Molecules Cells & Systems

TRENT UNIVERSITY MOLECULES, CELLS & SYSTEMS RESEARCH CENTRE

2nd Annual Research Symposium

PROGRAM

Wednesday, April 24, 2024

Trent University Student Centre
TSC 1.20
Peterborough, Ontario

TRENT UNIVERSITY

The Molecules to Systems Approach

The life sciences are a broad grouping of disciplines that study organisms across the tree of life. All organisms, whether unicellular (e.g., bacteria) or multicellular (e.g., plants, animals; including humans), require fundamental molecular building blocks. These building blocks facilitate the formation of a cell, which associates with other cells to form systems within a multicellular organism. Through evolution, organisms have become more complex, facilitated by the formation of specialized interdependent molecular and cellular systems. The Molecules to Systems Approach seeks to understand the mechanisms underlying each stage of this biological hierarchy.

Our Vision

Trent University has a wealth of expertise in various disciplines within the life sciences including molecular biology, cell biology, biochemistry, microbiology, developmental biology, physiology, psychology, and neuroscience. To exploit this competitive advantage, the Trent University Molecules, Cells & Systems Research Centre brings together researchers from the Departments of Biology, Chemistry, Forensic Science, Kinesiology, and Psychology to collaborate and apply the Molecules to Systems Approach in their research.

Organizing Committee

Dr. Robert Huber and Dr. Stephanie Tobin

Acknowledgments

Session moderators: Dr. Sanela Martic, Dr. Leslie Kerr, Dr. Neil Emery, and Dr. Neil Fournier
Talk evaluators: Debbie Lietz, Mark Seegobin, and Samantha Logan
Catering: Tracy Ross and Gillian Ferguson-Martin

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Schedule

Time	Activity
8:45 AM - 9:00 AM	Registration
9:00 AM - 9:05 AM	Introductory remarks
9:05 AM - 10:00 AM	Session 1: Dr. Ian Patterson (Brock University)
10:00 AM - 10:45 AM	Session 2: Student presentations
10:45 AM - 11:00 AM	Break
11:00 AM - 12:00 PM	Session 3: Student presentations
12:00 PM - 12:45 PM	Lunch (TSC 1.07)
12:45 PM - 1:45 PM	Session 4: Dr. Rene Harrison (University of Toronto Scarborough)
1:45 PM - 2:30 PM	Session 5: Student presentations
2:30 PM - 2:45 PM	Break
2:45 PM - 3:45 PM	Session 6: Student presentations
4:00 PM - 4:30 PM	Awards and wrap up

Session 1 (Moderator: Dr. Robert Huber)

9:05 AM – 10:00 AM
Beneficial viruses: engineering viruses to combat vector-borne diseases
Dr. Ian Patterson (Assistant Professor, Brock University)

Session 2 (Moderator: Dr. Sanela Martic)

10:00 AM – 10:15 AM
Aggregation inhibition mechanisms of pTDP-43: Monitoring protein structures and morphologies using common biophysical techniques
Josephine Esposto (Graduate student, Environmental & Life Sciences)

10:15 AM – 10:30 AM
Domain interactions in Giardia flavohemoglobin variants
Isabelle Decorso (Graduate student, Environmental & Life Sciences)

10:30 AM – 10:45 AM
The relationships among phytohormones and benzylisoquinoline alkaloids in Papaver rhoeas L.
Zeynab Azimychetabi (Graduate student, Environmental & Life Sciences)

BREAK (10:45 AM – 11:00 AM)

Session 3 (Moderator: Dr. Leslie Kerr)

11:00 AM – 11:15 AM
Cytokinins and encystation of Giardia intestinalis
Linh Tran (Undergraduate student, Biology)

11:15 AM – 11:30 AM
Optimization of rapid assays to test for flavohemoglobin inhibitors
Elias Henao (Graduate student, Environmental & Life Sciences)

11:30 AM – 11:45 PM
Selective determination of SARS-CoV-2 spike antigen by aptamers using localized surface plasmon resonance
Tyra Lewis (Graduate student, Environmental & Life Sciences)

11:45 PM – 12:00 PM
Magneto-priming provides no physiological advantage for soybean performance: additional evidence at the phytohormone level
Michael Capperauld (Undergraduate student, Biology)

LUNCH (12:00 PM – 12:45 PM)

Session 4 (Moderator: Dr. Stephanie Tobin)

12:45 PM – 1:45 PM
Microtubules from Manitoba to Mars
Dr. Rene Harrison (Professor, University of Toronto Scarborough)

Session 5 (Moderator: Dr. Neil Emery)

1:45 PM – 2:00 PM
Exploring the function of LITAF in Dictyostelium discoideum
Kyra Ball (Graduate student, Environmental & Life Sciences)

2:00 PM – 2:15 PM
Localization of a Giardia transcription factor by epitope tagging
Robert Ta (Undergraduate student, Biology)

2:15 PM – 2:30 PM
Assessing viral activity and changes in nuclei morphology in the presence of cytokinins
Galair Prevost (Graduate student, Environmental & Life Sciences)

BREAK (2:30 PM – 2:45 PM)

Session 6 (Moderator: Dr. Neil Fournier)

2:45 PM – 3:00 PM
Differential expression of cytochrome b5 isotypes during nitrosative stress in Giardia intestinalis
Aurielle Tobias (Undergraduate student, Biology)

3:00 PM – 3:15 PM
The effect of cytokinins (CKs) on skeletal myogenesis
Farnoush Kabiri (Graduate student, Environmental & Life Sciences)

3:15 PM – 3:30 PM
Long-lasting pain hypersensitivity after exposure to predator odor stress in rats
Kirkland Johnston (Graduate student, Psychology)

3:30 PM – 3:45 PM
Neurobiological mechanisms underlying stress-induced memory recall impairments
Lexi Thivierge (Graduate student, Psychology)

AWARDS AND WRAP UP (4:00 PM – 4:30 PM)

Keynote Speaker 1

Beneficial viruses: engineering viruses to combat vector-borne diseases

Ian Patterson

Department of Biological Sciences, Brock University, St. Catherines, Ontario

There is an increased threat of disease caused by viruses transmitted by mosquitoes because of changes in climate and land use. In general, two types of viruses are found in mosquitoes: arthropod-borne viruses (arboviruses) that are transmitted to humans and animals that can cause disease, like Zika virus and West Nile virus, and viruses that only infect the mosquito, called insect-specific viruses. Some insect-specific viruses may be beneficial because they can prevent mosquitoes from being infected by or transmitting arboviruses that cause disease. Negeviruses are a group of insect-specific viruses that may be particularly useful. Negeviruses have several characteristics that make them useful for biotechnology and controlling arbovirus infections. My research has evaluated the ability of negeviruses to block arboviruses from infecting mosquito cells and the potential to modify negeviruses to be even more effective at stopping arbovirus transmission in mosquito hosts. The long-term aim of these studies is to support new strategies to use insect-specific viruses to prevent mosquitoes from transmitting dangerous arboviruses and to inform release strategies of beneficial viruses in insect populations.

Keynote Speaker 2

Microtubules from Manitoba to Mars

Rene Harrison

Department of Biological Sciences, University of Toronto Scarborough, Scarborough, Ontario

Dr. Rene Harrison is a cell biologist with an expertise in the microtubule (MT) cytoskeleton which began as an undergrad at U of Winnipeg where her 4th year project looked at a crop herbicide that bound to specific isoforms of tubulin (the basic subunits of MTs). In her MSc at the University of Manitoba, Dr. Harrison studied MTs in a developmental context: in the ovarioles of the blood-feeding insect *Rhodnius prolixus*. For her PhD and postdoctoral work at Sick Kids Toronto, Dr. Harrison continued cytoskeletal analysis in breast cancer cells, then macrophage immune cells. She has continued studying macrophage MTs in her own lab at UTSC and extended this work to multinucleated osteoclasts, which form from macrophage fusion events. Osteoclasts are bone-degrading cells and of great interest clinically and this work led her to study bone loss in microgravity, where astronauts experience significant disuse osteoporosis.

Dr. Harrison has trained over 30 graduate students and postdocs and the lab has been supported by CIHR, NSERC and CSA grants. She now serves as the Vice-Dean, Graduate & Postdoctoral Studies at UTSC.

Aggregation inhibition mechanisms of pTDP-43: Monitoring protein structures and morphologies using common biophysical techniques

Josephine Esposto[1], Robert J. Huber[1,2], Sanela Martic[1,3]

[1]*Environmental & Life Sciences Graduate Program, Trent University, Peterborough, Ontario*
[2]*Department of Biology, Trent University, Peterborough, Ontario*
[3]*Department of Forensic Sciences, Trent University, Peterborough, Ontario*

TAR DNA-binding protein-43 (TDP-43) is a protein that has been implicated in several neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). In TDP-43 pathology, common characteristics include hyperphosphorylation, aberrant aggregation, and an increased cytoplasmic concentration in neuronal cells. Inhibiting the protein's aggregation mechanism has been suggested as a possible therapeutic target for treating ALS and FTLD. To date, research has demonstrated only a superficial understanding of the protein's structure and function, thus providing us with a critical knowledge gap to address. In this study, several biophysical techniques were used to characterize the morphology, structure, and antibody-based interactions of the *in vitro* aggregated proteins. Specifically, turbidity was used to monitor insoluble protein inclusions, thioflavin T (ThT) was used to monitor β-sheet formation, and transmission electron microscopy (TEM) for visualization of the protein aggregates. To understand its interactions and binding mechanisms, TDP-43 was incubated with epitope-specific antibodies and assessed for inhibition. As a result, the protein-antibody interactions showed a reduction in the formation of insoluble aggregates at differing antibody concentrations. This was consistent with the presence of hair-like fibrils from the TEM analyses and ThT-positive inclusions, as well as the negative results from additional antibody controls. The outcomes of the inhibition were highly dependent on the type and concentration of antibodies, indicating a dual functionality of the inhibitors. Further studies are required but provides a preliminary platform for TDP-43 proteinopathy treatments.

Domain interactions in Giardia flavohemoglobin variants

Isabelle Decorso[1], Steven Rafferty[1,2]

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[2]*Department of Chemistry, Trent University, Peterborough, Ontario*

Giardia flavohemoglobin (gFlHb) is the only heme enzyme of the microaerotolerant protist, *Giardia intestinalis*. Giardia causes giardiasis, the most common mammalian parasitic intestinal infection worldwide. gFlHb protects the parasite from nitrosative stressors encountered within the small intestines of mammals. As part of my characterization of the functional properties of gFlHb, I spectroscopically compared its NADH oxidase activity to that of Hmp, the flavohemoglobin of *E. coli* and observed that the activity of Hmp is twice that of gFlHb. Additionally, I found evidence that implicates gFlHb in oxidative stress response. This finding holds significance as Giardia lacks most conventional antioxidant mechanisms, such as superoxide dismutase and catalase. I predict that the functional differences between the two enzymes may be due to a pair of sequence inserts within the flavin and heme-binding domains of gFlHb that are predicted to interact with each other. Interestingly, metronidazole-resistant variants of Giardia are associated with alterations to the gFlHb gene, and many of these changes occur within these unique amino acid inserts. I will make destabilizing mutations within these inserts and study their effects on its NADH oxidase activity in comparison to the wildtype enzyme. This comparison will include the effects of prospective electron acceptors including oxygen, ferricytochrome c, and hydrogen peroxide. I also intend to replicate the metronidazole-related mutations and investigate their impact on the activity of three gFlHb variants.

The relationships among phytohormones and benzylisoquinoline alkaloids in *Papaver rhoeas* L.

Zeynab Azimychetabi[1], Scott C. Farrow[1,2], R.J. Neil Emery[1,3]

[1]*Environmental & Life Sciences Graduate Program, Trent University, Peterborough, Ontario*
[2]*Solar Biotech, Peterborough, Ontario*
[3]*Department of Biology, Trent University, Peterborough, Ontario*

Benzylisoquinoline alkaloids (BIAs) are widely distributed in the plant kingdom, playing essential roles in defense against pathogens and herbivores. These compounds are of great interest for both ecological and pharmaceutical research. The biosynthetic pathways of several BIAs in opium poppy (*Papaver somniferum*) have been well-characterized, however, how individual genes within these pathways are regulated remains largely unknown. Phytohormones are a class of naturally occurring, small organic molecules that coordinate a comprehensive suite of physiological processes in plants at very low concentrations. Because phytohormones may alter production of secondary metabolite defense compounds, I hypothesize that phytohormones regulate BIA metabolism. To date, phytohormones and BIA profiles have not been investigated simultaneously during ontogenesis in any member of the Papaveraceae family. Therefore, I investigated phytohormone and BIA profiles of Field poppy (*Papaver rhoeas* L.) during the first 5-days of in vitro culture. My data clearly showed that the production of BIAs depends on the developmental stage and starts between days three and four at shoot emergence. Phytohormone profiles changed during this time simultaneously, and directly correlated with changes observed in BIA levels. In addition, for the functional investigation of phytohormones that control the BIA pathway, silencing their biosynthesis, degradation, and response factor genes will help confirm their function. To knock down the genes related to phytohormones and BIA biosynthesis and/or regulation, I used virus-induced gene silencing (VIGS). The results from the VIGS experiment demonstrated that modifying the expression of genes associated with a class of phytohormones, cytokinins, leads to variations in the production of compounds across various branches of the BIA pathway.

Cytokinins and encystation of *Giardia intestinalis*

Linh Tran[1], Vedanti Ghatwala[2], R.J. Neil Emery[1,2], Janet Yee[1,2]

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[2]*Environmental & Life Sciences Graduate Program, Trent University, Peterborough, Ontario*

Giardia intestinalis is a protist that causes diarrheal disease in humans. The infection initiates when a host ingests infectious cysts in contaminated water. Once inside the host, the cyst hatches in the stomach to release the trophozoite form, which swims down and proliferates within the intestinal tract. In the small lower intestine, some trophozoites develop back into cysts, which are released in the feces where they become a source of new infections. The conversion of the trophozoite into the infectious cyst form is called encystation and this can be induced in laboratory cultures. Our lab is interested in studying the roles of cytokinins (CKs) in Giardia. CKs are hormones that are derived from adenosine. Previous work in our laboratory showed that Giardia can metabolize CKs during growth in both nutrient-rich and nutrient-poor conditions. My research is to determine if the addition of the cytokinin benzyladenine riboside (BAR) can inhibit the induction of Giardia encystation *in vitro*. Efficiency of encystation of the Giardia cultures was monitored by the appearance and localization of a cyst wall protein (CWP1) by using immunofluorescence microscopy, and by quantifying the increase of this protein by using immunoblotting. Those experiments were performed in parallel with endogenous measurements of the forms and levels of CKs inside Giardia cells during encystation using liquid chromatography-high resolution mass spectrometry.

Optimization of rapid assays to test for flavohemoglobin inhibitors

Elias Henao[1], Steven Rafferty[1,2]

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[2]Department of Chemistry, Trent University, Peterborough, Ontario

Although the parasitic protist *Giardia intestinalis* cannot make heme, it possesses genes that encode for hemoproteins. Among these, there is a flavohemoglobin (gFlHb) that differs from other flavohemoglobins because of two sequence inserts, each approximately 25 amino acid residues long, not found in any other members of this enzyme class. Flavohemoglobins act as nitric oxide dioxygenases (NOD) by catalyzing the reaction of globin-bound diatomic oxygen with nitric oxide to form nitrate, the uncatalyzed reaction would yield nitrite. This distinction is important as it is the basis for a means of screening for flavohemoglobin inhibitors that could be developed into drugs. There are no giardia-specific drugs, but certain azole-based compounds that bind to the heme iron can inhibit the NOD activity of other flavohemoglobins, such as Hmp of *E. coli*. The colorimetric Griess assay offers a cheap, rapid, and effective way to test a compound's ability to inhibit NOD activity of an enzyme, using two reagents that yield a color change in the presence of nitrite but not nitrate, with nitrate predominating as a product of the enzyme-catalyzed reaction. Despite being more expensive, a fluorescence assay, based on the change of intensity of the fluorescent probe 2,3-diaminonaphthalene (DAN) caused by the presence of nitrite in the solution, provides more sensitive and reliable measurements in an equally rapid manner. These assays work as rapid screening methods to identify potential giardia-specific inhibitors for FlHb by increasing the amount of detectable nitrite so we can identify what compounds are better at inhibiting NOD activity.

Selective determination of SARS-CoV-2 spike antigen by aptamers using localized surface plasmon resonance

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[2]Department of Forensic Science, Trent University, Peterborough, Ontario

The emergence and global spread of the SARS-CoV-2 virus has demonstrated the need for reliable, cost-efficient, and rapid detection methods. Traditionally, methods such as nucleic acid-based polymerase chain reaction (PCR) are used for the determination of viral infections, but it can be costly, time-consuming, and accompanied by high false -negative and -positive rates.[1] Thus, alternative methods are needed to improve the overall reliability of results and response time. The SARS-CoV-2 spike (S) glycoproteins play an important role in the function of the virus and can be used as diagnostic biomarkers for viral diseases.[1] Aptamers are suitable for biosensing applications, as they are highly specific, high-affinity binding partners toward their designated targets.[1] Localized surface plasmon resonance (LSPR) is an analytical technique that generates real-time, rapid results within minutes, which is also beneficial for point-of-care testing. In this work, a LSPR aptasensor was fabricated on a gold nanoparticle-streptavidin-biotin surface and aptamers used for the detection of SARS-CoV-2 antigens.[2] The S1 aptamer selectively bound the S1 antigen, with high affinity. Spiked samples achieved >90% recovery with the S1 aptasensor, and the sensor exhibited excellent shelf-life stability. Data indicate that LSPR is a viable analytical tool for measuring SARS-CoV-2 related aptamer-antigen interactions and such sensing platforms can be applied to other viral or non-viral antigen targets.

[1] Song, Y., Song, J., Wei, X., Huang, M., Sun, M., Zhu, L., Lin, B., Shen, H., Zhu, Z., Yang, C. *Anal. Chem. 92* (2020) 9895–9900.
[2] Lewis, T., Giroux, E., Jovic, M., Martic, S., *Analyst 146 (*2021) 7207-7217.

Magneto-priming provides no physiological advantage for soybean performance: additional evidence at the phytohormone level

Michael Capperauld[1], Daniel Palberg[2], R.J. Neil Emery[1,2]

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Over the last 60 years, magneto-priming (MP) has been reported as a sustainable method to enhance crop yield and resistance to adverse environmental conditions. Our study aims to determine whether the analysis of phytohormone flux can aid in the understanding of the altered physiological properties witnessed with MP. Soybean seeds were exposed to a static MF of 150 to 205 mT for 1 hour and used in either seedling or early vegetative growth (EVG) experiments. In seedling experiments, seedling weight radical length, and time to reach radical emergence were tracked to assess differences in the rate of growth. EVG experiments were used to examine other important agronomic properties such as the height, leaf area, chlorophyl content, and a LI-COR gas analyzer was used to assess the photosynthetic performance, transpiration, and conductance of the cotyledons. Biochemical level perturbations were investigated by harvesting cotyledons for phytohormone analysis using ultra-high-performance liquid chromatography high-resolution mass spectrometry (UHPLC-HRMS/MS). MP seedlings achieved radical emergence earlier; however, no other significant differences were found among the myriad of measurements. Our work provides a new perspective on MP and is in strong disagreement with previous claims that MP is an effective priming technique.

Exploring the function of LITAF in *Dictyostelium discoideum*

Kyra Ball[1], William D. Kim[1], Robert J. Huber[1,2], Craig R. Brunetti[1,2]

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Lipopolysaccharide induced tumour necrosis factor alpha-factor (LITAF) is a gene that plays important roles in the transport and delivery of proteins in the cell. In humans, LITAF localizes to endosomes and lysosomes and mutations in the *LITAF* gene cause a form of neuropathy called Charcot-Marie-Tooth disease type 1C (CMT1C). Although it is known that LITAF plays a role in endosomal trafficking, the precise mechanisms regulating its function are largely unknown. In this study, we examined the functions of normal and mutated LITAF in the model organism *Dictyostelium discoideum*, which has proven to be a reliable model for studying a multitude of neurodegenerative diseases. The *D. discoideum* genome encodes a homolog of mammalian LITAF known as Litaf. Our work shows that Litaf localizes to compartments involved in protein degradation and *D. discoideum* cells overexpressing mutated Litaf display an increased number of acidic compartments. Additionally, we show that mutated Litaf affects protein secretion, increases lysosomal enzyme activity, and decreases proteasome activity. Collectively, these data provide insight into the cellular affects of mutated LITAF and how they are linked to CMT1C.

Localization of a Giardia transcription factor by epitope tagging

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[2]*Environmental & Life Sciences Graduate Program, Trent University, Peterborough, Ontario*

Giardia intestinalis is an intestinal parasite that has a streamlined genome and minimized transcriptional machinery resulting from reductive evolution. The TATA-binding protein (TBP) is a critical transcription factor required for transcription by all eukaryotic RNA polymerases. Giardia encodes a single TBP (gTBP) that has the predicted protein fold common to other TBPs despite its high sequence divergence and substitutions in 3 out of 4 key phenylalanines that intercalate the DNA. Previous *in vitro* studies in our lab showed that gTBP preferentially binds single-stranded DNA over double-stranded DNA. Furthermore, its strongest mode of binding is to regions of single-stranded DNA with inherent flexibility. To determine if gTBP binding preference *in vivo* resembles that observed *in vitro*, an antibody that binds tightly and specifically to gTBP is needed. Since it was not possible to obtain such an antibody after multiple attempts, my goal is to HA-epitope tag the gTBP and express this fusion protein in transfected Giardia cells. Specific antibodies against the HA epitope are available from several commercial sources that could be used for future assays to examine the gTBP DNA binding *in vivo* and to identify its protein partners. One of my first uses for an epitope-tagged gTBP is to confirm its nuclear localization in transfected Giardia cells by using immunofluorescent microscopy.

Assessing viral activity and changes in nuclei morphology in the presence of cytokinins

Galair Prevost[1], Samantha Logan[1], Mark Seegobin[1], Shishir Siresh[2], R.J. Neil Emery[1,2], Craig R. Brunetti[1,2]

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Cytokinins (CKs) are a group of phytohormones best known from plant taxa. These N6 adenine derivative molecules are found across all kingdoms of life and are known for their role in the regulation of growth and development in plants. Frog virus 3 (FV3) is a type species of the Iridoviridae family, genus Ranavirus. FV3 is a main contributor to amphibian population declines in North America. The global impact of FV3 has led to extensive research making it a model virus for research. Previous work has demonstrated that CKs inhibit FV3 replication in a dose-dependent manner. We tested selected CKs and found that concurrent treatment of 20 □M of either N6-isopentenyl adenine (iP), N6- isopentenyladenosine (iPR), N6 furfurladenine/kinetin, and kinetin riboside (KR) showed decreases in FV3 replication. To expand on this, we examined the impact of CKs during viral replication on host cell nuclei. We found that concurrent infection with FV3 and treatment with iP or iPR produced larger host nuclei than FV3 infection alone. This may imply that CKs impact host cell nuclear structure. These results are the first to reveal insight into the potential mechanism in which FV3 replication is inhibited by iP and iPR.

Differential expression of cytochrome b5 isotypes during nitrosative stress in *Giardia intestinalis*

Aurielle Tobias[1], Anasofia Vargas[1], Melanie Marlow[2], Janet Yee[1,2]

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Giardia intestinalis is a protist that causes severe and chronic diarrhea in humans and is a common contaminant of freshwaters worldwide. Host immune responses includes the release of nitric oxide from the gut epithelium that results in nitrosative stress to the Giardia. One of the five heme proteins of Giardia is a flavohemoglobin (gFlHb) which counteracts nitrosative stress with its nitric oxide dioxygenase activity. The remaining four heme proteins in Giardia are isotypes of cytochrome b5 (gCYTB5-I, II, III, and IV). Our lab is interested in determining if any of these isotypes also have a role in Giardia's response to nitrosative stress. My research involves inducing nitrosative stress in laboratory cultures of Giardia with S-nitrosoglutathione (GSNO) at a sublethal concentration of this stressor that inhibits growth in the culture over 24 hours. Protein extracts from GSNO-treated cultures are collected and analyzed by immunoblotting with antibodies specific for each gCYTB5 isotype. The level of gFlHb will also be examined in these cultures to validate that nitrosative stress was induced. My hypothesis is that at least one of the gCYTB5-I isotype will increase in these GSNO cultures compared to an untreated culture grown over the same time period.

The effect of cytokinins (CKs) on skeletal myogenesis

Farnoush Kabiri[1], Lorna N. Phan[2], Stephanie W. Tobin[1,2]

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[2]Department of Biology, Trent University, Peterborough, Ontario

Myogenesis is a crucial process in embryonic development, postnatal growth, and adulthood, involving the formation and maturation of muscle tissue from precursor cells called myoblasts. Disruption of myogenesis can lead to various muscle disorders. Cytokinins (CKs) are adenine-derived signaling molecules with central roles in cellular growth, proliferation, and differentiation in various organisms, and occur in different structural forms and types. Among the different CK types, N6-furfuryladenine (kinetin) and N6-isopentenyladenine (iP) species are of significant interest in mammalian research. Although the roles of CKs in plants are well established, their functions and mechanisms of action in mammals are largely unknown. Recent studies have uncovered the potential functions of CKs in mammalian cells and in regenerative medicine, including skeletal muscle differentiation. iP is the building block for downstream CK types and may play a role in muscle cells, similar to its role in endothelial cells, mobilizing catabolic processes downstream of AMPK. Therefore, to investigate the effect of CKs on skeletal myogenesis, we focused on iP species. Our laboratory is currently working on the C2C12 myogenic cell line as a model for myogenesis research. Cells were treated with different concentrations of CKs, and Cell Counting Kit-8 and BrdU cell proliferation assays were used to observe the effect of different concentrations of CKs on cell viability and proliferation, respectively. Fluorescent images were captured and analyzed for differentiation and fusion indices as myogenic features for each treatment. Preliminary results demonstrated that iP-riboside has an inhibitory effect on C2C12 cell proliferation and myogenesis.

Long-lasting pain hypersensitivity after exposure to predator odor stress in rats

Kirkland Johnston[1], Neil M. Fournier[1]

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It is well known that pain can heighten sensitivity to stimuli that signal threat in most species. While evidence suggests an adaptive value of this enhanced sensitivity during states of acute pain, prolonged sensitivity of behavioural and neural responses to pain can be maladaptive if it persists in the absence of threat for extended periods. In humans, persistent sensitivity to both actual and perceived environmental threats often coincides with heightened sensations of pain and anxiety. In rodents, exposure to predator-derived kairomones, such as the odour component (2,3,5-trimethyl-3-thiazoline, TMT) found in fox feces, can trigger innate defensive reactions, such as avoidant behaviours, hypervigilance, and activation of the stress-hormonal axis. Alongside these defensive reactions, TMT typically results in analgesia. However, recent studies have shown that a brief (5 min) exposure to TMT can markedly enhance fear and nociceptive sensitivity in mice that have recently recovered from an injury. Building on these findings, this study aims to examine whether acute pain in rats similarly enhances sensitivity to threat, as observed in mice, potentially leading to a long-lasting enhancement of pain and anxiety behaviours.

Neurobiological mechanisms underlying stress-induced memory recall impairments

Lexi Thivierge[1], Neil M. Fournier[1]

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Several cognitive functions are known to be affected by stress, particularly memory retrieval. This susceptibility is believed to be mediated, at least in part, by stress-induced release of hormones and catecholamines which can interfere with neural processes associated with memory retrieval. Exposure to stress can promote rapid and significant changes in neural plasticity in brain regions critical in learning and memory, such as the hippocampus and medial prefrontal cortex. Thus, understanding these mechanisms is crucial for developing interventions to mitigate the adverse effects of stress on memory retrieval. In the present study, we found that sub-chronic exposure to thirty minutes of elevated platform stress over three consecutive days can significantly impair previous context fear memory in rats. Examination of neuronal activation marker, c-Fos, found that robust activation in brain areas involved in emotional regulation and threat behaviour following elevated platform stress. In addition, we also found reduced expression of reelin, an extracellular glycoprotein linked to dendritic spine formation and synaptic plasticity in the medial prefrontal cortex after platform stress. Interestingly, intravenous treatment of recombinant reelin before exposure to the stressor abrogated impairments in memory retrieval. These findings highlight a novel role for reelin as a potential target for treating memory impairments induced by stress.