UNIVERSITY OF TORONTO
FACULTY OF ARTS & SCIENCE
Office of the Dean

Project Code: MGY 1S

RESEARCH OPPORTUNITY PROGRAM
299Y/399Y PROJECT DESCRIPTIONS 2018-2019
SUMMER

Name and Title: Amy A. Caudy, Associate Professor
Department: Molecular Genetics

TITLE OF RESEARCH PROJECT: Discovery and Characterization of Novel Enzymes using Metabolomics and Genetics

Number of 299Y Spots: 3 **Number of 399Y Spots:** 3

OBJECTIVES AND METHODOLOGY:

Every cell on earth processes nutrients to release energy and form the chemical compounds needed for cellular maintenance and growth. The study of metabolism, how cells transform nutrients and produce energy, reaches back hundreds of years but is now undergoing a revolution due to the availability of new methods. Recent advances in analytical chemistry, particularly in small molecule mass spectrometry and 2D NMR (nuclear magnetic resonance) have demonstrated a far more complex small-molecule landscape than can be accounted for by current maps and models.

Our group uses mass spectrometry to identify and quantitate the chemicals. Our group is working to identify previously unknown metabolic pathways within cells by using mass spectrometry to measure the changes in intracellular metabolites that result from the deletion of previously uncharacterized enzymes.

There are two tremendous gaps in our understanding of metabolism. First, there are many chemical reactions that are known to occur as cells break down nutrients, yet we do not know the genes that enable these reactions. Second, recent technological advances in mass spectrometry have detected hundreds of chemicals in cells that are not predicted by the current knowledge of metabolism. My group has had success pursuing both types of questions (Cell. 2011 Jun 10;145(6):969-80., Anal Chem. 2010 Apr 15;82(8):3212-21.). We recently used these full scan mass spectrometric approaches to discover a major route for the synthesis of ribose, a key building block for DNA and RNA and are currently engaged in similar work understanding the synthesis of the redox carrier rhodoquinone.. This project combines cutting edge mass spectrometry approaches with the tools of genetics and biochemistry to discover the function of previously uncharacterized enzymes. We use budding yeast and the nematode worm *C. elegans*, for much of our work. Both are genetically tractable, fast growing organisms. The majority of metabolic reactions can be traced to the origins of life billions of years ago, so we then test our observations in yeast in mammalian cells to compare the roles of new pathways. An area of particular interest is the intersection of these uncharacterized metabolic pathways with the cell division cycle.

DESCRIPTION OF STUDENT PARTICIPATION:

The students will construct and design genetically engineered yeast and mammalian cell strains with targeted changes in candidate enzymes. This will involve PCR, DNA sequencing, and other molecular biology techniques to

create cells with desired characteristics. The students will be involved in the preparation, mass spectrometric measurement, and mathematical analysis of the data. The data will be compared with existing models of metabolism to identify the route of synthesis and degradation of novel compounds. For those students with the interest and aptitude, there are opportunities for design and fabrication of custom lab hardware and software to further extend our capabilities for high throughput metabolomics analysis.

MARKING SCHEME (assignments with weight and due date**):**

Oral examination on project background – scheduled within first 4 weeks[h] - 20%

Participation in group meeting and presentations including ROP299 poster session in March 2019 (2 times over term): 20%

Lab work (accuracy of work, evaluations of lab notebook, performed biweekly): 30%

Final project report: Due – last day of term: 30%

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Project Code: MGY 4S

RESEARCH OPPORTUNITY PROGRAM
299Y/399Y PROJECT DESCRIPTIONS 2018-2019
SUMMER

Name and Title: Marc Meneghini, Associate Professor
Department: Molecular Genetics

TITLE OF RESEARCH PROJECT: Identification of New Substrates for the Set1/MLL Histone Methyltransferase.

Number of 299Y Spots: 1 **Number of 399Y Spots:** 1

OBJECTIVES AND METHODOLOGY:

Methylation of histone H3 on lysine-4 (H3K4me) is conserved from yeast to human, and is one of the most prominently studied chromatin modifications impacting transcriptional control and epigenetic inheritance. In the budding yeast *Saccharomyces cerevisiae*, Set1 is the sole H3K4 methyltransferase. In humans, mutations in Set1 orthologs belonging to the MLL family cause devastating forms of leukemia and are associated with many other cancers. Substrates for the Set1/MLL family outside of H3K4 are unknown however, and given the ancient evolutionary origin of this protein family it seems likely that others exist. We have completed a synthetic dosage lethality screen (SDL) screen to find new candidate substrates of Set1. SDL screens identify overexpressed yeast proteins that cause reduced cell fitness specifically in a strain that has a gene of interest deleted. When the deleted gene encodes a protein controlling a post-translational modification, frequent screen hits have proven to be direct targets of the modification controlled by the deleted gene. We have identified dozens of proteins that cause reduced growth on strains lacking *SET1* (*set1Δ*). Each of these represents a candidate new target of Set1 methylation. The objective will be to systematically confirm the screen hits with independent experiments assessing the growth of wild-type (WT) and *set1Δ* strains overexpressing different proteins using plasmid-based inducible expression. Confirmed screen hits will be focused on for more detailed follow up studies, eventually leading to LC-MS/MS to identify methylated lysine residues on the top candidates.

DESCRIPTION OF STUDENT PARTICIPATION:

The student will be responsible for preparing plasmids and transforming them into a battery of strains including WT, *set1Δ*, and strains with other mutations in *SET1* and/or H3K4. These strains will then be used in "spotting assays" to evaluate the growth characteristics under conditions that induce over-expression from the selected plasmids. Following collection of this data, selected proteins will be chosen for follow-up molecular and genetic analysis.

MARKING SCHEME (assignments with weight and due date**):**

25%: Weekly reading assignments and group meetings.

This component will include, but not be restricted to, participation in weekly group meetings and journal clubs.

50%: Weekly progress evaluations.

The student will be expected to keep a notebook and discuss progress and anticipated experiments on a weekly basis. Meetings with the supervisor will be arranged to go over the progress.

25%: Final written report / poster presentation.

The student will provide a final 2-page written report and participate in a poster session to present their results (either in the MolGen summer program poster session or in the undergraduate research forum).

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Project Code: MGY 3S

RESEARCH OPPORTUNITY PROGRAM
299Y/399Y PROJECT DESCRIPTIONS 2018-2019
SUMMER

Name and Title: Dr. Thomas Hurd, Assistant Professor
Department: Molecular Genetics

TITLE OF RESEARCH PROJECT: Determining How Deleterious Mitochondrial DNA Mutations are Eliminated

Number of 299Y Spots: **1** **Number of 399Y Spots: 1**

OBJECTIVES AND METHODOLOGY:

Background

Unusual among organelles, mitochondria have their own genomes, which encode a small number of essential genes. Unlikely nuclear genes, we inherit these mitochondrial genes only from our mothers. Given the importance of these genes, mothers have evolved mechanisms to ensure they pass on good, mutant-free copies to their progeny. Without such mechanisms deleterious mutations would accumulate from one generation to the next ultimately causing the collapse of the species. Exactly what the molecular nature of these selection mechanisms is remains obscure, despite their fundamental importance. In this proposal we seek to understand how these selection mechanisms work on a molecular level.

The medical importance of these mechanisms is demonstrated by the damage caused later in life by mutations in the mitochondrial genome. While a mother may succeed in ensuring we start life with good mitochondrial genes, mutations nonetheless inevitably arise in those genes as we age. This causes disease in people, most often neurological, affecting on the order of 1 in 4,300. By understanding how mothers prevent deleterious mitochondrial genes from being inherited, we aim to develop strategies to eliminate these bad mutations and the diseases they cause as we age.

Objectives

The goal of this research project is to identify genes and pathways necessary for the elimination of deleterious mitochondrial DNA mutations.

Methodology

The students will assist in the development of a high-throughput quantitative PCR (qPCR) assay to measure wildtype and mutant mitochondrial DNA in a *Drosophila melanogaster* model. The students will then use this assay and RNAi to knockdown genes one by one to determine which are necessary for the elimination of

deleterious mitochondrial DNA mutations. Lastly, if time-permits, the students will validate the 'hits' from the above RNAi screen by generating null mutations in candidate genes using CRISPR/Cas9.

Suggested Reading

1. Stewart, J. B. & Larsson, N.-G. Keeping mtDNA in shape between generations. *PLoS Genet.* **10,** e1004670 (2014).
2. Hurd, T. R. *et al.* Long Oskar Controls Mitochondrial Inheritance in Drosophila melanogaster. *Dev. Cell* **39,** 560–571 (2016).

DESCRIPTION OF STUDENT PARTICIPATION:

This project is ideally suited to motivated students interested in gaining research experience for graduate school. The students will work under the direct supervision of Dr. Hurd and a graduate student in the lab. The students will be expected to participate in all aspects of the research process including: reading appropriate background literature; helping to design and plan experiments; conducting experiments on a semi-independent basis, with the expectation of increased independence as the project progresses; keeping accurate and thorough records of their experimental work; and participating in regular (bi-weekly) lab meetings.

The students will be exposed to a variety of genetic, biochemical and molecular biology methods that are widely applicable to a range of experimental disciplines. These include:

1. Quantitative PCR
2. Gene knockdown using RNAi
3. Molecular cloning
4. Drosophila genetics/husbandry
5. CRISPR/Cas9

Additionally, students should expect to learn how to present their data as publication-quality figures, and to improve their ability to communicate their research clearly and concisely, both orally and in writing.

MARKING SCHEME (% of grade; due date**):**

1. **Research Proposal (10%; due 2 weeks after project start):**
   Students will prepare a 2-4 page written summary of their research project, which will include background, hypothesis, aims, methods, predicted outcomes and significance.
2. **Attendance, work ethic and participation in the lab and in meetings (20%; throughout)**
3. **Experimental lab work (20%; throughout)**
   Students will be evaluated on their ability to conduct experiments, and to analyze and interpret the data generated.
4. **Lab notes and organization (20%; throughout)**
   Students will be evaluated on their ability to keep organized and detailed experimental records, which will include aims, methods, results and interpretation.
5. **Final Project Presentation (15%; due mid-July or mid-October)**

Students will present their research objectives, results and future directions to the lab (20 minutes), followed by a questions discussion period. Students will be evaluated on their presentation skills and ability to answer questions related to their research project.

6. **Final Research Report (15%; due at noon on the last day of term)**
   Students will provide a 5-10 page written report describing the background and rationale for the research project, experimental methods, results and interpretation, conclusions and future directions.

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Project Code: MGY 2S

RESEARCH OPPORTUNITY PROGRAM
299Y/399Y PROJECT DESCRIPTIONS 2018-2019
SUMMER

Name and Title: Dr. Alan Cochrane (Professor)
Department: Molecular Genetics

TITLE OF RESEARCH PROJECT: Regulation of HIV-1/Adenovirus RNA Processing: Insights into Novel Therapeutics

Number of 299Y Spots: 1 **Number of 399Y Spots:** 1

OBJECTIVES AND METHODOLOGY:

Multiple mammalian viruses (HIV-1, adenovirus, influenza, Herpes) are dependent upon the host cell for the processing and expression of their mRNAs. Work by my group has identified a number of host factors whose altered expression/function result in dramatic changes in viral RNA processing and inhibition of virus replication. To complement these findings, my group has also identified multiple small molecules which a suppress replication of multiple different viruses by inducing changes in host factor function. Greater understanding of how these small molecules act will provide important insights into the design of novel therapeutics for the treatment of multiple viral infections.

DESCRIPTION OF STUDENT PARTICIPATION:

The students will be involved in the screening and analysis of shRNAs to host factors or small molecules for their effects on HIV-1/adenovirus gene expression with particular focus on measuring the changes in viral RNA processing that underlay the observed effects. Work will involve the use of mammalian cell lines to measure changes in viral protein expression by western blot or ELISA followed by qRTPCR, RT-PCR and in situ hybridization to quantitate the accompanying changes in viral RNA levels and localization. To complement these findings, we also examine how changes in activity of putative targets of the compounds (by overexpression or depletion using shRNAs) mimic the effects of the compounds on viral RNA processing. Together, the information will provide a detailed understanding of the cellular pathways involved in controlling virus replication as well as identify pathways common to multiple viruses that will help in the development of pan anti-virals.

MARKING SCHEME (assignments with weight and due date**):**

Oral exam on project plan: scheduled one week before drop/add date: 20%
Participation in group meeting and presentations (2 times over term): 20%
Lab work (evaluations of lab notebook, performed biweekly): 30%
Final project report: Due – last day of term: 30%