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

Project Code: CSB 1

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

Name and Title: John Calarco, Assistant Professor
Department: Cell and Systems Biology

TITLE OF RESEARCH PROJECT: Exploring mRNA Diversity in the Nervous System

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

OBJECTIVES AND METHODOLOGY:

How have animals such as humans, fish, flies, and worms evolved the capacity to create the many diverse cell and tissue types found in their bodies? This is a far-reaching question in developmental biology. We are starting to understand that switching specific genes on and off in different tissues is emerging as a major determinant of cell identity during animal development. However, additional layers of processing occur in the path of converting genetic information from DNA to RNA to protein. For example, a process known as messenger RNA (mRNA) alternative splicing, where multiple mature mRNAs are produced from a single precursor, has enabled alternative evolutionary paths to cellular diversification. In particular, cells of the nervous system, called neurons, have evolved extensive use of alternative splicing relative to other cell types, and specific splice variants have been shown to play roles in the development and physiology of neurons. However, despite its importance, it remains challenging to study the mechanisms, evolution, and function of alternative splicing specific to the nervous system in the context of a living, developing organism.

Our group uses the tiny roundworm *Caenorhabditis elegans* as a model system to investigate how regulation of alternative splicing is achieved in individual neuron types. *C. elegans* is an excellent system to address this question on account of its simple but well differentiated nervous system and its genetic tractability. Using this powerful model organism, students will participate in the following project:

Using fluorescent reporters that monitor alternative splicing patterns at single cell resolution, we have identified a number of genes with mRNA transcripts that are differentially spliced in specific classes of neurons. We are now interested in identifying the factors responsible for this differential splicing regulation. In this project, a student will conduct genetic screens to search by fluorescence microscopy for mutants with defective neuronal splicing patterns. Mutations leading to these defects will be mapped to determine the underlying genes involved in controlling splicing patterns. If time permits, the student will then perform some initial experiments to characterize the mechanistic role of these newly discovered regulators of splicing.

Additionally, using recombinant DNA cloning, a student will introduce a random set of nucleotide sequence elements into a transgene reporter that is poorly spliced in neurons, creating a library of variants. This transgene library will then be introduced into *C. elegans* animals, expressed in neurons, and RNA splicing patterns resulting from inclusion

of random sequence elements will be analyzed by high-throughput sequencing and computational analysis. The student will then experimentally validate candidate RNA sequence elements identified to stimulate splicing.

DESCRIPTION OF STUDENT PARTICIPATION:

Interested students would have the opportunity to learn and utilize classical and molecular genetic techniques, PCR, DNA cloning, microinjection, genomics and computational analysis, and microscopy during this project.

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

Lab/Research Activity:

Bench work/attendance/participation – assessed continuously through interactions with Dr. Calarco (30%)

Lab notebook – assessed every two weeks by Dr. Calarco (15%)

mid-term report due November 2nd 2018 (15%)

Lab Presentation:

Lab meeting oral presentation on March 27th 2019 (20%)

Lab Report:

Final lab report due on April 3th 2019 (20%)

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

Project Code: CSB 2

RESEARCH OPPORTUNITY PROGRAM
299Y PROJECT DESCRIPTIONS 2018-2019
FALL/WINTER

Name and Title: Darrell Desveaux / Professor
Department: Cell and Systems Biology

TITLE OF RESEARCH PROJECT: Identifying Avirulence Factors of Plant Pathogens

Number of 299Y Spots: 2

OBJECTIVES AND METHODOLOGY: The goal of the research project will be to identify important avirulence determinants of the plant bacterial pathogen *Pseudomonas syringae*. The bacteria will be genetically modified and examined for their ability to cause disease in host plants. Any bacteria that have lost the ability to cause disease will be sequenced to identify the gene the gene conferring avirulence. The genes identified will represent those required for the ability of plants to recognize invading pathogens and will be promising targets for the development disease control strategies.

DESCRIPTION OF STUDENT PARTICIPATION: Students will be responsible for growing Arabidopsis plants and infecting them with bacteria that have been genetically modified. Bacteria will then be scored for their ability to cause disease in. Bacteria lacking this ability will be identified, retested and any reproducible strains will be sequenced to identify the transgene conferring resistance. The second round of analyses will involve understanding how the avirulence gene is recognized by plants using standard virulence assays developed in the laboratory. Students will be responsible for the growth and maintenance of plants required for infection. The project will require students to come in multiple days per week to grow plants, infect with bacteria, and score resulting disease symptoms (~20 hours per week total for summer project, ~10 hours per week total for Fall / Winter projects).

MARKING SCHEME (assignments with weight and due date**):**
Interim Report: 20% (Due December 21st, 2018)
Lab work: 20% (Due April 5th, 2019)
Lab Notebook: 20% (Due April 5th, 2019)
Final Report: 30% (Due April 5th, 2019)
Oral Presentation: 10% (Due April 5th, 2019)

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

Project Code: CSB 3

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

Name and Title: Prof. Tony Harris
Department: Cell & Systems BIology

TITLE OF RESEARCH PROJECT: Computer Simulations of Cytoskeletal Networks of the *Drosophila* Embryo

Number of 299Y Spots: 1

OBJECTIVES AND METHODOLOGY:

Under the surface membrane of a cell, cytoskeletal networks give the cell its particular shape. Cell shape is linked closely to cell activity (e.g. cell division, cell migration, cell-cell interaction, etc.), and is based on the molecular polymers and accessory proteins that form the cytoskeleton. These cytoskeletal networks can be observed by advanced microscopy, and the basic role of an individual component can be determined by removing it through genetic approaches. However, by microscopy and genetics alone we are unable to fully examine the physical properties of the cytoskeletal networks. To increase our understanding of the networks, we are generating mathematical models of the networks run as computer simulations using MATLAB software. These simulated networks are structured locally as nodes and edges and organized globally based on patterns we observe in embryos by microscopy. Our current wet-lab data provide only limited insight into the local structure and activity of the networks as the form, grow and impact cell shape. Our goal is to probe such parameters in our mathematical model to determine which values produce the most robust mimic of the whole-network behavior observed in the embryo, for both the normal embryo and a number of specific mutants in which a network component is lost and specific network abnormalities arise. Using our model, we are pursuing network properties that explain network behaviors important for controlling cell shape in the embryo.

DESCRIPTION OF STUDENT PARTICIPATION:

Working under the direct supervision of a PhD student in the lab, the 299Y student will formulate new versions of the model; test parameters of the model; and compare model results to data collected from live embryos using genetics and microscopy. The project will be computer-based and theoretical. Students with a solid background in computer programming and physics, and with a strong interest in applying this background to molecular and cellular biology are encouraged to apply.

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

Literature review	20%	One week before drop date - Jan 11, 2019
Research report	40%	Last day of classes - Apr 5, 2019
Oral presentation	10%	Second last week of classes - Mar 25, 2019
Lab work/interactions	30%	Throughout course

UNIVERSITY OF TORONTO
FACULTY OF ARTS & SCIENCE

Office of the Dean

Project Code: CSB 4

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

Name and Title: Shelley Lumba (Assistant Professor)
Department: Cell and Systems Biology

TITLE OF RESEARCH PROJECT: Molecular Characterization of Novel Signaling Networks in Parasitic Plants

Number of 299Y Spots: 2 **Number of 399Y Spots:** 2

OBJECTIVES AND METHODOLOGY:

Parasitic plants like *Striga hermonthica* cause significant yield losses ranging from 30 to 100% for African farmers and were identified to be the largest impediment to poverty alleviation by the UN. As a consequence of its parasitic lifestyle, *Striga* has evolved signaling pathways that are distinct from non-parasitic plants. To develop strategies to eradicate *Striga*, the Lumba lab aims to elucidate the molecular mechanisms of signaling pathways that are critical to *Striga* development. Because these mechanisms are poorly understood, we have developed novel strategies based on bioinformatics, transcriptomics, proteomics and genetics approaches to study *Striga*. The methodology is as follows: (1) identify signaling components in *Striga* by bioinformatics; (2) clone *Striga* genes into yeast two-hybrid (Y2H) vectors; (3) perform large-scale Y2H to detect protein interactions; (4) validate protein interactions by BiFCs and co-IPs; (5) test for gene function in a model system, Arabidopsis and (6) biochemically assay downstream outputs of signaling pathways in *Striga* and Arabidopsis. Finally, we apply systems biology strategies that integrate these "omics" data to construct novel signaling networks in *Striga*.

DESCRIPTION OF STUDENT PARTICIPATION:

The ROP student will participate in various aspects of the project proposed, which depends upon the progress of this ongoing research in my lab. The student could potentially acquire skills in the following: (1) sterile techniques for bacteria, yeast or plants; (2) cloning genes from *Striga*; (3) yeast transformation and two-hybrid; (4) plant transformation; (5) PCR-based genotyping of transgenic plants; (6) genetic crosses in plants; (7) Western blotting and (8) confocal microscopy.

The student will interact directly with members of the Lumba lab including graduate students and post-docs for training in various lab techniques as well as guidance in designing experiments. The student will be responsible for keeping a lab notebook, submitting an interim report, a project proposal and his/her findings in a write-up at the completion of the course. Part of the participation mark will include attendance, diligence in performing experiments in the lab and a presentation in our joint lab meetings.

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

Interim report (Protocols, Research experience)	16 November 2018	10%

Project proposal / literature review	16 November 2018	30%
Final write-up	5 April 2019	30%
Lab book	5 April 2019	10%
Lab participation		20%

UNIVERSITY OF TORONTO
FACULTY OF ARTS & SCIENCE

Office of the Dean

Project Code: CSB 5

RESEARCH OPPORTUNITY PROGRAM
299Y PROJECT DESCRIPTIONS 2018-2019
FALL/WINTER

Name and Title: Nicholas Provart
Department: Cell & Systems Biology

TITLE OF RESEARCH PROJECT: Bioinformatic Tools for Understanding Plant Biology

Number of 299Y Spots: 1

OBJECTIVES AND METHODOLOGY:

One of the foci of the Provart laboratory is the Bio-Analytic Resource, available online at http://bar.utoronto.ca. The BAR is used about 60,000 a month by plant researchers around the world. The "electronic fluorescent pictograph" or "eFP" Browser is the most popular of these tools - it allows researchers to quickly ascertain where their gene of interest is being expressed in the plant. This tool has been incorporated into our recently published “ePlant” (http://bar.utoronto.ca/eplant; Waese et al. 2017, http://dx.doi.org/10.1105/tpc.17.00073), which offers not only viewing of gene expression data at the centimeter scale but also natural variation data at the kilometer scale, all the way down to the gene product’s protein structure at the nanometre scale, with several other kinds of data viewable for hypothesis generation in between those levels. The idea is to provide a seamless environment for exploring biological data from plants. Aspects that we would like to add to ePlant include metabolic pathway data, GWAS information, etc.

DESCRIPTION OF STUDENT PARTICIPATION:

The CSB299Y student would be involved in several aspects of the above described project, which is ongoing in my laboratory, depending on the state of progress at the time of starting, and would thus acquire skills in programming, dynamic image generation, web-based interfaces and database design and query. The student would preferably have some experience in programming for the web using Python and/or Javascript/CSS/HTML5/jQuery.

The ROP student will interact on a one-on-one basis with Provart Lab research team (Prof. Provart, graduate students, post-docs and/or technicians) for training and assistance in carrying out experiments. The student will communicate his or her findings as decided upon signing the ROP project contract. Typically there is a mixture of a literature review, lab book note keeping, a poster presentation during the ROP fair, and a final report (with weightings decided at the start of the year). The student might also present in lab meetings, and in the past some students have actually published their results as part of a larger publication from the Provart Lab.

MARKING SCHEME (assignments with weight and due date):

Project proposal / literature review	20 Oct 2018	20%
Poster/undergrad research forum	March 2019	20%
Final write-up	4 Apr 2019	50%
Lab book/participation		10%

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

Project Code: CSB 6

RESEARCH OPPORTUNITY PROGRAM
299Y PROJECT DESCRIPTIONS 2018-2019
FALL/WINTER

Name and Title: Nicholas Provart
Department: Cell & Systems Biology

TITLE OF RESEARCH PROJECT: Molecular Characterization of Novel Environmental Stress-Associated Genes from Plants

Number of 299Y Spots: 1

OBJECTIVES AND METHODOLOGY:

Using the INTACT and TRAP systems for isolating mRNA populations from specific cell types (guard cells), we have identified several novel stress-associated genes (NSAGs) in *Arabidopsis thaliana*, the model system for plant research. Preliminary analysis of knock-out mutants of these genes indicates their necessity in response to drought. We have little knowledge about the precise molecular function of the gene products. To this end, we are applying several molecular and biochemical techniques, such as creating promoter::NSAG::GFP fusions to study tissue-specific and subcelluar expression patterns via confocal microscopy. Other possible venues for exploration include the overexpression of these genes in heterologous systems such as yeast or *E. coli* or identifying gene regulatory networks using the yeast one hybrid system in the case of NSAGs that are transcription factors.

DESCRIPTION OF STUDENT PARTICIPATION:

The CSB299Y student would be involved in several aspects of the above described project, which is ongoing in my laboratory, depending on the state of progress at the time of starting, and would thus potentially acquire skills in confocal microscopy, genotyping, phenotyping,
image analysis and other molecular methods.

The ROP student will interact on a one-on-one basis with Provart Lab research team (graduate students, post-docs and/or technicians) for training and assistance in carrying out experiments. The student will communicate his or her findings as decided upon signing the ROP project contract. Typically there is a mixture of a literature review, lab book note keeping, a poster presentation during the ROP fair, and a final report (with weightings decided at the start of the year). The student might also present in lab meetings, and in the past some students have actually published their results as part of a larger publication from the Provart Lab.

MARKING SCHEME (assignments with weight and due date):

Project proposal / literature review	20 Oct 2018	20%
Poster/undergrad research forum	March 2019	20%
Final write-up	4 Apr 2019	50%
Lab book/participation		10%

UNIVERSITY OF TORONTO
FACULTY OF ARTS & SCIENCE

Office of the Dean

Project Code: CSB 7

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

Name and Title: Arneet Saltzman, Assistant Professor
Department: Cell & Systems Biology

TITLE OF RESEARCH PROJECT: Chromatin Regulation in Development

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

OBJECTIVES AND METHODOLOGY:

Most of the cells in an organism share the same genome sequence, yet they are able to carry out many distinct functions. Our lab is interested in the role of chromatin regulation in the gene expression differences that underlie this cellular specialization. Chromatin, composed of genomic DNA wrapped around histones, can be altered by post-translational modifications, such as methylation, of histone proteins. Our broad goal is to understand the role of histone methylation and methyl-binding proteins, known as histone methyl 'readers', in how cells acquire and maintain different fates during development. In the long term, insights from this work may help us to understand how aberrant regulation of histone methylation contributes to the pathogenesis of several human diseases, including cancers.

Our approach is to use a tiny roundworm, *Caenorhabditis elegans*, as a model organism. The worm is transparent, grows quickly, has hundreds of progeny, and the lineages and fates of all its 959 cells have been mapped. In addition, many genetic pathways are conserved with humans, making it a simplified yet advantageous system to understand chromatin regulation in the context of a whole organism.

The first aim of this project is to investigate how deletion of histone methyl-reader protein genes, or mutation of the histone lysine residues that are modified by methylation, affects worm development. Students will use our lab's worm strains harbouring histone methyl-reader gene deletions. In addition, we are generating worm strains with histone lysine mutations. Notably, these histone lysine mutations play an oncogenic role in aggressive paediatric cancers, including glioblastoma.

To assay the impact of these deletions or mutations on worm development, students will examine (i) the expression of a panel of well-characterized fluorescent reporters for cell fates, (ii) the robustness of worm development upon forced expression of transcription factors, and (iii) the effect on the regulation of other histone modifications.

The second aim of this project is to learn which genes and cellular functions are regulated by histone methyl-reader proteins. We have used ChIP-seq (chromatin-immuno-precipitation and high-throughput sequencing) to identify genomic DNA fragments bound by the methyl-reader proteins, and RNA-seq to identify transcripts differentially

expressed in methyl-reader mutant animals. In this project, the student will analyse our data together with publicly available sequencing data for histone modifications and gene expression patterns. The objective will be to determine how the genomic binding sites of the methyl-reader proteins are related to patterns of histone modifications and gene expression regulation. In addition, the student will create protocols and pipelines based on their work to facilitate future analysis of similar data by other lab members.

DESCRIPTION OF STUDENT PARTICIPATION:

Students involved in the first project aim will gain experience in techniques including molecular biology (PCR, DNA cloning), genome editing (CRISPR/Cas9 or transposon-based), worm maintenance and strain construction (genetic crosses, genotyping, micro-injection), fluorescence microscopy of live and fixed samples, and assessing and quantifying worm phenotypes.

Students involved in the second project aim will focus on computational analysis of high throughput sequencing data. Experience with programming is an asset. In particular, familiarity with UNIX commands, R or Matlab, Python or Perl is helpful. Independent study skills will be essential for students to learn to use available open-source software (sequence manipulation, genome alignment, peak finding, visualization, statistics) and to integrate these tools with their own code to generate a data analysis pipeline.

In consultation with the supervisor, ROP students will be involved in planning and carrying out experiments as well as interpretation of data. ROP students will be responsible for maintaining accurate and detailed lab notebooks. They will develop presentation skills by participating in weekly informal 'round-table' lab meetings, where all lab members will discuss their work. With guidance from the supervisor, students will also prepare research reports as outlined in the marking scheme below.

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

Interim project report 1		
(project plan and literature review)	20%	Nov 2018
Interim project report 2	20%	Jan 2019
Final project report	20%	April 2019
Weekly lab meeting participation	15%	ongoing
Lab notebook	15%	ongoing
Skill development and attendance	10%	ongoing