved in analyzing and validating results from high-throughput RNA sequencing (RNA-seq); Analyze GBM xenografts by immuno-histochemistry and immunofluorescent microscopy; Analyze gene expression in mice and GBM cell lines, using a combination of gene expression analysis, western blotting, phospho-protein analysis and chromatin immunoprecipitation (ChIP). This will provide the opportunity to learn these approaches, although any knowledge of these techniques will be an advantage. The overall goal is to test the model (shown above), and to generate a better understanding of GBM progression.
Progressive kidney disease and ESRD are associated with high morbidity and mortality. Regardless of the cause of injury there is a stereotypical response leading to interstitial fibrosis. A key feature is the activation of extracellular matrix-producing myofibroblasts. Current therapies focus on nonspecific or supportive therapy. Understanding mechanisms of fibrosis will lead to new therapies. During the course of the current funding cycle we advanced our understanding of the role of sphingolipids in AKI. Sphingosine 1-phosphate (S1P), a pleiotrophic lysophospholipid that is involved in diverse functions such as cell growth and survival, lymphocyte trafficking, and vascular stability, has profound effects on the immune system and kidney injury. S1P is the product of sphingosine phosphorylation by two sphingosine kinase isoforms (SphK1 and SphK2) that have different subcellular localizations. Whereas SphK1 is cytoplasmic, SphK2 is localized in the nucleus, mitochondria and endoplasmic reticulum pointing to an intracellular/intranuclear signaling role of sphingosine 1-phosphate (S1P). We propose a series of in vitro and in vivo studies to better understand the role of SphK2 in kidney fibrosis. Our overall hypothesis is that: 1) the progression from AKI to fibrosis (or the process of repair and recovery after AKI) is regulated by SphK2 and 2) the absence or inhibition of SphK2 contributes to resistance to fibrosis. **Aim 1.** To test the hypothesis that pharmacological inhibition of SphK2 or genetic deficiency of SphK2 attenuates or reverses kidney fibrosis. **Aim 2.** To test the hypothesis that pharmacological inhibition of SphK2 blocks fibrosis or mice deficient of SphK2 are resistant to kidney fibrosis through regulation of local interstitial IFN-γ. **Aim 3.** To test the hypothesis that SphK2 mediates epigenetic regulation of expression of key genes that can cause kidney fibrosis. These studies will help us understand how S1P regulates fibrosis/repair but they also have the potential to uncover genes important for various aspects of disease progression that may serve as targets for therapy. The student will learn a variety of techniques: Surgical and experimental methods (ischemia-reperfusion injury, cisplatin, folic acid nephropathy) in genetically modified mice (tissue specific knockout using Cre-Lox technology, tamoxifen inducible systems, quantitative morphometric analysis using MBF stereology system, confocal and two photon microscopy, electron microscopy, SeaHorse, flow cytometry, hypoxic chamber, real time PCR, Western blotting.

Dr. Mark Okusa previously supervised three other students in the program. The last student, Marta Stremska (2013-14) received a Young Investigator Forum Award in 2014. (http://news.med.virginia.edu/medicinematters/uvasonrestriction/public/2014/04/02/yif-awards/)
Innate Immunity: Studies of a novel mechanism of microbial growth inhibition activated upon pathogen perception

Plants are continuously exposed to a myriad of fungal, viral and bacterial pathogens. Surprisingly, of all the microbial species that plants encounter throughout their lives, only a small fraction can cause infection and disease on a given plant host. To keep most microbes at bay, plants need to recognize them via the perception of microbe-specific molecules (MAMPs) mediated by the surveillance receptors of the innate immune system. This early perception allows plants to mount defense responses that prevent infection. Although several cellular and physiological responses that are activated upon MAMPs perception are well characterized, how plants actually inhibit microbe’s growth remain largely unknown.

Our lab recently discovered a novel mechanism of defense that we named Nutrient Withdrawal Response (NWR), which is sufficient to inhibit the growth of bacterial pathogens when bacteria are prevented from manipulating this response. Despite being photosynthetic organisms, not every single cell in a plant’s body has the capacity to produce its own food. Carbon and nitrogen, in the form of sugars and amino acids, are transported from the photosynthetic cells inside the leaves to the distal non-photosynthetic tissues. The first step in this transport process is the secretion of nutrients into the intercellular space that surrounds the photosynthetic cells inside the leaves, the apoplast. The nutrient-rich apoplast provides an excellent environment for microbial propagation and in fact most bacterial pathogens propagate in the apoplast without damaging the surrounding cells, their food factories. The Danna laboratory discovered that the elicitation of plant defense with MAMPs is sufficient to activate the withdrawal of nutrients from the apoplast and that this mechanism is required to inhibit the growth of bacterial pathogens (Danna et al. Current Biology – under revision). This plant defense mechanism is based on the reallocation of nutrients, which deprives the pathogens from the nutrients they need. To gain insight into this novel and likely broadly conserved mechanism of plant defense, we will: a) Identify and characterize genes required to activate and execute NWR in Arabidopsis thaliana and tomato; and b) Identify and characterize virulence factors that allow successful pathogens to manipulate NWR. The proposed research will take advantage of a validated high-throughput infection assay that enables direct measurement of pathogen growth in living plants (Danna et al, PNAS 2011). Additionally, we will use cutting edge genome approaches to generate candidate effector genes in tomato, and metabolomics, cell biological and physiological methods to characterize their function. Some of the specific technics/methods that will be used in this research are: global gene expression analysis via RNA sequencing, qRT-PCR, cloning of candidate genes and generation of GFP reporter fusions, confocal fluorescence microscopy, assessment of amino acid and sugar transport in oocytes, yeast and plant protoplasts, and metabolic profiling using high-end liquid chromatography and mass spectrometry.

Understanding how plants fence off most of their potentially harmful microbial neighbors will change the game in favor of plants, and will get humans one step closer to food security.
There is clear evidence that altered control of the differentiated state of vascular smooth muscle cells (SMC), or SMC phenotypic switching, plays a critical role in development of a number of major human diseases including atherosclerosis, hypertension, asthma, and cancer. However, the mechanisms and factors that regulate SMC phenotypic switching in these diseases are poorly understood. A major long-term goal of our laboratory has been to elucidate cellular and molecular mechanisms that control the growth and differentiation of SMC during normal vascular development, and to determine how these control processes are altered during vascular injury or in disease states [see review by Alexander et al.1]. For example, a major focus of previous studies has been to identify molecular mechanisms that control the coordinate expression of genes such as smooth muscle α–actin (SM α–actin), SM22α, and smooth muscle myosin heavy chains (SM MHC) that are required for the differentiated function of the SMC. Studies involve use of a wide repertoire of molecular-genetic techniques and include identification of cis elements and trans regulatory factors that regulate cell-type specific expression of SMC differentiation marker genes both in cultured cell systems and in vivo in transgenic mice. In addition, we use a variety of gene knockout, mouse chimeric, and gene over-expression approaches to investigate the role of specific transcription factors and local environmental cues (e.g. growth factors, mechanical factors, cell-cell and cell-matrix interactions, hypoxia, inflammatory cytokines, etc.) in regulation of SMC differentiation in vivo during vascular development, as well as following vascular injury, or with cardiovascular disease 2,3.

A particularly exciting recent development is that we have employed SMC specific promoters originally cloned and characterized in our laboratory to create mice in which we can target conditional knockout (or over-expression) of genes of interest specifically to SMCs and also perform rigorous SMC-pericyte lineage tracing experiments to define mechanisms that control phenotypic transitions of these cells during injury-repair and in diseases such as atherosclerosis 4. Remarkably, using these model systems, we have recently shown that SMC-pericytes de-differentiate, give rise to mesenchymal stem cell (MSC)-like cells, and trans-differentiate into alternative cell types during development of experimental atherosclerosis, as well as in models of myocardial infarction, lung injury, skin wounding, and partial hepatectomy. Moreover, we have shown that the phenotypic transitions of SMC-pericytes in these models is regulated by activation of stem cell pluripotency genes, including Oct4 (manuscript in review), and Klf4 5, factors also shown to be involved in reprogramming of somatic cells into induced pluripotent stem (iPS) cells.

Our lab has also pioneered studies of the role of epigenetic mechanisms in control of SMC differentiation and phenotypic switching 6, as well as lineage determination of multiple specialized cell types from embryonic stem cells (ESC) 7. Of major interest, we have shown that lineage determination of SMC, as well as other specialized cells from ESC, involves acquisition of locus- and cell-type selective histone modifications that influence chromatin structure and permissiveness of genes for transcriptional activation. Moreover, we have demonstrated that phenotypic switching of SMC into alternative cell types involves reversal of a subset of these histone modifications and transcriptional silencing of SMC marker genes. However, these cells retain certain histone modifications that we hypothesize serve as a mechanism for “cell lineage memory” during reversible phenotypic switching. That is, a mechanism that allows a SMC to undergo transient transitions to alternative phenotypes necessary for vascular repair, but which biases the cell into re-differentiating into a SMC once the repair...
is complete. Of major significance, we have recently developed a powerful new assay that allows assessment of specific histone modifications within single cells within fixed tissue specimens (i.e. a single cell chromatin immunoprecipitation assay) and using this system along with our SMC specific lineage tracing mice, have shown that de-differentiated (phenotypically modulated) SMC within advanced atherosclerotic lesions of ApoE-/- mice retain an epigenetic signature of SMC even when expressing no detectable expression of SMC marker genes such as Acta2 or Myh11.

Finally, a major long term emphasis of the lab is to translate results of our basic science studies into advancing clinical practice. Current projects in this area include testing how inhibition of IL-1β signaling may help promote increased stability of atherosclerotic plaques thus reducing the probability of a heart attack or stroke. In addition, we are investigating ways to therapeutically augment the stem cell like properties of SMC-pericytes as a means to treat a wide range of major human diseases.

Reference List


Iron transport in Kidney Injury

The main objective of my research program is to better understand how renal and systemic iron transport is regulated in kidney injury. Iron is an essential element with important physiologic roles including mitochondrial function and erythropoiesis. However, because of its ability to accept electrons from oxygen, it can catalyze generation of toxic reactive oxygen species such as hydroxyl radical and hydrogen peroxide (Haber-Weiss and Fenton reactions). So, iron absorption, metabolism and transport are tightly regulated in the body. Iron is transported with transferrin to the organs of iron storage. Intracellular iron levels are tightly regulated. Iron storage organs sequester or release iron and thereby exercise a fine control over plasma and tissue iron levels. This regulation occurs through the only known iron export protein in the body, ferroportin (FP). When FP is expressed on the cell surface, iron export results. FP exports ferrous (Fe\(^{2+}\)) species of iron that must be reduced to ferric species (Fe\(^{3+}\)) by ceruloplasmin before being accepted by transferrin for transport. FP expression is primarily regulated by hepcidin, an endogenous antimicrobial peptide produced by the liver. Hepcidin is also synthesized in smaller quantities by macrophages and kidney renal tubular epithelial cells.

Very little is known on the role of FP in kidney injury. We have recently demonstrated that hepcidin treatment prevents ischemic kidney injury in mouse models. The available project is to examine novel biochemical, molecular and therapeutic approaches to probe the role of hepcidin-FP-iron axis in diverse models of acute and chronic kidney injury. We are in the process of identifying drugs that could effectively target hepcidin-FP pathway. The student who embarks on this project will develop and test high throughout assays for drug discovery, test novel drugs in diverse kidney injury (animal) models including iron transport mutants, and will characterize the mechanisms of protection using immunofluorescence, immunohistochemistry, electron microscopy, RT-PCR, western blot, flow cytometry, and other biochemical assays that we have developed in the lab. In addition, we have ongoing collaborations with other research groups in at Virginia Tech, and the student will interact with members of these groups to develop new fluorescence-based assays for drug testing and development. If interested, travel to collaborating labs to conduct experiments is a possibility.
Through evolutionary history the human genome was optimized to promote survival in environments where food is mostly scarce. These survivor-genomes clash with an environment where calorie-rich foods are readily available. Based on the premise that the genes networks that allow animals to endure starvation are under strong selective pressure and consequently conserved, we use a combination of cutting-edge functional genomics, biochemical, cell biology, genetic, and physiological approaches to identify and characterize the conserved gene networks that allow the animal model *Caenorhabditis elegans* to adapt to changes in food availability. Ultimately, our research would contribute to better understanding of how dysfunctional gene networks affect or cause diseases like obesity and diabetes, and accelerate aging.

Our approaches allow us to tackle relevant biological and biomedical questions with unprecedented power. In collaboration with the Imaging Platform at the Broad Institute of Harvard and MIT, we developed what is currently the only available system to perform whole-living animal functional genomic screens (*Nature Methods*, 2012, and *Methods* 2014). Using this technology, we discovered that the previously uncharacterized transcription factors MXL-3 and HLH-30 coordinately activate a transcriptional program that is essential for survival to starvation from worms all the way up to humans (*Nature Cell Biology*, 2013). In addition, MXL-3 and HLH-30 regulate aging. *mxl-3* mutant animals are 40% longer lived than wild-type animals and this lifespan extension phenotype is independent of all lifespan extension pathways known to date. Thus, the mechanisms by which MXL-3 and HLH-30 regulate aging remain to be fully dissected and their study promises to uncover novel ways to regulate aging. The person joining our lab will use RNAseq to unveil the full set of genes controlled by these master transcriptional regulators. The genes found to be co-regulated by *mxl-3* and *hlh-30* will be tested for their ability to suppress the *mxl-3* extended lifespan phenotype through a high-throughput method to screen for aging genes currently under development. Because we found that HLH-30 coordinates the transcriptional activation of lipolysis and autophagy upon fasting in mammals too, it is possible that the aging regulators uncovered in *C. elegans* would also regulate aging in higher organisms. Alternatively, novel metabolic regulators coming out from our latest screens need excited minds interested in characterizing them.

We are looking for individuals with bright minds and hard-working hands passionate for science and discovery. We strive to broaden and strengthen the skills of students and postdoctoral fellows in the lab to produce future leaders in the fields of Metabolism and Aging.
Influenza continues to be a significant global public health problem, with 3-5 million severe cases annually, including 250,000 - 500,000 deaths worldwide. The vaccination program for influenza remains vulnerable to yearly genetic changes in the virus that limit vaccine effectiveness. In addition, newly emergent viral strains periodically cause pandemics, including the 2009 swine H1N1 pandemic. Currently there is only a single class of anti-influenza drug in clinical use. The target of this class of drugs is the viral neuraminidase protein. The potential for development of drug resistance to the neuraminidase inhibitors is high, which will minimize their efficacy in the human population. Therefore the development of new drugs that attack alternative viral targets is a necessity.

The Engel laboratory has discovered “small molecule” inhibitors of two important viral proteins, called NS1 and NP. The main role of NS1 during infection is to block the host cell interferon system, which is an important component of the host's innate immune response to virus infection. We have learned that our NS1 inhibitors restore the interferon response and thus block virus replication and spread. Another drug target is the viral nucleoprotein (NP), which is involved directly in replication of the viral RNA genome. We have identified and are currently developing a series of compounds that potently inhibit NP function and virus replication.

An important experimental approach in the design and study of small molecule inhibitors is X-ray crystallography. The crystal structures of NS1 and NP are known, so it should be possible to generate detailed structural data of these proteins in complex with their respective inhibitors. The proposed project for a visiting student is to express the NS1 and/or NP proteins in bacterial expression systems, and to establish the conditions for crystallization so that three-dimensional structural analysis can be performed on the drug-protein complex. The project will entail all aspects of this approach, including recombinant DNA cloning, protein expression, protein purification, crystallization, optimization of drug-protein complexes, binding assays, isothermal titration microcalorimetry and analysis of the structural data. The student who will join the project, will be supervised jointly by Drs Engel and Derewenda, and will have exposure to the research conducted in both Departments. The University of Virginia is superbly equipped for this purpose with all required equipment and access to synchrotron radiation beamline at the Argonne National Laboratory in Chicago.

Dr. Derewenda supervised to date more than 20 Masters students from various Polish Universities, many of whom went on to doctoral and postdoctoral fellowships at prestigious institutions, including the University of Virginia, University of Chicago, ETH Zurich, St. Andrews University (Scotland) and others. This is the first year of mentorship in the program for Dr. Engel.
We are trying to determine the basis for extreme drug resistance in bacterial infections. We use computational analysis to predict mutations to proteins that would increase resistance and then perform experiments to test these predictions. Students in the lab will help perform bacterial mutagenesis, testing for antibiotic resistance, and eventually functional characterization of resistance enzymes. We are also working on novel targets for drug-resistant infection, so there will be opportunities to help characterize expression profiles and changes in bacteria from clinical samples. All of this work will help discover new ways to diagnose and treat bacterial infections.
David S. Cafiso, PhD,  
Professor of Chemistry

Our lab is involved in the use of magnetic resonance techniques (NMR and site-directed spin labeling) to determine the molecular mechanisms of membrane transport and the molecular events underlying membrane fusion.

The prospective student will be studying the structure and structural transitions of a class of outer membrane bacterial transport proteins that function to bring rare nutrients such as iron and vitamin B12 into the cell. The student will learn to use both EPR and NMR methodologies in this work, and will get experience in site-directed mutagenesis, protein purification and membrane protein reconstitution.

Dr. Cafiso supervised to date two students from Poland, including Damian Dawidowski (UJ), currently a doctoral student at UVA, Chemistry Department, and Anna Cieslinska (UJ), who obtained Masters degrees from both UJ and UVA, and is a graduate (doctoral) student at Northwestern University.
Salem Faham, PhD, Assistant Professor of Molecular Physiology and Biological Physics

My lab is focused on two areas of research:

(1) Multidrug resistance.

The *mepRAB* operon in *staphylococcus aureus* has been shown to play a role in drug resistance. MepA is a multidrug efflux transporter, MepR is a transcriptional regulator that controls the expression of both MepA and MepB. MepB is the third protein in this operon classified as a protein of unknown function.

We are interested in studying the structure to function relationships for both MepA and MepB. We have been investigating how MepA is able to recognize a wide array of different substrates. And we have recently determined the structure of MepB and showed that it is a DNA binding protein. MepB’s exact role in drug resistance is not yet determined.

(2) Protein design.

We are working on using the inherent symmetries of protein oligomers to form fusion proteins that would assemble into higher order predictable patterns. Specifically we have working on making fusion proteins that would self-assemble into 2-dimensional and 3-dimensional protein lattices.

We also recently determined the structure of a designed protein fusion of a heterodimeric complex. This structure highlighted that heterodimeric structures contain a wealth of information that can improve structural predictions, yet they are currently unused.

**Dr Faham previously supervised Katarzyna Skorupka, who is currently a graduate (PhD student at the University of Virginia)**
Introduction: The majority of deaths due to cancer are caused not by the primary tumor but by metastases to distant organs. The process by which a primary tumor progresses to become metastatic involves a dramatic reprogramming of the tumor cell’s gene expression and chromatin modification patterns. Our lab is interested in understanding these changes in gene expression and chromatin structure across the entire genome. We aim to identify pro-metastatic gene expression and chromatin modification signatures to understand tumor progression. We are developing technology for specifically profiling tumor cells from solid tumors in mice, free from stroma and other contaminating cell types. This will allow the accurate comparison of gene expression and chromatin modification patterns in pure populations of nuclei from primary tumors and metastases.

The goal of this project is to biotin tag a nuclear envelope protein in human breast cancer cells, allowing isolation of biotin tagged nuclei from xenograft tumors in mice. We will compare gene expression and genome-wide chromatin modification profiles between primary tumors and metastases from this model.

Approach:

1. We will specifically biotinylate tumor cell nuclei by tagging an outer nuclear membrane protein with a biotin acceptor peptide in MDA-MB 231 breast cancer cells. These cells will be used to optimize methods for purifying biotin-tagged intact nuclei for gene expression analysis and determining chromatin modification states.

This will involve cell culture, basic biochemical and molecular biology methods and protein purification techniques, followed by chromatin immunoprecipitation and analysis of RNA expression by real time PCR.

2. We will generate xenografts in the mammary fat pad of nude mice with the MDA-MB 231 cell line expressing the biotin-tagged nuclear membrane protein. MDA-MB 231 cells metastasize to lung and liver, and we will isolate primary tumors and metastases for gene expression and chromatin modification profiling by RNA-seq and ChIP-seq. This will identify differences in transcriptionally active and repressed regions across the entire genome.

This will involve the isolation of tumor samples from mice, followed by purification of tagged nuclei by binding to streptavidin agarose. Purified nuclei will be analyzed by chromatin immunoprecipitation followed by real time PCR. RNA will be isolated from purified nuclei and analyzed by real time PCR. We will perform ChIP-seq using H3 K27Me3, H3 K9Ac and RNAPII antibodies.

These approaches will allow us to accurately determine which gene expression and chromatin modification changes are associated with breast cancer metastasis.
The structure and function of the TRIM/RBCC family of eukaryotic proteins

Background: The Pornillos laboratory is investigating the structure and function of cellular proteins that comprise the TRIM/RBCC family (about 67 different members identified so far). TRIM proteins have been implicated in many different cellular processes and pathways. They appear to have particularly important roles in anti-viral defense, activation of the cellular innate immune response, inflammation, and the development of cancer.

TRIMs share a common domain organization (see figure above), with an N-terminal RING domain, followed by a B-box 2 domain, a coiled-coil domain, and a C-terminal domain. (Some TRIMs contain a B-box 1 domain in between the RING and B-box 2 domains.) The RING/B-box/coiled-coil (RBCC) motifs mediate self-assembly of the TRIM proteins. TRIM C-terminal domains are variable in nature, and generally mediate interactions with other protein partners. We would like to understand the interplay between the self-assembly function of the RBCC domains and the various protein-binding activities of the C-terminal domains.

Students can join a number of active projects and will be assigned a specific aim that is expected to be completed within one year. Experimental approaches generally include any or all of the following methods: cloning of TRIM constructs into various plasmids, recombinant expression of the constructs in E. coli and baculovirus/insect cell systems, optimization of construct solubility by systematic testing of cell culture and biochemical parameters, protein purification using various chromatographic methods, protein crystallization, and structure determination by X-ray crystallography.

More information may be found at the laboratory website: http://people.virginia.edu/~owp3a.

Required Skills/Knowledge: Students are expected to have a strong understanding of the basic principles of molecular biology and biochemistry. Laboratory experience in cloning and protein purification will be very desirable.
PROJECT 1. **Prostate Cancer Progression.** We are studying how signal transduction pathways regulate prostate cancer progression from a highly localized, primary tumor to a metastatic tumors. Using a variety of model systems (genetically-engineered mice, xenografts, cultured cells), we are studying how loss of the tumor suppressor PTEN drives prostate cancer through a kinase pathway involving mTOR. The major methods associated with this project would be tissue culture, in vitro biochemical approaches such as kinase assays, gene expression analysis by PCR and RNA seq, and fluorescence microscopy.

PROJECT 2. **Androgen signaling and DNA repair.** We are studying how DNA double strand breaks, which, paradoxically are induced by androgen signaling, are repaired by the cell. We have recently identified a novel ubiquitin ligase complex that is recruited to sites of DNA damage, and the goal is to identify the ubiquitin-modified protein substrates and determined how they promote DNA repair. Chromosomal translocations are now known to occur frequently in prostate cancer, thus we are positioned to study a key step in the pathway. The major methods will be in vitro biochemical assays that reconstitute ubiquitination of chromatin proteins, chromatin immunoprecipitation, and fluorescence microscopy to analyze the dynamics of DNA repair. There is also an opportunity to establish a high throughput screen for inhibitors of the pathway, which could reveal lead compounds with therapeutic potential. A student with a strong background in computational biology would also have the opportunity to map DNA damage sites on a genome-wide scale, working in collaboration with others in my department.

PROJECT 3. **Aging mechanisms at the single cell level in Progeria.** We are studying how the mutant form of lamin A in Progeria induces changes at the cellular level. Our focus is on structural events in the lamina at the nuclear membrane, which induce three changes: (1) Disruption of nuclear transport by inhibiting nucleotide exchange on the Ran GTPase. (2) Production of reactive oxygen species (ROS) by an unknown mechanism. (3) Changes in histone post-translational modifications, in particular, reduced levels of histone H3K9-trimethylation. We would like to understand the molecular basis of each of these changes, and determine if they are separate features of Progeria or represent inter-related phenotypes. The major methods would involve tissue culture, recombinant protein expression, purification and characterization of the nucleotide exchange factor for the Ran GTPase, live cell imaging to measure ROS, and siRNA screens to identify the source and consequences of ROS production in Progeria, and analysis of chromatin-modifying complexes from Progeria patient cells.

PROJECT 4. **Nuclear transport in human epilepsy.** In collaboration with a group that has done exome sequencing, we identified a mutation in a nuclear transport factor that causes a specific form of epilepsy. By computational methods we have learned the mutation results in a single amino acid substitution in the domain the transport factor that binds to nuclear localization signals. The goals are to identify exactly what type of nuclear localization signal the transport factor recognizes and which cargoes (e.g. transcription factors) are imported into the nucleus via this factor, and how defects in this pathway impact on the biological function of nerve cells expressing this transport factor. The major methods to be used quantitative analysis of protein-protein interactions, mutagenesis, recombinant protein purification, molecular modeling of protein structure, fluorescence microscopy, siRNA knockdowns, cell culture of neurons, and gene expression analysis.