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*

NOAH PHIPPEN

on Wednesday, September 1, 2021 at 9:30 a.m. (online)

Thesis Title: **Establishing CRISPR-Cas9 and BioID workflows to probe molecular signaling pathways in kidney podocytes**

Examination Committee:

Dr. Jasmin Lalonde, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. Nina Jones, Dept. of Molecular and Cellular Biology
Dr. Marc Coppolino, Dept. of Molecular and Cellular Biology
Dr. Terry Van Raay, Dept. of Molecular and Cellular Biology

Advisory Committee:

Dr. Nina Jones (Advisor)
Dr. Marc Coppolino
Dr. Alicia Viloria-Petit

Abstract: Kidney podocytes are highly specialized epithelial cells that play an integral role in blood filtration, and they must withstand intense hemodynamic strain while maintaining tight adhesion to the underlying basement membrane. Podocyte detachment is a significant contributor to kidney disease progression, and it involves changes in signaling proteins which control actin and integrin activity. Integrins are a major component of focal adhesions, and their activity and matrix binding are negatively regulated by the adaptor proteins Dok1 and Dok2. We initially identified Dok1 as a protein of interest in podocyte adhesion signaling, and subsequently uncovered a protective effect of Dok1 and Dok2 loss during podocyte injury *in vivo*. To better understand the role of Dok proteins in podocytes, the goal of this thesis was to implement two new *in vitro* methodologies and adapt their use to cultured podocytes. Herein, we outline the development of workflows suited to the generation of CRISPR-Cas9 knockout (KO) podocytes as well as stable expressing bait-miniTurbo podocyte cell lines for proximity-dependent biotin identification (BioID). Synthesis of Dok1/2 CRISPR KO podocytes revealed a modest cell adhesion phenotype as well as increased cell migration. Dok1- and Dok2-FLAG-miniTurbo human podocyte cell lines were successfully generated and used for biotin labelling of associated proximity partners. Cellular component gene ontology analysis confirmed the association of Dok proteins with intercellular and adhesive junctions as well as actin cytoskeleton. Altogether, workflows established here can be extended to other signaling proteins to improve our understanding of podocyte biology.

Curriculum Vitae: Noah completed his Bachelor of Science Biochemistry (Co-op) at the University of Guelph in Fall 2018. After having worked in Dr. Jones' lab as a co-op student in 2017, Noah began his MSc in the lab of Dr. Jones during the Fall of 2019.