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

BRIDGET RYAN

BSc (University of Victoria, 2012)

“Investigating Direct and Cooperative MicroRNA Regulation of PAX6 in vivo Using a Genome Engineering Approach”

Division of Medical Sciences/Biology

Tuesday September 10, 2019
9:00 A.M.
Medical Sciences Building
Room 210

Supervisory Committee:
Dr. Robert Chow, Department of Biology, University of Victoria (Supervisor)
Dr. John Taylor, Department of Biology, UVic (Member)
Dr. Perry Howard, Department of Biochemistry and Microbiology (Outside Member)
Dr. Chris Nelson, Department of Biochemistry and Microbiology, UVic (Additional Member)

External Examiner:
Dr. Ruth Aslery-Padan, Human Molecular Genetics & Biochemistry, Tel Aviv University

Chair of Oral Examination:
Dr. Joseph Parsons, Department of Psychology, UVic

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

Cells must employ a diversity of strategies to regulate the quantity and functionality of different proteins during development and adult homeostasis. Post transcriptional regulation of gene transcripts by microRNAs (miRNAs) is recognized as an important mechanism by which the correct dosage of proteins is regulated. Despite this, the physiological relevance of direct regulation of an endogenous gene transcript by miRNAs in vivo is rarely investigated.

PAX6 is a useful model gene for studying miRNA regulation directly. PAX6 is highly dosage-sensitive transcription factor that is dynamically expressed during development of the eye, nose, central nervous system, gut and endocrine pancreas, and is mutated in the haploinsufficiency disease aniridia. Several miRNAs have been implicated in regulating PAX6 in different developmental contexts. Notably, miR-7 has been implicated in regulating Pax6 in the brain and endocrine pancreas.

Here, we generated a bioinformatics tool to enable selective mutation of candidate microRNA recognition elements (MREs) for specific miRNAs while ensuring that new MREs are not inadvertently generated in the process. We then performed the first comprehensive analysis of the Pax6 3’ untranslated region (3’UTR) to identify MREs that may mediate miRNA regulation of Pax6 and to identify miRNAs capable of interacting with the 3’UTR of Pax6. Using Pax6 3’UTR genetic reporter assay, we confirmed that two MREs for miR-7-5 located at 3’UTR positions 517 and 655 function together to regulate PAX6. We generated mice harboring mutations that disrupt either of these miR-7-5p MREs individually or in combination within the Pax6 3’UTR to explore the biological relevance of miRNA regulation directly. PAX6 protein was elevated in double miR-7-5p MRE mutants relative to wild type and single mutants in the ventral ventricular and subventricular zone (V-SVZ). However, this increase in PAX6 was not associated with an altered dopaminergic periglomerular neuron phenotype in the olfactory bulb.

Our findings suggest that, in vivo, microRNA regulation can be mediated through redundant interactions. This work also reveals that directly mutating predicted MREs at the genomic level is necessary to fully characterize the specific phenotypic consequences of direct miRNA-target regulation.