

**The following projects will be offered at the University of Virginia in 2011/2012:**



**David S. Cafiso, Professor of Chemistry**  
<http://ernst.chem.virginia.edu/dsclab>

**(confirmed)**

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 obtain Masters degrees from UJ and UVA, and is a graduate (doctoral) student at Northwestern University.***



**Edward Egelman, Professor Biochemistry & Molecular Genetics, University of Virginia Health Sciences Center**

**(confirmed)**

Our laboratory has focused on two main areas: 1) F-actin and complexes of actin with other proteins; 2) Nucleoprotein filaments formed by RecA/RadA/Rad51/Dmc1 on DNA. The main tools that we use are electron cryo-microscopy and computed image analysis. Because these filaments are poorly ordered and have great structural variability, we developed some general methods to be used for generating three-dimensional reconstructions of helical polymers from electron micrographs. These methods are now being applied to many other helical polymers in my lab: pili from pathogenic bacteria, filamentous bacteriophage, bacterial Type Three Secretion System components, and bacterial plasmid segregation system polymers.

We are looking for students who have some computational experience. Within a one year period, a student with a reasonable background might be expected to make some significant contribution to one of our existing projects, leading to a publishable paper. Prior experience with Linux and programming skills would be very helpful.

***Dr. Egelman supervised one doctoral student, Jakub Bielnicki, from Lodz University.***



**Marty W. Mayo , PhD, Professor of Biochemistry and Molecular Genetics**

**(Confirmed)**

A major focus in biomedical research is to understand how cancer cells invade healthy cells and metastasize to distant sites. Lung cancer in particular is more prone to aggressive forms of the disease that are difficult to treat effectively and have a poor clinical prognosis.

Non-small cell lung cancer (NSCLC) is the most common form of lung cancer. The 5-year mean survival for patients suffering from stage III lung cancer is less than 30%. Poor clinical prognosis for NSCLC is directly associated with late-stage diagnosis and high propensity for metastasis to liver and bone. For the last several years our laboratory has been working on a transcription factor called nuclear factor-kappa B (NF- $\kappa$ B). NF- $\kappa$ B is a protein complex that is found in all human cells. In non-cancerous cells NF- $\kappa$ B is tightly regulated, however, cancer cells have found ways to elevate the activity of NF- $\kappa$ B. Although it is known that cancer cells enhance NF- $\kappa$ B activity, the reasons for this are not fully understood. Our laboratory has discovered that NF- $\kappa$ B is one of the master-switch transcription factors required to induce phenotypic changes in cells referred to as epithelial to mesenchymal transition (EMT). EMT is a critical step in cancer metastasis. For the first time, our laboratory can demonstrate that following the induction of EMT, NF- $\kappa$ B is required to orchestrate changes associated with the induction and maintenance of cancer stem cells. Cancer stem cells are believed to act as a “seed” which is able to reestablish malignant disease. The overall goal of our laboratory is to understand transcription and transcription-independent mechanisms by which NF- $\kappa$ B induces and maintains cancer stem cells. Our laboratory uses two dimensional (2D) and 3D tissue culture systems, primary normal and tumor tissues, and small animal models as methods for studying EMT. Commonly employed techniques in our laboratory include, Western blot analysis, quantitative real-time PCR, Chromatin Immunoprecipitations (ChIP) and ChIP-sequence, ELISAs, invasion and migration assays, molecular subcloning and site-directed mutagenesis.

I would prefer to have student who is highly motivated and excited about learning or perfecting molecular biology techniques. A student who has firmly established wet-bench skills is preferred.

***Dr. Mayo supervised Anita Popko from the Technical University of Lodz. Anita co-authored a major paper, went on to join the lab as a Grad Student and later transferred to Karolinska Institute in Sweden. Dr Mayo is currently supervising Szymon Szymura, a PhD student from the Jagiellonian University, and Julia Krupa, a visiting student from the Technical University in Lodz.***



Mark Yeager, PhD, MD, Professor of Molecular Physiology and Biological Physics

(confirmed)

At the basic science level, we are intrigued by questions at the interface between cell biology and structural biology: How do membrane proteins fold? How do membrane channels open and close? How are signals transmitted across a cellular membrane when

extracellular ligand binds to a membrane receptor? How do viruses attach to and enter host cells, replicate, and assemble infectious particles? To explore such problems, we use high-resolution electron cryomicroscopy and computer image processing. With this approach, we can examine the molecular architecture of supramolecular assemblies such as membrane proteins and viruses.

In electron cryomicroscopy, biological specimens are frozen in a physiological state to preserve their native structure functional properties. A special advantage of this method is that can capture dynamic states of functioning macromolecular assemblies, such as open and closed states of membrane channels and viruses actively transcribing RNA. Three-dimensional density maps are obtained by digital image processing of the high-resolution electron micrographs. The rich in the density maps exemplifies the power of this approach to reveal the structural organization of complex biological systems that can be related to the functional properties of such assemblies.

Ongoing research projects include the structure analysis of (1) membrane proteins involved in cell-to-cell communication (gap junctions), water transport (aquaporins), ion transport (potassium channels), and transmembrane signaling; (2) viruses responsible for significant human diseases (HIV-1, hepatitis B [HBV], rotavirus, astrovirus); and (3) viruses used as model systems to understand mechanisms of pathogenesis (arenaviruses, reoviruses, nodaviruses, tetraviruses and sobemoviruses).

A rotation in our laboratory will allow students to gain experience in the design of constructs for expression of membrane proteins in *E. coli*, *Pichia pastoris* and SF9 insect cells via baculovirus. Metal affinity, ion-exchange and gel-filtration chromatography are used for purification. Detergents are screened to optimize the homogeneity and stability of the expressed protein. Students will receive personal tutorials in electron microscopy for routine screening of samples by negative-stain EM. Instruction will also be provide for preparation of frozen-hydrated specimens and the performance of electron cryomicroscopy. Depending on the duration of the rotation, students will pursue 2D and 3D crystallization trials for higher resolution structure analysis by electron and X-ray crystallography. Data are analyzed using software packages such as EMAN, 2DX and CCP4.

Ganser-Pornillos, B., Yeager, M. , and Sundquist, W.I. The Structural Biology of HIV Assembly. *Curr. Op. Struct. Biol.* **18**: 203-217 (2008).

Dryden, K.A., Coombs, K., and Yeager, M. Chapter 1. The Structure of Orthoreoviruses. In: Structure and Molecular Biology of Segmented Double-Stranded RNA Viruses, Patton, J., Ed. Horizon Scientific Press, Wymondham, Norfolk United Kingdom, pp. 3-25 (2007).

Yeager, M. and Harris. A. L. Gap Junction Channel Structure in the Early Twenty-first Century: Facts and Fantasies. *Curr. Op. Cell. Biol.* **19**: 521-528 (2007).

Adair, B.D. and Yeager, M. Electron Microscopy of Integrins. *Methods in Enzymol.* **426**: 337-373 (2007).

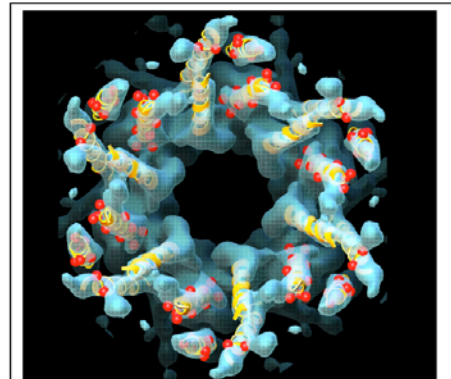


Figure 1. Intercellular gap junction channels have a diameter of about 65 Å and are formed by the end-to-end docking of 2 hemichannels, each composed of a hexamer of connexin subunits. A C<sup>α</sup> model (ribbons) for the membrane-spanning α-helices of the hemichannels was derived by combining the information from a computational analysis of connexin sequences, the results of more than a decade of biochemical studies, and the constraints provided by a 3-dimensional map derived by electron cryocrystallography. Although individually, none of these approaches provided high-resolution information, their sum yielded an atomic model that predicts how connexin mutations (spheres), which result in diseases such as nonsyndromic deafness and Charcot-Marie-Tooth disease, may interfere with formation of functional channels by disrupting helix-helix packing. From Yeager and Harris, 2007.

an

quick  
and  
we

detail

**Dr. Yeager currently serves as a mentor for Maciej Jagielnicki, Technical University of Wroclaw.**



**Salem Faham, PhD,  
Assistant Professor of Molecular Physiology and Biological Physics**

**(confirmed)**

Membrane proteins currently represent only a small fraction of the structures in the protein data bank (pdb). That is partly due to the difficulty in obtaining high quality crystals. Membrane proteins are typically solubilized and crystallized in detergent solutions. However, detergents are often blamed for the poor quality of membrane protein crystals. We have shown that membrane proteins can be crystallized from a specific lipid/detergent mix that forms bicelles. Bicelles offer an alternative medium for the crystallization of membrane proteins that holds a lot of promise. We are interested in the further development of the bicelle method for membrane protein crystallography. This project will involve cell growth, protein expression, protein purification, and protein crystallization.

**This is the first year for Dr. Faham in the program**



**Umesh S. Deshmukh, PhD, Assistant Professor of Medicine**

**(confirmed)**

My laboratory is interested in autoimmune disorders, specifically Systemic Lupus Erythematosus (SLE) and Sjögren's Syndrome (SS). The main emphasis is to understand how pathogenesis of these diseases is influenced by the activation of innate and adaptive immunity by microbial agents.

**Project 1. Role of molecular mimicry in activation of T cell responses in SLE.** Systemic lupus erythematosus is a complex autoimmune disorder affecting multiple organ systems such as kidneys, skin and lungs. The presence of autoantibodies reactive against multiple cellular proteins is a hallmark of SLE. We are interested in understanding how these autoantibody responses are initiated. The initiation of T cell responses against self-antigens is a critical step in the genesis of autoantibodies in lupus. My laboratory is investigating the role of molecular mimicry between self-antigens and foreign proteins as one of the mechanisms for activation of autoreactive T cells. We are using Ro60 as the model autoantigen. Ro60 is among the first autoantigens to be targeted in lupus, and immune responses against Ro60 are often present in SLE patients. The current studies focus on the mapping and detailed characterization of HLA-DR3 restricted T cell epitopes on Ro60. Identification of peptide mimotopes will be performed using different algorithms. The ability of selected peptide mimotopes to induce autoantibody responses against Ro60 will be investigated in mice transgenic for HLA-DR3. The data generated from this project will demonstrate that repetitive exposure to different microorganisms shapes the autoreactive T cell repertoire. In a lupus-susceptible individual, this leads to initiation of autoimmune responses. This project will aid in identifying novel environmental risk factors for the development of SLE.

**Project 2. Role of innate immunity activation in Sjögren's Syndrome.** SS is a chronic autoimmune disorder mainly affecting the salivary and lacrimal glands. A progressive lymphocytic infiltration within these glands causes glandular destruction. This is responsible for the dry mouth and dry eye symptoms of the disease. My laboratory is interested in understanding how activation of innate immunity influences the development of SS. We are using the Toll-like receptor 3 (TLR3) agonist poly(I:C) to activate innate immunity in NZB/NZW derived strains of mice. Our results demonstrate that poly(I:C) treatment causes acceleration of SS-like disorder in the NZB/NZW F1 mice. The salivary glands of poly(I:C) treated mice had severe lymphocytic infiltration. The mechanisms for this observation will be investigated in details. In our experimental mouse model system, poly(I:C) causes rapid upregulation of type I IFNs within the salivary glands, which causes rapid glandular dysfunction. Our studies in interferon receptor knock out mice show that type I IFNs are directly involved in this process. Paradoxically, systemic treatment of SS patients with type I IFN, has demonstrated beneficial effects on salivary gland function. Thus, another objective in this project will be to understand the contrasting mechanisms of IFN action on salivary gland function.

Students with keen interest in immunology, and an enthusiasm to investigate clinical diseases in experimental mouse models will be preferred. Some experience in tissue culture, animal work and basic immunoassays is desired. *Dominika Nackiewicz, who was a student in this program (2008-2009) is currently working as research assistant in our laboratory.*

**Dr Deshmukh currently supervises Agnieszka Szymula, from the Jagiellonian University.**



**David L. Brautigan, PhD, Professor of Microbiology and Director, Center for Cell Signaling.**

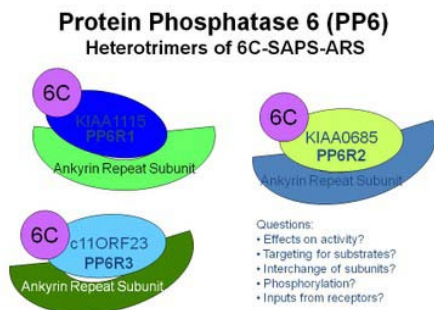
**Protein Phosphatase-6 in Cell Cycle, Inflammation and DNA damage responses (confirmed)**

Our goal is to discover how intracellular signaling pathways regulate cell proliferation, survival/apoptosis and cytokine production in response to stress signals and infections. This research is relevant to understanding normal physiology as well as the pathology encountered in human diseases such as cancer, diabetes and autoimmunity. Our focus is on **protein phosphorylation**, and especially the enzymes

called **protein Ser/Thr phosphatases** (the PPP and MPP Families). Genomics has shown that the PPP enzymes are extraordinarily conserved in all eukaryotes (e.g. mammals, *Xenopus*, *Drosophila*, *C. elegans*, *S. pombe*, *S. cerevisiae*). Humans and yeast have about the same total number of PPP genes, in separate functional classes (i.e. PP1, PP2A, PP4, PP6). Individual human PPP proteins can substitute in place of their yeast homologues, but not PPP of other functional classes, showing that individual PPP are functionally equivalent across evolution, but each class has a distinctive biological role. The conservation across species allows us to use the results from genetic experiments in various model organisms.

We primarily use cultured cells and combine functional genomics, biochemistry and cell biology. It is typical for students and fellows to learn the full array of molecular and cellular techniques while studying these signaling networks. (e.g. PCR, cloning, mutagenesis, protein expression and purification, tissue culture,

transfections, enzyme assays, immunoprecipitations, immunoblotting, microscopy, etc). Protein Phosphatase 6 (PP6) is a distinct member of the protein Ser/Thr phosphatase PPP family that is the mammalian homologue of yeast Sit4. The functions of Sit4/PP6 are conserved, because human PP6 rescues yeast sit4- mutations, whereas other PPP do not. In yeast Sit4 is genetically linked to cell cycle control. We have found that PP6 has effects on G1 to S phase progression in human cancer cells, influencing the levels of cyclin D1 and phosphorylation of Rb (*Cell Cycle*, 2007). A graduate student is testing how PP6 regulates levels of cyclin D1 in breast cancer cell lines. Other evidence points to the role of PP6 in cytokine signaling and pathways leading to activation of NF-kB. PP6 SAPS subunits



mediate association with I $\kappa$ B $\epsilon$  and alter the degradation of this regulator in response to TNF $\alpha$  stimulation (*J. Biol. Chem.* 2006). Proteomic results using mass spectrometry of immunoprecipitated SAPS complexes revealed a family of Ankyrin Repeat Subunits (we named ARS) that are functionally equivalent to the SAPS themselves in siRNA knockdown assays (*Biochemistry*, 2008). Thus, we propose that PP6 is a trimeric enzyme, composed of ARS, SAPS and a catalytic subunit (see Figure). We have analyzed the structure of SAPS subunits using modeling, to predict a helical repeat arrangement, and find charged residues are needed for PP6 association (*BMC Biochem.* 2009). Iga Kucharska from Wroclaw Technical University is working on co-expression, crystallization, and solving the structure of the PP6c:SAPS1(PP6R1) complex.

Another protein identified by mass spectrometry was DNA-PK, a kinase activated following damage to DNA and an initiator of DNA repair by the joining (NHEJ) pathway. We found that in glioblastomas (brain tumors) the PP6 and the SAPS1 subunit we call PP6R1 were recruited into the nucleus and into complexes with DNA-PK. PP6 and PP6R1 were both required for the activation of DNA-PK following ionizing radiation (gamma rays), and for the repair of double strand breaks in DNA, and for the survival of the cancer cells (*PLoS One*; 2009). Thus, inhibiting this action of PP6 makes cells more sensitive to radiation, and may provide new therapies to enhance radiation therapy for otherwise incurable brain tumors. We have a project underway that is looking to express dominant negative forms of SAPS1 to interfere with repair of DNA damage.

**Dr Brautigan currently supervises Iga Kucharska, student from the Technical University of Wroclaw.**



**Mark D. Okusa, MD, Chief, Division of Nephrology  
Director, Center for Immunity, Inflammation and Regenerative Medicine  
Immune Mediated Kidney Injury and Repair**

(confirmed)

**Projection Description:** My laboratory is interested in innate and adaptive immunity in acute and chronic kidney injury. Dendritic cells play an early role in activation of lymphocytes through antigen presentation of peptides to T cells or glycolipids to natural killer T cells. Through an understanding of the mechanisms that participate in the early activation and modulation of tissue injury we have developed pharmacological and cell based approaches to block these pathways. We use a variety of molecular, cell biological and immunological methods and in vivo models in our studies.

(1) *Kidney ischemia-reperfusion injury:* In vivo studies are aimed at determining the contribution of immune cells to ischemia-reperfusion injury and therapeutic strategies to reduce injury following acute kidney injury with the ultimate goal of bringing novel compounds to clinical trials. Current studies target adenosine 2A receptors and sphingosine 1 phosphate receptors as potential therapeutic approaches to block inflammation and tissue injury. These studies have led to a better understanding of the mechanisms of T cell activation by ischemia-reperfusion and tolerance induction by adenosine 2A compounds.

(2) *Diabetic nephropathy:* Our approach is to understand the immune mechanisms of injury in diabetic nephropathy and use novel compounds to reduce functional and morphological consequences of diabetic nephropathy.

(3) *Targeted delivery of novel compounds and small molecules.* Using nanotechnology and contrast enhanced ultrasound we are involved in target specific delivery of novel compounds and specific genes. Liposomes are nano sized artificial vesicles produced from phospholipids and cholesterol and form a bilayer sphere. An important property of these liposomes is that have the ability to trap water soluble and insoluble substances for their delivery to desired diseased targets.

**Methods Description:** We use a number of different in vivo models that include genetically deficient mice, chimeric mice, tissue specific gene knockout mice and transgenic mice. Cell culture, molecular biology, pharmacology, immunological methods that use Elispot, flow cytometry and confocal microscopy are routinely employed. A new area of investigation is the use of contrast-enhanced ultrasound to study noninvasively and in real-time renal function and in combination with liposomes the delivery of drugs and genes to specific targets.

**Clinical Problem:** Acute kidney injury is a burgeoning problem. Based upon the National Health Statistics and National Hospital Discharge Survey, between 1979 and 2002 there has been an increase in hospitalization for acute kidney injury (AKI) from 35,000 to 650,000 cases per year. Overall mortality has been reported to be 40-60% in critically ill patients. The estimated annual health care expenditures attributed to hospital-acquired AKI exceed \$10 billion Furthermore there is recognition that there is an increase in the end stage renal disease (ESRD) population due to AKI. In patients suffering from AKI, 13.4% of patients (or 30% of patients with AKI superimposed on chronic kidney disease) will progress to ESRD in 3 yrs. Thus to overcome barriers to successful treatment of AKI, well designed clinical trials will need to be based upon a precise understanding of the molecular, cellular and immunological basis of AKI. My laboratory focused on defining critical pathways of early activation of innate immunity and identifying novel therapies.

**Student Objectives:**

1. To learn basic immunological and/or advanced imaging methods
2. To apply methods in the study of relevant kidney diseases.
3. To develop skills in scientific principles, writing manuscript, and data presentation

**Reading List from the Okusa Laboratory:**

1. Jo SK, Rosner MH, Okusa MD. Pharmacologic Treatment of Acute Kidney Injury. Why Drugs Haven't Worked and What is on the Horizon. Clin J Am Soc Nephrol 2007;2:256-365.
2. Li L, Huang L, Vergis AL, et al. IL-17 produced by neutrophils regulates IFN-gamma-mediated neutrophil migration in mouse kidney ischemia-reperfusion injury. J Clin Invest 2010;120:331-42.

3. Bajwa A, Jo SK, Ye H, et al. Activation of Sphingosine-1-Phosphate 1 Receptor in the Proximal Tubule Protects Against Ischemia-Reperfusion Injury. *J Am Soc Nephrol*. 2010 Jun;21(6):955-65
4. Li L, Okusa MD. Macrophages, dendritic cells, and kidney ischemia-reperfusion injury. *Semin Nephrol* 2010;30:268-77.
5. Bajwa A, Kinsey GR, Okusa MD. Immune Mechanisms and Novel Pharmacological Therapies of Acute Kidney Injury. *Curr Drug Targets* 2009.
6. Kinsey GR, Li L, Okusa MD. Inflammation in acute kidney injury. *Nephron Exp Nephrol* 2008;109:e102-7.
7. Li L, Okusa MD. Blocking the Immune response in ischemic acute kidney injury: the role of adenosine 2A agonists. *Nature Clinical Practice Nephrology* 2006;2:432-44.
8. Kinsey GR, Sharma R, Huang L, et al. Regulatory T Cells Suppress Innate Immunity in Kidney Ischemia-Reperfusion Injury. *J Am Soc Nephrol* 2009;20:1744-53.
9. Li L, Huang L, Sung SJ, et al. NKT cell activation mediates neutrophil IFN-gamma production and renal ischemia-reperfusion injury. *J Immunol* 2007;178:5899-911.

**The following projects may be offered at the University of Virginia in 2011/2012, pending the clarification of funding prior to March 2011:**

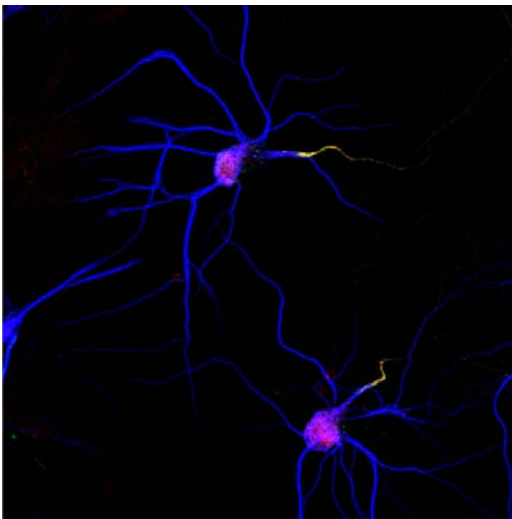


**Bettina Winckler, PhD, Associate Professor of Neurosciences**

**Neurofascin accumulation at the axonal initial segment is promoted by neurotrophin signaling and doublecortin (DCX).**

**(tentative, funding pending)**

Proper functioning of neuronal circuits depends critically on the correct wiring of large numbers of neurons. In addition, the electrical properties of the neurons in the circuit determine the ultimate output from the circuit. The electrical properties of a neuron are determined by the types of channels present, and by the distribution, and abundance of the channels in the neuronal plasma membrane. Changes in channel/receptor distribution and abundance can therefore lead to malfunctioning circuits. Several disorders are thought to be associated with changed receptor distribution or density,



including neuropathic pain, schizophrenia, myasthenia gravis, epilepsy, and multiple sclerosis. There are several subcellular locations in a neuron that contain high concentrations of channels and these domains are particularly important for determining the electrical properties of the cell. Synapses are one such place and much effort is directed at understanding their assembly and regulation. The axon initial segment (AIS), on the other hand, is an understudied domain despite its crucial importance in influencing neuronal excitability. Large numbers of voltage-gated sodium channels are clustered at the AIS, making it the spike initiation zone and the “gatekeeper” for action potential firing. The AIS is also the site of a large number of inhibitory synapses that modify its excitability. Loss of these AIS-resident GABAergic inputs might lead to serious disorders, such as epilepsy and schizophrenia. This project in my lab aims at understanding the cellular and molecular mechanisms that lead to assembly of the AIS-resident cell adhesion molecule neurofascin. This work has important implications for both

neurodevelopment of functional domains in neurons, as well as for disease states in which these domains fail to assemble properly or fail to be maintained.

Current models of how the axon initial segment assembles are solely based on steady-state analysis. While important insights have been gained from steady-state analysis, new approaches are necessary in order to elucidate the cellular pathways and molecular mechanisms responsible for proper assembly of the axon initial segment. The student will therefore combine steady state analysis with kinetic analysis, endocytosis assays, interference approaches, and live imaging to study the dynamics of axon initial segment assembly. Background in cell and molecular biology is desirable.

**Dr Winckler currently supervises Kamil Kruczek from the Jagiellonian University.**



**Dr. Lucy F. Pemberton , Associate Professor of Microbiology  
Center for Cell Signaling**

**(tentative, pending funding)**

Our lab is interested in how assembly and disassembly of chromatin regulates gene expression, replication and DNA repair. Correct regulation is fundamental to cellular processes such as cell division, differentiation and development, and misregulation can lead to genomic instability and ultimately cancer.

Histones are synthesized in the cytoplasm and imported into the nucleus. Our lab uses the model system *S. cerevisiae* and focuses on the early events of chromatin assembly, including understanding how nuclear import helps coordinate the assembly of chromatin. We are interested in questions such as how histones are imported into the nucleus and which transport factors and histone chaperones are important in this process. How do the histone chaperones coordinate the assembly of histones onto DNA and how does this regulate replication and transcription? Lastly, we are also trying to determine what role histone chaperones and the nuclear transport factors play in the removal of histones from chromatin?

We have several projects regarding the function of the histone chaperone Nap1p. Nap1p is part of an evolutionarily conserved superfamily of proteins. Human cells have 4 Nap1 proteins, the SET protein and the TSPY and TSPYL protein families. Different members of this superfamily have been shown to be upregulated or mutated in various cancers such as leukemias and gonadoblastoma, These proteins likely have a developmental role, as in mice loss of a neuronal-specific member of the family is embryonic lethal, and mutant embryos show overproliferation defects in neuronal tissues. We want to understand the mechanisms by which Nap1p is recruited to the promoters of different genes and its function there. We also want to determine how Nap1p mediates the exchange of variant histones with chromatin. This project will use a range of cell biological and biochemical techniques, as well as some yeast genetics.

**Recent Publications**

Del Rosario B.C and Pemberton, L.F. (2008) Nap1 links transcription elongation, chromatin assembly and mRNP biogenesis. *Mol. Cell. Biol.* in press (epub).

Calvert M, Keck K, Ptak C, Shabanowitz J, Hunt D and Pemberton, L.F. (2008). Phosphorylation by CK2 regulates Nap1 localization and function. *Mol. Cell. Biol.* 28: 1313-1325.

Blackwell Jr, J.S., Wilkinson, S.T., Mosammaparast, N. and Pemberton, L.F. (2007) Mutational Analysis of H3 and H4 Amino Termini Reveals Distinct Roles in Nuclear Import. *J. Biol. Chem.* 282: 20142-50.

Mosammaparast, N. Del Rosario, B. D, and Pemberton, L.F. (2005) Modulation of Histone Deposition by the Karyopherin Kap114. *Mol Cell Biol.* 25: 1764-1778.

***Dr. Pemberton supervised previously Kamila Grabowska from the Technical University of Lodz.***



**Jochen Zimmer, D.Phil, Assistant Professor of Molecular Physiology and Biological Physics**

**Mechanism of Biosynthesis and Membrane Translocation of Hyaluronan (tentative, pending funding)**

My group is interested in the mechanism of membrane translocation of biopolymers, in particular polysaccharides. A major emphasis in the laboratory is the functional and structural characterization of the Hyaluronan Synthase (HAS). Hyaluronan (HA) is a linear polysaccharide consisting of alternating glucuronic acid and N-acetyl glucosamine residues and can grow to several microns in length. The HA polymer is synthesized by a membrane embedded, processive glycosyltransferase (HAS) on the cytoplasmic side of the cell membrane and, coupled to its synthesis, is translocated across the membrane to form a major component of the extracellular matrix in vertebrates. HA affects a broad range of physiological processes, ranging from cell differentiation and proliferation to adhesion and mobility and altered expression levels of HA are involved in a number of pathological conditions, including breast and lung carcinoma. We are using the tools of molecular and structural biology to study the mechanism by which the HA polymer is translocated across the cell membrane. Our goal is to establish an in vitro HA translocation assay from purified components and to determine the structure of HAS using x-ray crystallography. The laboratory is very well set up for large-scale membrane protein expression and purification as well as their functional characterization in proteoliposomes.

**This is the first year for Dr. Zimmer in the program**