



March 23 and 24, 2019
at The University of Western Ontario

POSTER ABSTRACTS

Saturday March 23rd 2019

POSTER SESSION

1:00 – 2:30

PHYSICS & ASTRONOMY BUILDING - ATRIUM

1. Understanding the role of bile salt mixture in enterohemorrhagic *Escherichia coli*'s immune evasion strategies

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Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 is a food and water-borne pathogenic strain of *E. coli* that can cause severe gastrointestinal disease such as gastroenteritis, enterocolitis, hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) with potentially fatal consequences. EHEC responds to environmental cues such as bile salt mixes and ferric iron leading to the upregulation of the expression of genes that encode a two-component system (basRS) and a lipid A modification pathway (arnBCADTEF). The activation of this pathway results in the masking of negative charge on the pathogen's lipopolysaccharide, preventing interaction with cationic antimicrobial peptides produced in the gastrointestinal tract of the host. What is not yet known is how these lipid modifications affect the cytokine production of infected host cells. This study is focused on assessing the impact of EHEC treatment with different concentrations of bile salt mix on the cytokine production of EHEC-infected murine macrophages. Results will be obtained using quantitative real time PCR (polymerase chain reaction), PCR, and southern blots. The current focus of this work is on optimizing the real-time PCR and semi-quantitative PCR to evaluate the expression of three cytokines, TNF α , IL6 and IL10 in EHEC-infected murine macrophages. This study can contribute to our understanding of EHEC responses to host innate immune responses and how these responses enhance the virulence potential of this pathogen.

2. Identification of actinobacterial glycoproteins

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Ryerson University

Actinobacteria carry out a variety of complex metabolic activities like complex organic molecule degradation as well many small molecule biosynthetic activities. This group of bacteria are therefore useful for many biotechnological applications. Actinobacteria have been shown to have protein glycosylation which in higher organisms is critical to the function of those proteins. However, in Actinobacteria the function of this post-translational modification is not yet clear. In order to better understand this modification, we are examining *C. fimi*, *C. flavigena*, *C.*

glutamicum and *R. jostii* all of which carry genes for protein mannosylation, which is also seen in eukaryotes including humans. We have been examining the glycoproteins in these bacteria to identify common proteins which are central to the biology of these well known Actinobacteria. Our approach to glycoprotein characterization is first to enrich mannoproteins using affinity chromatography with two plant-based carbohydrate binding proteins (lectins) called concanavalin A (ConA) and Galanthus nivalis lectin (GNA). Using these lectins as detection reagents through a lectin blot, we are first visualizing these proteins in the proteome using various cell fractions. We will then identify these enriched proteins using proteomics analysis based on the capture of chemically modified glycoproteins. In this way we hope to identify both common and unique sets of glycoproteins critical for the biology of these important bacteria.

3. Glucose fluctuations alters survival in chemotherapy drugs

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Cell growth, DNA replication and chromosome division is tightly regulated to prevent mutation and cancer. Cell cycle checkpoints, including the DNA replication and DNA damage checkpoints, arrest the cell cycle and repair DNA before mitosis. Checkpoint mutants are missing key checkpoint proteins and die in DNA-damaging drugs. We use fission yeast to study how checkpoint mutant drug sensitivity is altered by environmental stress (eg glucose levels). We varied glucose levels in media containing 2 chemotherapy drugs. Hydroxyurea (HU) causes DNA replication instability; cells lacking the DNA replication checkpoint die in hydroxyurea (*rad3Δ*, *cds1Δ*, *mrcl1Δ*). Camptothecin (CPT) causes DNA breaks; cells lacking the DNA damage checkpoint die in CPT (*rad3Δ*, *chk1Δ*). We found that high glucose media decreases chronic CPT sensitivity, but increases HU sensitivity in replication checkpoint mutants. Under acute drug treatment, glucose starvation initially increases hydroxyurea sensitivity, while a bolus of glucose before hydroxyurea decreases mutant sensitivity to hydroxyurea. Since glucose fluctuations may occur in humans during chemotherapy treatment, understanding the relationship between cell glucose levels and drug sensitivity is important to direct effective therapeutics.

4. Site-directed mutagenesis of Cdc2p target sites in the fission yeast Adn2p transcription factor

Campbell, M., Karagiannis, J.

Department of Biology, The University of Western Ontario

In fission yeast, *Schizosaccharomyces pombe*, perturbation of the actin cytoskeleton leads to the activation of a cytokinesis monitoring system delaying cell cycle progression and stabilizing the cytokinetic actomyosin ring. A genome-wide screen using low doses of the actin depolymerizing drug LatA found deletion mutants lacking the *adn2* gene (adhesion defective), revealed hypersensitivity to LatA. Follow up research found Adn2p functions as a transcription factor in repair, re-establishment and subsequent constriction of the actomyosin ring. Proteomic data has also shown that Adn2p is a substrate of the cyclin dependent kinase, Cdc2p (on Serine-485 and Serine-519). Based on this data I examined whether Cdc2p mediated phosphorylation of Adn2p

might play a role in the regulation of the cytokinesis monitoring system. Fission yeast mutants expressing serine to alanine (to mimic the dephosphorylated state) and serine to glutamate (to mimic the constitutively phosphorylated state) mutations at positions 485 and 519 were created and analysed. Contrary to my hypothesis, these mutants did not exhibit any differences in LatA sensitivity relative to a wild-type control strain. Furthermore, the mutations did not affect the localization or stability of Adn2p as determined by fluorescence microscopy. I conclude that phosphorylation of Adn2p on Ser-485 or Ser-519 has no significant role in modulating the response of *S. pombe* cells to LatA mediated perturbation of the actin cytoskeleton.

5. Phenotypic and functional influence of Zeb1 knockdown in aggressive prostate cancer cells

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Prostate cancer is the 3rd most common cause of cancer death amongst Canadian men. These deaths are primarily a result of metastatic disease, as current therapies for metastasis are largely non-curative. The epithelial-to-mesenchymal transition (EMT) is thought to initiate the process of cancer metastasis. The mesenchymal-to-epithelial transition (MET) may lead to changes opposing EMT, and therefore, studying MET may provide insight into how cells become metastatic. The Allan lab has recently developed a pre-clinical model to study MET of aggressive prostate cancer (PC-3) cells. This was done by decreasing the expression of the EMT inducing transcription factor Zeb1 using an inducible lentivirus. It is not known whether MET will also induce changes in expression of other EMT related transcription factors Twist1, Twist2, Snail1, Snail2, and Zeb2 in PC-3 Zeb1 knockdown (KD) cells, or whether Zeb1 KD affects cell morphology and proliferation. Our study demonstrated that there was decreased protein expression for transcription factors Zeb2 and Snail1 in Zeb1 KD cells compared to control cells. When analyzing phenotypic changes, it was observed that the percent of cells with an epithelial morphology was significantly greater in Zeb1 KD cells than in the control cells, while the percent of cells with mesenchymal morphology was significantly higher in control cells compared to Zeb1 KD cells. In the end, we hope to have a better understanding of how MET contributes to metastasis.

6. Investigation of persistence in *Salmonella* isolated from a local watershed

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Salmonella are a gram-negative bacilli found around the world, are facultative anaerobes and are of serious medical concern, as they are enteric pathogens. Their ability to persist in the environment becomes crucial for both food and water system quality control. The aim of this study was to better understand the ability that isolated Salmonella from the Grand River watershed has to persist in stressing situations, directly influencing its likelihood of becoming “naturalized”. Using standard swab techniques, Salmonella isolates were collected from locations along the GR watershed. These isolates, once confirmed as Salmonella via biochemical testing, were subjected to stressors including low nutrient availability, low temperature, antibiotic presence, and competition with similar bacteria. Environmental isolates were shown to have a similar growth profile in these stress tests, especially low nutrient availability, when compared to lab-strain Salmonella isolates. Growth at 12 Degrees was much slower when compared to the optimal 37 Degrees, however was able to reach an optical density of similar proportion within 48 hours. Stress tests were also performed in combination, with growth profiles of low nutrient availability showing similar patterns at both 37 & 12 Degrees. This information is showing evidence that Salmonella which are found in the GR watershed have the capability to persist for periods of time, with potential for “naturalization” becoming more likely.

7. SOX2 ablation in the suprachiasmatic nucleus disrupts anxio-depressive processing.

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Circadian rhythms play a pivotal role in mood regulation. Mood disorders are linked to disrupted circadian rhythms, which are regulated by the Suprachiasmatic Nucleus (SCN) in the mammalian brain. While anxio-depressive behaviours are well characterized in human and mouse models, the precise pathways and their regulatory genes are not well understood. Here, we employ an SCN-specific, conditional knock-out (cKO) mouse model to investigate the role of Sox2 in regulating anxio-depressive behaviours. Behavioural assays with the Elevated Plus Maze (EPM) and Forced Swim Test (FST) demonstrate enhanced anxiety-like and reduced depressive-like behaviours in cKO compared to wild-type (WT) mice, respectively, and suggest reinterpreting the FST as an anxiety inducing paradigm. cKO mice spend less time in the open arms during the EPM and exhibit a shorter duration of immobility during the FST. We find that stress-induction by FST of neuronal activity in key anxiety-processing regions is observed in WT but not cKO mice. A pan-neuronal C-fos immunohistochemical stain after FST reveals neuronal activation in the Arcuate Nucleus of the Hypothalamus (ARH) and the Nucleus Incertus (NI) in WT mice, with significantly attenuated activation in cKO mice comparable to naïve controls. Altogether, our findings suggest that SOX2 expression in the SCN plays a critical role in modulating the anxio-depressive response in a pathway involving the ARH and the NI.

8. Uncovering the functions of genome stability protein Cbf11.

Hurst, D.N., Westwood, T. J.

University of Toronto, Mississauga

To ensure DNA is properly and fully copied with each cell division cycle, “genome stability” proteins regulate both DNA damage control and entry into mitosis. In collaboration with the Převorovský lab, we are investigating the fission yeast Cbf11 protein. Cbf11 is a transcription factor belonging to the family of yeast CBF1, Suppressor of Hairless, Lag-1 (CSL) proteins. Work in a *cbf11Δ* mutant has shown that Cbf11 plays a role to coordinate chromosome segregation and cell division; therefore, it likely plays a key role in genome stability. However, the exact function and mechanisms of Cbf11 are unclear. To better understand Cbf11 in *Schizosaccharomyces pombe* (*S. pombe*), we developed *cbf11Δ* yeast strains that express fluorescent markers of DNA metabolism and stability. We are testing chromosome segregation in *cbf11Δ* cells, both with and without drug treatment that may increase genome instability. Further, we are testing whether *cbf11Δ* strains carry low levels of DNA damage as marked with replication protein A (RPA) binding to single stranded DNA. Finally, we are testing how nuclear and nucleolar organization is altered in the *cbf11Δ* mutant. These fluorescent microscopy studies aim to highlight specific roles of the poorly understood CSL family in genome stability. This has significant implications to the development of human leukemias, where CSL protein mutations are reported.

9. Impact of temperature-dependent stress signaling on *cds1Δ* mutant yeast response to S-phase arrest

Benvenuto, V.E., Sabatinos, S.

Ryerson University

Cell cycle checkpoint mechanisms prevent genomic instability and cancer. The DNA replication checkpoint causes cell cycle arrest in response to replication stress, replication-events that cause slowing or stalled DNA replication forks. Chemotherapy drugs are known to cause replication stress. For example hydroxyurea (HU) slows replication forks through dNTP depletion. We use fission yeast, *Schizosaccharomyces pombe*, to investigate the consequences of checkpoint protein loss. In a checkpoint deficient strain (eg the *cds1Δ* mutant), HU treatment cannot be recognized by the checkpoint. There is no replication arrest in *cds1Δ* cells, forks collapse and cells die. The Sabatinos lab has found that *cds1Δ* viability in HU is dramatically increased following temperature treatment. We now test how the mitogen activated protein kinase (MAPK) and stress activated protein kinase (SAPK) pathways influence temperature shock response on checkpoint survival. Proteins in the MAPK pathway include *S. pombe* Wis1, Wis4 and Win1. These components respond to heat shock by activating downstream transcription to increase cell survival under environmental stress. I have constructed double mutants of checkpoint and MAPK components, and then tested HU sensitivity. Constitutively active *wis1* and *wis4* mutant proteins were tested in *cds1Δ* cells. Investigating the effects of environmental stress on replication stress response can be used to improve the efficacy of chemotherapy treatments offered.

10. Impacts of histone chaperone deletions on cell cycle regulation

Da Pra, V., Calhoun, L., Sabatinos, S.
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Histone chaperones are proteins that contribute to genome stability by assembling histone octamers, which are wound into compact nucleosomes. We hypothesized that histone chaperone proteins interact with DNA checkpoint mechanisms to promote cell death. We previously removed the Mug183 histone3-histone4 chaperone in fission yeast cells. These $mug183\Delta$ mutant yeast were crossed into checkpoint-deficient strains $rad3\Delta$, $cds1\Delta$, and $chk1\Delta$. I examined the sensitivity of double mutants relative to single mutant strains in the presence of various drugs: the replication stress drug hydroxyurea, and DNA damaging drugs camptothecin and phleomycin. We have found that the loss of $mug183$ decreases $rad3\Delta$ sensitivity to all drugs. While $mug183\Delta cds1\Delta$ were less sensitive to hydroxyurea than parental $cds1\Delta$ cells, $mug183\Delta chk1\Delta$ were more sensitive to hydroxyurea than parental $chk1\Delta$ cells, and were less sensitive to DNA damaging agents. These results suggest that loss of $mug183$ may minimize the effects of DNA damage. We are investigating the mechanisms for this, including potential cell cycle slowing in the $mug183\Delta$ strain. Further, we are generating a null mutant of the Chz1 histoneH2A-histoneH2B protein to further understand how histone chaperones effect drug sensitivities. Characterizing the roles of histone chaperones in cell cycle regulation is essential for further research in human cancer development and tumour treatments.

11. Understanding the role of intergenic DNA region of Hb9 gene during spinal motor neuron differentiation

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During the development of motor neurons, embryonic stem (ES) cells differentiate to spinal motor neurons by the activation of a unique combination of transcription factors. The Hb9 gene is the selective marker for the identity of post-mitotic spinal motor neurons. The previous research with Hb9 knockout mice has shown disruptions in the formation of spinal motor neurons. It has yet to be understood if modifications occurred in the intergenic regions of the genome, how would the chromatin organize and stabilize during differentiation, thereby the effect on gene expression. To study the effect on Hb9 gene expression from a change in chromatin organization, CRISPR-Cas9 was utilized to delete a 2.25 kb of the upstream intergenic region of Hb9 gene in motor neuron differentiation system using mouse ES cells. It was hypothesized with this deletion mutant, there would be downregulation in Hb9 gene expression in spinal motor neurons. Low efficiency of CRISPR was observed as out of 56 colonies, a single colony had the homozygous deletion of 2.25 kb intergenic region. After differentiation of these mutant ES cells to spinal motor neurons, real-time polymerase chain reaction results showed 35% of downregulation in Hb9 gene expression in nascent motor neurons and 39% of downregulation in maturing motor neurons for the mutant ES cells as compared to the wildtype. These results indicate that the 2.25 kb intergenic region plays a functional role in Hb9 gene expression.

12. Identification of three recessive variants for autism spectrum disorder and intellectual disability with bilateral frontoparietal polymicrogyria in consanguineous families

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Intellectual disability (ID) affects 1% of the population and is characterized by extreme genetic heterogeneity, where many gene loci contribute to a single phenotype, making molecular diagnosis complex. It is estimated that more than 2500 genes may be linked to ID. This project aimed to identify putative variants in autism and ID associated genes in two unrelated families, one from Pakistan, one from Iran. Regions of shared homozygosity by descent (HBD) were identified using FSuite and HomozygosityMapper, using the Illumina HumanCore Exome microarray genotyping platform. Exome sequencing was completed for the Iranian family using SureSelect V5 and the Illumina HiSeq 2500. Candidate variants were prioritized using MutationTaster, SIFT, Polyphen-2 and other evaluative software that predicted the mutation to be pathogenic. Variants that were deemed good candidates were validated via PCR amplification and Sanger sequencing. Two homozygous variants were detected, which segregated in the Iranian family with autism: an 8 base pair insertion (TATTCTGT) in exon 7 of LINS1 and a novel missense change (A>G) in exon 2 of SLC7A5. A known 7 base pair deletion (CCAGGAC) was identified in exon 5 of GPR56, which causes bilateral frontoparietal polymicrogyria (BFPP) and ID. Identification of likely disease-causing variants sets the stage for further downstream functional analysis in understanding the genetic basis of neurodevelopmental disorders.

13. Investigating the effects of EGF stimulation of TFEB localization and lysosomal biogenesis

Leung, R., Antonescu, C., Botelho, R.J.

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The lysosome is an acidic organelle that functions in cellular digestion, cell metabolism, and autophagy. Lysosomal biogenesis, therefore, is important for maintaining proper cell function and responding to cell stress. The transcription factor EB (TFEB) is a master regulator of lysosomal genes that is highly regulated by the mammalian target of rapamycin complex 1 (mTORC1). Under cellular conditions with high levels of nutrients and growth factors, TFEB is phosphorylated by mTORC1 and remains largely cytosolic. However, under nutrient starvation or cell stress, mTORC1 is inactivated, and TFEB translocates into the nucleus to induce the expression of lysosomal genes. One cell surface receptor that plays a critical role in cell signaling and proliferation is the epidermal growth factor receptor (EGFR), which is activated upon the binding of its ligand EGF. Although mTORC1 is known to be activated by growth factors, the response of TFEB through EGFR signaling remains unknown. We examined the effects of EGF stimulation on TFEB localization through immunofluorescence, and analyzed the level of TFEB

within cell nuclei. Cells that were stimulated with 20ng/mL of EGF for 2 hours, 1 hour, or 30 minutes following 1 hour of serum starvation showed nuclear translocation of TFEB, compared to cells that were only serum-starved. This data leads us to speculate that EGF may play a role in the activation of TFEB through a signaling pathway independent of mTORC1 activation.

14. Stimulating the innate immune response to combat viral infection

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The purpose of this thesis is to validate a novel nanoparticle's potential to carry a molecule capable of eliciting a broad-spectrum antiviral response. Two mammalian cell lines were used to test if the particle can increase protection against viral infection. First, Madin-Darby canine kidney (MDCK) cells were treated with varying concentrations of Poly I:C, a known immunostimulatory molecule or with Poly I:C bound to the nanoparticle. The cells were then infected with vesicular stomatitis virus tagged with GFP (VSVGFP), this allowed use of fluorescence microscopy to measure viral infection. Second, a human lung fibroblast cell line (MRC-5) was used in the same experiment to model how human cells may react to the particle. In MDCK cells there was a wide variation in responses to the nanoparticle bound to Poly I:C and Poly I:C alone, showing that this cell line may not be ideal for this type of research. In MRC-5, across all concentrations the Poly I:C bound to the nanoparticle consistently induced a stronger antiviral protection response than Poly I:C alone. Future directions include repeating these experiments with influenza H1N1 to test the particles efficacy against a more relevant human viral threat.

15. BHK-21 cell response to hyperosmotic stress through cytoskeletal reorganization and protein upregulation

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Many animal tissues (kidney, liver, spleen) can be exposed to local hyperosmotic stress and require activation of adequate mechanisms to prevent cell damage and maintain cellular functions. Accumulating evidence indicates that microfilaments play a key role in cellular response to osmotic stress. We used the BHK-21 cell line as a model system to study the relevant effects of hyperosmotic stress (300mM sucrose, 3h treatment) focusing on protein expression and the actin cytoskeleton. Actin-based structures (cortical actin, stress fibers, cytoplasmic networks) were visualized using fluorescence staining with phalloidin-TRITC. There was a significant remodelling of the actin cytoskeleton following hyperosmotic treatment including the disappearance of stress fibers and cytoplasmic networks while enhancing cortical actin staining. SDS-PAGE was used to analyze hyperosmotic-induced global changes in protein expression based on non-specific gel staining with Coomassie Brilliant Blue. Multiple protein bands showed higher intensity in hyperosmotic versus control cell lysate samples indicating accumulation of stress-sensitive proteins in cells. These findings provide further confirmation that the stabilization of

cortical actin is an essential response of cells to hyperosmotic stress, which might involve specific proteins required for remodelling of microfilaments. This study was conducted as part of Biology 3326F (Cell Biology Laboratory) with Dr. Alexander Timoshenko's supervision.

16. Evaluating the role of ferric iron in bile salt mix-induced resistance of Enterohemorrhagic *E. coli* to Polymyxin B

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Enterohemorrhagic *E. coli* (EHEC) is a foodborne pathogen which poses a serious threat to public health. EHEC infection generally resolves within 10 days however, it can sometimes lead to more serious complications. Therefore, it is vital that we study this pathogen in more depth to develop strategies for prevention and treatment. EHEC, like many enteric pathogens, is well adapted to surviving the innate immune responses within the gastrointestinal (GI) tract. Cationic antimicrobial peptides (CAMPs) are an important component of the host innate immune system. CAMPs destabilize bacterial membranes which results in increased membrane permeability. Bile salts and iron have each been shown to trigger an auto-phosphorylation of the BasS part of the two-component system BasRS, leading to activation of genes encoding enzymes that modulate LPS by masking LPS negative charge. This modulation prevents CAMPs from binding to the LPS. It is unclear whether iron present within physiological bile salt mixes (BSM) contributes to the resistance of EHEC towards CAMPs. The main goal of this study is to evaluate the effect of chelating iron in BSM on BSM-induced EHEC resistance to polymyxin B to better understand the role of iron within BSM. The importance of this project is to gain insight into the mechanisms that EHEC use to survive and colonize the human intestinal tract.

17. Role of *lsc* gene in *Erwinia amylovora* pathogenicity

Prosser, J.C.

Brock University

Erwinia amylovora is a gram-negative, highly virulent plant pathogen of rosaceous species. *E. amylovora* is the causative agent of fire blight disease that can destroy full orchards in a single growing season. Wilting of plant shoots is caused by the production of bacterial ooze, known as exopolysaccharides (EPSs). Production of the EPSs have been identified as a pathogenicity factor by Sjulín & Beer (1978). There are two main EPSs specific to *E. amylovora*, one neutral and one acidic: levan and amylovoran, respectively. Levan production is controlled by the *lsc* gene which encodes the levansucrase enzyme. Levansucrase hydrolyses sucrose into fructose and glucose and polymerizes fructose into levan. When levan production is deficient, the bacteria display delayed pathogenesis. Isolates of *E. amylovora* from Mexico have very recently been shown by visiting student Oscar Villanueva to have altered colony morphology and delayed pathogenesis. Based on literature, it was proposed that these altered characteristics were due to deficient amounts of levan production as a result of a mutation in the *lsc* gene. Levan and amylovoran production were quantified to determine the amount of EPS produced per cell. Amplification of

lsc gene for the Mexican isolates was attempted and was successful for 20 of the 25 isolates and amplicons were sequenced. Whole genome sequencing was performed for fifteen isolates as an alternative method for identifying mutations within the lsc gene.

18. Characterization of the immunity protein for a class IIb bacteriocin in *Streptococcus pyogenes*

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Streptococcus pyogenes is a human-specific bacterial pathogen that is among the top ten leading causes of infectious disease-related mortality globally. *S. pyogenes* possesses an arsenal of virulence factors that aid in niche competition. This includes an antimicrobial class IIb bacteriocin system comprised of the two *Streptococcus pyogenes* bacteriocin (Spb) M and N peptides, which were identified in the M18 serotype MGAS8232. The spbMN operon also encodes an immunity protein (Spbl) that protects the producing strain from the secreted bacteriocin. MGAS8232 is immune to SpbMN despite a single-base deletion that has produced a premature stop codon within the spbl gene. We hypothesized that the mutation creates a slippery sequence at which ribosome-bound tRNAs shift by one nucleotide in the 5' direction. To investigate whether this -1 frameshifting allows for expression of the full spbl, we examined the effect of a site-directed synonymous substitution mutation in the slippery sequence. Growth inhibition assays with recombinant expression of SpbMN demonstrated that growth of recombinant *Lactococcus lactis* expressing mutated spbl was impeded compared with *L. lactis* expressing wild-type spbl. Future work includes generating antibodies to Spbl to confirm the presence of programmed translational frameshifting. The influence of the slippery sequence on mRNA structure and regulation of frameshifting can provide a further understanding of the role of SpbMNI in *S. pyogenes* colonization.

19. The role of Rad18 in the resolution of aberrant R-loops

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R-loops are RNA-DNA hybrid structures that have normal function in transcription and replication. But, if R-loops accumulate uncontrollably, they can threaten the stability of the genome by stalling replication and transcriptional machinery, and thus can lead to the development of cancer or neurodegenerative diseases. In healthy cells, R-loops have been shown to be resolved by RNaseH1 and RNaseH2 ribonuclease enzymes. In addition, the Fanconi Anemia (FA) pathway, primarily known for its resolution of DNA interstrand crosslinks, has also been shown to resolve R-loops. In a yeast screen performed by Dr. Stirling, Rad18 was identified as another potential R-loop regulator despite no literature on its mechanism of involvement. Rad18 is an E3 ubiquitin ligase that is canonically involved in the activation of the DNA damage tolerance pathway, which allows for the bypass of the replisome past lesions in order to prevent

replication fork stalling and collapse. Here, we explore how Rad18 is involved in the regulation of R-loops via its effect on the activity of RNaseH2, the FA pathway and the role of Replication protein A as a sensor of R-loops. The results showed that the effect of Rad18 knockdown on R-loop levels was epistatic with the knockdown of members of the FA pathway, which suggests Rad18 is involved in the FA pathway mechanism. Additionally, we found that Rad18 may affect the ability of RPA to act as a sensor of R-loops and recruit and enhance RNaseH2 activity.

20. Examining the role of post-translational modifications of Crb in tissue growth

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Epithelial cells are structural units of the animal body, specialized to form sheets along the lining of surfaces, and act as physical barriers to separate the internal milieu of the body from the outside environment. The ability to form sheets and shape compartments is dependent on the polarized orientation of the epithelial cells. A key regulator of this epithelial apical basal polarity is the transmembrane protein Crumbs (Crb). In humans, mutations in CRB1 have been shown to cause severe forms of eye diseases such as retinitis pigmentosa and Leber congenital amaurosis, and CRB3 has been linked to cancer progression. Both *Drosophila melanogaster* and mammalian Crb proteins have been implicated in the regulation of growth and were shown to regulate the Hippo/Yap pathway that mediates growth control in both normal development and cancer. With this study, we aim to explore how additional post-translational modifications in the Crb intracellular region may impact its role as a growth regulator in *Drosophila* imaginal wing discs. Through both clonal cells and overexpression domains, we observed changes in the distribution of Crb binding partners such as Ex, Sdt and Yrt by immunostaining. To investigate Crb's function as an upstream regulator in cell signalling, we looked for changes in pathways such as growth regulatory Hippo pathway, and the cell survival pathway JNK.

21. Separation and characterization of bio-particles using low-cost microfluidic lab-on-a-chip

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Rapid separation and detection of bio-particles such as pathogenic bacteria and rare circulating tumor cells (CTCs) using a disposable and low-cost method can provide powerful tool in the world of science and medicine . Conventional methods such as flow cytometry, PCR and plating assays are extensive, time consuming and often require specialized training. LOCs are devices are stand-alone devices designed to integrate one or more functions onto a small-scale circuit, reducing what would be a large area into a few square centimeters, offering an attractive alternative to traditional methods. Microfluidic LOCs use microchannels to process volumes as small as picolitres allowing for reduced fluid consumption. LOCs have demonstrated the ability to characterize microparticles using spiral microchannels and the centrifugation effect. This simple method has a vast array of uses from detecting rare CTCs in blood to being determining bacterial

content in water. Despite research and development aimed at these particle separators, little has been done on using devices made from adhesive sealing film, allowing for lower cost, out of cleanroom manufacturing and flexibility on an optically clear platform. Using a spiral microfluidics along with fluorescent imaging we demonstrated the ability to separate 15 μ m and 5 μ m particles using an adhesive sealing film platform. We developed a reliable and cost-effective microfluidic lab on chip capable of particle separation and identification.

23. The characterization of COMMD2 and delineation of its role in ciliogenesis

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Primary cilia are molecular antennas used to facilitate cellular signalling. Defects in the primary cilia have shown to be associated with diseases such as Polycystic Kidney Disease. However, the exact mechanism of ciliopathy is still unknown. This demonstrates our insufficient knowledge in ciliogenesis and the need to understand the primary cilia for potential future treatments. The primary cilium structure consists of 9 microtubules doublets surrounded by the cilium membrane. Ciliogenesis begins at the basal body where nucleation, vesicle fusion, axoneme elongation and plasma membrane insertion of the basal body occurs. Since there is no protein synthesis at the cilium, it must be reliant on vesicle trafficking. However, little is known about these machineries that regulate ciliogenesis. Recent research has mapped the protein interaction landscape at the centrosome during ciliogenesis using proximal-dependent biotinylation for identification (BioID). Utilizing transfected short interfering RNA (siRNA), it was suggested that the COMMD complex, specifically COMMD2, are highly associated with ciliary proteins and cilia length. Hence, characterizing COMMD2 and its role in ciliogenesis is essential to understanding ciliopathies. This will be studied through immunocytochemistry for localization, siRNA for cilia function, and bioID to create an interactome. Recent progress has shown COMMD2 to localize as punctuates in the cell, possibly suggesting endosomal trafficking.

24. Non-specificity of transcription factor function in *Saccharomyces cerevisiae*

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Transcription factors (TFs) are proteins that regulate the rate of transcriptional initiation. In the traditional view of TF function, each transcription factor is thought to regulate a specific set of genes that give rise to a unique phenotype. However, recent studies in *Drosophila melanogaster* have shown that multiple TFs induce or rescue the same phenotype, a phenomenon referred to as phenotypic non-specificity. This study tests whether phenotypic non-specificity of TF function is also observed in *Saccharomyces cerevisiae*. Independently for both the GAL4 and GCN4 TF loci, ten yeast strains containing locus replacements with one of the ten randomly chosen TF coding sequences, a strain with reinsertion of the TF or a strain with the knock out of the TF locus were created. GAL4 strains were tested for growth on galactose media and GCN4 strains were tested for growth on aminotriazole containing media, which inhibits protein product of HIS3.

Phenotypic specificity is the inability to rescue the Gal4 or Gcn4 phenotype with a replacement TF. Phenotypic non-specificity is the rescue of the Gal4 or Gcn4 phenotype by any one of the replacement TFs. So far, we have found that putative replacement of GAL4 coding sequence with MOT3 coding sequence rescues growth on galactose medium indicating phenotypic non-specificity in yeast.

26. Uncovering the functions of genome stability protein Cbf11

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To ensure DNA is properly and fully copied with each cell division cycle, “genome stability” proteins regulate both DNA damage control and entry into mitosis. In collaboration with the Převorovský lab, we are investigating the fission yeast Cbf11 protein. Cbf11 is a transcription factor belonging to the family of yeast CBF1, Suppressor of Hairless, Lag-1 (CSL) proteins. Work in a *cbf11Δ* mutant has shown that Cbf11 plays a role to coordinate chromosome segregation and cell division; therefore, it likely plays a key role in genome stability. However, the exact function and mechanisms of Cbf11 are unclear. To better understand Cbf11 in *Schizosaccharomyces pombe* (*S. pombe*), we developed *cbf11Δ* yeast strains that express fluorescent markers of DNA metabolism and stability. We are testing chromosome segregation in *cbf11Δ* cells, both with and without drug treatment that may increase genome instability. Further, we are testing whether *cbf11Δ* strains carry low levels of DNA damage as marked with replication protein A (RPA) binding to single stranded DNA. Finally, we are testing how nuclear and nucleolar organization is altered in the *cbf11Δ* mutant. These fluorescent microscopy studies aim to highlight specific roles of the poorly understood CSL family in genome stability. This has significant implications to the development of human leukemias, where CSL protein mutations are reported.

28. Expression, purification and structural analysis of the Mβ subdomain of Toc159, a chloroplast import protein

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The integrity of the chloroplast relies on the import of many nuclear-encoded proteins that are post-translationally targeted to the organelle. This mechanism requires the utilization of specialized transport proteins known as translocons. The TOC and TIC complexes are located at the outer and inner membranes respectively and can be further divided into several subdomains. Toc159 contains 3 distinct domains (A, G and M) and is known to function as a regulatory receptor involved in preprotein recognition. The Toc159 M domain is known to function as a GTPase receptor and can be further segmented into 3 subdomains based on secondary structure. Prior research and bioinformatic analyses completed on the M2 domain have suggested that it may form a secondary structure rich in beta-strands, somehow being associated with the outer membrane and TOC complex assembly. Saying this, its role in protein targeting to the chloroplast, preprotein recognition and overall function are not well understood.

The current objectives of this project consist of optimizing the over-expression and increasing purity of the protein of interest, ultimately aiming to confirm the presumptive β -helix motif of Toc159 from the model plant *Arabidopsis thaliana*, recombinantly produced in *E. coli* BL21. These are major steps in contributing a structural characterization of the M-domain and its function in membrane association and the molecular mechanisms associated with TOC complex assembly.

29. Oxidative damage in perennial vs annual flax

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According to the oxidative theory of aging, aging is a result of damage accumulated over time by Reactive oxygen species (ROS). ROS are produced by regular cell metabolism, and they damage Lipids, Carbohydrates, amino acids, and DNA by reacting with them. We studied oxidative damage in Flax(Linum) because it had evolutionary closely related annuals (*L. usitatissimum* and *L. grandiflorum*) and perennials (*L. lewisii*, *L. flavum*, *L. pubescens*) variations of the species. Oxidative damage was measured by looking at the color change in the leaves, Chlorophyll A has absorption peaks at 430 nm and 660 nm and chlorophyll B has the highest absorption peak at 450 nm and 640 nm. As chlorophyll degrades the relative red and blue reflected should increase. When subjected to water, annuals exhibited 25% more damage. When subjected to added oxidative damage (Hydrogen Peroxide), we found that annual flax experienced 34% more oxidative damage compared to perennial flax. Since perennial live longer than annuals they should have more protective enzymes, therefore our results are consistent with the oxidative theory of aging. This also means that cells must have evolved to invest the minimum amount of protective mechanisms needed for their expected life.

30. Comparison of hamstring to quadriceps strength ratios pre and post a muscle fatiguing protocol

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The ACL is a stabilizing ligament of the knee that is more frequently injured in female athletes. ACL injuries usually result in extensive recovery, rehabilitation, and significant time away from physical activity. The main objectives of this study was to characterize the effects of muscle fatigue on isokinetic knee strength as well as the isokinetic knee strength during the last 20 degrees of a maximal knee flexion in young adult women. The hamstring:quadriceps ratio (HQR) surrounding the knee joint, can be used to assess injury risk. Participants underwent a muscle fatiguing protocol (MFP) which used a cycle ergometer and an isokinetic HQR testing pre and post fatigue. The MFP was 45 minutes in total which began with the Astrand maximal cycle test followed by 90 seconds of maximum effort on the ergometer bike. This gave us the maximum heart rate (HR) which was used to personalize the rest of the protocol next, the MFP ran through a circuit of max HR intensities. These intensities are meant to emulate the variable intensities of

a typical team sport like soccer. HQR was assessed using a 6th Edition Biodex Isokinetic Dynamometer and measurement of concentric hamstring and quadriceps peak torques, both pre and post a fatiguing protocol. Correlation between decreased HQR and MFP were recorded, additionally the HQR of last 20 degrees of flexion could be used as an assessment of knee injury risk as this is the most common anatomical position of a knee at the time of injury.

31. Clinical and environmental Salmonella virulence factors along with their antibiotic resistant genes present

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Salmonella is known to cause many types of infections in humans and will lead to fever, vomiting, diarrhea and even death. There is over 2000 different serotypes of salmonella. They have virulence factors present in both clinical and environmental that makes them more established along with being more persistent in their specific niche. These virulence factors can be inherited by horizontal gene transfer from other organisms. All 321 virulence factors that exist between 445 sequences (75 environmental and 370 clinical). The origins of these virulence factors come from 36 different species. With only 20 virulence factors coming from Salmonella or 6.2%, the most coming from species of E.coli (53 virulence factors) or 16.5% of all the virulence factors. Environmental Salmonella had a higher average of virulence factors per sequence with 250 per, while Clinical only 225 on average per sequence. Furthermore, 29 of the 321 virulence factors only came from Clinical Salmonella. None of these 29 were present in Environmental Salmonella. Thus, making the Clinical Salmonella more variety of virulence factors that present. There were 16 sequences of the 445 that did not have any virulence factors present. Only 1 sequence of the Environmental had no virulence factors and 15 Clinical sequences had no virulence factors present. All Environmental sequences came from the SALFOS database and all Clinical sequences came from the PATRIC database.

32. Exploring the impacts of increased salinity on zooplankton communities in Sturgeon Lake

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Salt is used in the colder regions of North America as a method of deicing roadways and improving safety in the winter. However, road salt can leach into freshwater systems and expose freshwater organisms to increased salt concentrations. This study aims to investigate the impacts of increased salinity from road salt application on the community structure, species richness, abundance and diversity of freshwater zooplankton. I hypothesize that increased salinities will result in a shift towards halotolerant species in the community as well as reduce species richness, abundance and diversity. I ran a 6 week field experiment in Sturgeon Lake to test the effects of sodium chloride (NaCl). Multivariate analyses showed that salinity significantly decreased both richness and abundance, showing a clear threshold around 1.0 ppt. Additionally, no species consistently persisted in salinities above 2.0 ppt. These results indicate salinity

decreased the number of salinity tolerant species in a community to virtually zero. However, applying these results to the actual salinity changes occurring in North America, it seems the impacts of road salt on zooplankton species richness and abundance may not be as immediately detrimental as original studies suggested. This study is part of a larger project called the Global Salt Experiment organized by Dr. Shelley Arnott at Queen's University.

33. Influences of biotic factors on the behaviour of a trematode parasite infectious stage

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Free-living parasite infectious stages need to be able to find suitable hosts for successful transmission and should use cues to maximize their odds. For instance, the swimming cercariae of parasitic flatworms in the class Trematoda are known to exhibit a response to various abiotic factors corresponding to likely encounter with hosts, such as shadows and vibrations, but little research has addressed the impact of biotic factors such as chemical cues. Here, our goal was to examine whether cercariae of *Echinostoma* sp. that commonly infect larval amphibians responded to various chemical cues by altering their spatial orientation in an experimental arena with four choices consisting of a control (dechlorinated water), spinach, water in which dragonfly larvae were kept, and water that had housed tadpoles. The number of cercariae found in each chamber was recorded and analyzed for 15 trials consisting of 50 cercariae in each. We found no difference in *Echinostoma* sp. cercariae preference for any of the four treatments, indicating that these motile infectious stages may not primarily use chemical cues when seeking suitable hosts to infect. Future work is thus needed to better understand host-seeking behaviour by free-living parasite infectious stages, and should consider aspects such as chemical cue intensity, additional cercariae behaviours, and the possible interaction of abiotic and biotic cues.

34. Impact of trematode infection on foraging selection in snails

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Infections by trematode (flatworm) parasite in snails represent a severe burden to the host that may lead to behavioural changes in order to mitigate the costs of infection. Previous studies have indicated that parasitized individuals can change their foraging preferences as an anti-parasite behavioural strategy, and that dietary antioxidants can promote immune function. Due to this, infected hosts may selectively forage upon antioxidant-rich foods as an anti-parasite behaviour. Here, we tested whether food selection by a freshwater snail (*Stagnicola elodes*) differed based on parasitism by the trematode *Plagiorchis* sp. The foraging choices of both infected and uninfected snails (12 replicates each) were examined with a Y-maze under two conditions: i) control food versus no food; and, ii) control versus antioxidant-rich (β -carotene) food. There was no significant difference in the foraging selection between infected and uninfected snails, and no overall preference for the antioxidant-rich food. These results indicate that selection of antioxidant-rich foods does not seem to be a strategy used by infected snails to

increase tolerance of trematode parasitism, nor do infected snails compensate for infection through an increase in foraging. Further studies are required to explore how trematode infection may lead to changes in snail foraging behaviour, as well as the possible benefits of consuming certain dietary compounds for parasitized animals.

35. Factors influencing planktonic rotifer community structure in fresh water lakes

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The Northwest Territories (NWT) is experiencing rapid environmental changes due to a warming climate and human development. These stressors are causing significant changes in aquatic habitats, including increases in water temperature, conductivity, calcium concentrations, and water clarity. Water quality changes may impact biota in lakes and ponds, but little is known about how some important aquatic organisms, such as rotifers, may respond. This study aims to identify whether changing environmental conditions might impact rotifer community structure in small Arctic lakes. Baseline data on water chemistry, lake bathymetry and rotifer communities were collected and analyzed from a total of 20 lakes located along the highway running between Fort McPherson, Inuvik and Tuktoyaktuk. A total of 21 rotifer genera were identified, with an average of 12 genera per lake. Analyses indicated that pH, conductivity, water clarity, total organic carbon (TOC) and calcium have a significant impact on rotifer richness, while diversity is significantly influenced by conductivity, water clarity and calcium concentrations. Several of the factors we identified as important for structuring rotifer communities are expected to be affected by climate change, permafrost thaw, and human development, including nutrient levels, conductivity, temperature, and calcium and TOC concentrations. As a result, we expect the richness and diversity of rotifer communities in NWT lakes will be significantly affected.

36. Histological impacts in white sucker (*Catostomus commersoni*) after long term exposure to bleached-kraft pulp mill effluent in Jackfish Bay, ON

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Jackfish Bay, on the north shore of Lake Superior (48°50'N, 86°58'W), was identified as an Area of Concern (AOC) due to the large volume of effluent that discharged from a pulp mill in Terrace Bay, ON. Studies dating back to the late 1980s have documented a variety of impacts to white sucker (*Catostomus commersoni*) populations exposed to bleached Kraft mill effluent including, reduced reproductive fitness, delayed sexual maturation and altered levels of circulating sex steroid hormones. In 2012, under new management, the mill switched process types from bleached-Kraft to dissolving pulp. The aim of this study was to utilize histochemical analysis to determine whether there were histological changes in tissue morphology of white sucker at Jackfish Bay as compared to a reference site (Mountain Bay) respectively. Forty individuals were collected in late August 2018 using gill nets, and measured for size, condition and relative organ size (GSI, LSI, fecundity, condition factor, length and weight); 10 males and 10 females from both

sites, were dissected for the following tissues: anterior kidney, gonad(male), proximal intestine, spleen and liver. The preliminary results show that males were larger in terms of mass at Jackfish Bay and both sexes showed larger condition factors at Jackfish Bay, and also possessed smaller relative gonad sizes. Histological studies and age analyses are ongoing.

37. Active learning in anatomy and physiology: Evaluating the impact of interactive engagement in student success in a university setting

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Pedagogical tools, such as 3D applications and physical models, are utilized when teaching human anatomy courses. Previous literature shows that tools based on interactive engagement (in which students use physical models or activities to learn a concept) augment academic performance. We conducted a study assessing the effectiveness of such interactive engagement (IE) in a large Human Anatomy & Physiology course (BIO210) at the University of Toronto Mississauga (UTM). Specifically, we introduced IE when reviewing upper and lower body muscles in BIO210 and expected that doing so will lead to higher academic performance. Students were randomly assigned to two groups of IE: physical models and flashcard activities. The materials provided to students in both groups were shared with the entire class and were used in group study sessions before the term test. Participants were assessed on several occasions to check for performance: 1) immediately after the session; 2) on the term test; and, 3) on the midterm exam. Students who completed an IE (N=128) performed better on term test and the exam than those who attended a question and answer review lecture (N=164). However, no statistical significance was found amongst both groups in long-term retention of upper and lower body muscles. The lack of retention differences could be attributed to varying student study habits or how frequently students used IE after being introduced to it. This is a potential area of study for the future.

38. Inquiry-based learning experiences for the promotion of higher order skills

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Inquiry learning encompasses a variety of techniques, including structured and guided inquiry (Prince and Felder, 2006). Both strategies begin with presenting a problem. Structured inquiry is followed with a solution 'model', whereas in guided inquiry, the student must devise a solution independently. Much research has emphasized the positive relationship between inquiry learning and the promotion of higher- order skills. This association holds especially true when the learning experience is engaging, knowledge- and student-centered (Freeman et al., 2014; Martin et al., 2007; Prince and Felder, 2006). While learning cycles have been designed to optimize such parameters (Martin et al., 2007), the role of structured inquiry in conjunction with or independent of guided inquiry has not been explored in genetics. Our work explores the differences between structured inquiry, guided inquiry, and combinations in promoting higher

order skills. The learning experience was designed in the context of Alzheimer's disease, a topic that is welcome within the population of biology students aiming for medical school. Through the accomplished progress, we hope to share our findings with the teaching and learning community in an effort to promote the development of evidence-based teaching practices that foster higher order skills.

39. A model for promoting course and curricular integrative teaching and learning

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One indicator of advanced intellectual development is the ability to integrate new information in the context of prior knowledge. Such integrative learning promotes the establishment of relationships between concepts, priming the mind for innovation when the opportunity arises.¹ Current research highlights case studies and problem-based learning as key activities that promote integrative thinking and hence. This is especially the case when these experiences are designed to be engaging, knowledge-centered, and student-centered. Although case studies and problem-based learning are frequently employed in genetics and molecular biology education, students continue to fall short in their initiative and ability to integrate knowledge. These learning experiences, however, are usually discontinuous in nature and integrate material from proximally related material. Our work has focused on creating a knowledge- and student-centered learning experience that spans a full semester in order to attain the following features: 1) increased student engagement through semester-long story characters that form the basis of various case studies and 2) semester-long extensive integration of concepts within the course and curriculum. A small-scale pilot was designed to assess the success of such a model. Through our achieved progress, we hope to share our findings with the teaching and science community to promote the development of course and curricular integrative teaching and learning.

41. Developing geo-caching multimedia resource for UTM Ornithology Trail

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The University of Toronto Mississauga (UTM) campus is a home to many non-migratory and migratory birds. As such, UTM campus presents unique educational resources for students, staff and faculty. The objective of this project was to contribute to development of the UTM ornithology Trail by collecting multimedia resources, educational materials and bird abundance data on campus. This is an ongoing project, which will result in the material being more accessible to the public through a physical QR codes positioned along the trails on campus and a website leading to accessible online materials regarding birds at the UTM campus. Educational materials in the form of still images, videos and audio recordings were collected starting in early May to the end of July of 2018 at UTM campus as well as Erindale Park and Riverwood Conservancy, which are both in close proximity to UTM campus. The audio recordings were later

enhanced using Audacity to eliminate background noise. Edits to the still images and videos were done through Adobe Photoshop. Bird abundance data was collected through point counts at UTM and Erindale Park.

45. How to draw a scientific illustration

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There are various skills that undergraduate Biology students develop in a science laboratory in order to make effective observations. One of these skills is drawing a scientific illustration to make observations. Currently, the existing process of learning how to make a scientific illustration involves following detailed instructions in a laboratory manual for first year and second year undergraduate biology students. A supplemental video was created outlining instructions for creating scientific drawings to present the information in a different manner. This teaching tool has been provided to first year biology students to evaluate the usefulness and solicit their feedback. Depending on student responses the video may be modified.