RESEARCH OPPORTUNITY PROGRAM
299Y/399Y PROJECT DESCRIPTIONS 2019-2020
SUMMER

Name and Title: Joshua Currie, Assistant Professor
Department: Cell and Systems Biology

TITLE OF RESEARCH PROJECT: Self Organization of the Regenerative Blastema

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

OBJECTIVES AND METHODOLOGY:
We are interested in the how certain animals are able to completely regrow their organs, including their limbs. This requires that cells from the stump must migrate to the injury site and self-organize into the regenerative blastema. Our objective is to understand how the recruitment and organization of cells scales during regeneration based on what tissue is missing. This project will investigate the role of various signaling pathways in controlling the scaling of cell recruitment and patterning across scales of regeneration. Students will learn basic laboratories techniques and salamander husbandry as a foundation for experiments in the lab. Students will perform regeneration timecourses under control conditions as well as in the presence of pharmacological perturbations and observe the macroscopic and molecular alterations that happen to regeneration at different scales of regenerative growth. This will include histology and microscope imaging of the regenerating blastema to determine how cell migration, cell proliferation, and coordination are altered. Our goal is to understand how scale impacts different parameters of blastema self-organization.

DESCRIPTION OF STUDENT PARTICIPATION:
Students will work as a team together with Professor Currie and a member of the lab to support the lab, prepare reagents for experiments, care for animals, design and execute experiments, and analyze results. Students will be expected to be contributing lab members and responsible for successfully designing and performing experiments (with their team and supervisor(s)). Teams will present their results in lab meetings and do some supervised reading of primary literature and literature reviews to understand the context and direction of their project. Teams will have individual meetings with Professor Currie and supervisor(s) on a regular basis.

MARKING SCHEME (assignments with weight and due date):

Lab/Research Activity:
Project written proposal – A short, written proposal covering background, objectives, and any preliminary data.
20% of final grade
Due July 15th 2019
**Written Lab documentation** – Evaluation of lab notebook including written documentation of experiments, protocols, and results.
30% of final grade
Evaluated at end of ROP term

**Lab citizenship and performance** – Student’s contribution to support your student team and broader Currie lab. Effort of the student to perform rigorous and careful experimentation with properly created reagents, controlled experiments, and careful adherence to reproducible protocols
30% of final grade
Evaluated at end of ROP term

**Final presentation in lab meeting and/or ROP Research Forum**
20% of final grade
Evaluated one week prior to end of ROP term
Name and Title: Darrell Desveaux
Department: Cell and Systems Biology

TITLE OF RESEARCH PROJECT: Identifying Avirulence Factors of Plant Pathogens

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

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 projects).

MARKING SCHEME (assignments with weight and due date):

Interim Report: 20% (Due June 14th, 2019)
Lab work: 20% (Due August 12th, 2019)
Lab Notebook: 20% (Due August 12th, 2019)
Final Report: 30% (Due August 12th, 2019)
Oral Presentation: 10% (Due August 12th, 2019)
Name and Title:  Tony Harris, Professor  
Department:  Cell and Systems Biology 

TITLE OF RESEARCH PROJECT:  Identifying Avirulence Factors of Plant Pathogens 

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 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 or quantitative computational tools to evaluate how changes to parameters affect model output. 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):  
Progress Report  (10%)  Due June 24  
Journal/lab book  (10%)  Due June 24  
Final Report  (50%)  Due Aug 12  
(includes literature review, results, discussion, figures, tables, references)
Progress meetings  (15%)
Lab work and interactions  (15%)
Name and Title: Shelly 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* ORFs into yeast two-hybrid (Y2H) vectors; (3) perform large-scale pairwise Y2H to detect protein interactions; (4) validate protein interactions by BiFCs and co-IPs; (5) test for gene function in a model system, Arabidopsis; (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 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 CSB299/399Y student(s) 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 and a presentation in lab meetings.

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NAME AND TITLE: Shelly Lumba, Assistant Professor

DEPARTMENT: Cell and Systems Biology

TITLE OF RESEARCH PROJECT: Bioinformatics Analyses of NGS Applications in Cannabis and African Parasitic Weeds

OBJECTIVES AND METHODOLOGY:
In order to develop solutions to critical agricultural challenges, the research community is required to translate our basic knowledge of plant biology into non-model plants. Our research group is focused on understanding the signaling pathways of a parasitic weed, *Striga hermonthica*. *Striga* infestations result in devastating yield losses for subsistence farmers in sub-Saharan Africa. The obligate hemi-parasitic and outcrossing nature of *Striga* have prevented the use of traditional genetics techniques to study gene function. As a result, we have turned to Next-Generation Sequencing (NGS) applications such as whole genome sequencing (WGS) and RNA-seq based transcriptomes to make inroads in understanding *Striga* germination. The ROP student(s) will use bioinformatics approaches to integrate NGS datasets from model and non-model plants to identify critical genes involved in the germination of *Striga* seeds. These genes are potential targets to combat *Striga*.

In a more industrial application, we have begun our goal to make cannabis into a horticultural crop. NGS has paved the way to accelerating our progress in mapping the genetic differences amongst commercially important cannabis strains. The ROP trainee(s) will integrate WGS and RNA-seq to create an atlas of the genomic and transcriptomic landscape of cannabis varieties. The goal is to “fingerprint” or identify specific cannabis varieties using sequence data obtained from the latest NGS technologies. In the long-term, the student(s) will contribute to developing prognostication tools for cannabis varieties.

DESCRIPTION OF STUDENT PARTICIPATION:
The ROP student(s) will learn coding and apply bioinformatics approaches to integrate NGS datasets from model and non-model plants to identify critical genes involved in the germination of *Striga* seeds as well as identify molecular “fingerprints” for a range of cannabis varieties. They will also be responsible for contributing to the establishment of a bioinformatics pipeline, which would analyze sequence data from multiple cannabis samples. The student(s) will acquire bioinformatics skills analyzing large-scale datasets, which are in high demand in the biotech sector.

The CSB299/399Y student(s) will interact directly with members of the Lumba lab including students and post-docs for training in various bioinformatics analyses. The student will be responsible for keeping a record of code
generated, 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 and presentations in lab meetings.

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Name and Title: Sergey Plotnikov, Assistant Professor
Department: Cell and Systems Biology

TITLE OF RESEARCH PROJECT: Regulation of Pro-Inflammatory Signaling by Focal Adhesions.

Number of 299Y Spots: 1

OBJECTIVES AND METHODOLOGY:
The ability of cells to sense and correctly respond to their microenvironment is a fundamental phenomenon that is critical for life of unicellular and multicellular organisms. For over a decade it was widely accepted by the cell biology community that cell signaling is mediated by a complex network of cytosolic proteins activated by cell surface receptors. However, recently it has been shown that activity of signaling networks can also be regulated by focal adhesions (FAs) - integrin-based protein complexes that physically connect the cell’s cytoskeleton to the extracellular matrix (ECM). Several major signaling pathways, such as epidermal growth factor signaling, Wnt and cytokine signaling, are all regulated by FAs and the biological role of such regulation has been well documented. Among these signaling pathways the effect of FAs on inflammatory responses due to IL-1, a proinflammatory cytokine, has been the most heavily studied. Despite this, the exact mechanistic link between FAs and IL-1 signaling is unknown. Here, we propose to use IL-1 pathway as a model to elucidate how FAs modulate the activity of signaling networks.

DESCRIPTION OF STUDENT PARTICIPATION:
The CSB299Y student will be involved in several aspects of experimental research described above. After obtaining initial training in cell biology techniques and cell imaging, the student will assess how the effect of phraseological manipulations on focal adhesion maturation investigate how suppression and enhancement of focal adhesion maturation affects the activity of the extracellular signal-regulated kinase, a major downstream effector of the IL-1 pathway, by Western blot and immunostaining. After identifying a subpopulation of FAs (nascent or mature adhesions) that are essential for IL-1 signaling, the student will conduct an siRNA screen to determine focal adhesion components that regulate the extent and dynamics of IL-1 signaling.

The ROP student will interact with members of Dr. Plotnikov’s lab for training and assistance in carrying out experiments. The student will communicate his/her experimental results at lab meetings, give a poster presentation during the ROP fair, and provide a written report at the end of the project.

MARKING SCHEME (assignments with weight and due date):
Interim report (Lab meeting) 25 June 2019 20%
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RESEARCH OPPORTUNITY PROGRAM
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Name and Title: Sergey Plotnikov, Assistant Professor
Department: Cell and Systems Biology

TITLE OF RESEARCH PROJECT: Molecular Analysis Cell Mechanosensing by Focal Adhesion

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

OBJECTIVES AND METHODOLOGY:
Tissue stiffness is an important factor regulating cell behavior, but how the cells sense stiffness of the microenvironment is unknown. Recently, we discovered a novel mechanism that allows migrating fibroblasts to sense stiffness by exerting fluctuating forces reminiscent of repeated tugging on the extracellular matrix (ECM). However, the molecular mechanisms that drive force fluctuations and transduce force dynamics in cellular behavior remains elusive. To address this question, we are applying live-cell imaging combined with biophysical and cell biology techniques to identify focal adhesion component that are essential for fluctuating force dynamics and to reveal signaling pathways that are activated by the fluctuating forces.

DESCRIPTION OF STUDENT PARTICIPATION:
The CSB299Y/399Y students will be involved in several aspects of experimental research described above. After obtaining initial training in cell biology techniques and cell imaging, the students will investigate how depletion of specific focal adhesion proteins affects the dynamics of traction force. The students will also assess how changes in cellular force dynamics induced by pharmacological manipulations affect the activity of major cellular signaling pathways. These experiments will require the students to culture the cells, to visualize movement of individual cells by time-lapse imaging and to quantify cell migration by custom-built software (MatLab). The students would preferably have some experience in laboratory work. Experience in programming is beneficial, but not required.

The ROP students will interact with members of Dr. Plotnikov’s lab for training and assistance in carrying out experiments. The students will communicate his/her experimental results at lab meetings, give a poster presentation during the ROP fair, and provide a written report at the end of the project.

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