University of Victoria | Graduate Studies

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

of

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BSc (McMaster University, 2007)

"The Requirements of ARS2 in RNA Processing and Retina Development"

Department of Biochemistry and Microbiology

Friday, August 19, 2016
1:00 P.M.
Engineering and Computer Science Building
Room 130

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

External Examiner:
Dr. Stephen Rader, Department of Chemistry, University of Northern British Columbia

Chair of Oral Examination:
Dr. Ulrike Stege, Department of Computer Science, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies

Abstract

ARS2 is a stable component of the nuclear cap-binding complex (CBC) and is critical for RNA Polymerase II transcript processing. As such, ARS2 functions in numerous RNA Polymerase II transcript processing events, which happen co-transcriptionally from initiation to termination, and post-transcriptionally during maturation and export into the cytoplasm. Developmentally, ARS2 is essential for stem cell maintenance and differentiation during embryogenesis and in neural stem cells. Two major questions in the field were: 1) how does ARS2 function in stem cell maintenance and/or differentiation? and 2) how does ARS2 distinguish between disparate RNA classes and processing complexes? In chapter 2, I show that ARS2 is required for the proliferation and cell fate decisions of progenitors in the mouse retina. Specifically, ARS2 knockdown delays cell cycle progression and leads to premature cell cycle exit. Additionally, ARS2 knockdown increases expression of rod photoreceptor marker Nrl, and decreases Müller glial marker expression. Similarly, knockdown of FLASH, an essential component for replication-dependent histone transcript processing and cell cycle progression, increases Nrl expression, suggesting ARS2's role in histone processing is contributing to cell cycle progression and fate specification in the developing retina. In chapter 3, I used bioinformatics analysis and homology modeling to classify four structural domains of mammalian ARS2, including a newly identified RNA recognition motif (RRM), and performed mutagenesis to assess their functions. The unstructured C-terminus is required for interaction with the CBC, the Mid domain is implicated in binding DROSHA, which is required for microRNA biogenesis, while the zinc finger and RRM are involved in binding FLASH. Moreover, the zinc finger is required for interacting with RNA. Collectively, this work establishes a model where ARS2 acts as a scaffold, using multiple domains to interact with distinct processing complexes in a mutually exclusive manner. It is also the first study describing the requirements of ARS2 in the developing retina. Understanding the molecular mechanisms governing progenitor proliferation and cell fate specification is crucial in order to design therapies for retinal degenerative diseases.