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*

GAELAN MELANSON

on Wednesday, December 15, 2021 at 1:30 p.m. (online)

Thesis Title: **Interferon-stimulated gene 15 – mediated restructuring of the hypoxic mRNA translation initiation machinery is implicated in ribonucleoprotein dynamics by gene ontology enrichment analysis**

Examination Committee:

Dr. Ray Lu, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. Jim Uniacke, Dept. of Molecular and Cellular Biology
Dr. Nina Jones, Dept. of Molecular and Cellular Biology
Dr. Andrew Bendall, Dept. of Molecular and Cellular Biology
Dr. Bruce McKay, Dept. of Biology and Institute of Biochemistry, Carleton University (External Examiner)

Advisory Committee:
Dr. Jim Uniacke (Adv)
Dr. Nina Jones
Dr. Scott Ryan
Dr. Sarah Wootton

Abstract: Tumor hypoxia is a major barrier to therapeutic intervention and disease-free survival. Cancer cells exploit an ancient hypoxia-response program mediated by the hypoxia-inducible transcription factors (HIF) 1α & 2α that promote angiogenesis, metastasis, and epithelial-to-mesenchymal transitions. Interestingly, HIF-2α also participates in hypoxia-mediated cap-dependent translation initiation as a member of the eIF4F^{H} complex which is an important axis for gene expression in solid tumors. Therefore, there is a need for characterization of the underlying biology of the HIF-αs to develop anti-HIF therapeutic interventions. Interferon-stimulated gene 15 (ISG15) is a ubiquitin-like modifier implicated in inflammatory-mediated tumor progression that restricts HIF-1α *trans*-activity via proteasomal degradation in a negative feedback loop, however, ISG15's interaction with HIF-2α remains uncharacterized. We identify HIF-2α as a substrate for ISG15 conjugation, and ISGylation disrupts formation of the eIF4F^{H} complex on the 5' m^{7}-cap of mRNA via conjugation-dependent and -independent mechanisms. Surprisingly, ISGylation enhances the polyribosome association of eIF4F^{H} factors isolated by sucrose density fractionation with no alterations to translation of select mRNA transcripts. Gene ontology enrichment analysis of hundreds of ISG15 substrates identified in published ISGylome datasets indicate a significant enrichment of functions related to ribonucleoprotein dynamics and stress granule formation. Polysome profiles of HCT116 cells overexpressing the ISGylation system and treated with puromycin, an inducer of stress granule formation, shows enhanced co-fractionation of eIF4F^{H}-associated initiation factors with heavier polyribosome fractions despite translation arrest which is a classic indication

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of stress granule formation. Therefore, our results implicate ISGylation as a translation regulating pathway of HIF-2α-dependent translation in hypoxia.

Curriculum Vitae: Gaelan completed his B.Sc. in Molecular Biology and Genetics at the University of Guelph in 2015 and began his Ph.D. in Molecular and Cellular Biology that same year in the lab of Dr. Jim Uniacke.