Notice of the Final Oral Examination
for the Degree of Master of Science
of

STEWARD AUSTIN HAMMOND

BSc (University of Victoria, 2006)

“Modulation of Thyroid Hormone Action by Environmental Temperature”

Department of Biochemistry and Microbiology

Thursday, December 17, 2015
1:00 P.M.
Engineering and Computer Science Building
Room 128

Supervisory Committee:
Dr. Caren C. Helbing, Department of Biochemistry and Microbiology, University of Victoria
(Supervisor)
Dr. Christopher J. Nelson, Department of Biochemistry and Microbiology, UVic (Member)
Dr. Leigh Anne Swayne, Division of Medical Sciences, UVic (Outside Member)

External Examiner:
Dr. Juergen Ehlting, Department of Biology, UVic

Chair of Oral Examination:
Dr. Mohsen Akbari, Department of Mechanical Engineering, UVic

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

Thyroid hormone (TH) signaling is conserved across vertebrates, where it is important for normal growth and development, particularly in the perinatal period. TH has an additional critical role in amphibian metamorphosis as the sole signal that initiates the transition from a larval tadpole to juvenile frog. Premetamorphic tadpoles have a thyroid gland but are functionally athyroid, yet can be induced to undergo precocious metamorphosis by exogenous TH administration. This essential dependence upon TH makes amphibian metamorphosis an excellent model to study TH signaling.

Metamorphosis is sensitive to environmental stimuli such as temperature. Low temperature delays or slows metamorphosis, whereas high temperature advances or accelerates it. Whether a temperature is considered low or high varies by species and is related to its natural habitat. In temperate climes the North American bullfrog, *Rana catesbeiana*, does not undergo natural or precocious metamorphosis at low winter temperatures of 4-5°C. Tadpoles injected with TH at low temperature essentially clear it from their bodies after 60-80 days, but some manner of TH signaling has occurred such that they rapidly execute metamorphosis if returned to 20-25°C. This apparent molecular memory is poorly understood, but there is evidence that components of gene expression programs may be involved.

This thesis investigated the role of these factors in the molecular memory of TH formed at low temperature in the liver, brain, lung, back skin, and tail fin of *Rana catesbeiana*. The results suggested that TH receptor beta (*thrb*), CCAAT/enhancer binding protein 1 (*cebp1*), and Krüppel-like factor 9 (*klf9*) may contribute to the molecular memory to different extents in each tissue, and that TH-induced basic leucine zipper-containing protein (*thibz*) may have an important role in this process for every tissue examined. Assessment of additional genes was hampered by the limited genetic resources available for this species, so *de novo* high throughput RNA sequencing (RNA-seq) techniques were explored to alleviate this limitation. Trans-ABySS sequence assembly software produced a high quality *Rana catesbeiana* liver transcriptome that was annotated by BLAST alignment to established sequence databases and resulted in a more than ten-fold increase in *Rana catesbeiana* sequence information. This approach was supplemented with original software for Transcriptome Expression and Characterization (TRENCH) that was used to refine replicate *Rana catesbeiana* back skin assemblies, and by construction of a Bullfrog Annotation Resource for the Transcriptome (BART) that was used to quickly annotate more than 97% of the assembled back skin sequences.

In the future, the *Rana catesbeiana* transcriptome sequence resources can be leveraged to identify additional genes that may be involved in formation of the TH molecular memory, and chromatin immunoprecipitation could help characterize the factors and epigenetic marks in the promoter regions of these genes. Elucidation of the molecular memory mechanism provides a means to uncover key events in TH signaling.