



University
of Victoria

Graduate Studies

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

of

SHIREEN H. PARTOVI

BSc (Utah Valley University, 2014)

**“Transcriptomic Analysis of Thyroid Hormone Effects on *Rana*
[Lithobates] Catesbeiana Tadpole Tissues with Special Emphasis on
the Innate Immune System”**

Department of Biochemistry and Microbiology

Friday, December 8th, 2017
10:00 A.M.

Engineering and Computer Science Building
Room 130

Supervisory Committee:

Dr. Caren Helbing, Department of Biochemistry and Microbiology, University of Victoria (Supervisor)

Dr. Caroline Cameron, Department of Biochemistry and Microbiology, UVic (Member)

Dr. Juergen Ehling, Department of Biology, UVic (Outside Member)

External Examiner:

Dr. Steve Perlman, Department of Biology, UVic

Chair of Oral Examination:

Dr. Peter Stahl, Department of Anthropology, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies

Abstract

Amphibian metamorphosis is facilitated solely by thyroid hormones (THs), L-thyroxine (T4) and 3,5,3'-triiodothyronine (T3). TH modulates the remodeling of many different organs and systems in the body of developing tadpoles, including the immune system. Previous research found evidence of T4 action on direct-response genes in outer ring deiodinase-poor premetamorphic tadpole tail fin and liver without the required conversion to T3 described by current TH dogma. The mechanisms of environmental endocrine disrupting chemicals (EDCs) may be better understood by expanding our understanding of the transcriptomic effects of both forms of THs and how they relate to estrogen signaling. Furthermore, analysis of TH-modulation of the immune system may enable a greater understanding of the devastating effects of amphibian pathogens such as Ranavirus. Premetamorphic *Rana (Lithobates) catesbeiana* tadpoles were exposed to physiological concentrations of T4, T3, or 17-beta-estradiol (E2) through water bath immersion. qPCR analysis was performed to assess the response of canonical TH-responsive genes *thra*, *thrb*, and *thibz* to these hormones in the liver and tail fin tissues of bullfrog tadpoles. E2 treatment did not elicit a response in these gene transcripts in either tissue. T3 treatment in the tail fin elicited an overall stronger response than T4, while T4 treatment in the liver recapitulated results consistent with non-genomic mechanisms of T4 signaling for *thrb* and *thibz* transcripts. Illumina HiSeq2500 was used to sequence RNA isolated from hormone-treated premetamorphic tadpole liver and tail fin tissues to assess differential transcriptomic responses and identify TH-responsive immune system-associated transcripts. The impact of TH-treatment on the general immune system in the liver and tail fin transcriptomes was also analyzed using RNA-seq data. We found that E2 modulates at least some shared TH pathways in the liver, but none in the tail fin and that the tail fin transcriptome is more affected by T3, while the liver transcriptome is more affected by T4. Additionally, evidence of immune system modulation by both THs was found in both the liver and tail fin transcriptomes.

Antimicrobial peptides (AMPs) are an important component of the amphibian immune response. Details regarding the regulation, synthesis, and expression of AMPs remain obscure, although evidence of TH-modulation of specific AMPs has been identified, as well as evidence of increased expression of AMPs throughout metamorphosis. Frog skin is a prolific source of AMPs that may prove useful in the quest for alternative antimicrobial agents in the face of antibiotic resistance. Identification of new AMPs is hindered by the practical limitations of classical protein-based discovery approaches. By using known AMP characteristics and common AMP properties, we developed a high throughput bioinformatics approach predicated on the use of *R. catesbeiana* genome resources. We mined these resources and identified novel and known AMPs that exhibited verified antimicrobial activity against various bacterial organisms.

This thesis sought to elucidate the differential and modulatory effects of both forms of TH on a transcriptomic level and in the context of immunity, and to examine the utility of the bullfrog transcriptome and genomics resources in identifying and characterizing novel bullfrog-derived AMPs and elucidating aspects of AMP expression.