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 ***Doctor of Philosophy*** *of*

MORGAN STYKEL

on Friday, September 10, 2021 at 9:30 a.m. (online)

Thesis Title: **Cellular pathologies in SNCA mutant neurons: implications for Parkinson's disease**

Examination Committee:

Dr. Ray Lu, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. Scott Ryan, Dept. of Molecular and Cellular Biology
Dr. Richard Mosser, Dept. of Molecular and Cellular Biology
Dr. Shaun Sanders, Dept. of Molecular and Cellular Biology
Dr. Joel Watts, Dept. of Biochemistry, University of Toronto (External Examiner)

Advisory Committee:
Dr. Scott Ryan (Adv)
Dr. Elena Choleris
Dr. Richard Mosser
Dr. John Vessey

Abstract: Parkinson's disease (PD) is associated with oxidative stress, a genetic susceptibility to agrochemical exposure, and impaired proteostasis which primarily affect dopaminergic neurons that populate the substantia nigra pars compacta, the region of the PD brain that most prominently degenerates. The discovery that mutations in alpha-synuclein (aSyn) causes inherited forms of PD has dramatically improved our understanding of PD at a cellular level. Herein, we use human stem-cell derived-dopaminergic neurons harboring the A53T or E46K aSyn mutation and isogenic controls to assess PD-related pathologies. We show that early accumulation of aSyn in PD neurons results in impairments to the antioxidant response and mitochondrial dynamics. We determined that these impairments can be attributed to mutant aSyn's inability to associate with PKC, resulting in decreased Nrf2 phosphorylation and activation, consequently reducing the antioxidant response. We demonstrate that forced activation of Nrf2 by pharmaceutical modulation with dimethyl fumarate (DMF) rescues antioxidant enzyme expression in PD neurons. Further, our findings highlight the importance of genetic vulnerability to toxin exposure, including the agrochemicals paraquat, maneb and rotenone. Using these toxins below the reported EPA-lowest observable effects levels we demonstrate that anterograde mitochondrial transport was impaired in mutant aSyn neurons, but not in control neurons. We determined that this was due to the nitration of alpha-tubulin which inhibited the association of aSyn and kinesin 5B with microtubules. We showed that we could rescue mitochondrial anterograde trafficking by using the nitric oxide synthetase inhibitor L-NAME. In addition, we demonstrate that aSyn accumulates on multi-vesicular bodies and lysosomes in PD neurons. As such, aSyn is secreted by exosomes which promotes the spread of disease from cell-to-cell. We demonstrate that constitutive expression of LC3B reduces the accumulation of aSyn (PS129) as well as the amount of secreted aSyn in exosomes. Since transmission of aSyn is coincident with

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mitochondrial pathology and oxidative stress in previously healthy cells, our findings suggest that targeting aSyn or exosomes might slow disease progression. Altogether this research offers mechanistic insight as to the development and spread of PD-pathology.

Curriculum Vitae: Morgan obtained her B.Sc. with honours in Psychology and Philosophy (emphasis in ethics) at Trent University in 2013. Following this, Morgan completed her M.Sc. in Neuroscience at the University of Calgary under the supervision of Dr. Jeff Biernaskie. In 2016, Morgan began her Ph.D. in Molecular and Cellular Biology with a specialization in neuroscience under the supervision of Dr. Scott Ryan here at the University of Guelph.

Awards: Dr. Donald R. Phillips Molecular and Cellular Biology Scholarship (2019), Vanier Canada Graduate Scholarship (2018), Natural Sciences and Engineering Research Council - PGS D (2018*declined), Ontario Graduate Scholarship (2018*declined), First Place Graduate Student Poster Award at the American Association for Anatomy (AAA) annual meeting at Experimental Biology (2018), Ontario Graduate Scholarship (2017), and a Genetic Tools Workshop Award & Travel Stipend through the Ontario Institute of Regenerative Medicine (2017).

Publications:

Stykel MG, Humphries KM, Kamski-Hennekam E, Buchner-Duby B, Porte-Trachsel N, Ryan T, Coackley CL, Bamm VV, Harauz G, Ryan SD. α-Synuclein mutation impairs processing of endomembrane compartments and promotes exocytosis and seeding of α-synuclein pathology. Cell Rep. 2021 May 11;35(6):109099. doi: 10.1016/j.celrep.2021.109099. PMID: 33979611.

Stykel MG, Humphries K, Kirby MP, Czaniecki C, Wang T, Ryan T, Bamm V, Ryan SD. Nitration of microtubules blocks axonal mitochondrial transport in a human pluripotent stem cell model of Parkinson's disease. FASEB J. 2018 Oct;32(10):5350-5364. doi: 10.1096/fj.201700759RR. Epub 2018 Apr 24. PMID: 29688812.

Czaniecki C, Ryan T, Stykel MG, Drolet J, Heide J, Hallam R, Wood S, Coackley C, Sherriff K, Bailey CDC, Ryan SD. Axonal pathology in hPSC-based models of Parkinson's disease results from loss of Nrf2 transcriptional activity at the Map1b gene locus. Proc Natl Acad Sci U S A. 2019 Jul 9;116(28):14280-14289. doi: 10.1073/pnas.1900576116. Epub 2019 Jun 24. PMID: 31235589; PMCID: PMC6628831.

Ryan T, Bamm VV, Stykel MG, Coackley CL, Humphries KM, Jamieson-Williams R, Ambasudhan R, Mosser DD, Lipton SA, Harauz G, Ryan SD. Cardiolipin exposure on the outer mitochondrial membrane modulates α-synuclein. Nat Commun. 2018 Feb 26;9(1):817. doi: 10.1038/s41467-018-03241-9. PMID: 29483518; PMCID: PMC5827019.

Drolet J, Buchner-Duby B, Stykel MG, Coackley C, Kang JX, Ma DWL, Ryan SD. Docosahexanoic acid signals through the Nrf2-Nqo1 pathway to maintain redox balance and promote neurite outgrowth. Mol Biol Cell. 2021 Apr 1;32(7):511-520. doi: 10.1091/mbc.E20-09-0599. Epub 2021 Jan 27. PMID: 33502893; PMCID: PMC8101469.