UNIVERSITY of GUELPH

COLLEGE of BIOLOGICAL SCIENCE

DEPARTMENT OF MOLECULAR AND CELLULAR BIOLOGY

Announcement: All interested members of the university community are invited to attend the Final Oral Examination for the degree of **Master of Science** of

KAYLA HUMPHRIES, on Monday, December 11, 2017 at 1:00 p.m. in SSC 3317
(Advisor: Dr. S. Ryan)

Thesis Title: **A53T alpha-synuclein induces mitophagy through MAM-induced fission and reduced impairment of LC3B to promote alpha-synuclein degradation**

Examination Committee:

Dr. A. Bendall, Dept. of Molecular and Cellular Biology (Chair)
Dr. S. Ryan, Dept. of Molecular and Cellular Biology
Dr. G. Harauz, Dept. of Molecular and Cellular Biology
Dr. J. Lalonde, Dept. of Molecular and Cellular Biology

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized by mitochondrial dysfunction and aggregated α-synuclein (α-syn). Some cases of PD are caused by mutations in α-syn, resulting in an increased propensity for aggregation. To investigate the link between mitochondrial dysfunction and the α-syn mutation A53T, human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), each consisting of an A53T line and a wildtype or corrected control line, were differentiated into dopaminergic human neurons (hNs). Transmission electron microscopy demonstrated that A53T cells had elongated mitochondrial-associated membranes (MAMs) at early timepoints, which were shortened to normal lengths by day 60 of differentiation, concurrent with mitochondrial fragmentation, Golgi dilation and increased vacuolar area, all indicative of increased autophagic load. Aggregated α-syn has a greater colocalization with lysosomes in A53T hNs, suggesting lysosomes attempt to degrade aggregated α-syn. This degradation could occur alongside mitophagy, as knockdown of the apoptotic protein beclin1 decreased cell survival in the A53T line, indicating mitophagy is protective. Further, *in vitro* experiments demonstrate that A53T α-syn has a reduced ability to impair binding of the mitophagy-initiating protein LC3B to mitochondria, suggesting that A53T cells would be more vulnerable to mitophagy. Potential therapies should focus on preventing MAM-induced fission and restoring the ability of A53T cells to impair LC3B-mediated mitophagy.

Curriculum Vitae: Kayla obtained her B.Sc. (Hons) in Biochemistry at the University of Guelph in 2015. She then began her M.Sc. (Neuroscience Specialization) in the lab of Dr. Scott Ryan in January 2016.