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Gene Expression Analysis Of Upregulated Genes By 20-OH Ecdysone in *Brugia malayi*

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by

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Dedication

I dedicate this thesis to my family. I thank you for loving me unconditionally. You have taught me the meaning of determination and persistence. I would not have made it this far without your support.

Acknowledgments

I am very thankful for my academic advisor, and primary committee member, Dr. Thomas Unnasch for giving me the opportunity to conduct research in his laboratory as well as motivating and inspiring me as a student. I owe my sincerest gratitude to Dr. Amruta Mhashilkar for her dedicated mentorship and guidance throughout the course of my project.

Table of Contents

List of Tables

List of Figures

Abstract

Brugia malayi is a filarial nematode causing lymphatic filariasis in humans characterized by swelling of the lower extremities. The aim of this study was to conduct a real time PCR (qRT-PCR) to verify gene expression levels of *Brugia malayi* nematodes treated with 20 hydroxyecdysone. Transcriptome analysis was previously performed resulting in the identification of 44 genes that were upregulated by exposure to 20-hydroxyecdysone. Based on transcriptome results, known GO Terms and functions, four genes and one endogenous housekeeping gene were chosen for validation by RT-PCR. Induced samples showed a mean increase of microfilarie by 2.2 fold. Induced wells exhibited a 2.8 fold increase of pre- microfilarie production. On day two adult females treated with 20-HE displayed 3.8-fold increase of microfilaria production as compared to uninduced controls. Overall, all four genes showed upregulation with treatment of 20-hydroxyecdysone at levels that corresponded to the results obtained from the transcriptome analysis. Findings in this experiment expand on the understanding of the ecdysone response system in *Brugia malayi*,

which could serve as a potential drug target against filarial disease.

Chapter One: Introduction

Lymphatic filariasis, also known as elephantiasis is a neglected tropical disease caused by one of the three filarial nematodes, *Brugia malayi*, *Wuchereria bancrofti*, or *Brugia timori.* This particular disease is extremely important as a cause of morbidity due to the immobilizing and life altering side effects that occur later on in life as a consequence of the infection. Currently there are 120 million infected in tropical and subtropical parts of the world. An astonishing 1.4 billion are at risk in 73 different countries including South America, Africa, Asia, parts of the Carribean and the Pacific (CDC, 2013). The vast majority of infections are caused by *Wuchereria bancrofti*. *Brugia malayi* infects 13 million people specifically in South and Southeast Asia (WHO, 2014).

Filarial nematodes have a complex life cycle that begins with a necessary vector. For this particular nematode vectors are mosquito species from the genera Culex, Mansonia or Aedes. Infection occurs when a mosquito transmits infectious L3 larvae to a human host upon taking a blood meal. Full-grown males are thirteen to twenty three millimeters in length by seventy to

eighty micrometers in width while females measure forty three to fifty five millimeters in length by one hundred thirty to one hundred seventy micrometers in width. The adult worms then migrate to and lodge themselves in the lymphatic system where they compromise the functionality of the lymph system (Biology 2010).

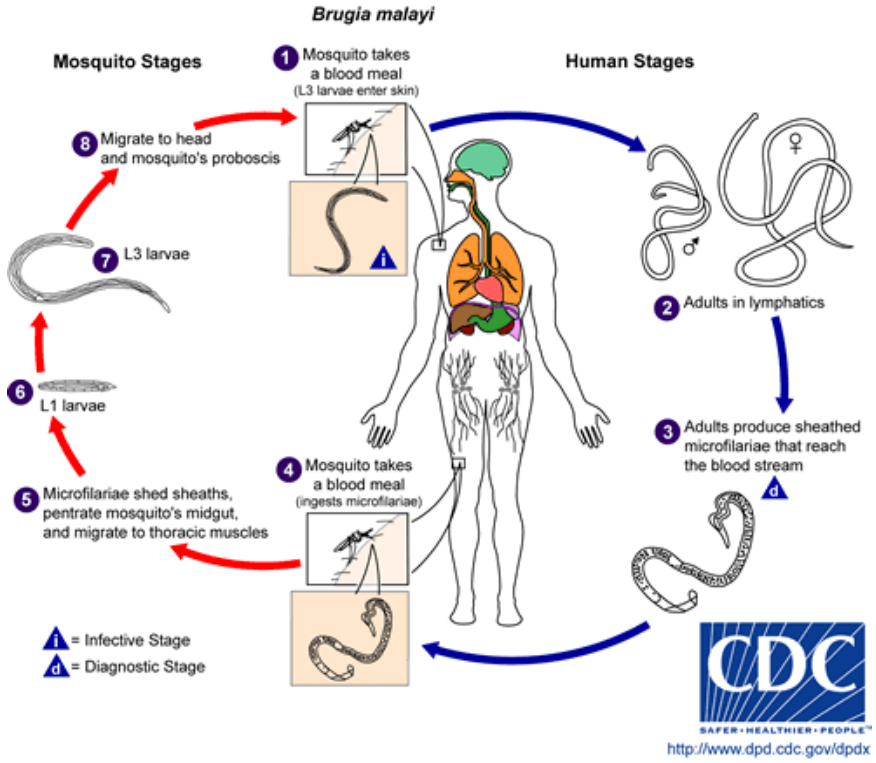

Figure 1.1. *Life cycle of Brugia malayi (Biology 2010).*

Adult female worms produce sheathed microfilariae, exhibiting nocturnal periodicity. Microfilariae enter the blood stream and ultimately into the peripheral blood. The cycle is perpetuated when another mosquito takes a blood meal from an infected human. The mosquito then ingests the microfilariae,

which undergo developmental molting from first stage larvae (L1) to third stage larvae (L3). The process of molting from L1 to infective L3 larvae takes 10-12 days. Stage three larvae migrate to the mosquito's proboscis where upon blood meal are able to be to be transmitted to humans. The infectious life cycle is thus perpetuated (World Health Organization, 2010).

The pathology of lymphatic filariasis is classified as asymptomatic, acute, or chronic. During asymptomatic infection patients may not show any clinical manifestations, but upon taking a blood test, analysis will be positive for thousands, even millions of microfilariae. The gold standard for testing for filarial disease is performing a blood smear and identifying microfilariae through microscopic evaluation. Asymptomatic infection, even though it may cause no outwardly clinical symptoms will still compromise the immune system as well as the kidneys. During acute infection an individual experiences bouts of fever that are correlated to the inflammation of skin, lymph nodes, as well as the lymphatics. (WHO, 2014.).

Infection with *Brugia* species causes increased episodes of fever and inflammation while infection with *W. bancrofti* are known for fewer episodes of acute symptoms (S. Babu). Chronic infection is marked as the most severe of all three pathological classifications as well as the most debilitating. Chronic symptoms develop years after initial infection and more commonly

in males than in females. Ten to fifty percent of males develop hydrocele. Both females and males suffer from general elephantiasis of limbs and lymphoedema. In females, symptoms include edema of the breast and genital regions (WHO, 2014).

Most of the time individuals are infected during childhood. This early infection allows for unseen harm to be done to the lymphatic system throughout the 6-8 years or more that the adult parasites are lodged in the lymph nodes. Manifestations of lymphatic filariasis include social stigma, enlarged body parts, weakened immune system, pain, and severe disability (WHO, 2014). Furthermore, disability adjusted life years (DALYs) have been calculated to 4.4 millions years in men and over 1.3 million years in women (WHO,2014). Due to this pathology, individuals are unable to work and thus have to bear the economic impact of healthcare burdens as well as lack of productivity (DNDi, 2014). These burdens along with the debilitating effects of the disease make the need for effective drug treatments of utmost public health importance.

In the year 2000 the World Health Organization established the Global Programme to Eliminate Lymphatic Filariasis (GPELF) with the goal to put an end to filarial disease as a public health concern by 2020. The World Health Organization program involved two key points. Point one involved mass drug administration to hinder transmission of filarial disease in

regions where infection was present. Point two was to mitigate the pain and suffering presently being caused by those infected by filarial disease through morbidity control (WHO, 2014).

Another means of eliminating filarial disease is to prevent transmission altogether. Mosquitoes infected with parasites known to cause filarial disease are more likely to take blood meals between the hours of dusk and dawn; therefore this is the time to be most aware and adhere to strict preventive measures if living in areas where filarial disease is present. During the hours of the night, it is recommended to sleep in a room that is air-conditioned or at least sleep under the protection of a mosquito net. Other preventative measures include; wearing long sleeve shirts and pants, as well as wearing insect repellent on exposed skin (CDC, 2013).

Mass drug administration is key for prevention of filarial disease and entails two single dose medications given annually to the vulnerable population in this specific order; 400 mg of albendazole in conjunction with 6 mg/kg of diethylcarbamazine or 150-200 mcg/kg of ivermectin. Although these drugs do not kill the adult parasite they have been proven effective at eliminating microfilariae from the blood stream and thus are able to prevent transmission from human to mosquito. This strategy has proven successful, more than 4.4 billion therapies have been dispatched to necessary regions and approximately 984

million humans have been treated. The latest data shows that since the beginning of the GPELF program, populations that were once at risk for lymphatic filariasis have decreased by 43%. The economic burden has also decreased; during the years 2000-2007 the program has benefited financially US$24 billion (WHO, 2014).

Although much progress has been made towards eliminating filarial disease, it still remains a neglected tropical disease with 120 million infected globally (WHO, 2014. Despite the general success of the MDA program, there are severe adverse side effects of the drugs available, which is one of the driving forces behind the need for novel drug therapies. In countries where *Loa loa* is endemic, treatment with ivermectin can cause serious adverse side effects such as neurological problems (Twum-Danso NAY, 2003). Diethylcarbamazine (DEC) should not be given to patients that are co infected with onchoceriasis as this drug is known to cause high fever and may lead to loss of vision. In individuals co-infected with *Loa loa*, adverse side effects include, encephalopathy, renal failure and possible death (CDC, 2014).

There are various factors that impact an individual's compliance to the MDA program such as cultural norms and illiteracy. Another factor affecting noncompliance is the lack of education of disease transmission and causality. Non-compliant individuals will most likely not have heard of the MDA

program or were not aware that drug administration would provide a cure for the disease (Talbot et al, 2008).

Furthermore, drugs currently available do not kill the adult worm, which is responsible for the severe pathology. Thus, microfilaria reappear after a period of time, resulting in the need of repeated treatments over a period of years. Evidence suggests that the most promising targets for drug design include the reproductive system as well as molting and cuticle development (Scott, A. L., & Ghedin, E. 2009). Due to the need for novel therapeutic drug therapies, growth of research and development of treatment and preventive measures is a priority.

In *Drosophila melanogaster*, 20-hydroxyecdysone is the main steroid hormone involved in regulation of cell proliferation, apoptosis, and differentiation during development. Developmental stages controlled by 20-hydroxyecdysone pulses include molting, metamorphosis and pupation (Riddiford, 1993). This particular steroid hormone classified as an ecdysteroid is produced and released by the prothoracic gland (Zitnan et al. 2007).

The ecdysone receptor heterodimerizes with Ultraspiracle (USP), its activity partner. 20-HE, its cognate ligand, activates transcription of the cascade of genes that control molting. USP displays similarities to the homologous mammalian retinoid receptor (RXR)(Koelle et al., 1991; Thomas et al., 1993; Yao et al., 1992; Yao et al., 1993). EcR and USP are both

ligand-dependent transcription factors that regulate the activity of specific target genes modulated by hormone expression (Mangelsdorf et al., 1995; Riddiford et al., 2000; Thummel, 1995).

In *Drosophila melanogaster*, the EcR/USP structure activates developmental hierarchies. Research has been completed regarding developmental hierarchies and the unceasing 20-OH pulses that activate metamorphosis. Of the two pulses that have been investigated, the first symbolizes that larval progression has come to an end and metamorphosis has commenced. The second pulse is necessary for development into the pupal stage, in the course of which adult maturation is complete (Richards, 1997; Russell and Ashburner, 1996; Thummel, 1996). Each pulse leads to the initiation of early genes, which are known to control more than a hundred late genes (Ashburner et al., 1974). The three early genes consist of the broad complex (Br-C), E-74, and E-75, which encode various formations of homologous transcription factors. BR-C specifically encodes multiple zinc finger proteins and exhibits a multigene locus (Burtis et al., 1990; DiBello et al.,1991; Segraves and Hogness, 1990).

Previous studies revealed a functional ecdysone receptor present in *Brugia malayi*. The investigation began with in-vivo metabolic labeling of adult female worms in the presence and absence of 20-hydroxyecdysone followed by analysis of proteins

from cultured parasites through gel electrophoresis, which revealed changes in the expression of various proteins and thus revealed presence of an ecdysone-responsive gene network (Canhui, Liu, Tracy Enright, George Tzertzinis, and Thomas R. Unnasch, 2012).

Caenorhabditis elegans, reveals no presence of EcR or USP receptor components (Slauder, 2001). NHR-25 has a role in larval metamorphosis (Lavorgna et al., 1993). NHR-25, NHR-23, and NHR-41 alter molting and morphogenesis in *C.elegans*. Nuclear hormone, NHR-67 has an unforeseen role in molting as well as morphogenesis. NHR-67 is homologous to *Drosophila melanogaster* TLL that in contrast plays no role in the molt cycle. Previous studies revealed that declines in levels of NHR-6 resulted in ovulation abnormalities (Gissendanner et al., 2004,Strecker et al., 1988).

Ecdysis is a characteristic specific to invertebrates and thus the ecdysone receptor has a high pharmacological significance. By gaining understanding of the function of the ecdysone signaling system in Brugia malayi, this can also help gain knowledge of the signaling system as a potential drug target.

Chapter Two: Materials and Methods

Brugia malayi culturing

All worm culture protocol was done under sterile conditions. The media recipe was composed of CF-RPMI which includes; RPMI-1640 (Gibco) with 25mM of Hepes, 10% FBS, 1.8g Glucose, 1g NaHCO3, 1% Pen-Strep (100x= 10,000 u/ml of penicillin and streptomycin, 1% Amphotericin B at 250ug/ml and 400ul of 50mg/ml Gentamycin. The pH was adjusted to 7.52. The media was filter sterilized and stored at 4°C (Lok JB, Unnasch TR, 2005).

Embryogram

The adult female worms were cultured invitro using CF-RPMI. 3ml media was added to each well of a sterile 6 well plate. 3-5 worms were transferred into each well. The maximum number of worms per well was 5. Time was then recorded making sure the process was completed within one hour of receiving the worms.

On day one of culturing *Brugia malayi* parasites, the experiment began at approximately the same time as on day zero. The wells were divided into induced and controls. The induced

wells received 20HE at 5uM final concentration. The controls received ethanol, vehicle for 20HE.

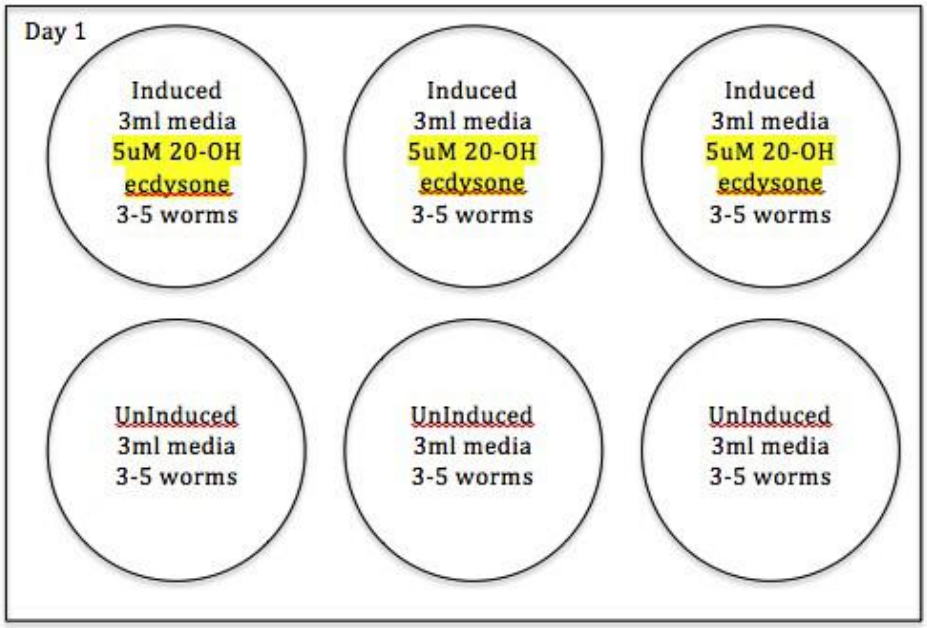

Figure 1.2: *Embryogram layout for experiment.*

Figure 1.2 depicts layout for embryogram experiments. Each well consists of 3-5 worms. The induced wells received 20-HE and the control received ethanol.

The parasites were observed for phenotypic changes by counting all microfilariae, eggs and pre-microfilariae. The counting method gives us an approximate estimate of the microfilariae in the media. Results were recorded.

Total RNA Isolation from Filarial Parasites Using the Trizol LS Reagents

After inducing the worms for 2 days, the worms were flash

frozen in liquid nitrogen. On the day of the extraction, the worms were thawed over ice and transferred to a 2ml round bottom eppendorf tube. Worms were extracted using 600 ul of Trizol LS reagent followed by 3 freeze/thaw cycles at 80 °C. A 5mm stainless steel bb was added to a 2ml round bottom tube and attached to the TissueLyse II at a frequency of 1/30 for 7 minutes. The tube was vortexed briefly. 200 ul of chloroform was added, followed by a brief vortex and 3 minutes of incubation on ice.

The entire sample was transferred to a pre spun phase lock gel tube and centrifuged at 4°C for 15 minutes at 11,900xg. RNA was precipitated using isopropanol. The precipitated RNA pellet was washed twice with 75% cold ethanol. The pellet was air dried and resuspended in 0.1 X TE buffer consisting of Tris and EDTA.

10X DNase buffer and DNase I was added to total RNA and mixed gently by inversion. The reaction was incubated for 30 minutes at 37 °C. DNase inactivation reagent was added to inactivate DNase I to prevent degradation of RNA and mixed by inversion. Sample was then spun at 10,000x g for 3 minutes to precipitate the inactivation reagent and Supernatant RNA was then transferred to a new tube. RNA was stored at -80°C.

The total RNA was incubated with 5M Ammonium acetate at -80°C for 30 minutes. After spinning, the pellet containing RNA was washed with 75% cold ethanol. The pellet was then air dried

and resuspended in 0.1 X TE buffer. RNA was stored at -80°C.

RNA Analysis

RNA was quantified using the Agilent 2100 Bioanalyzer. This system allows for sizing, quantitation and quality control of RNA through the use of microcapillary electrophoresis (Agilent Technologies, Palo Alto, CA). RNA purity was measured by Qubit 2.0 Fluorometer. This system allows for RNA quantification through the use of a fluorescent dye that only releases a signal when bound to the target (Life Technologies, Grand Island, NY).

Transcriptome:

In collaboration with Dr. Steven Williams at Smith College, a Miseq RNA library was constructed using NEBNext Ultra RNA Library Prep Kit for Ilumina (New England Bio Labs, Ipswich, MA). The constructed library was loaded into the 300-cycle Mi-seq cartridge. The cycles ran were single ended on a Mi-seq Sequencer (Illumina). The data generated was analyzed using Tophat and differentially expressed genes were identified.

Primer and Probe Design

Primers and probes were made for four target genes, chosen from the transcriptome analysis. Genes were chosen based on GO Terms (Gene Ontology) as shown on Table 1.2. One endogenous housekeeping gene, NADH ubiquinone oxidoreductase was used as an internal control. For each chosen gene, unspliced sequences were accessed through Wormbase Database using given GeneIDs. Real-time PCR primers and probes were created using Integrated DNA Technologies Software (Integrated DNA Technologies, Inc, Coralville, Iowa). Primer criteria included exon-exon spanning, GC content between 40-60%, amplicon length of less than 150 base pairs and probe length between 18-30 base pairs.

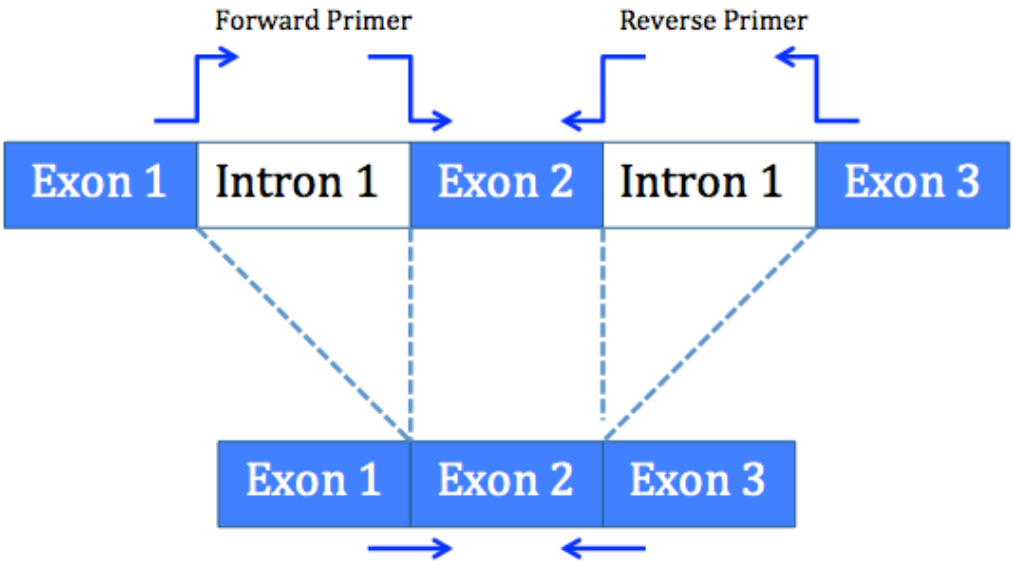

Figure 1.3: *Exon-Exon spanning allows for introns to be spliced out and inhibits genomic DNA from being amplified.*

Primers were made to span two exon-exon junctions. Upon amplification, introns will get spliced out, leaving only coding regions. Table 1.1 illustrates the primers and probes used for this experiment.

Table 1.1: *Primers and probes designed to conduct qRT-PCR.*

Gene ID	Primer/ Probe		Size In Bps
Bm58	Forward Reverse Probe	5'-GAGCAACAGGACTTGTTGGA-3' 5′-ACGACCACCTTCAGGTGA-3′ 5′(FAM)-TCACTTGTACATCGCTATTTAACTGGTGCT-(BHQ1)p3′	104
Bm 12555	Forward Reverse Probe	5′-CTTTCGTAGAACGGTTCGTG-3′ 5′-CGTTACGACCAGCTTTATCAA-3′ 5′(FAM)-ACGTGTGTCGTTATGAGCAGAAGTGT-(BHQ1)p3′	83
Bm 11454	Forward Reverse Probe	5′-GTGGAGGATACGGTTATGGTAAA-3′ 5′-CTGATAGCAATATCATACCCTCTATAT-3′ 5′ (FAM)-AAATTGCCGAGATGGGTGCCTACG-(BHQ1)p3′	103
Bm 2121	Forward Reverse Probe	5′-GCTTATAAAGCTGGATTTATTGGC-3′ 5′-TGTTTCTTCACCACATATATAGGC-3′ 5′ (HEX)-ACGTATTCGTTCATCGAGGTGCTGG-(BHQ1)p3′	105
Bm 7925	Forward Reverse Probe	5′-GAACCAGAAACGCTGAAAGTG-3′ 5′-CCGATATGATGGTCGTCGATT-3′ 5′ (FAM)-AATCAGTGACGATGAGGCACGA-(BHQ1)p3′	123

Real- Time PCR

Real time PCR (qRT-PCR) was performed to establish levels of gene expression in Brugia malayi parasites induced and uninduced with 20 hydroxyecdysone. Primers were confirmed using 2x Reaction Mix, One Step RT-PCR Kit (Qiagen, Valencia,CA). A gradient PCR was performed to determine optimum annealing

temperature. The cycling conditions consisted of 30 minutes at 50°C for reverse transcription, 5 minutes at 95°C for initial PCR activation. This was followed by denaturation for 20 seconds at 94°C, annealing for 30 seconds at 55°C and, extension for 2 minutes at 68°C. These three steps were repeated for 45 cycles followed by a final extension for 20 minutes at 72°C. The sample was held continuously at 4°C. Gel electrophoresis was performed to verify amplicon size.

Biorad Iscript One Step RT-PCR Kit for Probes was used to perform real time PCR experiment on induced and uninduced *Brugia malayi* samples. The cycling conditions consisted of 10 minutes at 50°C for reverse transcription reaction, 5 minutes at 95°C for polymerase activation and DNA denaturation. Additionally, samples were held for 15 seconds at 95°C denaturation followed by 30 seconds at 60°C, these two steps were repeated for 45 cycles. The samples were held continuously at 4°C. Real time PCR (qRT-PCR) reactions were run on Bio-Rad iCycler Thermal Cycler.

When performing relative quantification PCR efficiencies must be established for all genes. Efficiencies must be homologous and recommended to be at ninety percent or higher. Efficiencies are established by performing a 5-fold serial dilution with 8 samples followed by plotting the CT value as a function of log [5] concentration of template. Comparative CT analysis (ΔΔCt) for relative quantification was performed to

quantify gene expression levels of four genes and one reference gene. This particular technique normalized the target gene to the endogenous housekeeping gene. The 20-hydroxyecydsone induced samples were quantified relative to the uninduced target samples.

Chapter Three: Results

Based on previous research, it was found that a functional ecdysone-signaling pathway exists in *Brugia malayi*. The purpose of this experiment was to confirm the results of transcriptome analysis by executing real time PCR (qRT-PCR). Transcriptome analysis was performed to establish gene expression of four genes and one endogenous housekeeping gene treated with 20-hydroxyecdysone.

An embryogram was performed and distinct morphological stages were recorded. Phenotypic changes were observed when performing embryogram. All treated adult *Brugia malayi* parasites exhibited an increase in microfilariae, eggs, and pre-microfilariae count relative to untreated samples. This phenotypic change was consistent with all biological replicates.

Figure 1.4 shows data recorded from samples displaying phenotypic changes dependent upon exposure to 20-HE. Overall, induced samples demonstrated an increase in microfilariae production by 2.2 fold as compared to uninduced samples. Pre-microfilarie in induced wells had a fold increase of 2.8 as compared to uninduced controls. Production of eggs displayed a fold increase of 2.51 for induced samples with 20-HE. Adult females treated with 5uM of 20-HE showed a 3.49 fold increase of

microfilariae on day 2 and were thus chosen as the day for RNA extraction.

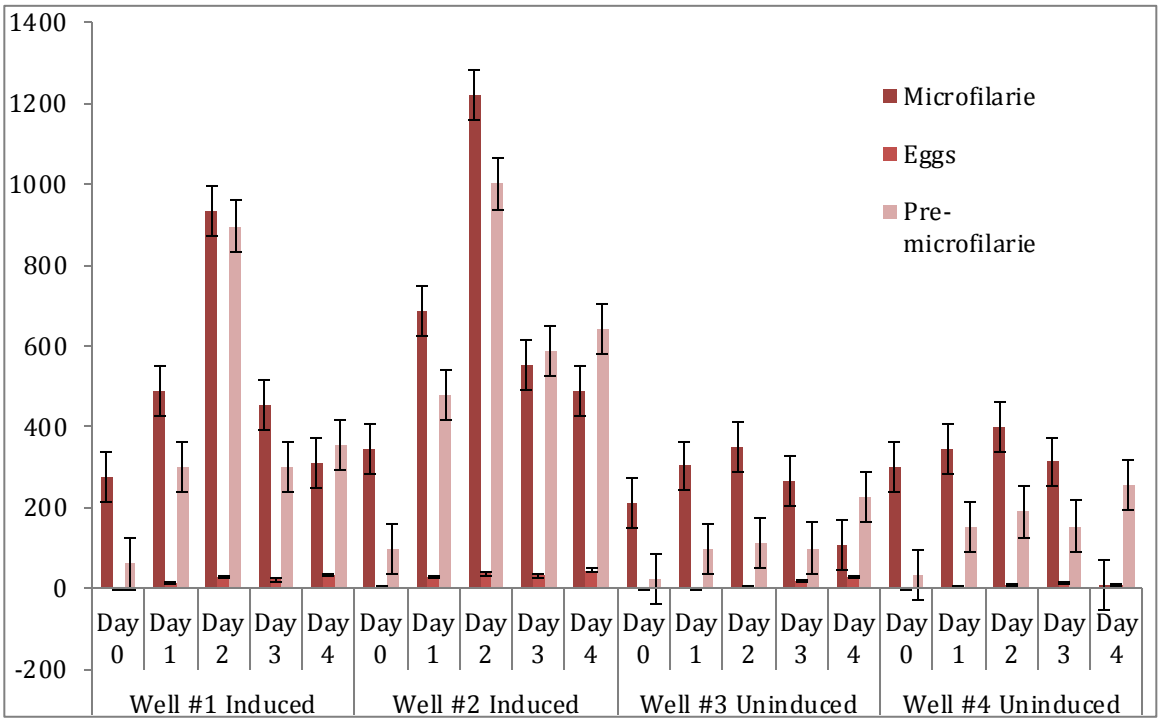

Figure 1.4 *Phenotypic changes of treated and untreated adult female parasites. This graph depicts the phenotypic changes of microfilariae, eggs and pre-microfilariae of treated and untreated samples with 20-OH.*

Previous research involving transcriptome analysis revealed differential expression of genes. Specific genes were further investigated on Wormbase database. Function was established based off GO Terms (Gene Ontology Term) located on the Wormbase database. The annotations file used to download the latest entry from wormbase was: Bioproject PRJNA10729, WS244.

Table 1.2 *Gene IDs for four genes and one endogenous housekeeping gene of treated and untreated Brugia malayi RNA samples.*

Gene ID	Go Terms
Bm58	Signal transduction
Bm12555	Nuclear Receptor
Bm11454	RNA binding
Bm7925	Regulation of transcription
Bm2121	Oxidation Reduction Process

Table 1.2 shows the functions for each gene chosen for this experiment. As an internal control, Bm2121 has an oxidative-reductive process while both Bm7925 and Bm12555 are regulators of transcription.

Table 1.3 *shows induced and uninduced FPKM averages and transcriptome (fold difference) levels from transcriptome analysis.*

Gene ID	FPKM Induced Avg	FPKM Uninduced Avg	I/UI
Bm 58	63.3	7.6	8.3
Bm 12555	103.8	20.0	5.2
Bm11454	392.018	1.0	392.0
Bm7925	159.9	1.0	159.9

The table above generated from transcriptome results depicts FPKM averages for treated and untreated samples. Bm 11454 showed the most significant FPKM level relative to uninduced of 392.018 followed by BM7925 with a FPKM level of

159.9. The results consistently demonstrated a positive trend of FPKM levels for treated samples with 20-OH in proportion to untreated adult *Brugia malayi* females.

Table 1.4 *Gene Expression Levels of Brugia malayi parasites*

Gene ID	**$2^{-\Delta\Delta CT}$**	**I/UI**
Bm58	7.82±0.43	8.3
Bm12555	5.16± 1.79	5.2
Bm11454	345.69± 41.6	392.0
Bm7925	161.27± 15.72	159.9

Gene expression levels of adult female *Brugia malayi* filarial nematodes treated with 20-OH relative to untreated controls for four genes. As well as transcriptome fold upregulation.

Real time quantitative PCR (qPCR) was performed to confirm the results of transcriptome analysis. $2^{-\Delta\Delta CT}$ and standard deviations were calculated through triplicate qPCR experiments. As depicted in table 1.4, Bm 11454 is shown to have the most notable increase in gene expression after treatment with 20-OH with a value of 345.69, followed by Bm 7925 with a value of 161.27. These values are correlated with transcriptome analysis

as depicted in figure 1.5. Below is a representation of fold induction for four individual genes and transcriptome analysis.

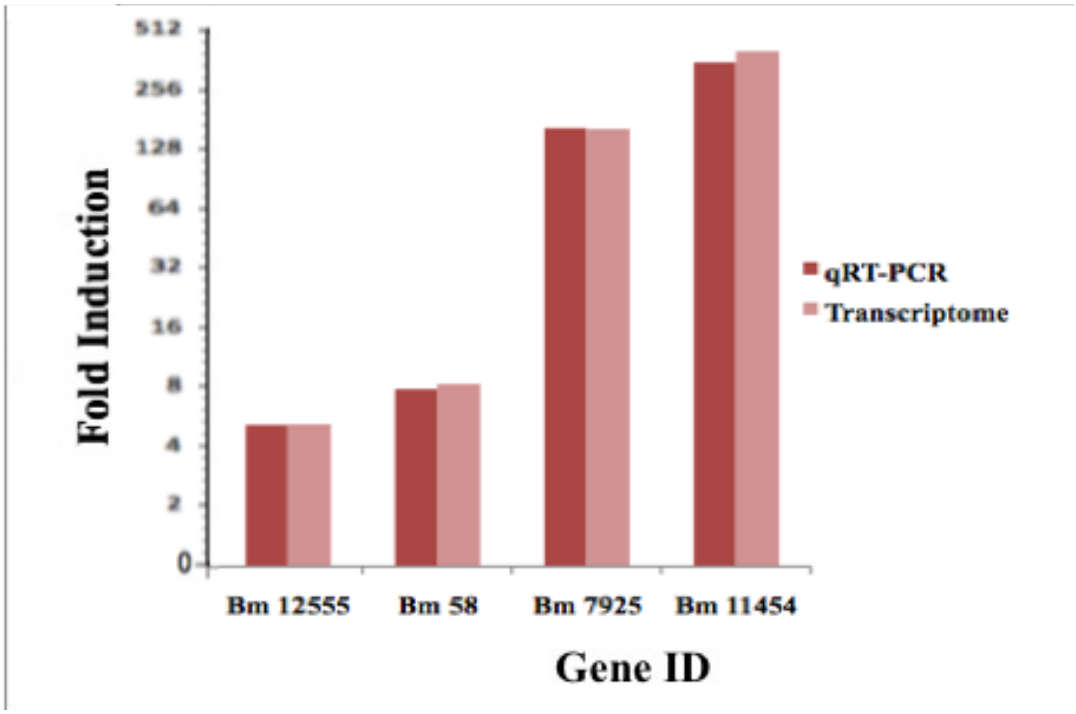

Figure 1.5: *Quantitative real time PCR (qRT-PCR) showing fold induction between induced and uninduced samples as well as transcriptome fold upregulation.*

Figure 1.5 represents a $2^{-\Delta\Delta CT}$ of gene expression levels of 20-hydroxyecdysone induced *Brugia malayi* adult females. Samples treated with 20-OH showed an increased fold induction relative to untreated adult *Brugia malayi* females. Results from qRT-PCR were consistent with transcriptome anaylsis.

Chapter Four: Discussion

A functional ecdysone receptor was previously found to exist in *Brugia malayi.* Edysone has been hypothesized to be a master regulator of downstream genes. Upon heterodimerization between ultrapsiracle and ecdysone receptor, 20-HE activates a cascade of genes that are hypothesized to play a key role in molting and thus potentially have a high pharmacological importance. Molting is a characteristic unique to invertebrates making the ecdysone response element a potential novel chemotherapeutic drug target (Canhui., et al 2012).

In this experiment a real-time PCR was employed to quantify gene expression levels of adult *Brugia malayi* parasites induced with 20-hydroxy ecdysone relative to uninduced controls. The results from this study support that there is a functional ecdysone inducible gene expression network. Upon executing a six-day embryogram, analysis of results showed that on day two there was a 3.49 fold increase of microfilariae count relative to uninduced controls. The fact that 20-hydroxyecdysone induces a phenotypic change is vital for this particular experiment. This further supports the hypothesis that there is a biological effect of 20-hydroxyecdysone on adult *Brugia malayi* parasites,

although the exact mechanism remains unknown.

As hypothesized, the results from the real-time PCR experiments demonstrated significant increase in gene expression levels of treated adult *Brugia malayi* worms relative to untreated controls. Bm7925 showed a fold induction of 161.27 relative to the uninduced control samples. This gene is a regulator of transcription. As a regulator of transcription these genes may have the ability to play a role in controlling the rate at which DNA is transcribed into mRNA. Bm 12555 displayed upregulation of gene expression by 5.16 fold compared to uninduced samples and is categorized as a nuclear receptor. The ecdysone receptor is a member of the nuclear receptor family and as aforementioned it is hypothesized to regulate development, thus this upregulation is significant.

The gene that showed the most significant increase was Bm 11454 with a fold induction of 345.69. This gene codes for RNA binding, which interacts selectively with portions of RNA. Research has been performed on RNA binding proteins of C. elegans and it was found that RNA binding proteins have many functions in development and post-transcriptional control of RNA metabolism (Lee MH, Schedl T, 2006). Bm58 displayed an increase of 7.82 fold relative to uninduced controls and codes for signal transduction. Signal transduction occurs when an extracellular cue activates a receptor thus creating a biochemical response.

In Brugia malayi the cue is 20-HE and the biochemical response is increase in gene expression of four genes and one endogenous housekeeping gene (Lodish H., et al 2000). The results of this experiment supported the findings from the transcriptome analysis. As Compared to transcriptome analysis results, real-time PCR was consistent in each of the four genes.

By gaining a better understanding of the ecdysone gene network this can also help gain knowledge of the signaling system as a pharmacological agent against lymphatic filariasis. As a potential drug target that is not toxic to humans, the ecdysone developmental pathway is indeed an important prospective therapeutic target against filarial disease and may one day replace the therapies currently being used today (Nakagawa Y. et al., 2005,Canhui L. et al., 2012).

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