

**URA's, USRA's, MCB-SRA's 2012  
in the Department of Molecular and Cellular Biology**

The Department of Molecular and Cellular Biology is offering 6 URA's, 8 USRA's and 3 \*\*MCB-SRA for Summer 2011 (scroll down for MCB project proposals). University-wide proposals, application information and award criteria are available on-line at <http://www.uoguelph.ca/registrar/studentfinance/index.cfm?aid/index>.

\*\*MCB-SRA's are offered in conjunction with the University's URA and NSERC's USRA programs. The criteria and value of these awards is the same as that of the USRA's.

For information on award criteria and the application process, please visit the Student Financial Services website:

USRA's - <http://www.uoguelph.ca/registrar/studentfinance/index.cfm?usra/index>

URAs - <http://www.uoguelph.ca/registrar/studentfinance/index.cfm?ura/index>

The Chair's Office in the Department of Molecular and Cellular Biology (MCB) is now accepting applications in SCIE 4478. **All application materials must be received in hard copy by 4p.m. on Friday, February 3, 2012. Applications not received in the Chair's Office will not be considered.** Please direct all queries to Laleh Hatefi at [lhatefi@uoguelph.ca](mailto:lhatefi@uoguelph.ca).

MCB Faculty Research Profiles may be viewed at [http://www.uoguelph.ca/mcb/people/mcb\\_faculty.shtml](http://www.uoguelph.ca/mcb/people/mcb_faculty.shtml).

Faculty Offering Awards for Summer 2012:

**MCB-SRA's**

Anthony Clarke  
Annette Nassuth  
Janet Wood

**URA's**

Mark Baker  
Steffen Graether  
George Harauz  
David Josephy  
Frances Sharom  
Roz Stevenson

**USRA's**

Andrew Bendall  
John Dawson  
Cezar Khursigara  
Matt Kimber  
Baozhong Meng  
Terry Van Raay  
Chris Whitfield  
Joseph Yankulov

### 3 MCB-SRA's

#### 1. MCB-SRA Dr. Anthony Clarke New antibiotic targets in peptidoglycan metabolism

Studies in my laboratory pertain to the metabolism of peptidoglycan with the aim of finding new targets for antibacterial therapy. Peptidoglycan is the rigid cell wall polymer present in most bacterial cells and its continued biosynthesis is essential for their growth and reproduction (division). On-going projects designed to investigate this area of bacterial physiology include the characterization of the autolysins (enzymes involved in the lysis of peptidoglycan during its biosynthesis and turnover), and studies on the O-acetylation and de-O-acetylation of peptidoglycan. Depending upon technical experience, the successful applicant will participate in our investigations on the structure and function relationship of peptidoglycan O-acetyltransferase, O-acetyl peptidoglycan esterase and/or lytic transglycosylases. These experiments will involve: the application of aseptic technique to culture the various bacterial species and strains of interest; isolation and purification of peptidoglycan; cloning and over-expression of genes of interest; and the purification of over-produced proteins by high-pressure liquid chromatography. In addition, assistance will be required for the genetic engineering of bacterial strains for the expression of mutant forms of the enzymes and/or the development of HPLC-based assays for enzymatic activity.

#### 2. MCB-SRA Dr. Annette Nassuth Grape transcription factor localization and regulon

Induction of the CBF pathway leads to stress tolerance in many plants. The pathway induction is thought to activate transcription factors (TFs), called ICE and CBF that are part of this pathway. This activation might affect 1) synthesis and/or stability of the TF, 2) localization of the TFs to the nucleus, and/or 3) binding to regulatory elements and subsequent activation of genes with such elements in their promoter (the regulon).

The Nassuth lab investigates the ICE and CBF transcription factors from wild and cultivated wine grape. Constructs expressing these proteins can be delivered in tobacco leaves by *Agrobacterium* and the subsequent transient expression can be monitored. Stability (1) and localization (2) can be assessed using coding sequence fused to a reporter gene such as GFP; activation of genes, and identification of differences between different ICEs or CBFs can be assessed by RT-PCR analysis of gene expression after agroinfiltration with ICE/CBF expressing constructs. Subsequent experiments can assess the effect of mutations in the ICE/CBF sequences and test the hypothesis that certain protein domains are important for function.

The summer students will participate in the above experiments.

#### 3. MCB-SRA Dr. Janet Wood Osmoadaptation by *Escherichia coli*: osmosensory mechanisms and implications for bacterial pathogenesis

Adaptive mechanisms permit animal, plant and bacterial cells to survive and grow in diverse environments. The Wood lab uses bacterium *Escherichia coli* as a model to study how cells sense and respond to osmotic pressure changes. ProP is one of four transporters that prevent dehydration of *E. coli* cells by catalyzing organic osmolyte accumulation. We showed that ProP, alone, can both sense and respond to osmotic shifts by reconstituting pure ProP in lipid vesicles. But what is the sensory mechanism? Does ProP sense changes in osmotic pressure directly, or does it sense osmotically-induced changes to the cytoplasm or the membrane? *E. coli* adapts to growth in high osmotic pressure media by increasing the cardiolipin (diphosphatidylglycerol) content of its cytoplasmic membrane. The osmotic pressure response-range of ProP is determined by its C-terminal coiled-coil domain and its co-localization with phospholipid cardiolipin at the cell poles. What mechanisms locate cardiolipin and ProP at the cell poles, thereby tuning ProP function? We use biophysical, biochemical and molecular biological to answer these questions by correlating osmotic effects on ProP structure, dynamics, activity and subcellular localization with effects of osmotic pressure changes on the cytoplasm and the membrane in which ProP resides. Those techniques include fluorescence spectroscopy, radiotracer techniques, protein purification and covalent modification, gene deletion, allelic replacement and site-directed mutagenesis. *E. coli* tolerates environments of fluctuating osmotic pressure while surviving transmission between hosts and colonizing mammalian tissues. We have characterized contributions of known osmoadaptive mechanisms to pathogenesis and now seek evidence that additional transporters and osmolytes contribute to bacterial osmotic, thermal, urea and oxidative stress tolerance. These studies will also contribute to *E. coli* "metabolomics" and the functional genomics of *E. coli* membrane proteins. A student recruited through the U(S)RA program will contribute to our research in an area indicated by our recent progress and by the student's interests and academic background.

**1. URA Dr. Mark Baker Role of the human Brca2 tumor suppressor in homologous recombination**

The breast cancer susceptibility 2 (*Brca2*) gene is one of two known breast cancer susceptibility genes. It is a classic tumour suppressor in that individuals carrying a single mutant allele require inactivation of the second allele for cancer to develop. Cells bearing *Brca2* mutations are characterized by gross chromosomal rearrangements (GCR). Since a key function of *Brca2* is control of the central homologous recombination protein, Rad51, defects in the repair of DNA damage by homologous recombination are believed to be the basis for the GCR observed in *Brca2* deficient cancer cells. The *Brca2* gene was discovered over a decade ago, yet many of its functions remain unclear. For several years, our laboratory has been investigating the mechanisms of homologous recombination. As part of these studies, we have developed mouse cell lines that are depleted for mouse *Brca2* through expression of small inhibitory RNA (SiRNA)<sup>1,2</sup>. The *Brca2*-depleted cell lines are deficient in DNA repair and recombination responses. In order to study how the DNA repair deficiency depends on functional *Brca2*, DNA transfection procedures have been exploited to express the full length human *Brca2* gene in the *Brca2*-depleted mouse cells. The human *Brca2* gene is able to “rescue” the homologous recombination deficiency in the *Brca2*-depleted cells. Currently, site-directed mutagenesis procedures are being used to construct mutant forms of the human *Brca2* gene with the aim of testing whether the mutant *Brca2* alleles are able to function in the restoration of homologous recombination in this system. Thus, we have developed a model genetic system in which we can rigorously test the significance of individual *Brca2* amino acid residues for their capacity to function in DNA repair. The URA summer student project will assist in the construction of these mutant *Brca2* alleles and testing their ability to function in homologous recombination. This research will help contribute to our understanding of how *Brca2* functions in the repair of DNA damage in mammalian cells.

1. Lee, S.A. and Baker, M.D. 2007. *DNA Repair* 6:809-817.

2. Lee, S.A. *et al.* 2008. *DNA Repair*- (epub available on-line).

**2. URA Dr. Steffen Graether Protection of membrane damage by an intrinsically disordered protein**

Cold-blooded organisms have evolved several mechanisms to prevent death by freezing and dehydration. In plants, a class of proteins called dehydrins are expressed to inhibit the growth of ice and to prevent proteins and membranes from changing their structure. What is unusual about dehydrins is that they are intrinsically disordered proteins (IDPs), i.e. they have no stable three-dimensional structure, which breaks the central dogma of biochemistry that the function of a protein is determined by its structure. Our lab is interested in understanding how dehydrins carry out their function, with the proposed research project focussing on the protection and interaction of a model dehydrin with membranes and membrane mimetics. This research will help us to better understand how plants tolerate cold and drought, and will provide insight into how disordered proteins can carry out specific biological functions.

The student will learn biochemical techniques such as how to recombinantly express the protein in bacteria and purify the protein using cation exchange FPLC and reversed-phase HPLC. The model membranes, consisting of liposomes and micelles, will also be made and purified by the student. The interaction will be characterized using nuclear magnetic resonance (NMR) and circular dichroism, while membrane protection will be assayed using fluorescence spectroscopy. The long term goal of this project is to understand how the structure of the membrane is stabilized. Students will learn many practical biochemical skills that can be applied to diverse research areas.

**3. URA Dr. George Harauz Molecular architecture of the myelin sheath of the central nervous system and its degeneration in multiple sclerosis**

Canada has one of the highest incidences in the world of multiple sclerosis (MS). An “inside-out” model suggests that MS is caused by a cytodegenerative process aimed at the oligodendrocyte-myelin complex. In other words, the initial pathological event happens inside the myelin or myelinated axons in the central nervous system and leads to a chronic inflammation and autoimmune response. It is thus essential to understand myelin architecture to decipher the onset and pathogenesis of MS. The objectives of this project are to use spectroscopic techniques (FRET – Förster resonance energy transfer, and EPR – electron paramagnetic resonance) to determine how a key basic protein of the myelin sheath is structured and how it may interact with membranes and other proteins to form and stabilize healthy myelin. Recombinant variants of the protein, representing “healthy” and “diseased” forms, will be modified by site-directed mutagenesis to generate single- and double-cysteine substitutions to enable site-specific labeling with fluorescent and spin probes. Proteins will be reconstituted into myelin-mimetic environments for spectroscopic investigations, to be complemented by molecular dynamics simulations. The completion of either Analytical Biochemistry or Laboratory Methods in Molecular Biology I, or equivalent, is expected of applicants. Training in general biochemical and molecular biological techniques such as recombinant protein over-expression and purification, SDS-PAGE, Western blotting, and fluorescence spectroscopy, will be provided.

#### 4. URA Dr. David Josephy Quinone-glutathione adducts: preparation and metabolism

The reactions of quinones with glutathione are important processes for the detoxication of many drugs (e.g., acetaminophen) and toxicants. However, quinone-glutathione conjugates are usually rather difficult to isolate, because the reaction occurs at several positions on the aromatic ring, and the various products (regio-isomers) must be separated by chromatography. The compound 2,3,5-trimethylquinone (TMQ) should circumvent this problem, because only one position (the 4 position on the ring) is available for glutathione addition. The student will prepare this quinone by a published method (McKillop *et al.*, *Tetrahedron* 26: 4031-9, 1970) and study its reaction with glutathione (Butler and Hoey, *Free Radical Biol. Med.* 12, 337-345, 1992) catalyzed by the enzyme glutathione transferase. The product (the TMQ-glutathione adduct) will be isolated and examined as a substrate for the enzyme gamma-glutamyltranspeptidase, using HPLC analysis for the enzyme assay. These studies will allow the student to gain valuable experience in organic synthesis, enzymology, and instrumental analysis.

#### 5. URA Dr. Frances Sharom Investigating the catalytic cycle of the bacterial ABC transporter MsbA using site-directed mutagenesis

The ABC superfamily transporter MsbA plays an essential role in assembly of lipopolysaccharide in the bacterial outer membrane, by acting as a cytoplasmic membrane flippase for lipid A. The protein is also thought to be an efflux pump capable of transporting amphipathic drugs out of the cell. MsbA is the only essential ABC protein in *E. coli*, and is thus a potential novel antibiotic target. It is proposed to function as a homodimer, and go through alternating cycles of ATP hydrolysis at its two nucleotide binding domains (NBDs) to power transport. Site-directed mutagenesis of amino acids in the NBDs and the transmembrane domains is a useful approach to investigate the role of specific residues in the ATP hydrolysis and lipid flippase/drug transport activity of MsbA. The published X-ray crystal structure of MsbA will be used to guide residue selection. The student will express His-tagged native and selected mutant MsbA proteins in *E. coli*, extract them from the membrane with detergent, and purify them using Ni-chelate chromatography. The ATPase activity and ATP binding affinity of each mutant MsbA protein will be determined using enzyme assays and fluorescence spectroscopy, respectively, and compared with native MsbA. The purified MsbA mutant proteins will be reconstituted into lipid bilayer model membranes (proteoliposomes), and fluorescence-based flippase/transport assays will be used to assess their function. These experiments will assist in understanding fundamental aspects of the catalytic and transport cycles of MsbA.

#### 6. URA Dr. Roz Stevenson Environmental reservoirs of fish-pathogenic bacteria

Bacterial pathogens of fish may survive as asymptomatic infections in host fish, but they likely have other environmental reservoirs or habitats in water, sediments, biofilms, and aquatic biota. Amoeba graze on bacteria as a food source, but amoeba-resistant bacteria (ARB) exist which are not digested when taken up by amoeba but survive, and may even multiply, in the host. **This project aims to design and test a pathogen screening system based on the ability of environmental amoeba to selectively extract and enrich bacterial pathogens of fish and animal hosts.** Amoeba can select specific strains of a pathogen that have important virulence traits, and also resuscitate damaged bacteria and facilitate detection of viable-but-not-culturable pathogens. Encysted amoeba may also allow pathogens to survive disinfectant treatments used in aquaculture. Rapid PCR techniques with bacteria-specific primers allow detection of target organisms in amoeba even when they are present only at low levels in populations of other, innocuous, aquatic microbiota. **Student work on this project will involve:** (1) collection, culture and identification of amoeba from aquatic sources; (2) testing amoeba and cysts for ARB by microscope examination and PCR amplification of prokaryotic ribosomal RNA sequences; (3) evaluation of interaction of amoeba isolates (stock cultures and new isolates) with known fish pathogens: *Aeromonas salmonicida*, *Yersinia ruckeri*, *Renibacterium salmoninarum*, *Flavobacterium* spp. **Skill-requirements and development:** The student should already have some basic skills in (or aptitude for) aseptic technique, microbial culture and light microscopy. There will be opportunities to enhance these skills and to learn and apply molecular techniques including DNA isolation, gel electrophoresis, PCR and microbe identification.

## 8 USRA's

### 1. USRA Dr. Andrew Bendall Molecular mechanisms of *Dlx* gene function

Vertebrate *Dlx* genes are expressed in a variety of embryonic tissues with a broad range of biological functions that are context-specific. For example, *Dlx5* expression demarcates the anterior limit of the neural plate early in neurogenesis and later controls the maturation of specific subsets of forebrain neurons. *Dlx5* also regulates differentiation in osteoblasts and chondrocytes. At the molecular level, there is accumulating evidence that a given Dlx protein may act as either an activator or repressor of transcription, depending upon the promoter. It is now well appreciated that the assembly of gene-specific transcription factors on the regulatory regions of target genes depends on the cellular context and is the result of multiple protein-protein as well as protein-DNA interactions. The cell-type and promoter specificity of action of Dlx proteins is therefore likely to be the result of interactions with cell-specific and promoter-specific protein partners that, together, define the Dlx-dependent response of individual gene targets in different cell types. The successful applicant will contribute to our ongoing investigations into the context-dependent transcriptional properties of Dlx proteins, for which we are using a combination of *in vitro*, cell-based, and *in vivo* functional assays.

### 2. USRA Dr. John Dawson Building Actin Filaments for Structural Biology

Actin is the most abundant protein in the human body, comprising a major component in all types of muscle and in the cytoskeleton that provides strength to the plasma membranes of all of our cells. Despite its major biological role, the atomic details describing actin protein polymers remain unclear because isolating short filamentous actin (F-actin) structures for structural determination is a major problem.

Our lab has pursued several strategies to build and characterize short F-actin structures and is in a position to take advantage of our discoveries. This summer, we need a hard-working and excited person to be part of our team to learn how to produce short F-actin species using mutant and wild-type actin proteins purified from a variety of sources using affinity chromatography and classic protein purification. The successful candidate will also learn how to test the properties of these short F-actin species using a variety of cutting-edge assays involving the use of actin binding assays.

A successful summer student will gain deep experience with protein biochemistry and will make a major contribution toward solving one of muscle physiology / biochemistry's biggest remaining problems.

### 3. USRA Dr. Cezar Khursigara Probing the structure and function of bacterial cell division proteins

Cellular microbiology has seen a renaissance in the past several years, spurred in part by advances in imaging techniques. The ability of researchers to peer into cells and visualize the localization and organization of macromolecular complexes involved in fundamental biological processes has advanced our understanding of how bacterial cells divide, move, associate and infect. The primary focus of our laboratory is to develop and use novel cryo-electron microscopy, electron tomography and correlative light-electron microscopy techniques to advance our understanding of how bacteria perform complex cellular processes. Specifically, we are investigating the organization and structures of macromolecular protein complexes involved in bacterial cell division.

In *E. coli*, the cell division plane is defined by the presence of the Z ring, which is a polymer of the tubulin homologue FtsZ (filamentous temperature sensitive protein Z). The primary function of the Z ring is to recruit and assemble a series of proteins involved in cell division, collectively called the divisome. Although several models have been proposed to explain how FtsZ filaments form at the site of division, the mechanism by which the Z ring assembles and applies constrictive forces remains unclear.

This studentship offers an excellent opportunity to participate in a multidisciplinary research effort by cloning relevant cell division genes, expressing fluorescently tagged divisome proteins and monitoring the localization of these proteins within bacterial cells. The resulting protein localization patterns will be further probed at high-resolution using cryo-electron microscopy. This project is intended to provide students with strong research skills in molecular biology, biochemistry and microbiology and will make a significant contribution towards our understanding of bacterial cell division. With the emergence of a growing number of multi-drug resistant strains of bacteria there is a pressing need to identify new drug targets. Accordingly, the process of cell division and the divisome complex provides a number of exciting candidates.

**4. USRA Dr. Matt Kimber Structural and interaction studies of a carboxysomal  $\square$ -carbonic anhydrase**

Carboxysomes, which fix inorganic carbon in cyanobacteria, are ecologically important in that they mediate about a quarter of the planet's overall carbon fixation. Carboxysomes are biochemically unusual objects, being extremely large (up to 400 nm) and organized as an enzymatic core of RubisCO and carbonic anhydrase (CA which catalyses the interconversion of CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup>), encapsulated by a thin (~5 nm) protein shell.  $\square$ -Carboxysomes use either the  $\square$ -CA, CcaA, or a version of the scaffolding protein CcmM that contains an active  $\square$ -CA, as their carbonic anhydrase. Interestingly, these two unrelated proteins can both be present and active, and should form a complex, but the interaction mode and kinetics consequences of this complex are unknown. The goal of this project is to clone, purify, kinetically characterize and crystallize a CcaA homolog. Structure determination is also possible if sufficient progress is made. In addition, the student will work towards purifying a well-defined complex between CcaA and CcmM for biochemical and structural characterization. The trainee will have the opportunity to learn a wide variety of generally useful techniques, including cloning, protein purification, protein crystallization, characterization of protein interactions through co-purification and FRET techniques, and enzyme kinetics.

References: Espie GS, Kimber MS. *Photosynth Res.* 2011 Sep;109(1-3):7-20.  
Peña KL, Castel SE, de Araujo C, Espie GS and Kimber MS. *PNAS.* 2010; 107(6):2455-60.  
Kimber MS and Pai EF. *EMBO J.* 2000;19(7):1407-18.

**5. USRA Dr. Baozhong Meng Molecular mechanisms that govern virus replication, *in vitro* virion assembly and proteolytic processing of *Grapevine rupestris stem pitting-associated virus***

*Grapevine rupestris stem pitting virus* (GRSPaV) is a positive strand, single-stranded RNA virus in the family *Betaflexiviridae*. GRSPaV is widespread among commercial table and wine grape varieties throughout the world and is implicated in several important diseases. As a newly discovered virus, molecular mechanisms that govern different aspects of virus replication and virion assembly are not well understood. Recently, we have established full-length cDNA clones for GRSPaV and have shown that these viral clones are infectious in the model plant *Nicotiana benthamiana* upon delivery through *Agrobacterium*- based inoculation. Several related projects are being carried out in our laboratory, which include (1) optimization of conditions for testing the infectivity and replication kinetics of these viral clones in grapevine protoplasts; (2) determination of the catalytic domain responsible for autocleavage the virus replicase polyprotein and the sites of cleavage; (3) over-expression of the viral capsid protein in *E. coli* and *in vitro* assembly of virion structures; and (4) determination of the 3-D structure of the capsid protein through X-ray crystallization.

The successful student receiving this award will be involved in one or more of these projects and will learn and practice a wide range of experimental technologies that are important for investigations in virology, molecular biology, as well as biochemistry. These techniques include construct design, molecular cloning, over-expression and purification of proteins, PCR and reverse transcription (RT)-PCR, Northern blotting, Western blotting, protoplast generation and electroporation.

**6. USRA Dr. Terry Van Raay Regulation of Wnt signalling in development and disease**

Wnt signalling is important for proper development of the embryo as well as maintaining homeostasis in certain stem cell populations in the adult. How Wnt signalling is turned on and off during development is not well understood and in the adult, when the Wnt signalling pathway can't be turned off it will cause cancer, colon cancer is the most common. In fact, most colon cancers are caused by mutations in the Wnt pathway that keep the pathway on. I am studying how this pathway is normally turned off, to gain insight into how this pathway regulates itself, with the potential to gain insight into how this shutting down mechanism may go awry in disease. One way the Wnt pathway can control itself is through the use of negative feedback regulators: genes that are turned on in response to a Wnt signal, that turn around and shut the pathway down. One such gene is called Naked, named for the mutant phenotype in the fruit fly, *Drosophila*. I am working on the Naked (Nkd) gene in zebrafish because we can do many *in vivo* experiments to obtain a better working knowledge of how this protein works in vertebrates. The project will involve analyzing Nkd1 mutations in the zebrafish embryo. Some techniques will include: gene cloning, sequencing, RNA synthesis, micro manipulation of zebrafish embryos, zebrafish husbandry and protein biochemistry to name a few. MBG\*3350 Laboratory Methods in Molecular Biology (or equivalent) is required.

**7. USRA Dr. Chris Whitfield Assembly of Bacterial Cell Surfaces**

Complex carbohydrates such as lipopolysaccharides and capsular polysaccharides play critical roles in membrane function and cell surface structure, and they are essential for virulence of bacterial pathogens. As a result, their synthesis is being investigated as a target for the design and development of novel antibacterial therapeutics. The assembly of complex macromolecules on bacterial cell surfaces requires the coordinated activity of multi-enzyme complexes that span the cell envelope and export of the macromolecules must happen without compromising the permeability barrier imposed by the cell envelope. To identify key targets for intervention, we are investigating the structure and function of prototype enzyme complexes. This studentship offers an opportunity to participate in this effort. Depending on progress, the student will clone relevant genes, express and purify the key enzymes, generate precise mutations to test predictions about mechanism of action, and make constructs with epitope tags to study the cellular locations of the proteins. This summer project is intended to provide a broad base of research skills in molecular biology, biochemistry and microbiology, as well as make a significant contribution to understanding the processes in bacterial surface assembly. In addition, the student will have the opportunity to work within a larger research group working towards a common goal. To learn more about the lab and its activities, please visit <http://www.uoguelph.ca/~cwhitfie/index.html>.

**8. USRA Dr. Joseph Yankulov Analysis of epigenetic conversions: role of the histone chaperone CAF-I**

Epigenetic regulation refers to the control of gene expression via chromatin structure. Normally, compact heterochromatin represses genes while relaxed euchromatin allows for expression. This type of regulation is critical during stem cell differentiation and has been linked to mental disorders and cancer. We use the yeast *S.cerevisiae* to study the mechanisms of this type of control. In particular, we are focusing on the sub-telomeric loci to study the epigenetic stability of genes over multiple generations. We are also studying how the passage of replication forks influences the epigenetic state of genes.

In eukaryotes, the passing of replication forks disperses the existing chromatin, which is then rebuilt on the two new DNA strands from "old" and "new" histones. Normally, the pre-existing state of chromatin is preserved, but regulated apparent conversions from euchromatin to heterochromatin state and vice versa have been observed. We know very little about the mechanisms of these conversions.

Recently my lab has implicated a histone chaperone (called CAF-I) in the control of epigenetic stability at telomeres. In the current project we explore CAF-I and decipher its mechanism of action. We test if CAF-I is associated with replication forks at the telomeres and if this association correlates to epigenetic stability. We will also test if subtelomeric heterochromatin and telomere length are affected in CAF mutants. The project provides general understanding of epigenetic regulation and practical skills in the analysis of DNA and chromatin, in yeast genetics and in the analysis of gene expression.