The Chair’s Office in the Department of Molecular and Cellular Biology (MCB) is now accepting applications in SCI 4478. All application materials must be received by 4p.m. on Friday, February 10, 2017. Applications not received in the Chair’s Office will not be considered.

MCB is offering 7 URAs, 8 USRAs and 1 *MCB-SRA for Summer 2017 (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-SRAs 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. These projects are listed on the Student Financial Services website as both URAs and USRAs. Faculty with MCB-SRAs are also eligible to supervise student recipients of the College of Biological Science Undergraduate Research Assistantships.

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

USRAs - http://www.uoguelph.ca/registrar/studentfinance/index.cfm?usra/index
URAs - http://www.uoguelph.ca/registrar/studentfinance/index.cfm?ura/index

Application Process in MCB:

If you apply to multiple projects within MCB, you must rank the projects in order of interest. Please do not do this on the application materials; just put it into the text of your email submission or on a separate paper/post it note (for hard copy applications).

Electronic applications are acceptable and should be submitted to mcb@uoguelph.ca. A complete application must be received for each project of interest. Documents must be in 1 PDF in the following order:

1. Form 2 - Student’s Application to Department
   This form must be on 1 page and must be complete. ‘See attached’ is not acceptable.
   Electronic signature is acceptable
2. FORM 202 - Part I is required for USRAs only
   OR
   A Confirmation of Financial Need document is required for URAs only
   Neither Form 202 nor Confirmation of Financial Need are required for MCB-SRAs
3. A current unofficial transcript
4. Any other documents you wish to submit although not required (ie cover letter, resume, etc.)

Hard-copy applications are also acceptable and must be received in SSC 4478. A complete application is required for each project of interest.

Please direct all queries to mcb@uoguelph.ca.

MCB Faculty Research Profiles may be viewed at http://www.uoguelph.ca/mcb/people/mcb_faculty.shtml.

Faculty Offering Awards for Summer 2017:

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1. URA  Dr. Joe Colasanti, Associate Professor  Characterization of a Novel Synthetic Gene that Alters Flowering Time in Diverse Plant Species

The plant life cycle consists of two basic parts: a vegetative phase when plants make leaves and stems, and a reproductive phase when plants produce flowers and seeds. The switch from vegetative to reproductive growth is called the floral transition - a key developmental event in the life of most plants on earth. Understanding the floral transition is especially important for agriculture because a precise balance between vegetative growth and flower production is directly correlated with plant yield. A key regulatory molecule found in all plants that induces the floral transition is called florigen. Florigen is a small protein synthesized in leaves and transported to stem cell clusters in the plant (meristems) where it causes the floral transition. This research project investigates the function of a novel synthetic variant derived from florigen, called anti-florigen, that has the opposite effect of florigen; i.e. it prevents flowering rather than promotes it. The student researcher will characterize this anti-florigen by molecular manipulation of the protein coding sequence to determine how this variant inhibits flowering. This will be done by recombinant DNA techniques followed by transformation into different plant species to determine the effect on plant flowering. In the course of this research the student will develop basic skills in recombinant DNA manipulation, plant transformation, quantitative PCR and high-throughput RNA sequence expression analysis.

2. URA  Dr. Steffen Graether, Associate Professor  Preventing cold damage in plants – the role of dehydrins in protecting the cytoskeleton

The inability of plants to move away from danger has resulted in the expression of large number of proteins to protect them from damage caused by drought, cold, and high salinity. Research in the Graether lab involves the characterization of a family of proteins called ‘dehydrins’ that protect plants from desiccation damage. Their functional characterization is particularly challenging since dehydrins are intrinsically disordered proteins, that is, they do not have a defined structure, such that traditional biochemical approaches have failed to identify their biological target(s).

The main goal of the project is to examine whether dehydrins are able to stabilize microtubules during cold stress. Microtubules are critical parts of the cytoskeleton that are involved in maintaining the cell’s shape and in intracellular transport. They are composed of α-tubulin and β-tubulin monomers. Analysis of gene expression data suggests that some dehydrins in Arabidopsis thaliana are upregulated along with microtubule-binding proteins during cold stress. Preliminary analysis of binding data suggests that dehydrins do not interact with the individual monomers but may interact with the microtubule.

In this project, students will learn how to purify tubulin from natural tissue and purify dehydrins from recombinant bacteria. In addition, they will learn how to use several biochemical techniques (pull-down assays, circular dichroism and fluorescence) to characterize the dehydrins-microtubule interaction.

3. URA  Dr. Baozhong Meng, Associate Professor  Construction of full-length clone for Grapevine leafroll-associated virus 3 for molecular biology and virus-host interaction studies

Grapevines, an economically important fruit crop in the world including Canada, are host to a large number of viruses that belong to diverse taxonomic groups with distinct genome structure and expression strategies. One of the most damaging viruses is Grapevine leafroll-associated virus 3 (GLRaV-3), a member of the genus Ampelovirus in the family Closteroviridae. GLRaV-3 has a single stranded RNA genome of nearly 19 kb containing 13 open reading frames, one of the largest RNA viruses ever identified. Unfortunately, little is known about the function of each of its gene products, molecular mechanisms of replication and its interaction with the host. Recently, we identified two new distinct strains of GLRaV-3 and obtained the complete genome sequences of them. It would be highly desirable to establish an infectious cDNA clone for these two new strains for the purpose of a range of fundamental studies on GLRaV-3.

The main objective of this project is to construct a full-length viral clone for one of these two new strains we have identified. This will involve long-distance RT-PCR, overlap PCR, restriction digest, and cloning into plasmid vectors. In addition to the student’s own project, the student will have the opportunity to be exposed to, and master, a wide range of experimental systems and techniques that are essential for future research in virology, molecular biology, as well as evolutionary biology, which include RNA isolation, RT-PCR, quantitative PCR, restriction digest, molecular cloning, vector engineering, and bioinformatics. Our laboratory is currently composed of three graduate students, a PDF, a research associate, and three undergraduate research project students. The recipient will have plenty of opportunities to interact with others engaged in diverse projects including next generation sequencing, genetic diversity, bioinformatics, subcellular localization and organelle targeting of viral proteins, fluorescence microscopy, as well as diagnostic technologies for major grape viruses. Students with strong interest in virology will receive special consideration for the position.
4. URA Dr. Robert Mullen, Professor Characterization of proteins involved in lipid droplet biogenesis: Development of novel bioengineering strategies for plant biofuels

Lipid droplets (LDs) are subcellular organelles found in essentially all eukaryotic organisms. Their role in sequestering lipids within the cytosol of cells has led to the concept that LD compartments serve as a stable depot for the temporary and efficient storage of high-energy carbon reserves. However, in the last decade there has been an increasing appreciation for the prevalence of LDs in a much broader range of functions, including lipid signaling, trafficking of intracellular components, etc. LDs have also become an attractive target for the enhanced production of biofuels in plants, whereby new bioengineering strategies are being developed based on the formation of LDs in vegetative tissues (e.g., leaves), wherein LDs normally do not accumulate. Recently, we used a combination of lipidomic, proteomic and transcriptomic studies to identify a new class of LD-associated proteins (LDAPs) that are involved in the general process of binding and perhaps the stabilization of LDs in plant cells. We are now interested in identifying and characterizing the molecular mechanisms underlying the function of these proteins. Toward this end, the project will involve the characterization of several novel proteins that we have identified as potential binding partners of LDAP proteins. Collectively, this study will provide an opportunity to engage in cutting edge molecular and cellular biology research, with the long-term goal of better understanding of the process of LD biogenesis and, by extension, enhancing the production of biofuels in plants via bioengineering.

5. URA Dr. Melissa Perreault, Assistant Professor GSK-3 disruption of neuronal firing synchrony in a schizophrenia animal model system

There exists a strong relationship between the coordinated activity of neural networks in specific regions of the brain, such as cortex and hippocampus, with learning and memory. Cognitive decline in neuropsychiatric disorders such as schizophrenia (SZ) is accompanied by a dysregulation in this synchronous neuronal oscillatory activity. Yet, uncovering the molecular signalling deficits that contribute to changes in neuronal oscillations in SZ has proven elusive. Glycogen synthase kinase-3 (GSK-3), a protein that shows upregulated activity in cortex and hippocampus of persons with SZ has been widely shown to negatively impact cognitive performance in a number of CNS diseases, yet the relationship between GSK-3 and neuronal oscillatory activity has not been examined. Our preliminary data indicates an improvement in cortical and hippocampal network activity following short-term administration of GSK-3 inhibitors in Wistar rats. Using a developmental rat model system of SZ, the overall focus of the project will be to examine the effects of GSK-3 inhibition (through the use of inhibitors or AAV-mediated GSK-3 knockdown) on neuronal oscillations in these animals and to associate these changes with improvements in learning and memory. The student will have an opportunity to gain experience in potentially a number of in vivo methodologies by assisting with stereotactic surgeries, drug administration, behavioural analysis and/or immunochemistry. This project will introduce the student to animal models of neuropsychiatric disease and provide a very strong knowledge base for any future research in this area.

6. URA Stephen Seah, Associate Professor Structure-function studies of Ltp2, an enzyme involved in cholesterol degradation in Mycobacterium tuberculosis

Tuberculosis (TB) is the leading cause of mortality by an infectious agent. A hallmark of infection by Mycobacterium tuberculosis, the causative agent of TB, is the ability of the bacteria to survive for long periods of time within nutrient limited granulomas in the lungs by utilizing host cholesterol as sole carbon and energy source.

The focus of this proposal is to determine the structure and function of a key aldolase enzyme, Ltp2, involved in cholesterol degradation in M tuberculosis. The gene encoding Ltp2 is within the "igr, intracellular growth" operon as deletion of this operon in M. tuberculosis led to the inability of this bacteria to replicate within mouse macrophages. We hypothesize that Ltp2 is a novel enzyme that catalyzes a retro-aldol reaction in the last step of cholesterol D-ring side chain reaction. The student involved in the project will receive broad training in a variety of molecular biology and biochemical techniques. These include expression and purification of Ltp2 and site-specific mutagenesis to replace key residues. Purified enzymes will be characterized using various spectroscopic and HPLC based assays. Crystallization trials may also be attempted to enable structural determination of the enzyme. The project will facilitate the development of new antibiotics against drug-resistant strains of M. tuberculosis by inhibiting the cholesterol degradation enzymes.
### USRA's (8)

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<tr>
<th>USRA</th>
<th>Dr. Tariq A. Akhtar, Assistant Professor</th>
<th>Plant Polysoprenoids – Secondary metabolites or physiologically important superlipids?</th>
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<td>This USRA studentship offers an exciting opportunity to gain valuable practical experience in plant natural product biochemistry. We are looking for an enthusiastic and hard-working student to help us better understand a class of compounds known as polysoprenoids, a unique class of hydrophobic natural products that reside in cellular membranes in all kingdoms of life. Yet despite their widespread occurrence, the biological relevance of these compounds is almost completely unknown, particularly in plants. Moreover, the enzymes that are thought to synthesize plant polysoprenoids, which are known as cis-prenyltransferases (CPTs), remain largely uncharacterized. Accordingly, the goal of this summer project is to characterize a recently discovered CPT from tomato (<em>Solanum lycopersicum</em>) and begin to address the physiological role of this enzyme’s product. The student will utilize standard molecular techniques (i.e. PCR, cloning, DNA &amp; RNA isolation) coupled with protein biochemical methods (heterologous expression and protein purification) and advanced analytical approaches (i.e. HPLC-based enzyme assays) in order to accomplish this goal. This summer project is intended to provide a broad base of research skills that are ideally transferable to other disciplines of biology as well as shed light onto the largely unexplored area of plant polysoprenoid metabolism. For further information on this project, interested students are encouraged to review our recently published work in this area (Akhtar et al. 2013. <em>The Plant Journal</em>. 73: 640-652; Brasher et al. 2015. <em>The Plant Journal</em>. 82: 903-914).</td>
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<th>USRA</th>
<th>Dr. Emma Allen-Vercoe, Associate Professor</th>
<th>Development of genetic tools for <em>Fusobacterium nucleatum</em>, a fastidious anaerobic microbe</th>
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<td><em>Fusobacterium nucleatum</em> is an unusual bacterial species with many enigmatic properties. Genome sequence analysis of several strains has revealed highly AT-rich genomes with a very large percentage of predicted genes of unknown function. <em>F. nucleatum</em> is a component of the oral microbiome of most people, and has also been detected in the gut microbiota. Recently, work from our lab in conjunction with our collaborators has shown that some strains of <em>F. nucleatum</em> may behave pathogenically, playing a causative role in colorectal cancer. However, because of the cryptic nature of the <em>F. nucleatum</em> genome, we do not yet understand the genetic basis of the difference between benign and pathogenic strains. There are as-yet no genetic tools that may be effectively used to transform pathogenic <em>F. nucleatum</em> strains in order to start answering questions about the molecular biology behind the characteristics of these strains. Analysis of one of the most well-characterized <em>F. nucleatum</em> genomes has shown the presence of a small, 4kb cryptic plasmid as part of its genome. The successful candidate for this position will work to purify this plasmid, hybridize it with an <em>E.coli</em> p15A origin to create a novel shuttle vector, and then add various components (for example, resistance cassettes, multiple cloning sites, fluorescent gene markers), while determining the dispensability of the 4 cryptic ORFs of the native plasmid, as well as confirming the presence of the fusobacterial origin of replication. The candidate will be expected to have a solid understanding of bacterial genetics, and will be trained in various techniques including anaerobic bacteriology.</td>
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### 7. URA

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<th>Ian Tetlow, Associate Professor</th>
<th>Developing Glycogen Branching Enzymes for Industrial Processing of Starch-Based Polymers</th>
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<td>The ability to manipulate glucan branching in starch-based bio-products offers many industrial end-users in the food and non-food sectors (e.g. paints/coatings) with superior performance products and avoids costly post-harvest modification of starch using environmentally hazardous chemical agents. Glycogen branching enzymes from heat-tolerant prokaryotic sources are promising sources of industrial enzymes for modifying harvested plant starch to improve functional and physicochemical characteristics. This project aims to develop novel branching enzymes from both plant and bacterial sources which will combine the advantages of thermo-tolerance from bacteria with the specific glucan chain transfer capabilities unique to starch branching enzymes from plants. Protein structure and bioinformatics information on plant and bacterial branching enzymes will be employed to guide targeted mutations in specific amino acids and various functional domains in order to modify thermostability and substrate specificity. Mutated proteins will be expressed in heterologous bacterial systems, purified, and biochemically characterized, and glucan products analyzed. It is anticipated that application of modified glycogen branching enzymes with promising properties will be conducted in close collaboration with an existing industrial partner. The student will be involved in cutting-edge research in an important area of biology and biotechnology and learn a range of widely applicable experimental techniques in molecular biology (site-directed mutagenesis, PCR), glucan chemistry, and microbiology, including bioinformatics, protein purification, use of radio-isotopes and carbohydrate extraction and analysis.</td>
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### 3. USRA Dr. Anthony Clarke, Professor

**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.

### 4. USRA Dr. John Dawson, Professor

**How do changes in cardiac muscle proteins lead to heart disease?**

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 our cells. There are 16 known mutants of the human cardiac actin gene (ACTC) that are related to the development of heart disease.

Our lab is one of only three in the world that knows how to produce human cardiac actin employing baculovirus in insect cells. A summer student in our lab will gain experience with this cutting-edge protein expression technique, producing mutant ACTC protein for biochemical characterization to understand the molecular basis of human heart disease development. Specifically, a summer student will learn how to produce recombinant baculovirus through insect cell transfection and cell culturing. You will then purify mutant ACTC protein from larger volumes of infected insect cells using affinity chromatography and follow the purification through SDS-polyacrylamide electrophoresis and quantitation with Bradford assay.

Once purified a summer student will help characterize variant cardiac actin protein interactions with important sarcomeric proteins, such as myosin, alpha-actinin, troponin and tropomyosin to help understand how defects in critical interactions may translate into the development of disease. These interactions will be studied using a variety of biophysical techniques. The student will gain experience with fluorescence- and absorbance-based kinetic assays, fluorescence microscopy-based assays, and *in vitro* motility assays.

### 5. USRA Dr. Scott Ryan, Assistant Professor

**Stem cells and regenerative medicine in the treatment of Parkinson’s Disease**

Parkinson disease (PD) is the second most prevalent neurodegenerative disease worldwide and the most common human movement disorder, affecting over 100 000 Canadians. Characterized by a progressive decline in voluntary movement, motor function eventually dissipates and patients lose the ability to both move and speak.

To better understand the disease, we created a novel stem cell model of PD, wherein we have reprogrammed patient cells to induced pluripotent stem cells. We then used genome editing to correct the genetic mutation that caused the disease in this patient, giving us two genetically identical stem cell systems, one with the disease and one that was effectively normal. Using this system, this project will assess PD pathology by high-resolution live cell imaging coupled with biochemical analysis patient derived neurons. This study will provide a better understanding of disease progression so that we can identify molecular targets for therapeutically development. Our goal is to use this system to establish a drug-screening platform that will identify new drugs able to protect from further neuronal loss in PD or potentially regenerate lost tissue.

### 6. USRA Dr. Jim Uniacke, Assistant Professor

**Investigating the regulation of ribosome composition and alternative splicing by low oxygen in human cancer**

Living organisms are characterized by their ability to respond to stimuli and adapt. The fundamental biological process of protein synthesis, also known as translation, is a crucial step in the flow of genetic information from messenger ribonucleic acid into protein through the use of molecular machines called ribosomes. Stressful environmental changes lead to a reconfiguring of translation efforts away from the maintenance of basic cell functions and toward the production of stress response proteins. This project will investigate the reorganization of ribosome composition for the adaptation to hypoxia (low oxygen) in human cancer cells. The acquisition of adequate oxygen is crucial to produce enough ATP for the proper functioning of cells, especially in those that constitute multicellular animals. Recently, specialized ribosomes have been observed in stressed yeast cells and are a trending topic in molecular biology. Could specialized ribosomes be recruited by the hypoxic translation machinery as an adaptive response for survival under harsh conditions? The student will analyze the global expression and alternative splicing of 78 ribosomal proteins genes between normal and hypoxic conditions by using real-time PCR techniques and capillary electrophoresis. The importance of hypoxia-induced ribosomal proteins will be examined through a variety of classic and modern molecular and cell biology approaches including immunoprecipitation, immunofluorescence, microscopy, human cell culture, polysome profiling, gene cloning and DNA sequencing.
7. USRA  Dr. George van der Merwe, Associate Professor  Delineating the role of the Vid30c in the regulation of the AMPK/Snf1 pathway in *Saccharomyces cerevisiae*.

The mammalian AMP activated kinase (AMPK) and its yeast counterpart, the Snf1 kinase, serve as intracellular “energy gauges” that allow these cells to adapt to changing nutrient and cellular stress environments. AMPK/Snf1-mediated adaptation involves transcriptomic and proteomic adjustments to ultimately coordinate a response to the available cellular energy, which is often dictated by the available carbon sources. Despite the critical function within eukaryotic cells, the molecular mechanism(s) that control adaptation has not been fully elucidated. Our lab has uncovered the Vid30 complex, a ubiquitin ligase, as a pivotal component in Snf1-mediated adaptation in yeast. This project is aimed at gaining further insight into the role(s) of the Vid30c in Snf1-mediated adaptation. The successful candidate will generate a series of mutant yeast strains using integrative transformation and strain confirmation techniques. Mutants will be analysed for its ability to regulate the transcription of specific target genes (qRT-PCR), control the subcellular localization of specific regulatory proteins (fluorescence microscopy) and impact the post-translational modification of these regulatory proteins (western blotting) in comparison to the parent strain. This information will allow us to gain insight into the operation of a highly conserved eukaryotic signalling pathway that ultimately controls metabolism. In yeast this signalling pathway impacts the efficiency with which wine and beer yeast performs alcoholic fermentations.

8. USRA  Dr. John Vessey, Assistant Professor  Investigating Asymmetric RNA-Localization in Neural Stem Cells

Stem cells have the unique ability to divide and produce two daughter cells with different identities. In the mammalian brain, this allows for neural precursor cells to divide and produce a differentiated neuron as well as one recurring stem cell, ensuring that the stem cell population is maintained. How these asymmetric divisions occur is largely unknown, however clues come from model organisms such as Drosophila. In fly neural stem cells (pictured), it’s been shown that certain mRNAs are transported to one region of the dividing cell whereby only one of the daughters inherits them. Typically, these mRNAs encode for proteins that send the receiving daughter cell down the differentiation pathway. I have recently demonstrated that this mechanism is, at least in part, conserved in mammalian neural precursor cells and I found that if the pathway is disrupted, these precursors lose the ability to divide asymmetrically and generate only neurons. I am now setting up my own laboratory with the goal of further investigating asymmetric RNA localization in neural stem cells and how this mechanism contributes to the development of the mature brain. I would like to know how mRNAs destined for localization are identified and how these mRNAs are kept “off” until they arrive at their destination and receive the appropriate signal to commence protein production. To answer these questions, the lab will utilize primary cell culture, molecular biological and biochemical techniques. Applicants should have some exposure to a laboratory setting, either via course work (ex. MGB*3350 – Laboratory Methods in Molecular Biology) or previous experience working/volunteering in a similar setting.

MCB-SRAs (1)

1. MCB-SRA  Dr. Lucy Mutharia, Associate Professor  Developing Genomic Approaches to Diagnostic Tests for Pathogens of Freshwater Fish

Diagnosis of bacterial pathogens that affect freshwater fish has relied heavily on basic microbiology culture and microscopy techniques. However, increasingly, genetic approaches are been used to provide rapid identification and to differentiate strains of a species with different antigenic and virulence characteristics.

The gram-negative bacterium, *Yersinia ruckeri* is the causative agent of enteric redmouth disease in salmonid fish, and several serotypes have been identified. This research project will use existing complete genome sequences, partial sequences along with phenotypic and serological characteristics in order to define and test sequences that could be used for rapid differentiation of serotypes in a diagnostic laboratory.

The work will involve both genetic and microbiology techniques. The student involved in this work should have good basic microbiology laboratory skills (aseptic technique, bacterial culture, basic microscopy) and some knowledge and interest in modern genomic tools and approaches.