Notice of the Final Oral Examination
for the Degree of Doctor of Philosophy

of

JACOB ALEC McPHAIL

BSc Honours (University of Victoria, 2015)

“Molecular mechanisms of phosphatidylinositol 4-kinase III beta (PI4KB) regulation and their role in human disease”

Department of Biochemistry and Microbiology

Wednesday, March 11, 2020
1:20 P.M.
Clearihue Building
Room B017

Supervisory Committee:
Dr. John Burke, Department of Biochemistry and Microbiology, University of Victoria (Supervisor)
Dr. Alisdair Boraston, Department of Biochemistry and Microbiology, UVic (Member)
Dr. Caroline Cameron, Department of Biochemistry and Microbiology, UVic (Member)
Dr. Fraser Hof, Department of Chemistry, UVic (Outside Member)

External Examiner:
Dr. Julie Brill, Molecular Genetics, University of Toronto

Chair of Oral Examination:
Dr. Christopher Lalonde, Department of Psychology, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies
Abstract

The lipid signalling molecule phosphatidylinositol 4-phosphate (PI4P) is an essential factor in the coordinated regulation of membrane trafficking, lipid transport and cytokinesis. At the Golgi, a key generator of PI4P is the type III phosphatidylinositol 4-kinase beta isoform (PI4KIIIβ), which has been identified as a host factor necessary for the replication of numerous devastating pathogenic viruses. Crucial to the regulation of PI4KIIIβ are interactions with a variety of both host and viral protein-binding partners. Additionally, parasitic variants of PI4KIIIβ have been established as essential enzymes in the proliferation of the malaria and cryptosporidiosis parasites. Therefore, study of PI4KIIIβ and its regulatory proteins is of great importance in understanding normal cellular signalling and the proliferation of viral and parasitic pathogens.

To study PI4KIIIβ regulation, I utilized a multifaceted approach of biochemistry, hydrogen-deuterium exchange mass spectrometry (HDX-MS), and X-ray crystallography to elucidate molecular mechanisms of PI4KIIIβ regulation by the key protein binding partners ACBD3 and c10orf76, and viral proteins that manipulate these complexes. This synergistic approach provided a unique opportunity to study the structure and dynamics of both natural PI4KIIIβ regulation and inhibition by small molecules. This dissertation will consist of an introduction to signalling of PI4KIIIβ and its role in disease, followed by two data chapters wherein I investigate ACBD3 and c10orf76 regulatory complexes required for viral replication. A third data chapter summarizes my efforts in defining the molecular basis of inhibitor selectivity towards PI4KIIIβ and related lipid kinases. A conclusion and discussion of future directions will be presented in the final chapter.

Fundamentally understanding how PI4KIIIβ is regulated, and how viruses manipulate PI4KIIIβ signalling, will expand our knowledge of PI4KIIIβ biology and facilitate development of novel therapeutic strategies targeting this pathway. My work provides novel insight into the complex regulation of PI4KIIIβ and elucidates molecular mechanisms of selective inhibition by therapeutic small molecule inhibitors. Altogether this dissertation contributes significant advances in our understanding of the role of PI4KIIIβ in signalling and human disease.