ABSTRACT

USE OF MICROARRAY HYBRIDIZATION TO IDENTIFY
BRUGIA GENES INVOLVED IN MOSQUITO INFECTIVITY

By Kathryn Greer Griffiths

Filarial nematodes are a family of insect-borne parasitic worms that cause a number of diseases in man and animals. The most widespread filarial disease of humans is lymphatic filariasis, caused by worms in the genera *Wuchereria* and *Brugia*. Lymphatic filariasis is an economic and social burden in endemic countries and affects approximately 119 million people worldwide. *Brugia malayi and B. pahangi* microfilariae (mf) require a maturation period of at least five days in the mammalian host before they can infect mosquito vectors. This maturation process correlates with changes in the surface composition of mf that likely are associated with changes in gene expression. To test this hypothesis, we verified the differential infectivity of immature (<3 day) and mature (>30 day) *Brugia* mf for black-eyed Liverpool strain of *Aedes aegypti* (LVP), and then assessed transcriptome changes associated with microfilarial maturation by competitively hybridizing microfilarial cDNAs to the *B. malayi* oligonucleotide microarray. We identified transcripts that were more abundant in immature (94 in *B. pahangi* and 29 in *B. malayi)* and mature (64 in *B. pahangi* and 14 in *B. malayi*) mf. In each case, >40% of *Brugia* transcripts shared no similarity to known genes, or were similar to genes with unknown function; the remaining transcripts were categorized by putative function based on sequence similarity to known genes/proteins. Microfilarial maturation was not associated with demonstrable changes in the abundance of transmembrane or secreted proteins; however, differences in transcript abundance were observed for many that have predicted functions. For example, immature mf were enriched for transcripts putatively associated with immune modulation, neurotransmission, transcription and cellular cytoskeleton elements. In mature mf, there was an increase in transcripts potentially encoding hypodermal/muscle and surface molecules, i.e., cuticular collagens and sheath components. These finding lend support to the underlying hypothesis that changes in microfilarial gene expression drive surface modifications that influence the parasite to begin development in compatible vectors. *Brugia malayi* genes corresponding to differentially abundant transcripts were identified, and transcript abundance validated by quantitative polymerase chain reaction. These studies serve as a starting point towards gaining a better understanding of the parasite side of the intricate parasite/mosquito relationship.

USE OF MICROARRAY HYBRIDIZATION TO IDENTIFY *BRUGIA* GENES INVOLVED IN MOSQUITO INFECTIVITY

by

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A Thesis Submitted
in Partial Fulfillment of the Requirements
for the Degree of

Master of Science-Biology

Microbiology

at

University of Wisconsin Oshkosh
Oshkosh, Wisconsin 54901-8621

May 2009

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ACKNOWLEDGEMENTS

I greatly appreciate the guidance of my advisor, Dr. Michelle Michalski, through the duration of this thesis project. I would also like to thank my thesis committee members, Dr. Lisa Dorn and Dr. Toivo Kallas for their advice and time. We also thank Dr. Bruce Christensen and his lab members of UW-Madison, Sara Erickson, George Mayhew, and Jeremy Fuchs without whom this project would not have been possible. I also appreciate the opportunity to participate in and help instruct a filarial nematode workshop put on by the Filariasis Research Reagent Resource Center at University of Georgia and those that facilitated it: Mike Dzimianski, Prasit Supakorndej, Ray Kaplan, John McCall, and Andy Moorhead. Finally, I would like to thank my parents, Marty Greer and Dan Griffiths, for their continuing support of my career goals.

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INTRODUCTION

Filarial nematodes are arthropod-borne obligate parasites that cause a variety of economically important diseases such as onchocerciasis (river blindness), lymphatic filariasis, and heartworm disease. The most widespread filarial disease of humans is lymphatic filariasis, caused by worms in the genera *Wuchereria* and *Brugia*. Lymphatic filariasis is an economic and social burden in endemic countries and affects approximately 119 million people worldwide (Michael *et al.* 1997). In humans, the worms live in and block the lymph vessels, causing improper flow of lymph, and inflammation of the lymphatic system. Symptoms include: fever, swollen limbs and genitals, generalized malaise; and disease can progress to a debilitating condition known as elephantiasis (Figure 1). Lymphatic filariasis is a major cause of psychosocial and psychosexual problems worldwide, because chronic morbidity limits productivity, and physical deformity prevents marriage of affected people in cultures where familial prosperity depends on marriage.

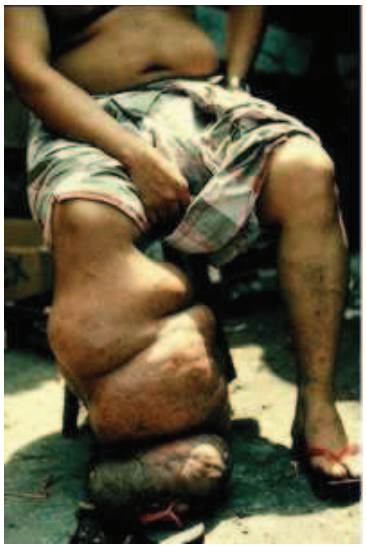

Figure 1. Elephantiasis of the leg caused by lymphatic filariasis. Source, University of California Davis.

The life cycle of the parasites that cause LF is complex, involving sequential development in invertebrate and mammalian hosts (Figure 2). Adult male and female worms live in the lymphatics of infected humans and copulate to produce larval offspring, or microfilariae (mf), that are sheathed with a vestige of the chitinous eggshell, and circulate in the bloodstream (Zaman 1987). Peripheral microfilariae ingested by blood-feeding mosquitoes are carried to the mosquito midgut (intestine), and quickly penetrate it to escape into the hemocoel (some with in as few as 10 minutes) (Figure 3). Worms enter the thoracic muscle cells of the mosquito and develop to the second larval stage in about eight days and to the infective third stage (L3) two to four days later. Upon the mosquito's next bloodmeal, third stage larvae migrate to the head of the mosquito, escape the proboscis and enter through the bite wound of the mammal.

Introduced L3smigrate to the lymphatics, undergo two molts, and sexually mature in approximately three months; thus completing the life cycle. Each larval transition throughout the life cycle is associated with molt of the external cuticle. Although *Wuchereria bancrofti* causes most cases of LF worldwide, it is strictly a human parasite and can not be raised in laboratory animals. Because of this restriction, *B. malayi* (a parasite of humans in Malaysia) and *B. pahangi* (a parasite of cats in Malaysia) are used for experimental maintenance of the life cycle, because they can be passaged using the Mongolian gerbil (*Meriones ungulates*) in place of the human host, and a genetically susceptible strain of laboratory mosquito (*Aedes aegypti* black-eyed Liverpool strain).

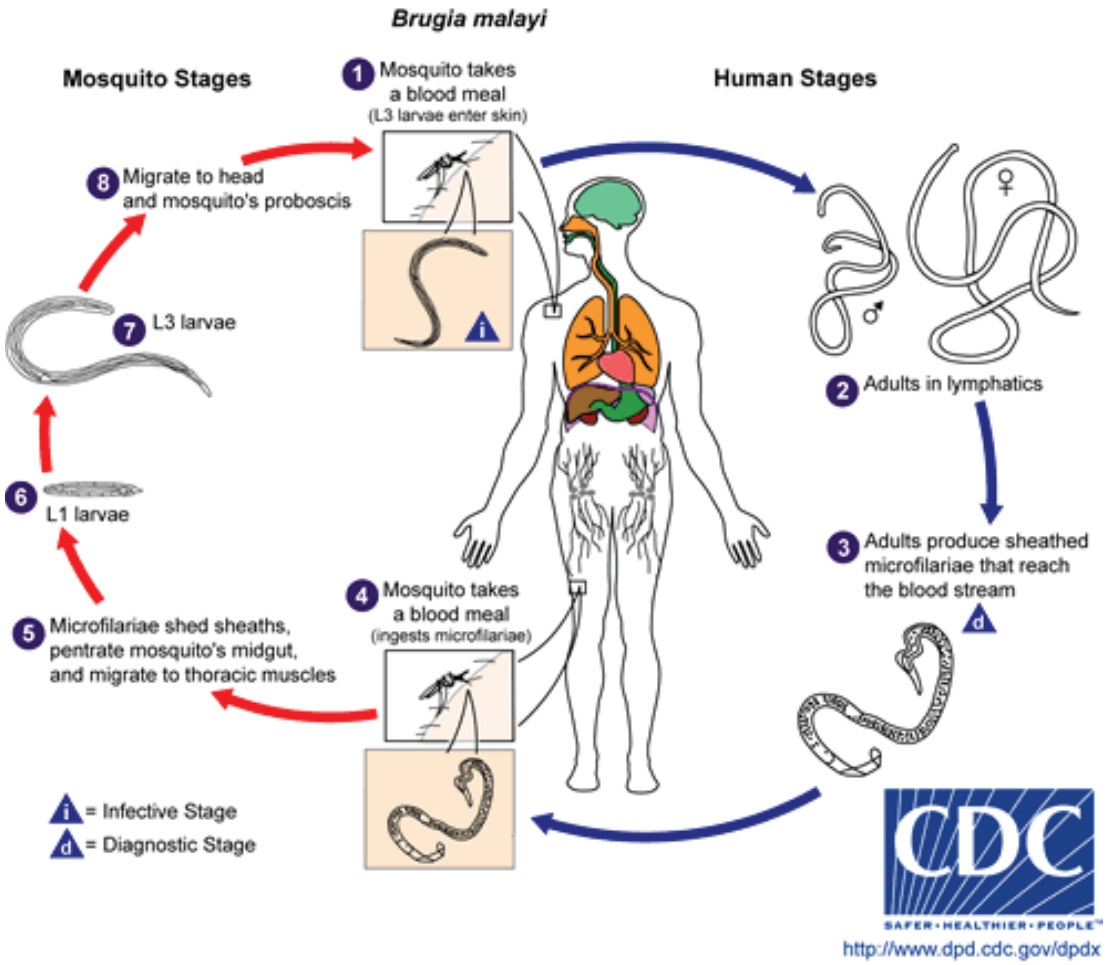

Figure 2. Life cycle of *Brugia* spp. Source, Centers for Disease Control.

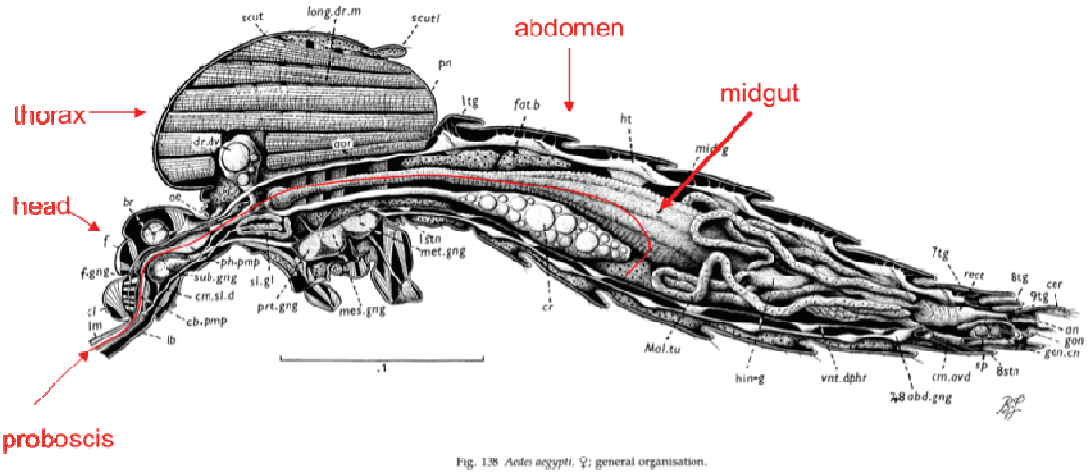

Figure 3. Mosquito midgut penetration by filarial worms. This is a longitudinal section of a mosquito. The red line indicates the passage of blood meal to the midgut, and midgut penetration and entry into the hemocoel by microfilariae. Further larval development occurs in muscle cells of the thorax.

Many lines of LF research are currently aimed at breaking disease transmission in endemic areas. Our research focuses on the transmission of microfilariae to the disseminating mosquito host, and is based on the interesting observation that mf must be at least 7 days old to successfully infect the mosquito (de Hollanda *et al.* 1982). Newborn mf that have not 'matured' cannot successfully penetrate the midgut of the mosquito, and subsequently cannot develop to the L3 stage; apparently requiring at least 5 days in the mammalian host to mature (Fuhrman *et al.* 1987). Glycosidic changes occur to the microfilarial surface with maturation, an observation of great interest because the sheath

and underlying cuticle are the worm surfaces that directly interact with the mosquito and mammalian hosts (Furman and Ash 1983 a and b).

This project takes advantage of recent advances in filarial nematode genomics to examine maturation–induced gene expression changes. The chromosomal genome of *B. malayi* is sequenced and though not yet mapped, has been karyotyped to show 4 autosomes and 1 sex determining pair (XY) (Sakaguchi *et al.* 1983), with a projected size of ~90 Mb with ~11,500 protein coding genes (Ghedin *et al*. 2007). *Wuchereria* and *Brugia* have an endosymbiotic relationship with *Wolbachia pipientis,* a rickettsia family bacterium that is present mainly in the reproductive tract of the worm (Kramer *et al.* 2003). This relationship is apparently required for worm survival and reproduction, because treatment of worms *in vivo* and *in vitro* with antibiotics that kill *Wolbachia* cause reduced worm fertility and induce worm death (Foster 2005 *et al*.; Pfarr and Hoerauf 2006). The filarial *Wolbachia* genome of *B. malayi* has also been sequenced (Foster 2005 *et al*.). The efforts of the World Health Organization-funded Filarial Genome Project (FGP) in generating filarial expressed sequence tags (ESTs) and genomic sequences led to the production of the *Brugia malayi* microarray (now in its second version), a spotted oligonucleotide array representing ~80% of *B. malayi* genes, as well as putative open reading frames and transcripts of the *Wolbachia* endosymbiont, and other medically relevant filarial nematodes including *W. bancrofti.*

Microarray technology, first employed in the 1980s, allows for the examination of gene expression levels of thousands of genes in one experiment (Sasik *et al.* 2004). Microarray analysis is widely used in scientific research and can be applied to a variety

of complex questions that require global relative transcript abundance data for paired biological samples. This thesis describes the microarray analysis we employed to characterize changes in gene expression that take place during the *Brugia* microfilarial maturation process in the mammalian host (Chapter I), and identification of corresponding *B. malayi* genes with validation of the microarray by quantitative polymerase chain reaction (Chapter II). It is our hope that describing gene expression changes that occur as mf mature will allow us to understand the nature of the physiological transition that allows mf to transition from the human to the mosquito host. With this information in hand, we hope to eventually identify parasite molecules that could be targeted to prevent successful infection of the mosquito by mf; thereby curtailing parasite transmission.

Chapter I

INTRODUCTION

This manuscript, submitted to Molecular and Biochemical Parasitology in 2009 April, describes the microarray analysis we conducted to compare gene expression profiles of immature and mature *Brugia* mf. The premise of microarray hybridization is that, in one experiment, the relative abundance of individual transcripts can be determined by hybridization of sample cDNAs to the microarray, followed by careful analysis of the very large resulting dataset (Causton *et al.* 2003). The raw data contains fluorescence intensity readings for each oligonucleotide spotted on the microarray platform (Figure 4), which are converted into ratios to compare the samples. Data analysis involves Lowess normalization and the comparison of results as ratios, combining replicate measurements, and performing t-tests to determine if hybridizing transcripts are statistically different between samples. These data are then used to compile a list of differentially abundant transcripts for each condition that serves as the starting point for understanding how the differences in transcript profiles might affect the biology of the organism of interest. Transcripts identified by the microarray are termed 'differentially abundant' and not 'differentially expressed' because microarray hybridization quantifies mRNA levels and does not measure transcriptional activity.

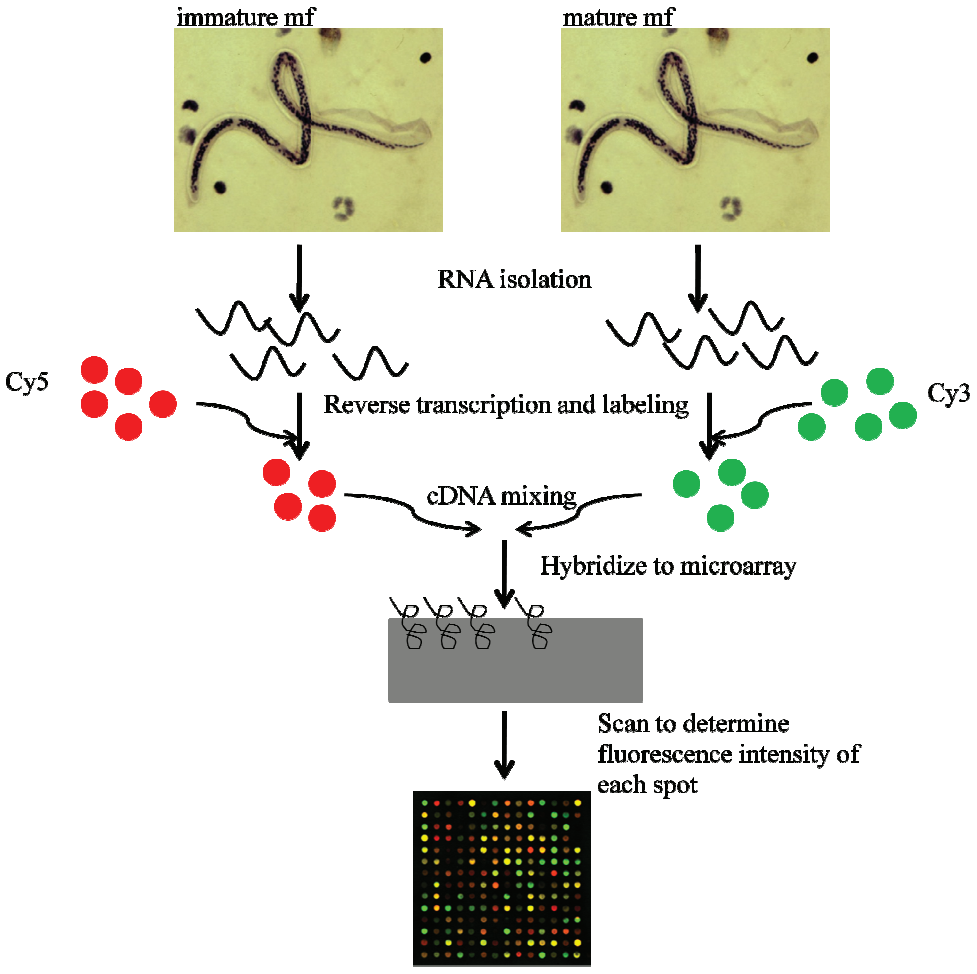

Figure 4. Microarray experiment design and execution. Briefly, RNA is isolated from two biologically different populations, cDNA is made by reverse transcription which is labeled with fluorescent probes. Labeled cDNAs from the two populations are mixed in equal amounts and applied to the microarray to allow them to hybridize to the DNA probes on the chip. The array is then scanned to determine intensity values for each sample at each spot. Sources, mf picture: http://www.stanford.edu, microarray image: http://www.imbb.forth.gr.

USE OF MICROARRAY HYBRIDIZATION TO IDENTIFY *BRUGIA* GENES INVOLVED IN MOSQUITO INFECTIVITY

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INTRODUCTION

Arthropod-borne filarial nematodes cause a variety of economically important tropical diseases such as onchocerciasis and lymphatic filariasis (LF).The most widespread filarial disease of humans is LF, that burdens approximately 119 million people in endemic areas (Michael *et al*. 1997) and is caused by worms in the genera *Wuchereria* and *Brugia*. In humans, the adult worms block lymphatic vessels, causing improper flow of lymph and inflammation of the lymphatic system, and leading to chronic morbidity and, in some cases lymphadema and hydrocole. *Brugia malayi*, a human pathogen, and the closely related cat parasite *Brugia pahangi*, are often used as laboratory models for LF because, unlike *W. bancrofti*, they can be maintained in laboratory animals. Both *B. malayi* and *B. pahangi* can be maintained in the dark-clawed Mongolian jird (*Meriones unguiculatus*). The mosquito most often used for laboratory maintenance of these parasites is the Liverpool strain of *Aedes aegypti* that was genetically selected for susceptibility to filarial worms in the classic studies of Macdonald (Macdonald 1962; Macdonald 1965).

To date, most investigations of vector competence and mosquito immunity against filarial worms in this system have focused primarily on the mosquito responses to filarial infection, with the goal of identifying vector determinants that control infection outcome and the parasite molecules targeted by the mosquito immune system (Christensen *et al*. 2005; Beerntsen *et al*. 2000; Townson and Chaithong 1991). Little attention has been paid to the parasite's role in this complex interaction even though it is equally important in determining infection outcome. This was elegantly demonstrated by the repeated passage of *Brugia patei* in a partially permissive vector, *Aedes togoi*, that resulted in increased infectivity over generations (Laurence and Pester 1967). These findings provide clear evidence that the complex interaction of *Brugia* with its vector undoubtedly is mediated genetically by both the vector and the parasite, and provide a model for successful adaptation of filarial worms to new vector species. Our understanding of the worm contribution to the parasite-vector interaction is quite limited, and leaves many questions unanswered regarding the physiological transition that mf undergo as they leave the mammalian host environment and enter that of the mosquito. This report represents our initial attempt to characterize the first step of this process, namely to identify gene expression patterns that correlate with the ability of *Brugia* microfilariae to penetrate the mosquito midgut.

Our approach is based on the intriguing observation that *Brugia* mf must be at least 5-7 days old to successfully infect *Ae. aegypti* (de Hollanda *et al*. 1982; Fuhrman *et al*. 1987). Newborn mf that have not 'matured' do not successfully penetrate the mosquito midgut, and subsequently cannot enter the thoracic musculature where larval

development occurs. Biochemical and immunological experiments have shown that microfilarial maturation is accompanied by changes in the composition of the microfilarial surface (Furman and Ash 1983 a and b), and we hypothesize that surface changes are driven by changes in gene expression. Our hypothesis is based on the concept that gene expression changes can explain differential infectivity, and is supported by evidence for transcriptional changes accompanying developmental transitions and growth (Brehm *et al.* 2003; Buckingham *et al.* 2003; Paba *et al.* 2004; Storey 2003; Yamazaki and Saito 2002). In the present study, we used microarray analysis to examine gene expression changes that occur with maturation of mf in the mammalian host.

MATERIALS AND METHODS

Generation and collection of Brugia *microfilariae*

To collect mature mf, male *M. unguiculatus*, with patent intraperitoneal infections of *B. malayi* or *B. pahangi* (for a minimum of 30 days) were sacrificed and adult worms and mf were removed by peritoneal flush with sterile, prewarmed RPMI-1640 medium (Lonza, Walkersville, MD USA). Flushes containing adults and mature mf were maintained in petri dishes in RPMI at 37°C with 100 µg/mL penicillin and 100 µg/mL streptomycin (P/S) (Cambrex, Rockland, ME USA). Adult worms were removed from the dishes and randomly assigned for surgical transplantation into the peritoneal cavities of uninfected jirds to generate immature mf. Mature mf (>30 days) remaining in the peritoneal flushes were isolated by centrifugation through a density gradient (Histopaque 1083, Sigma Chemical Co., St. Louis, MO USA) (Chandrashekar *et al.* 1984). Immature mf (<3 days) were collected by peritoneal flushing from jirds containing transplanted

adults at three days post transplantation. Microfilariae pellets of both immature and mature worms were resuspended in 250 ul of sterile phosphate buffered saline, flash frozen, and stored at -80° C. *Brugia pahangi* transfers and harvests were performed in triplicate to generate three biological replicates for microarray hybridization, and *B. malayi* transfers and harvests were performed in duplicate. Microfilariae collected for mosquito infectivity assays were pelleted directly from peritoneal flushes and washed twice with P/S free RPMI.

Mosquito infectivity assays

Aedes aegypti (LVP) was reared as previously described (Christensen and Sutherland 1984), and 3- to 4-day-old females were sucrose starved overnight prior to blood feeding. Microfilariae were reconstituted in uninfected gerbil blood at a concentration range of 114–377mf/20µl blood and fed to mosquitoes using a water-jacketed glass membrane feeder (Rutledge *et al.* 1964) fitted with a paraffin membrane (Parafilm M, ThermoFisher Scientific Wilmington, DE USA). Fully engorged mosquitoes were separated from non-bloodfed mosquitoes in each mf treatment group, and maintained as described (Christensen and Sutherland 1984). To record parasite development, cold anesthetized mosquitoes were dissected in *Aedes* saline (Hayes 1953), and larvae observed using phase-contrast optics (Olympus, BH-2, Center Valley, PA USA). At 5 hours post exposure (PE), the mosquito midgut was removed and dissected separately in order to assess the ability of mf to penetrate the midgut, and representative mosquito groups were dissected at 6 or 7 days and 12 days PE to count surviving mf, L2 and L3 stages and determine prevalence and mean intensities.

RNA isolation and microarray hybridization

Total mf RNA was isolated by organic extraction with Trizol LS (Invitrogen, Carlsbad, CA USA), followed by column purification using Ambion RNAqueous-Micro® Kit (Applied Biosystems, Foster City, CA USA). RNA integrity was confirmed visually by agarose gel electrophoresis (data not shown), and purity and concentration determined spectrophotometrically (NanoDrop ND-1000, ThermoFisher Scientific Wilmington, DE USA); samples were stored at -80° C. Total RNA was lyophilized under vacuum for transport to the Washington University Genome Sequencing Center, where RNA quality was verified using an Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). First strand cDNA was generated by oligo-dT primed reverse transcription (Superscript II; Invitrogen, Carlsbad, CA USA) using the 3DNA Array 900 kit (Genisphere, Hatfield, PA USA). Hybridization of mf cDNA to the *B. malayi* Version 2 (V.2) array was performed as previously described by Li *et al.* (2005). For each species, competitive hybridization between mature and immature mf cDNA were completed so that each biological replicate was represented by two technical replicates by dye swap method. The array is composed of 18,153 oligonucleotides: 878 based on *Wuchereria bancrofti* expressed sequence tags (ESTs); 1,016 based on O*nchocerca volvulus* gene indices; 804 from the *Wolbachia* complete genome; and the remaining 15,455 based on *B. malayi* ESTs, The Institute for Genomic Research (TIGR) gene indices, TIGR gene models, and L3 ESTs. The *B. malayi* microarray platform has been extensively validated by quantitative reverse transcriptase polymerase chain

reaction (Li *et al.* 2005) and tissue localization by in situ hybridization of a panel of gender-associated transcripts (Jiang *et al*. 2008).

Data collection and analysis

Slides were scanned on a Perkin Elmer ScanArray Express HT scanner to detect Cy3 and Cy5 fluorescence as described by Li *et al.* (2005). Data were analyzed with GeneSpring software (Agilent Technologies, Santa Clara, CA, USA). Volcano plots (Appendix A) were used to identify differentially abundant transcripts using a 95% confidence interval over twofold values (Bartholomay *et al.* 2007). To reduce false positives from only two biological replicates in the *B. malayi* study, results were filtered using Benjamini and Hochberg false discovery rate (Benjamini and Yekutieli 2001).

Differentially expressed microfilarial transcripts were classified by Gene Ontology (http://www.geneontology.org) (Ashburner *et al.* 2000) based on sequence similarity. Basic local alignment search tool (BLAST) similarity analysis searches were performed using the National Center for Biotechnology Information (NCBI) BLASTN against the nucleotide collection database (http://blast.ncbi.nlm.nih.gov/Blast.cgi), WU-BLASTX against the Wormpep (*C. elegans*) database (http://www.sanger.ac.uk/cgi-bin/blast/submitblast/c_elegans), and NemaBLASTN (http://www.nematode.net/BLAST/) against all available nematode libraries excluding *B. malayi*. A p-value or E-value of (<0.10) was used as the cutoff for BLAST analysis. Putative secreted or transmembrane proteins were identified by sequence analysis using Signal P v3.0 (Bendtsen *et al.* 2004), Phobius (Käll *et al.* 2004), and TopPred-2 (Claros and von Heijne 1994).

RESULTS

Mosquito infectivity assays

At days 1, 7 and 12-post exposure (PE), mature *B. pahangi* mf infected >90% of bloodfed LVP, and immature *B. pahangi* mf infected ≤10% of bloodfed mosquitoes at the same time points (Table 1-1). Similar results were noted for *B. malayi* (Table 1-1), confirming earlier observations (de Hollanda *et al*. 1982; Fuhrman *et al*. 1987) and demonstrating that McCall *Brugia* strains have maintained their infectivity characteristics despite repeated passage through laboratory mosquitoes and mammals.

Microarray

The *B. malayi* microarray is constructed with oligonucleotides representing genes/transcripts from several different filarial species, and the *Brugia* prokaryotic *Wolbachia* endosymbiont. Heterologous hybridization with *B. pahangi* cDNA identified 94 transcripts that were more abundant in immature mf (83 of these were designed from *B. malayi* sequences, 4 *Wuchereria bancrofti* and 7 *Wolbachia)*, and 64 in mature mf (59 *B. malayi* sequences, 2 *W. bancrofti* and 3 *Wolbachia).* The parallel experiment with *B. malayi* revealed 29 differentially abundant transcripts in immature mf (28 represented *B. malayi* and 1 *W. bancrofti)* and 14 in mature mf (14 *B. malayi* sequences). Microarray data (Appendix B) were posted at the NCBI GEO database (Edgar *et al.* 2002) accession number GSE15017 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE15017). The consensus sequences to which the array oligonucleotides were designed were analyzed for functional clues by sequence similarity, and approximately half were classified as unknown or conserved unknown because they had no similarity to known

genes (unknown), or had sequence similarity to genes of unknown function (conserved unknown) (Figure 1-1). Transcripts identified as uniquely abundant in immature mf were in the functional categories: heat shock protein, immune modulation, membrane transport, protease inhibitor, and protein sorting while those unique to mature mf were: hypodermal/muscle, RNA binding, and retrotransposon (Figure 1-1). Transcripts assigned to the categories of collagen/sheath/surface and metabolic were more abundant in mature mf while those of cytoskeleton and neurotransmission were more abundant in immature mf (Figure 1-1). Remaining transcripts identified as more abundant in *B. malayi* mature mf showed sequence similarity to *C. elegans* genes/proteins (10), *W. bancrofti* (3), *O. volvulus* (3) and to other nematodes (4); and transcripts from the immature mf were similar to *C. elegans* (10), *W. bancrofti* (16) and other nematodes (3). From the *B. pahangi* experiment, sequence similarity of the mature transcripts was to *C. elegans* (37), *W. bancrofti* (3), *O. volvulus* (10) and other nematodes (16); immature transcripts had similarity to *C. elegans* (48), *W. bancrofti* (9), *O. volvulus* (12), and other nematodes (31).

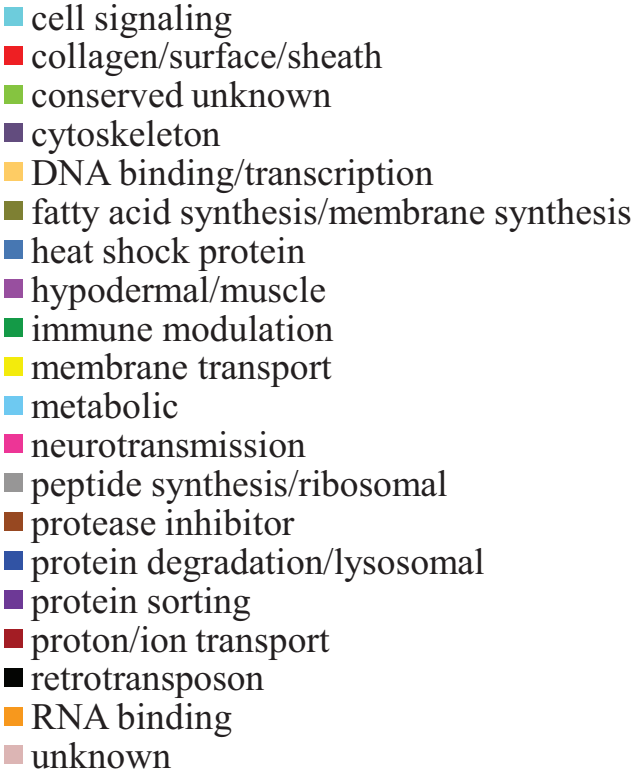
Eukaryotic signal sequences were present in 21 and 34% of immature transcripts *B. pahangi* and *B. malayi*, respectively and 26 and 29% of mature transcripts *B. pahangi* and *B. malayi*, respectively. Putative transmembrane regions were detected in 67% of *B. pahangi* and 62% of *B. malayi* immature mf transcripts, and 67% of *B. pahangi* and 57% of *B. malayi* mature mf transcripts. Six transcripts were identified as differentially abundant by both microarray experiments, namely a putative cytoskeleton-associated protein with a CAP-Gly domain (consensus sequence BMC11473), large ribosomal

subunit (BMC04649) and an unknown (BMC00072) in immature mf; and a putative sodium: hydrogen antiporter (14965.m00428), sorbitol dehydrogenase (WB-contig_242), and an unknown transcript (14961.m05058) in mature mf. Of these six transcripts, all but BMC00072 showed sequence similarity to *C. elegans* genes and had RNAi determined phenotypes, but none of the RNAi data provided definitive functional clues beyond previously determined putative function (Tables B-5–8).

Table 1-1. Mature *Brugia* microfilariae develop to infective stage parasites but immature microfilariae are not infective to mosquitoes.

Species	mf age	DPE[a]	mfa[b]	Number of mosquitoes dissected	Prevalence[c]	mfa	Number of mosquitoes dissected	Prevalence
Brugia								
pahangi	immature	1	143	10	10% (1)	203	n.d.[d]	n.a.[e]
	immature	7		n.d.	n.a.		5	0%
	immature	12		135	0%		80	0%
	mature	1	114	10	90% (3.6 ± 2.4)	377	n.d.	n.a.
	mature	7		n.d.	n.a.		3	100% (15.0 ± 7.2)
	mature	12		25	96% (10.3 ± 4.5)		9	100% (7.4 ± 3.2)
Brugia								
malayi	immature	1	143	10	10% (1)		n.d.	n.a.
	immature	6		10	0%		n.d.	n.a.
	immature	12		20	0%		n.d.	n.a.
	mature	1	342	10	100% (6.3 ± 4.2)		n.d.	n.a.
	mature	6		10	100% (4.8 ± 2.9)		n.d.	n.a.
	mature	12		20	100% (8.6 ± 3.4)		n.d.	n.a.

[a]Days post exposure (DPE)
[b]mfa= microfilaremia of blood meal (mf/20 μL)
[c]Prevalence (mean intensity ± standard deviation)
[d]not done (n.d.)
[e]not applicable (n.a.)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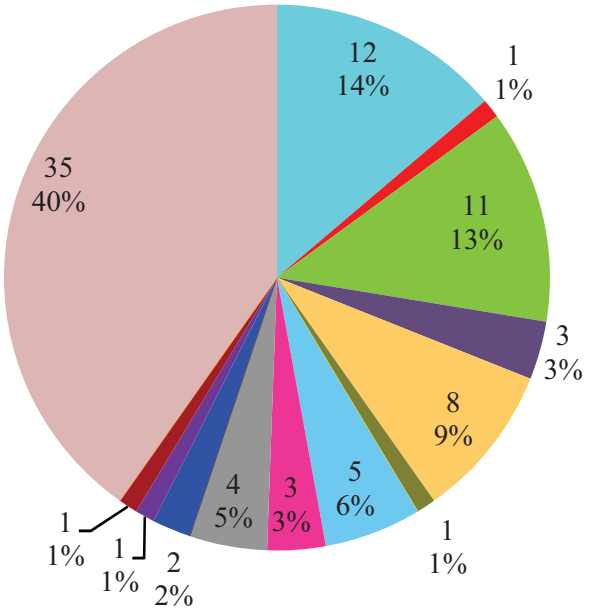
collagen/surface/sheath
conserved unknown
cytoskeleton
DNA binding/transcription
fatty acid synthesis/membrane synthesis
heat shock protein
hypodermal/muscle
immune modulation
membrane transport
metabolic
neurotransmission
peptide synthesis/ribosomal
protease inhibitor
protein degradation/lysosomal
protein sorting
proton/ion transport
retrotransposon
RNA binding
unknown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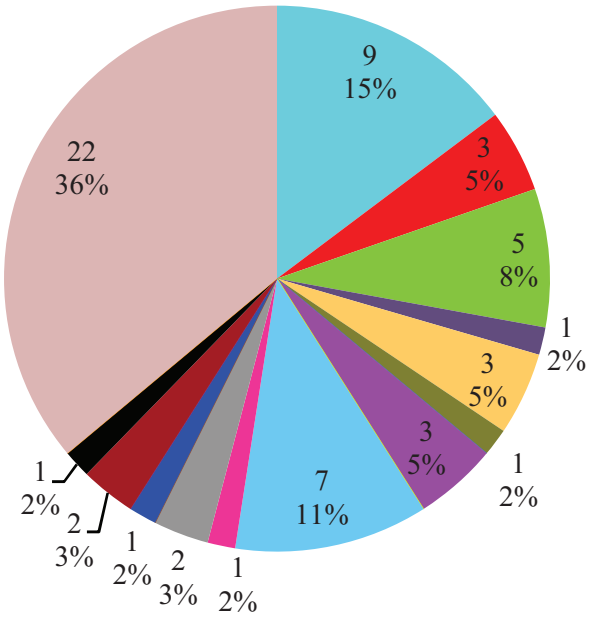
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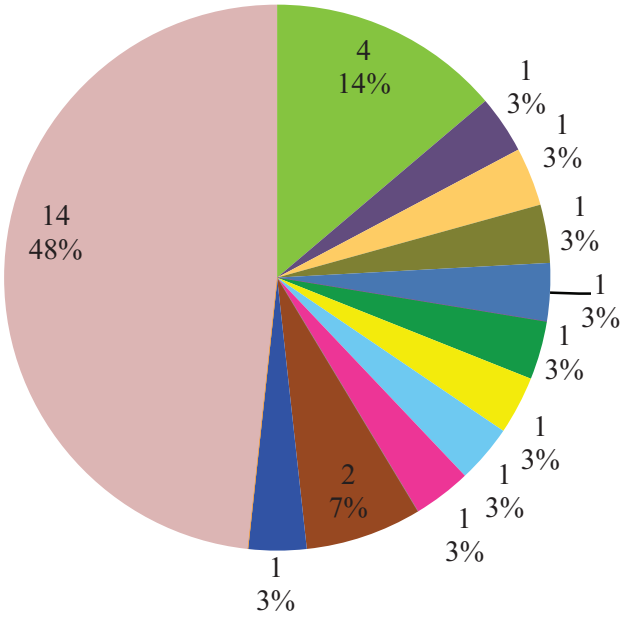
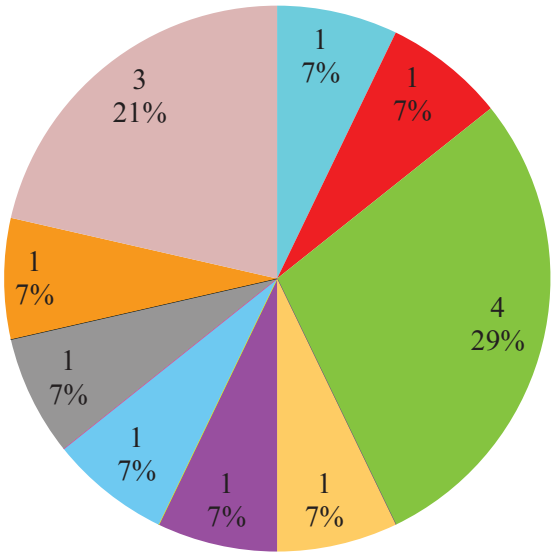


2A



2B

Figure 1-1. Functional categorization of abundant transcripts in *Brugia mf* based on sequence similarity to known genes/proteins. Panel 1A. *B. pahangi* immature (< 3 day old) mf (n=87). Panel 1B. *B. pahangi* mature (>30 day old) mf (n=61). Transcripts in the following functional classes increased in abundance with mf maturation: collagen/surface/sheath, and metabolic, while transcripts in the functional class of DNA binding/transcription account for a higher percentage of transcripts in immature mf. Protein sorting transcripts were identified as differentially abundant only in immature mf. Transcripts in the hypodermal/muscle category were only identified as differentially abundant in mature mf. Panel 2A. *B. malayi* immature (< 3 day old) mf (n=29). Panel 2B. *B. malayi* mature (>30 day old) mf (n=14). Transcripts in the functional classes DNA binding/transcription and metabolic account for a higher percentage of the differentially abundant transcripts in mature mf than in immature mf. Transcripts present only in mature mf fall into the following functional categories: RNA binding, peptide synthesis/ribosomal, cell signaling, collagen/surface/sheath, and hypodermal/muscle. Transcripts present only in immature mf are in the functional categories: immune modulation, cytoskeleton, membrane transport, membrane synthesis, heat shock protein, neurotransmission, protein degradation and protease inhibitor.

DISCUSSION

Our findings demonstrate that there are detectable transcriptomic differences in mature and immature mf. We have generated gene expression profiles for uninfective and infective *Brugia* mf, and examined them for evidence of a dominant physiologic process driving maturation. Despite the high proportion of seemingly filarial worm-restricted or uninformative genes that lack functional sequence-based clues, we observed several differences in abundance for transcripts identified with certain functional gene categories. Most notably, the transcriptome of mature mf is enriched for transcripts identified with the microfilarial cuticle and sheath, providing support for the hypothesis that surface changes during maturation of mf might determine their infectivity for the mosquito vector (Fuhrman *et al.* 1987).

The sheath and underlying cuticle that make up the microfilarial surface are critically important structures because they mediate the interaction of the worm with both hosts. Inside the mammalian host, developmentally-arrested *Brugia* mf circulate in the peripheral blood until they are taken up by a mosquito in a bloodmeal. Within the mosquito, infective mf quickly penetrate the midgut and resume development in the hemocoel. The sheath, a remnant of the eggshell membrane, is cast from the worm during or after midgut penetration in a successful infection (Fuhrman *et al.* 1987; Sutherland et al. 1984; Yamamoto et al. 1983). The sheath is acellular and insoluble, and is composed of chitin (Laurence and Simpson 1974; Simpson and Laurence 1974; Paulson *et al.* 1988; Fuhrman and Piessense 1985), matrix proteins (elastin, fibronectin, collagen type IV, laminin), and shp2, an insoluble and cross-linking protein (Hirzmann *et al.* 2002); as well

as sulfur-containing proteins, proteoglycans, phospholipids and acidic mucopolysaccharides (Laurence and Simpson 1974; Simpson and Laurence 1974; Araujo *et al.* 1993; Araujo *et al.* 1994). Enzymatic activities associated with the sheath include acid phosphatase and 5' nucleotidase (Sayers *et al.* 1984). There is evidence that the microfilarial sheath is modified during maturation; for example, immature mf differ from mature mf in that they cannot be chemically exsheathed with calcium, the sheaths of immature and mature mf differ in their ability to bind various lectins (Furman and Ash 1983 a and b; Paulson *et al.* 1988; Fuhrman and Piessens 1989) and a sheath-directed monoclonal antibody (Fuhrman *et al.* 1987; Canlas *et al.* 1984), and unlike mature mf, immature mf do not bind complement factors and promote granulocyte adherence *in vitro* (Johnson *et al.* 1981). Chitinase activity in the sheath increases during maturation and coincides with mf infectivity for mosquitoes (Fuhrman *et al.* 1987; Fuhrman *et al.* 1992). We have identified transcripts encoding two known filarial sheath proteins, SXP-1 (GenBank accession no. **XM_001900001**) (BMC06144) and major microfilarial sheath protein SHP-1 (GenBank accession no. **XM_001895241**) (BMC11831), that were more abundant in mature mf. The antigen designated SXP-1 is diagnostic for human infection for several filarial species, and is localized to the hypodermis, where it is possibly secreted to the sheath (Dissanayake *et al.* 1992; Sasisekhar *et al*. 2005). Major microfilarial sheath protein SHP-1 was previously identified as one of six major polypeptide constituents of the sheath (Hirzmann *et al.* 2002), and RNAi phenotypes for SHP-1 in *B. malayi* consist of reduced mf release by adult females, and shortened sheaths

of released mf (Aboobaker and Blaxter 2003). The infectivity phenotype of this mutant is unfortunately unknown.

The cuticle underlying the sheath is absorptive and composed mainly of disulfide-linked collagens and lipids (i.e. α-tocopherol); it also is associated with alkaline phosphatase and calcium-activated ATPase activities (Araujo *et al.* 1993; Sayers *et al.* 1984; Smith *et al*. 1998). Filarial developmental transitions induce changes in the protein, lipid and carbohydrate composition of the cuticle (Hirzmann 2002; Apfel *et al.* 1992; Ham *et al*. 1988; Lewis *et al.* 1999), and our transcriptome data indicate that mature mf differentially produce transcripts involved in cuticular structure, including two encoding cuticular collagens, one (TC2854) that is highly similar to *C. elegans* col-34, and the other an uncharacterized *B. malayi* gene (GenBank accession no. **XM_001894466.1**) (14154.m00011) with a nematode cuticle collagen N-terminal domain. An ShK domain-containing transcript (AA635198) that is highly similar to the *C. elegans* predicted secreted surface ZK673.1 gene was identified in immature mf and may likely be associated with the cuticle.

Furthermore, we found evidence for transcriptomic changes of genes potentially involved in other relevant biological processes. Immature mf displayed more transcripts potentially related to the cellular cytoskeleton, transcription, immune modulation and neurotransmission; while mature mf were enriched for transcripts related to hypodermis/muscle structure. The abundance of transcription factors in immature and mature mf suggests that gene expression actively occurs despite developmental arrest. Potential immune modulation factor transcripts identified in immature mf include that for

Bm-SPN-2 (GenBank accession no. **AA991131**), a serpin precursor with disputed inhibitory activity for human neutrophils (Zang *et al.* 1999; Stanley and Stein 2003), and another (GenBank accession no. **XM_001895491**) (BMC00466) that potentially encodes a complement control protein with characteristic short complement-like repeats. It is interesting that immature mf displayed several transcripts from genes potentially involved in neurotransmission, namely several voltage-and ligand-gated ion channels (13156.m00099, 14972.m07453, 14974.m00802, 14977.m04875) and a netrin receptor shown to be involved in axon migration in *C. elegans* development (14974.m00802); the only potential neurotransmission gene identified in mature mf was a neuronal intracellular sodium proton exchanger (14965.m00428). These data are provocative in that they raise the possibility that the microfilarial maturation process may in part involve a neurological transition that ultimately allows mf to respond to environmental cues in the mosquito midgut. This transition may also involve structural changes that allow a physical response, supported by the observation that mature mf express several transcripts with predicted functions in hypodermis and muscle; such as a putative orthologue of *C. briggsaemup*-4 that mediates junctional attachments of hypodermal epithelia with underlying muscle and overlying cuticular matrix (13207.m00046); *C. elegans lev*-11 that encodes tropomyosin (12695.m00041), an actin-binding contractile structural protein of body wall muscle; and *C. elegans let*-805 that encodes myotactin (14429.m00009), a novel single-transmembrane protein of the hypodermis that is essential for proper association between hypodermal fibrous organelles and the contractile apparatus of muscle cells.

In conclusion, these experiments have successfully identified several genes that are potentially critical for successful infection of vectors by *Brugia* mf and proved a starting point towards gaining a better understanding of the parasite side of the intricate parasite/mosquito relationship. Most interesting for our purposes are the transcripts putatively associated with surface modification, hypodermal/muscular structure, and neurotransmission. With further study, the products of these genes and their interactions could be determined to reveal the genetic basis of filarial worm compatibility with their vectors.

ACKNOWLEDGEMENTS

We are grateful for the assistance with worm transplants provided by Marty Greer and Jessica Nerenhausen. We also thank Juliet Fuhrman, Steven Williams, and Lori Saunders for helpful discussion. This project was funded by National Institutes of Health Grants 1 R15 AI067295-01A1, and AI 19769. Microfilaraemic blood and infected jirds were supplied by the Filariasis Research Reagent Repository Center at the University of Georgia. K. Griffiths is a recipient of a University of Wisconsin-Oshkosh 2008 Graduate Student Collaborative Research Grant. All animal protocols were reviewed and approved by UWO and UWM Institutional Animal Care and Use Committees.

Chapter II

BRUGAI MALAYI GENE CORRELATIONS AND VALIDATION OF MICROARRAY

INTRODUCTION

Microarray analysis is a widely used and powerful tool for globally characterizing gene expression, and has been used to address a wide variety of complex biological questions in many different systems including *Homo sapiens* (Bechelli *et al.* 2009), *Drosophila melanogaster* (Palanker *et al.* 2009), and *Caenorhabditis elegans* (Jeong *et al.* 2009). Microarray results have historically been verified by other techniques such as Northern blots, quantitative reverse transcriptase polymerase chain reaction (qPCR), or ribonuclease protection assay because , they are theoretically prone to Type I statistical errors due to large datasets. These types of errors result from rejection of a true null hypothesis, and can mistakenly lead to the conclusion that a transcript is differentially abundant between two conditions; largely because the sheer size of the datasets increases randomness and incorrectly strengthen correlations (Causton *et al*. 2003). Recent thought is that validation of proven microarray platforms is unnecessary because the strict statistical analysis of the primary microarray data is validation in and of itself (Yang and Speed 2002; Morey *et al.* 2006). In our case, the *B. malayi* microarray platform has been extensively validated through a combination of microarray, quantitative reverse transcriptase PCR (qPCR), and tissue localization by *in situ* hybridization studies (Li *et*

al. 2005; Li *et al.*2004; Jiang *et al.* 2008), therefore we did not report further validation of the results in the submitted manuscript (Chapter I). We did, as a matter of course, verify the differential expression of two candidates from the *B. malayi* dataset by qPCR, and provide these data as well as correlations of candidate transcripts to *B. malayi* genes' these data are the subject of Chapter II.

Quantitative RT-PCR is a quantitative method for examining relative transcript abundance between two or more biologically different groups of organisms. The reaction is conducted in the presence of a fluorescent dye so that the abundance of the amplicons of interest can be quantitatively determined after each PCR cycle (Figure 2-1). The fluorescence measurements are used to construct an amplification plot (Figure 2-4), and these data are analyzed by drawing a threshold line through the exponential phase of amplification. The PCR cycle number at which the plot crosses the threshold is used to determine the starting abundance of each transcript, relative to an endogenous control. We employed this technique to verify differential abundance of a limited number of transcripts identified by our *Brugia malayi* microarray experiment. Specifically, we identified in our dataset (a) a transcript correlating to ribosomal subunit S40 that apparently does not change in abundance between immature and mature mf, for use as an expression control; (b) a putative voltage-gated calcium channel transcript identified as differentially abundant in immature mf; and (c) a putative ground-like domain containing protein identified as differentially abundant in mature mf.

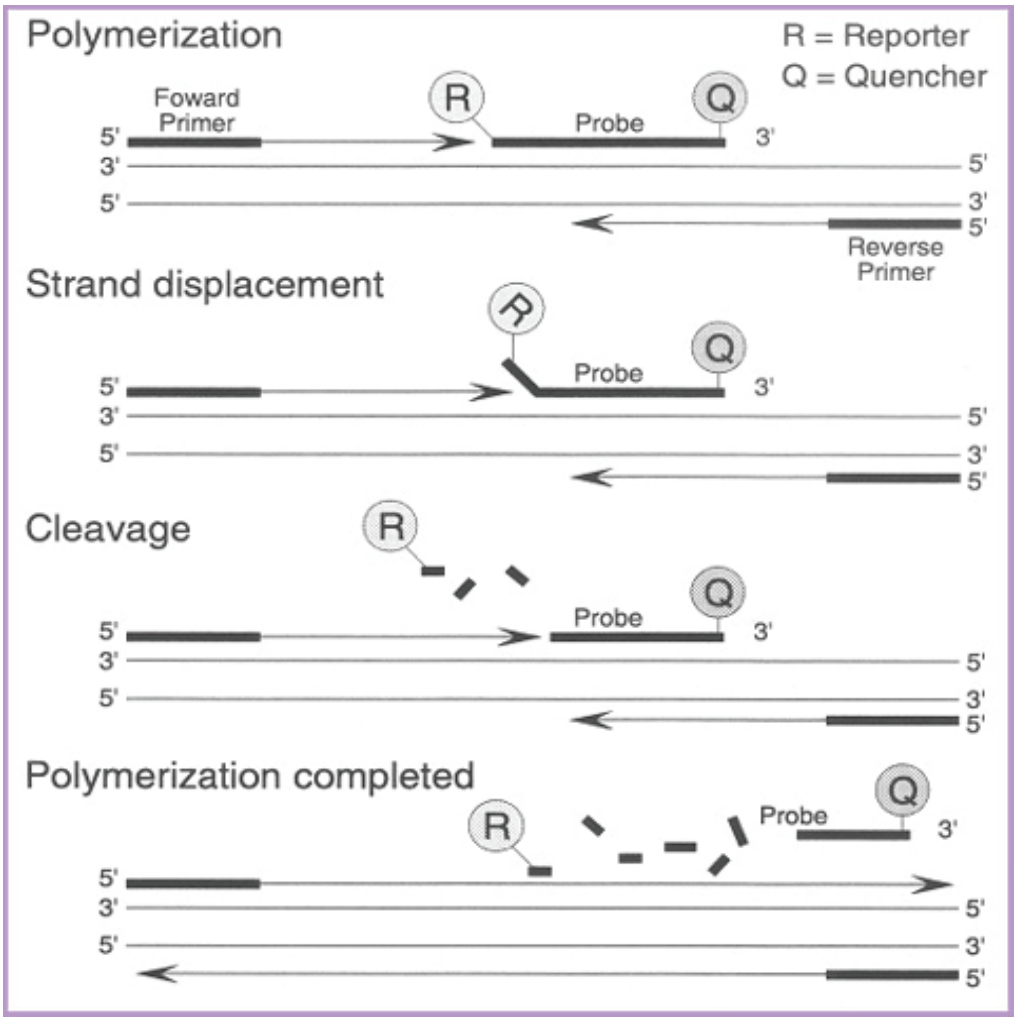


Figure 2-1. Mechanism of qPCR. Amplicons are generated in the same way as in general PCR using a forward and reverse primer with the addition of a fluorescently labeled probe. As copies of the amplicon are produced the quencher is cleaved and its physical separation from the reporter allows the reporter dye to fluoresce. Fluorescence level of the reaction is measured after each cycle which here is designed as 'polymerization completed'. Source, www.hgbiochip.com/eservices-3.html

After establishing that our microarray results were valid, we identified transcripts belonging to three putative functional categories that we consider biologically interesting in the context of infectivity: proteins putatively expressed in hypodermal/muscle and on the microfilarial surface and those potentially involved in neurotransmission. These transcripts interest us for several reasons. Those associated with the surface, sheath and cuticle are important because these surfaces come into direct contact with both the mosquito and vertebrate host and could be essential for mediating host/parasite interactions (Figure 2-2). Potential hypodermal proteins are also of interest because of the role of the hypodermis in secretion of cuticular components, and its junctional attachments to underlying longitudinal muscle that enable locomotion (and possibly midgut penetration). We also hypothesize that changes in neurotransmission-associated transcripts could reflect a neurological maturation event in circulating mf that allows them to receive stimuli from the mosquito midgut and initiate a behavioral response that promotes midgut penetration (see Figure 2-3).

Before we can initiate further study of these proteins, however, we must identify the genes that gave rise to each of these transcripts. This is not necessarily straightforward, because the oligonucleotide probes representing filarial genes on the *B. malayi* microarray were designed to conserved regions of predicted genes or expressed sequence tags, therefore, it is possible that a 'differentially abundant' transcript arose from a single, as of yet unidentified, gene from within a gene family. To accurately identify the protein that could arise from such a transcript, it is necessary to correlate the transcript to a particular gene. Eukaryotic genes are composed of coding regions, or

exons, separated by non-coding introns that are spliced out prior to translation. Our final goal for this chapter was to correlate each transcript of interest to open reading frames from the sequenced *B. malayi* genome, determine if they arose from single genes or from gene families, and to map the location of conserved and unique regions in exons of gene family members to facilitate future studies of gene expression.

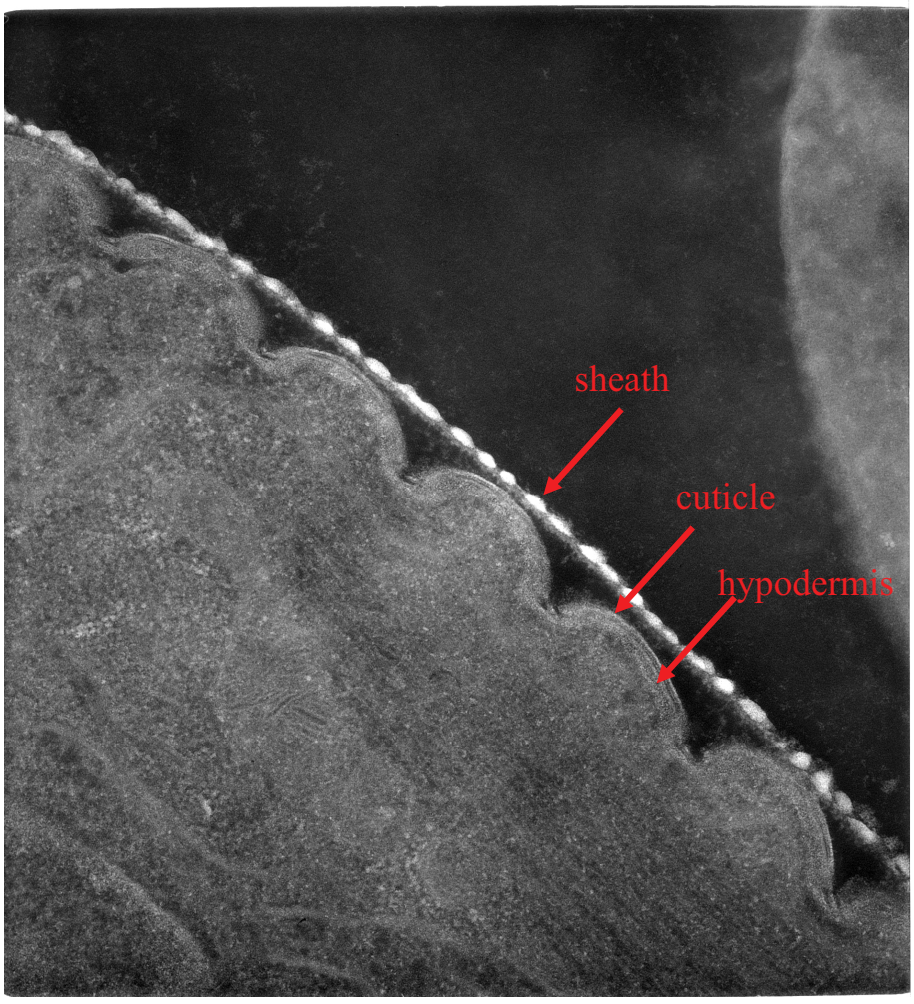


Figure 2-2. Transmission electron micrograph (TEM) of the *B. malayi* mf surface. The sheath, cuticle and hypodermis are shown. (TEM image by Dr. Todd Kostman)

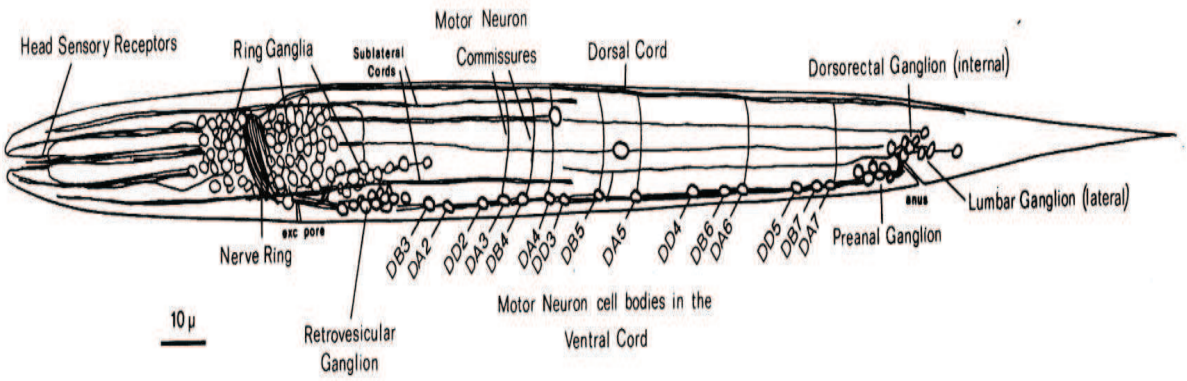


Figure 2-3. Depiction of the nematode nervous system. Major components of the nervous system are the nerve ring, a cluster of axons which surrounds the pharynx, and the dorsal and ventral nerve cords with associated peripheral nerves. The dorsal and ventral nerve cords are the main longitudinal ganglia that underlie the epidermis. Source, http://www.wormatlas.org

MATERIALS AND METHODS

Complementary DNA synthesis

A portion of each RNA preparation (2µg each) used for microarray hybridization was retained for qPCR analysis. Samples were treated with DNase I (Ambion, Austin, TX USA) according to the manufacturer's instructions. Complementary DNA synthesis was carried out with 200 µM oligo (dT) primer and incubating at 85 °C for 3 minutes in the presence of 20U RNase inhibitor (RNasin, Ambion, Austin, TX USA), followed by incubation at 42 °C for 60 minutes then 92 °C for 10 minutes with 80 mM dNTPs (New

England Biolabs, Ipswich, MA USA), reverse transcriptase buffer (1X final concentration), 20U RNasin (Ambion, Austin, TX USA), and 200U M-MLV reverse transcriptase (Ambion, Austin, TX USA) then stored at -20 °C.

Validation of differential transcript abundance

Unlabeled-oligonucleotide primers and minor groove binding probes containing FAM reporter dye were designed from sequences obtained from GenBank using Primer Express software under default parameters (Version 3.0, Applied Biosystems, Foster City, CA). Primer/probe sequences are posted on the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO) database with the microarray data (Edgar *et al.* 2002) (http://www.ncbi.nlm.nih.gov/geo/) (accession number GSE15017). Polymerase chain reactions were carried out in 48 well microtiter plates in 20 µl reactions with a final concentration of 1X TaqMan Master Mix, 250nM probe and 900nM of both forward and reverse primers (Applied Biosystems, Foster City, CA). Cycling conditions were: 50°C for 2 min, 95°C for 10 min and then 95°C for 15 sec and 60°C for 1 min for 40 cycles- each followed by capture of the fluorescence. Primer/probe sets were validated so that efficiencies of target and reference were approximately equal (93.6%–101.7%). Efficiency of amplification was determined by producing a standard curve using a 5 point 1:5 dilution series of mature *B. malayi* mf cDNA. The qPCR products were also run on a 1% agarose gel and visualized to verify amplification of only one product for each primer/probe set. Additionally, the amplicons were cloned into a TOPO® vector according to the manufactures instructions (Invitrogen, Carlsbad, CA USA), plasmids were purified with the QIAprep Miniprep kit according to the manufacturers instructions

(Qiagen Valencia, CA USA) and the products were sequenced and analyzed with the ClustalW2 tool (http://www.ebi.ac.uk/Tools/clustalw2/index.html) to verify that the transcript of interest was amplified. Relative transcript abundance differences were calculated by comparative C_t method (Heid *et al*. 1996 and Li *et al*. 2004), which compares experimental samples to an endogenous control. The control or calibrator is used to account for differences in amount of cDNA added to each sample to be compared by pipetteing error and a ΔC_t value (cycle at which the amplification curve crosses the set threshold) is generated for each sample. To calculate fold change the formula used was $2^{-\Delta\Delta Ct}$, the $\Delta\Delta C_t$ value is the difference in ΔC_t obtained for test and calibrator samples. We used *B. malayi* ribosomal subunit S40 as the endogenous control (BMC00146, GenBank:**XM001902078**); a transcript found to be equally abundant in mature and immature mf in both the *B. malayi* and *B. pahangi* microarray experiments. The endogenous control was identified as equally abundant in mature and immature mf of both *B. malayi* and *B. pahangi* because the mature: immature fluorescence ratio for both was ~1, indicating that the transcript was equally abundant in both age groups of mf.

Gene correlation analysis

Fourteen *B. malayi* transcripts belonging to the surface, neurotransmission, and hypodermis/muscle functional groups were compared using the BLAST algorithm and default parameters to open reading frames in the *B. malayi* genome database at The Institute for Genomic Research (TIGR) (http://www.tigr.org/db.shtml). Transcripts subjected to BLAST analysis were the consensus sequences generated by the Filarial Genome Project and derived by cDNA library construction for all life cycle stages and to

which the array oligonucleotides were designed. Putative genes identified as significantly similar to each transcript (p-value <0.01) were compared to the transcript and corresponding microarray probe sequences by generating multiple sequence alignments using the ClustalW2 tool (http://www.ebi.ac.uk/Tools/clustalw2/index.html). These alignments were visually inspected to identify regions of similarity, and were used to determine which sequenced *B. malayi* open reading frames correspond to each partial transcript sequence.

RESULTS

Quantitative RT-PCR

A putative voltage-gated calcium channel gene, 13156.m00099, that was identified as differentially abundant in immature *B. malayi* mf by microarray analysis was shown to be 2.1 fold more abundant in immature mf than mature by qPCR; similarly, 13895.m00081, a putative ground-like domain containing protein, in mature *B. malayi* mf by was verified by qPCR to be 1.1 fold more abundant in mature mf than immature (Table 2-1 and Figure 2-4). Average C_t values for each target and both of the samples were determined by qPCR. The C_t values for the validation of the calcium ion channel gene are as follows: immature mf 33.23±0.31 and mature mf 34.28±0.14; these data were normalized to the values for the endogenous control (immature mf 24.59±0.14, mature mf 24.65±0.06). The ground-like domain containing protein gene C_t values were: 33.17±0.22 for immature mf normalized to the control 21.18±0.15 and 32.37±0.12 for mature mf normalized to the

control 21.25±0.05. Validation data are posted on the NCBI GEO database (Edgar *et al.* 2002) (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE15017).

Table 2-1. Summary of qPCR correlation to microarray for candidate *B. malayi* transcripts. While qPCR validated the microarray results qualitatively, there was quantitative disagreement.

Microarray systematic name	**GenBank**	**PCR efficiency**	**Microarray result**	**qPCR result**
BMC00146	XM_001902078.1 (40S ribosomal protein)	99.90%	equally abundant in both ages	equally abundant in both ages
13895.m00081	XM_001893955 (Ground-like domain containing protein)	93.59%	4.3 fold more abundant in mature mf	1.1 fold more abundant in mature mf
13156.m00099	XM_001892541. (Voltage-gated calcium channel, T-type, alpha subunit)	101.72%	13.0 fold more abundant in immature mf	2.1 fold more abundant in immature mf

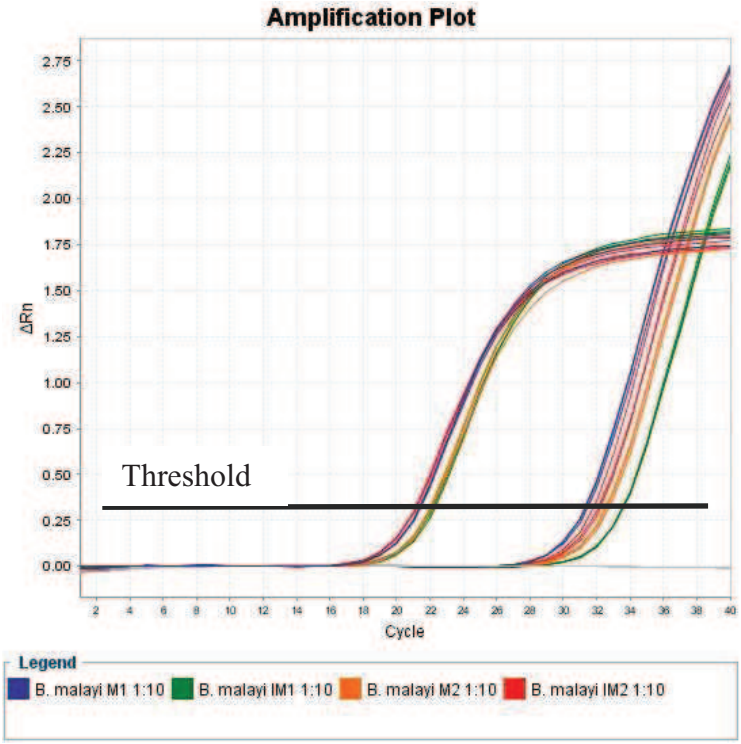


Figure 2-4. Representative amplification plot from a qPCR experiment. Regions from two transcripts are amplified: a control transcript (Ribosomal-set of curves that cross threshold at ~ cycle 21) that does not change in abundance, and a test transcript (Ground-set of curves that cross threshold at ~cycle 30) that is more abundant in mature than immature mf. The reaction was run in duplicate, M1 and M2 designate mature mf cDNA samples; IM1 and IM2 designate immature mf samples. The threshold is set in the exponential phase of amplification to provide the most accurate estimation of amplicon abundance, namely a window where amplified product is detectable but the reaction is not yet limited by decreasing amounts of primer or probe. The threshold is used to determine the starting amount of cDNA present. For example: we conclude that ribosomal cDNA was initially more abundant than ground because it reaches the threshold at cycle 21 while ground reaches it at about cycle 33. The comparative C_t method is then used to statistically calculate the relative abundance of the transcripts of interest. The amplification curves do not plateau at the same point as reagents become limited earlier in amplification for the ribosomal transcript as the template was more abundant to begin with and this is not a point of concern because the amplification efficiency of the test transcript was within ±10% of the control (Table 2-1) (Gaj *et al.* 2008).

Gene correlations

Of the fourteen candidate transcripts identified by microarray, eight apparently originate from multi-gene families. The number of genes in each gene family ranged from 2-5 members, as follows; neurotransmission transcripts: 13156.m00099 (2 genes represented), 14972.m07453 (3), 14974.m00802 (2), and 14965.m00428 (2); Collagen/sheath/surface: TC3150 (4), 14154.m00011 (5) (Figure 2-5), and TC2854 (2); and BMC12124 represented the only hypodermis/muscle associated transcript that seems to be a member of a multi-gene family and appears to have 4 gene family members. Gene correlations for the two transcripts validated by qPCR (13156.m00099 and 13895.m00081) were also evaluated, both of which appear to be members of two gene families. The members of these families were evaluated by sequence to determine if the qPCR probe/primers used for validation might have detected another transcript in the same gene family, but in both cases probes/primers were only complementary to the transcript they were designed to amplify.

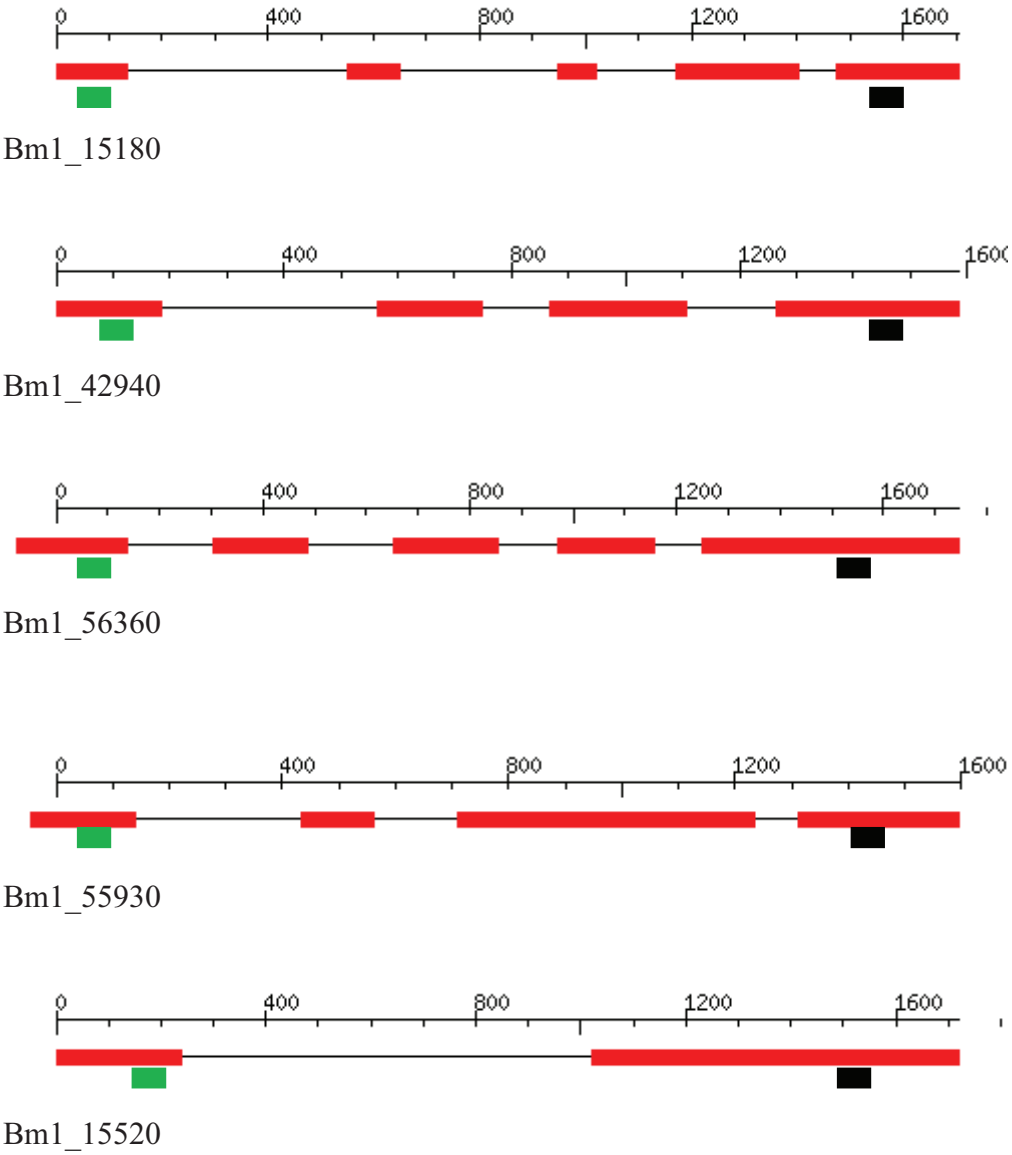


Figure 2-5. Alignment of the *B. malayi* collagen transcript consensus sequence with the microarray oligonucleotide sequence, and those of collagen gene family members. Transcript 14154.m00011 is putatively identified as a collagen in mature mf. It is represented by a five-member gene family (Bm1_15180, Bm1_42940, Bm1_56360, Bm1_55930, and Bm1_15520), in which exons are indicated by red boxes. The oligonucleotide designed for use on the microarray is represented by a green box. Regions of exons unique between individual genes that are good candidates for designing discriminatory qPCR primers and probes are designed by the black boxes.

DISCUSSION

Quantitative reverse transcriptase polymerase chain reaction (qPCR) is often used to validate microarray data by confirming the differential abundance of a subset of identified transcripts (Gaj *et al*. 2008). It is debatable whether microarray or qPCR provides a more accurate account of gene abundance (Gaj *et al.* 2008) however their tandem use provides a very powerful set of molecular techniques to determine relative transcript abundance between two different groups of organisms. We chose to validate transcripts identified by homologous *B. malayi* microarray hybridization because we had fewer biological replicates that experiment than the heterologous *B. pahangi* hybridization, which made it necessary to employ additional statistical manipulations of the data. The direction of change in transcript abundance, but not the magnitude change, was validated by qPCR and largely confirms the findings of others that have validated the *B. malayi* array, and arrays from unrelated species (Li *et al.* 2005; Morey *et al.* 2006), thereby allowing us to confidently compile a list of transcripts that encode proteins potentially involved in microfilarial infectivity for the mosquito vector. Currently, microarray data are considered to be valid if the array and qPCR results agree on the direction, and not necessarily the quantitation, of transcript abundance (Morey *et al.* 2006). The exact reason or reasons for quantitative variation between microarray and qPCR have yet to be identified, but may be related to spot fluorescence intensity, p-value, different priming methods for array and qPCR; and transcripts with low fold changes levels (<1.4) show lower correlation with qPCR results than those with greater differences (Morey *et al.* 2006). At least one additional transcript identified as more

abundant in mature *B. malayi* mf will be tested by qPCR to strengthen the validation of the microarray results. Our analysis suggests that 8 of the 14 transcripts apparently arise from gene families, therefore to continue our work it will be necessary to design qPCR assays directed at the unique regions of theoretical transcripts arising from gene family members to elucidate the identities of genes giving rise to individual transcripts of interest. Our future plans are to use this information as a springboard to further investigate the roles of encoded proteins in microfilarial maturation and mosquito infectivity, possibly by confirming differential protein expression, localizing expressed proteins in worm tissues, and using recombinant proteins to create competition assays for abrogation of midgut penetration. These findings will deepen our knowledge about the symbiotic relationship of *Brugia* with its mosquito host, and potentially lead to identification of gene products can be targeted to block the transmission cycle of lymphatic filariasis.

ACKNOWLEDGEMENTS

We thank Sara Erickson, George Mayhew, Matthew Nelson, Steven Williams and Lori Saunders for helpful discussion and technical advice. This project was funded by National Institutes of Health Grant1 R15 AI067295-01A1.

CONCLUSIONS

A maturation period of at least 5 days in the mammalian host is apparently required for *Brugia* mf to be infective for the mosquito vector (Fuhrman *et al.* 1987). Based on observations that surface composition changes during maturation we hypothesized that molecules involved in infectivity are produced by a change in gene expression that temporally coincides with maturation. After verifying the differential infectivity of *Brugia* mf, we conducted microarray comparison of mature and immature mf global transcript abundance. This analysis produced a list of 191 transcripts that are potentially critical for *Brugia* mf maturation. We validated our microarray data using quantitative RT-PCR, and identified genes of origin for transcripts potentially encoding anatomical proteins involved in the sheath/surface and hypodermal/muscle categories, and for neurotransmission. We conclude that the transcript profiles of mature mf and immature mf are different, preliminarily supporting our hypothesis and suggesting that gene expression changes are correlated with differential infectivity. This study serves as a starting point for understanding compatibility of filarial worms for mosquitoes, which could possibly lead to intervention strategies that prevent parasite transmission. Future investigations include determining the biochemical pathways the proteins of interest are included in, determining the location of protein expression, and designing *in vivo* competition assays to abrogate infection in laboratory mosquitoes.

APPENDIX A
Volcano Plot

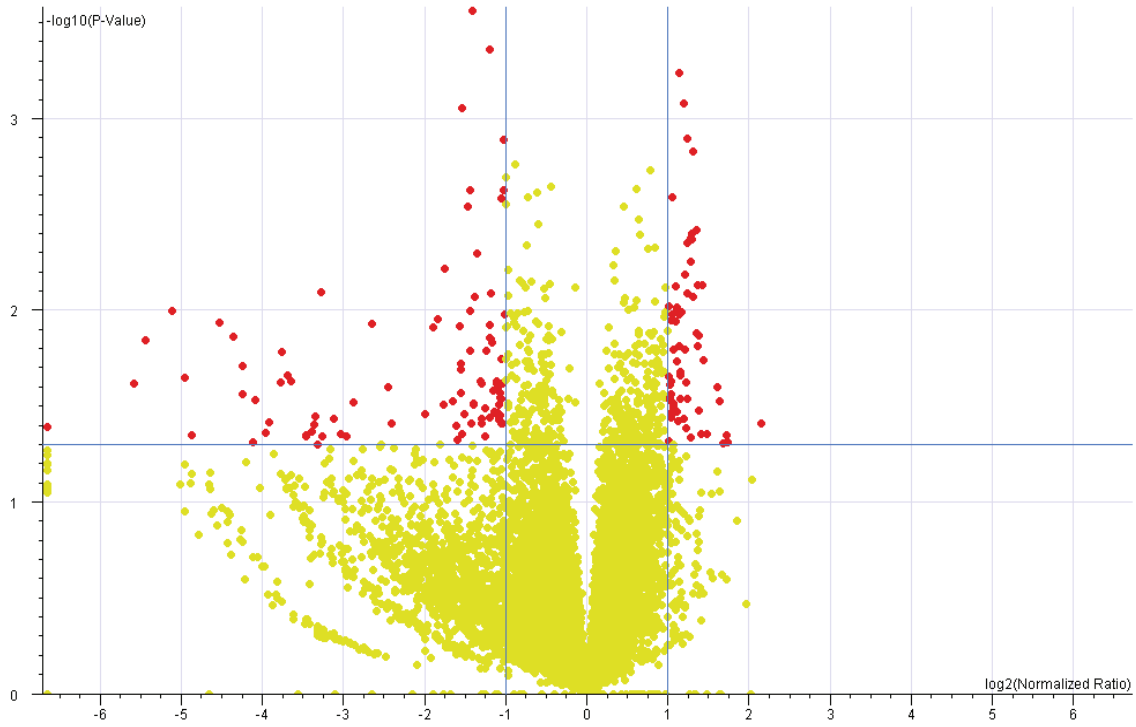


Figure A-1. Differential abundance of *B. pahangi* microfilarial transcripts. Transcripts in red have differential abundance twofold or greater with 95% or greater confidence. Each spot represents the normalized value of hybridization of an oligonucleotide target on the slide. On the y-axis, significance is plotted as the negative logarithm of a p-value, and on the x-axis, the magnitude of change is plotted in relation to the control sample. Transcripts with a normalized ratio >1 are more abundant in mature mf and those <-1 are more abundant in immature mf. Transcripts under the approximate '1' value on the y axis have a statistically insignificant fold change due to high variability, those above the region has biologically significant changes in transcript numbers (low variance).

APPENDIX B
Microarray Results

Table B-1. *Brugia malayi* immature mf differentially abundant transcripts.

Systematic Name	mature/ immature normalized ratio	t-test p-value	top BLAST hit (NCBI)	Gene Ontology (Uniprot)	Top WormPep BLAST hit	E-value	functional category
BMC02019	0.586	2.80E-05	Brugia malayi Myosin VI XM_001901311	myosin/ ATP binding	F47G6.4	4.60E-19	cell structure
TC9567	0.0788	6.90E-06	fungal protease inhibitor-1 precursor AM939570	none	F43A11.3	0.059	conserved unknown
BMC00805	0.184	0.000108	hypothetical protein [Brugia malayi] XM_001896722	none	no sig hit		conserved unknown
14824.m00117	0.192	6.15E-06	hypothetical protein [Brugia malayi] XM_001896722	none	no sig hit		conserved unknown
BMC01892	0.42	1.12E-05	Brugia malayi BCL7, N-terminal conserver region family protein XM_001899635	contains a conserved N-terminal domain which is found in the BCL7 family. BCL7 proteins may be involved in early developm ent.	C28H8.1	1.90E-31	conserved unknown
14967.m01578	0.308	7.70E-06	Brugia malayi Zinc finger, C2H2 type family protein XM_001898464	zinc ion binding	C34H3.2	4.70E-09	DNA binding/transcri ption

14952.m01375	0.249	7.68E-05	Brugia malayi Hsp20/alpha crystallin family protein XM_001897480	heat shock protein	F52E1.7a	3.10E-12	heat shock protein
BMC00466	0.469	1.35E-05	Brugia malayi hypothetical protein XM_001895491	none	F36H2.3	1.40E-09	immune modulation
AA545881	0.154	3.82E-06	Rattus norvegicus RT1 class I, CE16 BC090028	integral to membrane	no sig hit		membrane synthesis
BMC11738	0.432	1.57E-05	Brugia malayi Tim44-like domain containing protein XM_001900661	P-P-bond-hydrolysis-driven protein transmembrane transporter activity	Y119D3B.16	2.70E-15	membrane transport
14979.m04604	0.619	1.46E-05	Brugia malayi Suppressor/enhancer of lin-12 protein 9 precursor XM_001900517	transport/integral to membrane	W02D7.7	7.50E-80	membrane transport
13156.m00099	0.0766	1.73E-06	Brugia malayi Voltage-gated calcium channel, T-type, alpha subunit. XM_001892541	voltage-gated calcium ion transport	C48A7.1a	9.20E-103	neurotransmission
AA991131	0.18	7.05E-05	B. malayi serpin precursor (Bm-spn-2) AF009825	serine-type endopeptidase inhibitor	no sig hit		protease inhibitor

14946.m00534	0.411	2.09E-05	Brugia malayi Kunitz/Bovine pancreatic trypsin inhibitor domain containing protein XM_001897319	serine-type endopeptidase inhibitor activity	K10D3.4	1.00E-72	protease inhibitor
13830.m00006	0.245	8.37E-05	hypothetical protein [Brugia malayi] XM_001893892	metalloprotease	F54E4.4	0.0052	protein degradation
AA056807	0.0359	8.00E-06	hypothetical protein [Brugia malayi] XM_001894908	none	no sig hit		unknown
BMC00522	0.0398	1.46E-06		none	no sig hit		unknown
14294.m00131	0.042	1.82E-06	hypothetical protein [Brugia malayi] XM_001894908	none	no sig hit		unknown
15375.m00008	0.0505	1.48E-05	hypothetical protein [Brugia malayi] XM_001902686	none	no sig hit		unknown
BMB_gene_11.564	0.0549	2.11E-05		none	no sig hit		unknown
WB-contig_267	0.0556	8.09E-05	hypothetical protein [Brugia malayi] XM_001902686	none	no sig hit		unknown
14274.m00231	0.0564	7.87E-05	hypothetical protein [Brugia malayi] XM_001894831	none	no sig hit		unknown

BMC11770	0.156	0.000104	Brugia malayi hypothetical protein XM_001894235	none	no sig hit		unknown
BMB_gene_55.331	0.156	3.49E-06	no sig hit	none	no sig hit		unknown
14046.m00189	0.191	8.10E-06	hypothetical protein [Brugia malayi] XM_001894235	none	no sig hit		unknown
14824.m00118	0.249	8.34E-05	hypothetical protein [Brugia malayi] XM_001896723	none	no sig hit		unknown
BMC00873	0.263	0.000101	Brugia malayi hypothetical protein XM_001895461	none	no sig hit		unknown
14992.m11307	0.366	0.000112	Brugia malayi hypothetical protein XM_001901957	none	no sig hit		unknown
BMC05977	0.46	0.000128	no sig hit	none	no sig hit		unknown

Table B-2. *Brugia malayi* mature mf differentially abundant transcripts.

Systematic Name	mature/immature normalized ratio	t-test p-value	top BLAST hit (NCBI)	Gene Ontology (Uniprot)	Top WormPep BLAST hit	E-value	functional category
13895.m00081	4.372	4.74E-05	Brugia malayi Ground-like domain containing protein XM_001893955	ground-like domain containing proteins may bind and modulate the activity of Patched-like membrane molecules	T16G1.8	1.50E-20	cell signaling
AI096276	1.607	9.65E-05	no sig hit	none	Y40H7A.9	0.11	conserved unknown
BMC05170	1.836	6.71E-05	Brugia malayi hypothetical protein XM_001894084	none	C48E7.1	1.90E-41	conserved unknown
BMC01609	2.39	4.70E-05	Brugia malayi Histone H3.3 XM_001897381	DNA binding	F45E1.6	1.30E-66	conserved unknown
BMC05233	5.703	5.58E-06	no sig hit	none	no sig hit		conserved unknown
14982.m02218	1.639	2.10E-05	Brugia malayi Conserved hypothetical protein XM_001900871	none	ZK632.9	9.60E-10	DNA binding
BMC12124	2.276	3.21E-05	Brugia malayi EF hand family protein XM_001901190	calcium ion binding	F54C1.7	3.40E-78	hypodermal/muscle
BMC03479	2.286	4.27E-05	no sig hit	Transthyretin-like protein 16 (extracellular region)	T07C4.5	4.00E-20	metabolic
14990.m08053	1.524	0.000105	Brugia malayi 60S acidic ribosomal protein P2 XM_001901358	translational elongation	C37A2.7	4.10E-17	peptide synthesis

12787.m00387	1.901	1.16E-05	Brugia malayi RNA binding motif protein XM_001892076	RNA binding	R05H10.2	4.20E-82	RNA binding
BMC06144	1.6	1.85E-05	Brugia malayi antigen XM_001900001	filarial antigen SXP-1	C06A8.3	5.40E-13	sheath
BMB_gene_50.122	1.735	9.85E-05	so sig hit	none	no sig hit		unknown
BMC02493	2.567	9.83E-05	no sig hit	none	no sig hit		unknown
BMC01963	7.582	5.40E-05	no sig hit	none	no sig hit		unknown

Table B-3. *Brugia pahangi* immature mf differentially abundant transcripts.

Systematic name	mature/ immature normalized ratio	t-test p-value	top BLAST hit (NCBI)	Gene Ontology (Uniprot)	Top WormPep BLAST hit	E-value	functional category
14224.m00316	0.455	0.034	XM_001894615. Brugia malayi RasGEF domain containing protein	guanyl-nucleotide exchange factor activity	F25B3.3	2.50E-77	cell signaling
14529.m00203	0.368	0.0162	XM_001895549. Brugia malayi Ras-related protein Rab-14	small GTPase mediated signal transduction	K09A9.2	2.50E-97	cell signaling
14641.m00157	0.337	0.0122	XM_001895978.Brugia malayi Protein kinase domain containing protein	protein serine/threonine kinase activity	ZK930.1	1.10E-155	cell signaling
14779.m00765	0.439	0.00818	XM_001896639. Brugia malayi Protein kinase domain containing protein	protein serine/threonine kinase activity	F52F12.3	9.00E-36	cell signaling
14992.m11321	0.25	0.035	XM_001901971. Brugia malayi RhoGAP domain containing protein	diacylglycerol binding	K08E3.6	3.60E-54	cell signaling
15168.m00059	0.0665	0.0386	XM_001902343. Brugia malayi serine/threonine protein kinase	protein serine/threonine kinase activity	no sig hit		cell signaling
BMC01868	0.189	0.0391	no sig hit	kinase activity	F11D5.3a	2.00E-14	cell signaling
TC3375/TC4329	0.481	0.0392	AW257743 Onchocerca volvulus molting L3	kinase activity	B0495.2	6.70E-22	cell signaling

12575.m00210	0.326	0.0399	XM_001891767. Brugia malayi Protein kinase domain containing protein	protein serine/threonine kinase activity	T05G5.3	2.20E-71	cell signaling
12903.m00103	0.475	0.0269	XM_001892243. Brugia malayi Serine/threonine protein phosphatase C34C12.3 in chromosomeIII	hydrolase activity	C34C12.3	5.60E-114	cell signaling
14917.m00328	0.418	0.0452	XM_001896949. Brugia malayi Cyclin, N-terminal domain containing protein	regulation of progression through cell cycle	ZC168.4	1.40E-16	cell signaling
14282.m00452	0.341	0.019	XM_001894864. Brugia malayi hypothetical protein	none	R05D11.9	4.80E-42	cell signaling
BMC05064	0.466	0.0252	no sig hit	actin binding	Y66H1B.3	5.40E-32	cell structure
BMC05176	0.492	0.00237	no sig hit	actin binding	R31.1	1.30E-29	cell structure
BMC11473	0.405	0.0233	no sig hit	none	M01A8.2a	6.80E-07	cell structure
TC3150	0.468	0.0345	AA635198 Onchocerca volvulus infective larva cDNA	ShTK domain containing protein	ZK673.1	0.00036	collagen/sheath/surface
13093.m00035	0.0775	0.0218	XM_001892449. Brugia malayi hypothetical protein	none	F38A5.6	1.90E-07	conserved unknown
13247.m00705	0.442	0.014	XM_001892699. Brugia malayi	'SD27140p	ZC132.5	5.40E-30	conserved unknown
13486.m00113	0.053	0.0196	XM_001893358 Brugia malayi hypothetical protein	none	C56C10.12	3.30E-99	conserved unknown

13945.m00126	0.409	0.0243	XM_001894046. Brugia malayi hypothetical protein	none	no sig hit		conserved unknown
14222.m00062	0.332	0.0471	XM_001894596. Brugia malayi PPE FAMILY PROTEIN	PPE FAMILY PROTEIN	Y39B6A.1	1.70E-20	conserved unknown
14954.m01646	0.0972	0.0393	XM_001897586. Brugia malayi gag-pol polyprotein precursor	Gag-pol polyprotein, putative.	ZC132.5	0.00038	conserved unknown
15349.m00011	0.0796	0.0234	XM_001902659. Brugia malayi WH2 motif family protein	WH2 motif family protein	T24B8.4	5.70E-09	conserved unknown
15472.m00011	0.464	0.0236	XM_001902829. Brugia malayi hypothetical protein	none	M01E11.3	1.00E-08	conserved unknown
BMC05863	0.369	0.01	no sig hit	none	R06C7.6	5.70E-18	conserved unknown
BMC06914	0.0341	0.0451	no sig hit	none	W01C9.5	0.015	conserved unknown
BMC12453	0.0209	0.0243	no sig hit	none	F55H12.4	3.90E-15	conserved unknown
13644.m00285	0.476	0.0287	XM_001893597 Brugia malayi protein F10G7.2	nucleic acid binding	F10G7.2	1.40E-14	DNA binding/remodeling
14707.m00128	0.491	0.00129	XM_001896285. Brugia malayi Bromodomain containing protein	'Bromodomain containing protein	F57C7.1a	2.10E-35	DNA binding/remodeling
14971.m02789	0.115	0.0368	XM_001898562 Brugia malayi Steroid receptor seven-up type 2	regulation of transcription	F55D12.4b	4.30E-51	DNA binding/remodeling

14977.m04904	0.438	0.0363	XM_001900086 Brugia malayi Zinc finger, C2H2 type family protein	nucleic acid binding	F56D1.1	5.30E-18	DNA binding/remodeling
WB-contig_1557	0.436	0.0119	no sig hit	zinc finger	no sig hit		DNA binding/remodeling
12612.m00253	0.423	0.0164	XM_001891843. Brugia malayi ethanolamine-phosphate cytidylyltransferase, putative	nucleotidyltransferase activity	Y37E3.11a	9.20E-130	fatty acid synthesis/membrane synthesis
13555.m00076	0.123	0.0439	XM_001893463. Brugia malayi uridine phosphorylase family protein	uridine phosphorylase activity	ZK783.2	1.80E-72	metabolic
14450.m00179	0.104	0.0452	XM_001895357. Brugia malayi hypothetical protein	steroid metabolism	Y47D3A.17d	2.30E-24	metabolic
14975.m04355	0.27	0.0123	XM_001899846. Brugia malayi cytochrome P450	oxidoreductase activity	F08F3.7	0.019	metabolic
BMC05678	0.341	0.0271	no sig hit	none	C01G12.5	1.10E-16	metabolic
BMC11992	0.104	0.008	no sig hit	none	R04B5.5	2.20E-54	metabolic
15476.m00018	0.0905	0.0456	XM_001902835 Brugia malayi Myb-like DNA-binding domain containing protein	pancreatic ribonuclease activity	R151.8	7.70E-53	mRNA splicing/transcription
WB-contig_1081	0.383	0.00846	no sig hit	none	F28D9.1	0.055	mRNA splicing/transcription

14972.m07453	0.0324	0.0226	XM_001899172. Brugia malayi Neurotransmitter-gated ion-channel ligand binding domain containing protein	extracellular ligand-gated ion channel activity	F58H7.3	6.30E-106	neurotransmission
14974.m00802	0.0955	0.0427	XM_001899761. Brugia malayi Uncoordinated protein 40		T19B4.7	7.30E-07	neurotransmission
14977.m04875	0.437	0.0139	XM_001900059. Brugia malayi Voltage gated chloride channel family	voltage-gated chloride channel activity	B0491.8b	1.30E-239	neurotransmission
BMC00336	0.293	0.0313	no sig hit	ribonucleoprotein complex	no sig hit		peptide synthesis/ribosomal
BMC04649	0.0577	0.0486	no sig hit	none	C06B8.8	0.0024	peptide synthesis/ribosomal
BMC12066	0.483	0.029	BMC12066	ribonucleoprotein complex	F28C6.7a	3.50E-32	peptide synthesis/ribosomal
WB-contig_239	0.0733	0.0239	no sig hit	ribonucleoprotein complex	F53A3.3a	1.30E-56	peptide synthesis/ribosomal
13282.m00272	0.159	0.0117	XM_001892836. Brugia malayi Serine carboxypeptidase S28 family	serine-type peptidase activity	ZK112.1	1.60E-105	protein degradation/lysosomal
14992.m11208	0.1	0.0499	XM_001901858. Brugia malayi Zinc finger protein-like 1	ubiquitin-protein ligase activity	Y45G12B.2a	1.20E-63	protein degradation/lysosomal
14240.m00034	0.462	0.0241	XM_001894697. Brugia malayi hypothetical protein	none	C09H6.2b	0.00038	protein sorting

14958.m00357	0.482	0.0026	XM_001897790 Brugia malayi Major Facilitator Superfamily protein	tetracycline:hydrogen antiporter activity	F55A4.8a	3.40E-103	proton/ion transport
14990.m07820	0.494	0.0106	XM_001901124 Brugia malayi LD23056p	none	Y37H9A.3	5.20E-102	transcription
12867.m00020	0.0979	0.036	XM_001892184. Brugia malayi hypothetical protein	none	no sig hit		unknown
14681.m00081	0.342	0.0204	XM_001896148 Brugia malayi hypothetical protein	none	no sig hit		unknown
14747.m00204	0.37	0.00238	XM_001896451. Brugia malayi hypothetical protein	none	no sig hit		unknown
14787.m00176	0.318	0.03	XM_001896654. Brugia malayi hypothetical protein	none	no sig hit		unknown
14977.m04918	0.0905	0.0447	XM_001900100. Brugia malayi hypothetical protein	none	no sig hit		unknown
14977.m05087	0.128	0.0456	XM_001900265. Brugia malayi hypothetical protein	none	no sig hit		unknown
14990.m07788	0.47	0.0373	XM_001901092 Brugia malayi hypothetical protein	binding	no sig hit		unknown

14992.m10867	0.409	0.0391	XM_001901528 Brugia malayi hypothetical protein	none	no sig hit		unknown
15003.m00144	0.0735	0.0166	XM_001902062. Brugia malayi hypothetical protein	none	no sig hit		unknown
15515.m00012	0.476	0.0353	XM_001902892. Brugia malayi hypothetical protein	none	no sig hit		unknown
AA161574	0.029	0.0101	AA161574. Brugia malayi adult female cDNA	none	no sig hit		unknown
AA508953	0.0433	0.0115	AA508953. Brugia malayi adult female cDNA	none	no sig hit		unknown
AA756934	0.023	0.0144	AA756934. Brugia malayi adult male cDNA	none	no sig hit		unknown
AA840846	0.343	0.00088	AA840846 Brugia malayi day 6 post-infection third stage larvae	none	no sig hit		unknown
AA841172	0.0487	0.0138	AA841172 Brugia malayi day 6 post-infection third stage larvae	none	no sig hit		unknown
BMC00072	0.372	0.0391	no sig hit	none	no sig hit		unknown
BMC00368	0.362	0.00288	no sig hit	none	no sig hit		unknown
BMC00575	0.467	0.0354	no sig hit	none	no sig hit		unknown
BMC02140	0.435	0.000436	no sig hit	none	no sig hit		unknown

BMC02185	0.345	0.0444	no sig hit	none	no sig hit		unknown
BMC03379	0.392	0.00507	no sig hit	none	no sig hit		unknown
BMC03559	0.488	0.039	no sig hit	none	no sig hit		unknown
BMC07164	0.379	0.0305	no sig hit	none	no sig hit		unknown
BMC11407	0.35	0.0346	no sig hit	none	no sig hit		unknown
BMW0004 0.662	0.183	0.025	no sig hit	none	no sig hit		unknown
BMW0008 3.153	0.0642	0.0437	no sig hit	none	no sig hit		unknown
BMW0008 9.455	0.444	0.0148	no sig hit	none	no sig hit		unknown
CB328783	0.136	0.0304	CB328783. Brugia malayi 2D L3 irradiated pAMP1 v2	none	no sig hit		unknown
H21280	0.448	0.0261	H21280. Brugia malayi infective L3	none	no sig hit		unknown
N43135	0.01	0.0406		none	no sig hit		unknown
TC3121	0.376	0.000275	AI374092 Onchocerca volvulus microfilaria cDNA	none	no sig hit		unknown
TC3606	0.297	0.0061	BE669093 Onchocerca volvulus L2 larvae cDNA	none	no sig hit		unknown
TC8887	0.0589	0.0294	no sig hit	none	no sig hit		unknown
TC9506	0.279	0.0111	no sig hit	none	no sig hit		unknown
WB-contig_453	0.475	0.0271	no sig hit	none	no sig hit		unknown

Table B-4. *Brugia pahangi* mature mf differentially abundant transcripts.

Systematic name	mature/ immature normalized ratio	t-test p-value	top BLAST hit (NCBI)	Gene Ontology (Uniprot)	Top WormPep BLAST hit	E-value	functional category
12491.m00043	2.165	0.00997	H21265. Brugia malayi infective L3	Variant SH3 domain containing protein	F55C7.7b	0.00037	cell signaling
12621.m00167	2.008	0.0221	no sig hit	transferase activity	F53H8.4	1.20E-30	cell signaling
13227.m00139	2.578	0.00741	XM_001894466. Brugia malayi Nematode cuticle collagen N-terminal domain containing protein	protein tyrosine/serine/threonine phosphatase activity	F26A3.4	3.70E-22	cell signaling
14356.m00518	2.08	0.0105	AA841675. Brugia malayi day 6 post-infection third stage larvae	IQ calmodulin-binding motif family protein.	no sig hit		cell signaling
14667.m00131	2.367	0.0291	XM_001897976. Brugia malayi hypothetical protein	'EGF-like domain containing protein	R05G6.9	3.50E-08	cell signaling
14961.m05239	2.368	0.00127	XM_001896092. Brugia malayi EGF-like domain containing protein	protein serine/threonine kinase activity	F17E5.1a	4.50E-35	cell signaling
14975.m04377	2.058	0.0342	XM_001892765. Brugia malayi hypothetical protein	phosphoinositide phospholipase C activity	T01E8.3	2.60E-253	cell signaling
BMW01175.503	2.31	0.00656	AA990945. Brugia malayi microfilaria cDNA	PDZ domain	ZK1321.4	9.10E-18	cell signaling

BMW01417.440	2.035	0.0283	XM_001898393. Brugia malayi sodium/hydrogen exchanger 3 family protein	none	F33D4.2g	9.80E-10	cell signaling
13472.m00068	2.432 (0.1 to 6.4)	0.0056	AA563555. Brugia malayi microfilaria cDNA	tubulin-tyrosine ligase activity	ZK1128.6a	4.60E-146	cell structure
14154.m00011	2.562	0.0132	XM_001900908. Brugia malayi hypothetical protein	structural constituent of cuticle	Y51H7C.1	4.80E-06	collagen/sheath/surface
BMC11831	2.162	0.0184	XM_001892453. Brugia malayi hypothetical protein	major microfilarial sheath protein	Y49E10.25	0.041	collagen/sheath/surface
TC2854	2.599	0.0332	XM_001892650. Brugia malayi hypothetical protein	collagen col-34	F36A4.10	3.60E-43	collagen/sheath/surface
12720.m00024	2.137	0.0115	XM_001902731. Brugia malayi hypothetical protein	GYF domain containing protein	R10D12.14a	2.30E-19	conserved unknown
13261.m00253	2.054	0.0364	XM_001893203. Brugia malayi Fatty acid elongation protein 3	none	M176.5	0.00063	conserved unknown
14961.m05058	2.361	0.00445	XM_001900709 Brugia malayi hypothetical protein	none	B0393.4	3.10E-11	conserved unknown
14992.m11108	2.211	0.0153	XM_001892623.Brugia malayi Muscle positioning protein 4	TB2/DP1, HVA22 family protein	T19C3.4	8.80E-20	conserved unknown

14992.m11320	2.189	0.038	XM_001891637 Brugia malayi Variant SH3 domain containing protein	none	no sig hit		conserved unknown
13628.m00024	2.427	0.0464	XM_001898155. Brugia malayi CASK	DNA recombination	no sig hit		DNA binding/remodeling
14630.m00084	2.099	0.0161	XM_001891555 Brugia malayi protein R52.2	nucleic acid binding	2L52.1	1.60E-14	DNA binding/remodeling
14980.m02807	2.354	0.00812	no sig hit	none	F43C1.3	6.70E-14	DNA binding/remodeling
13415.m00446	2.049	0.0275	no sig hit	integral to membrane	D2024.3	3.70E-78	fatty acid synthesis/membrane synthesis
12695.m00041	3.061	0.0252	no sig hit	none	Y105E8B.1e	0.0063	hypodermal/muscle
13207.m00046	2.21	0.000577	AF532606 Onchocerca volvulus enolase	calcium ion binding	K07D8.1	1.40E-92	hypodermal/muscle
14429.m00009	2.06	0.0112	XM_001899869. Brugia malayi Variant SH3 domain containing protein	Lethal protein 805, isoform d	H19M22.2d	2.40E-52	hypodermal/muscle attach
13258.m00170	2.474	0.00148	XM_001902365 Brugia malayi hypothetical protein	oxidoreductase activity	ZK1251.3	1.60E-29	metabolic
14992.m11165	2.326	0.0413	XM_001900902 Brugia malayi Abnormal growth rate protein 1	Rhodanese-like domain containing protein	R186.6	7.10E-80	metabolic
BMC06319	2.783	0.0445	XM_001898286 Brugia malayi hypothetical protein	none	MTCE.25	2.30E-11	metabolic

BMC11984	2.245	0.0102	XM_001892627. Brugia malayi hypothetical protein	Cathepsin L-like cysteine proteinase	T03E6.7	6.10E-13	metabolic
BMW00144.486	2.011	0.0481	XM_001891871 Brugia malayi Phosphatidylcholine:ceramide cholinephosphotransferase 1	'3'5'-cyclic nucleotide phosphodiesterase family protein	Y95B8A.10b	1.20E-55	metabolic
TC2871	2.197	0.0106	XM_001901970. Brugia malayi Conserved hypothetical protein	glycolysis	T21B10.2a	9.50E-183	metabolic
WB-contig_242	2.646	0.0441	AA294478 Onchocerca volvulus molting L3 larva cDNA	none	R04B5.5	4.00E-51	metabolic
14965.m00428	2.026	0.0296	XM_001900716. Brugia malayi vacuolar proton pump	sodium:hydrogen antiporter activity	F57C7.2b	6.30E-153	neurotransmission
13270.m00027	2.281	0.0366	AY533167.1	ubiquitin-protein ligase activity	F10G7.10c	1.00E-19	peptide synthesis/ribosomal
14982.m02252	2.316	0.016	no sig hit	tRNA isopentenyltransferase activity	ZC395.6	5.50E-59	peptide synthesis/ribosomal
14968.m01478	2.454	0.00425	XM_001893331. Brugia malayi Tubulin-tyrosine ligase family protein	ShTK domain containing protein	F39D8.4	0.00077	protein degradation/lysosomal
13096.m00015	2.152	0.00964	XM_001892002. Brugia malayi GYF domain containing protein	integral to membrane	F52F12.1a	2.20E-60	proton/ion transport
14980.m02814	2.025	0.00948	no sig hit	proton transport	VW02B12L.1	2.20E-235	proton/ion transport

12434.m00015	2.094	0.0308	AA231999. Brugia malayi microfilaria cDNA	Protein R52.2, putative.	R52.2	6.60E-37	retrotransposon
13210.m00165	2.235	0.0289	XM_001901521. Brugia malayi hypothetical protein	none	no sig hit		unknown
13828.m00099	2.481	0.00852	XM_001892748. Brugia malayi 3-oxo-5-alpha-steroid 4-dehydrogenase 1	none	no sig hit		unknown
14961.m05186	2.217	0.0209	XM_001901760. Brugia malayi TB2/DP1, HVA22 family protein	none	no sig hit		unknown
14961.m05369	2.71	0.0182	no sig hit	none	no sig hit		unknown
14973.m02618	3.102	0.0297	no sig hit	none	no sig hit		unknown
14982.m02259	2.55	0.00381	XM_001893886 Brugia malayi hypothetical protein	none	no sig hit		unknown
14990.m08136	3.317	0.0451		none	no sig hit		unknown
14992.m10861	2.221	0.0219	XM_001898102 Brugia malayi hypothetical protein	none	no sig hit		unknown
15182.m00048	2.463	0.004	XM_001898500. Brugia malayi ShTK domain containing protein	none	no sig hit		unknown
15409.m00015	2.136	0.00749	XM_001895916. Brugia malayi Zinc finger, C2H2 type family protein	none	no sig hit		unknown
AA231999	2.093	0.0319	XM_001895085. Brugia malayi IQ calmodulin-binding motif family protein	none	no sig hit		unknown

AA246059	3.342	0.0485	XM_001901441. Brugia malayi hypothetical protein	none	no sig hit		unknown
AA257580	2.069	0.0346	AW561859. Brugia malayi infective larva cDNA	none	no sig hit		unknown
AA563555	2.43	0.00423	XM_001893567. Brugia malayi resolvase	none	no sig hit		unknown
AA841675	2.071	0.00259	AA257580. Brugia malayi adult female cDNA	none	no sig hit		unknown
AA990945	2.284	0.000837	XM_001892796 Brugia malayi hypothetical protein	none	no sig hit		unknown
AW561859	2.062	0.0235	XM_001895325. Brugia malayi Lethal protein 805, isoform d	none	no sig hit		unknown
BMC03110	2.334	0.0237	XM_001901816. Brugia malayi Rhodanese-like domain containing protein partial	none	Y55D5A.3	2.50E-17	unknown
BMC06644	3.193	0.0498	XM_001899647. Brugia malayi hypothetical protein	none	no sig hit		unknown
H21265	2.163	0.034	no sig hit	none	no sig hit		unknown
TC3617	2.61	0.0136	BF942764 Onchocerca volvulus molting L3 larva cDNA	none	no sig hit		unknown
WB-contig_1303	4.449	0.0391	AA246059. Brugia malayi microfilaria cDNA	none	no sig hit		unknown

Table B-5. Abundant immature mf transcripts from *Brugia malayi* that share sequence similarity on the peptide level with *C. elegans* genes displaying RNAi phenotypes, Source; Wormpep.

Systematic Name	**Top WormPep BLAST hit**	**RNAi result in *C. elegans***
13156.m00099	C48A7.1a	protruding vulva, sterile progeny, egg laying abnormal, clear
13830.m00006	F54E4.4	slow growth, postembryonic development abnormal, embryonic lethal, larval lethal, larval arrest, maternal sterile, sterile progeny, abnormal morphology
14952.m01375	F52E1.7a	postembryonic development abnormal, embryonic lethal, maternal sterile, abnormal morphology
14967.m01578	C34H3.2	early larval arrest, pharyngeal pumping abnormal, feeding behavior abnormal, abnormal intestinal morphology, larval arrest, larval lethal, reduced brood size, slow growth, locomotion abnormal
14946.m00534	K10D3.4	postembryonic development abnormal, embryonic lethal, maternal sterile, abnormal morphology
BMC01892	C28H8.1	embryonic lethal, abnormal morphology, maternal sterile, lethal, transgene subcellular localization abnormal, protein aggregation abnormal, postembryonic development abnormal, slow growth, sterile progeny, larval arrest, larval lethal
BMC11738	Y119D3B.16	postembryonic development abnormal, embryonic lethal, maternal sterile, abnormal morphology
BMC00466	F36H2.3	postembryonic development abnormal, embryonic lethal, maternal sterile, abnormal morphology
BMC02019	F47G6.4	reduced brood size, sterile, oocyte morphology abnormal, embryonic lethal
14979.m04604	W02D7.7	abnormal locomotion, slow growth, protruding vulva, larval arrest

Table B-6. Abundant mature mf transcripts from *Brugia malayi* that share sequence similarity on the peptide level with *C. elegans* genes displaying RNAi phenotypes.

Systematic Name	**Top WormPep BLAST hit**	**RNAi result in *C. elegans***
14990.m08053	C37A2.7	slow growth, embryonic lethal, development abnormal
BMC06144	C06A8.3	postembryonic development abnormal, lethal, maternal sterile, larval arrest, larval lethal
AI096276	Y40H7A.9	embryonic lethal, abnormal morphology, maternal sterile, lethal, transgene subcellular localization abnormal, protein aggregation abnormal, postembryonic development abnormal, slow growth, sterile progeny, larval arrest, larval lethal
14982.m02218	ZK632.9	postembryonic development abnormal, lethal, maternal sterile, larval arrest, larval lethal
BMC05170	C48E7.1	egg laying defective
12787.m00387	R05H10.2	slow growth, patchy coloration, embryonic lethal, larval arrest, late larval arrest, pattern of transgene expression abnormal, receptor mediated endocytosis defective, maternal sterile
BMC12124	F54C1.7	embryonic lethal, larval arrest, maternal sterile, pattern of transgene expression abnormal, receptor mediated endocytosis defective, slow growth, paralyzed
BMC03479	T07C4.5	postembryonic development abnormal, lethal, maternal sterile, larval arrest, larval lethal
BMC01609	F45E1.6	embryonic lethal, larval lethal, morphology abnormal, slow growth, age associated fluorescence increased, shortened life span, spontaneous mutation rate increased
13895.m00081	T16G1.8	postembryonic development abnormal, lethal, maternal sterile, larval arrest, larval lethal

Table B-7. Abundant immature mf transcripts from *Brugia pahangi* that share sequence similarity on the peptide level with *C. elegans* genes displaying RNAi phenotypes.

Systematic name	**Top WormPep BLAST hit**	**RNAi result in *C. elegans***
14990.m07820	Y37H9A.3	Sterile progeny, slow growth, larval and embryonic arrest/lethal
BMC05176	R31.1	At hatching, sma-1 mutant larvae are short and wide owing to a proportionate decrease in the entire body length. sma-1 larvae also have a blunt nose and crooked tail.
14707.m00128	F57C7.1a	embryonic lethal, abnormal morphology
BMC12066	F28C6.7a	larval arrest, embryonic lethal, maternal sterile, slow growth, pleiotropic defects severe early emb, organism morphology abnormal, transgene subcellular localization abnormal
14958.m00357	F55A4.8a	embryonic lethal, morphology abnormal, larval arrest, larval lethal, post embryonic development abnormal, slow growth
TC3375/TC4329	B0495.2	spontaneous mutation rate increased, slow growth, embryonic lethal, sterile
13644.m00285	F10G7.2	transgene subcellular localization abnormal, protein aggregation abnormal, slow growth, sterile, embryonic lethal
12903.m00103	C34C12.3	cortical dynamics abnormal early emb, aldicarb resistant, slow growth
TC3150	ZK673.1	Slow growth/lethal
BMC05064	Y66H1B.3	embryonic lethal, abnormal morphology, sterile progeny
15472.m00011	M01E11.3	embryonic lethal
14240.m00034	C09H6.2b	embryonic lethal, abnormal morphology, sterile progeny
14224.m00316	F25B3.3	embryonic lethal, abnormal morphology, sterile progeny, post embryonic development abnormal
13247.m00705	ZC132.5	not reported
14779.m00765	F52F12.3	embryonic lethal, protein subcellular localization abnormal, transgene subcellular localization abnormal, protein aggregation abnormal
14977.m04904	F56D1.1	sterile progeny, slow growth, larval and embryonic arrest/lethal
14977.m04875	B0491.8b	sick, embryonic lethal, maternal sterile
12612.m00253	Y37E3.11a	embryonic lethal, maternal sterile, abnormal morphology
14917.m00328	ZC168.4	removing maternal product in cyb-1(RNAi) embryos causes multiple nuclei, and affected embryos arrest development at an early embryonic stage.

BMC11473	M01A8.2a	embryonic lethal, maternal sterile, abnormal morphology, postembryonic development abnormal
WB-contig_1081	F28D9.1	embryonic lethal, locomotion abnormal, sick
BMC05863	R06C7.6	Sterile progeny, slow growth, larval and embryonic arrest/lethal, postembryonic development abnormal
14529.m00203	K09A9.2	sterile progeny, slow growth, larval and embryonic arrest/lethal, postembryonic development abnormal
BMC05678	C01G12.5	slow growth
14282.m00452	R05D11.9	embryonic lethal
14641.m00157	ZK930.1	fat content reduced
14222.m00062	Y39B6A.1	transgene expression increased
12575.m00210	T05G5.3	blistered, embryonic lethal, maternal sterile, protruding vulva, one cell arrest early emb, abnormal locomotion, sterile progeny, cell cycle slow early embryo, embryo osmotic pressure sensitive early emb, passage through meiosis abnormal early emb, oogenesis abnormal, nuclear envelope breakdown abnormal
14975.m04355	F08F3.7	Slow growth, embryonic lethal, maternal sterile, larval lethal/arrest, abnormal morphology
14992.m11321	K08E3.6	organism morphology abnormal, embryonic lethal, postembryonic development abnormal, embryonic development abnormal, pattern of transgene expression abnormal, receptor mediated endocytosis defective, cytokinesis abnormal emb, exploded through vulva, locomotion abnormal, P0 spindle position abnormal early emb, cytokinesis fails early emb, ectopic cleavage furrows early emb, multiple nuclei early emb, pronuclear migration failure early emb, pronuclei meet centrally early emb, pronuclear breakdown asynchronous early emb, pseudocleavage absent early emb
BMC01868	F11D5.3a	Slow growth
13282.m00272	ZK112.1	embryonic lethal, abnormal growth, abnormal morphology, larval lethal, larval arrest, abnormal sterile progeny, post embryonic development abnormal
13555.m00076	ZK783.2	embryonic lethal, abnormal growth, abnormal morphology, larval lethal, larval arrest, abnormal sterile progeny, post embryonic development abnormal
14971.m02789	F55D12.4b	abnormal locomotion
14450.m00179	Y47D3A.17d	embryonic lethal
BMC11992	R04B5.5	embryonic lethal, abnormal growth, abnormal morphology, larval lethal, larval arrest, abnormal sterile progeny, post embryonic development abnormal
14992.m11208	Y45G12B.2a	embryonic lethal, abnormal morphology, abnormal, maternal sterile

14954.m01646	ZC132.5	embryonic lethal, abnormal growth, abnormal morphology, larval lethal, larval arrest, abnormal sterile progeny, post embryonic development abnormal
14974.m00802	T19B4.7	fat content reduced, slow growth, adult lethal, egg laying abnormal, paralyzed, cord commissures fail to reach target, axon fasciculation abnormal, locomotion abnormal, ventral cord patterning abnormal
15476.m00018	R151.8	embryonic lethal, abnormal growth, abnormal morphology, larval lethal, larval arrest, abnormal sterile progeny, post embryonic development abnormal
15349.m00011	T24B8.4	embryonic lethal, abnormal growth, abnormal morphology, larval lethal, larval arrest, abnormal sterile progeny, post embryonic development abnormal
13093.m00035	F38A5.6	embryonic lethal, abnormal growth, abnormal morphology, larval lethal, larval arrest, abnormal sterile progeny, post embryonic development abnormal
WB-contig_239	F53A3.3a	slow growth, sick, embryonic lethal, sterile progeny, extended life span, abnormal organism heat response, larval arrest, molt defect
BMC04649	C06B8.8	sick, larval arrest, sterile, slow growth, sterile progeny, reduced brood size, protruding vulva
13486.m00113	C56C10.12	locomotion abnormal, sluggish, clear
BMC06914	W01C9.5	has no obvious function in mass RNAi assays
14972.m07453	F58H7.3	slow growth, postembryonic development abnormal, embryonic lethal, larval lethal, larval arrest, maternal sterile, abnormal morphology
BMC12453	F55H12.4	lethal, postembryonic development abnormal, slow growth, larval lethal, larval arrest, abnormal sterile progeny, abnormal morphology

Table B-8. Abundant mature mf transcripts from *Brugia pahangi* that share sequence similarity on the peptide level with *C. elegans* genes displaying RNAi phenotypes.

Systematic name	**Top WormPep BLAST hit**	**RNAi result in *C. elegans***
12695.m00041	Y105E8B.1e	Inactivating the body wall and pharynx-specific isoforms by RNA-mediated interference causes 50-75% embryonic lethality for either isoform, indicating that tropomyosin is essential for development. worms that survived RNAi of the body wall isoform had abnormal body morphology and uncoordinated movement; those surviving RNAi of the pharyngeal isoform had deformed pharynges and gut regions
WB-contig_242	R04B5.5	Slow growth, larval/embryonic lethal, maternal sterile, larval arrest
TC2854	F36A4.10	Abnormal morphology, dumpy, abnormal locomotion
13227.m00139	F26A3.4	Extended life span
13258.m00170	ZK1251.3	slow growth, abnormal postembryonic development, embryonic lethal, larval lethal, larval arrest, maternal sterile, abnormal sterile progeny
14968.m01478	F39D8.4	embryonic lethal, abnormal morphology, slow growth, larval arrest, larval lethal, abnormal postembryonic development, sterile progeny
13472.m00068	ZK1128.6a	TTLL-4 has no obvious function in mass RNAi assays
14961.m05239	F17E5.1a	lin-2(e1309), lin-2(e1424), and lin-2(e1453) mutant animals are egg-laying defective and exhibit abnormal postembryonic cell lineages, including those that lead to development of the hermaphrodite vulva.
14667.m00131	R05G6.9	lethal, embryonic lethal, morphology abnormal, post embryonic development abnormal
14961.m05058	B0393.4	lethal, embryonic lethal, morphology abnormal, post embryonic development abnormal, larval arrest, larval lethal, slow growth
14980.m02807	F43C1.3	loss of F43C1.3 activity via RNAi results in slower than normal growth rates
BMC03110	Y55D5A.3	lethal, embryonic lethal, morphology abnormal, post embryonic development abnormal, larval arrest, larval lethal, slow growth
14992.m11165	R186.6	lethal, embryonic lethal, morphology abnormal, post embryonic development abnormal, larval arrest, larval lethal, slow growth
14982.m02252	ZC395.6	larval arrest, maternal sterile, slow growth, reduced brood size
BMW01175.503	ZK1321.4	lethal, embryonic lethal, morphology abnormal, post embryonic development abnormal, larval arrest, larval lethal, slow growth

13270.m00027	F10G7.10c	lethal, embryonic lethal, morphology abnormal, post embryonic development abnormal, larval arrest, larval lethal, slow growth
BMC11984	T03E6.7	embryonic lethal, slow growth, abnormal locomotion
14992.m11108	T19C3.4	Embryonic development abnormal
13207.m00046	K07D8.1	body muscle displaced, molt defect, muscle attachment abnormal, slow growth. mup-4 embryos arrest development either with their hypodermis failing to enclose the embryo, or during the threefold stage with defects in hypodermal cell organization and muscle cell positions. mup-4 is required for normal morphogenesis of the embryonic epidermis (hypodermis) and for maintenance of muscle position, epithelial cell adhesion mediating mechanical coupling between the body wall muscles and hypodermal cells
TC2871	T21B10.2a	embryonic lethal, extended life span, larval arrest, slow growth, small, pattern of transgene expression abnormal, receptor mediated endocytosis defective
12491.m00043	F55C7.7b	embryonic lethal, slow growth, paralyzed, cord commissures fail to reach target, axon fasciculation abnormal, ventral cord patterning abnormal, locomotion abnormal, distal tip cell migration abnormal, sluggish
BMC11831	Y49E10.25	lethal, embryonic lethal, morphology abnormal, post embryonic development abnormal, larval arrest, larval lethal, slow growth
13096.m00015	F52F12.1a	Slow growth, embryonic lethal, larval arrest/lethal
12720.m00024	R10D12.14a	embryonic lethal, lethal
14630.m00084	2L52.1	lethal, embryonic lethal, morphology abnormal, post embryonic development abnormal, larval arrest, larval lethal, slow growth
12434.m00015	R52.2	lethal, embryonic lethal, morphology abnormal, post embryonic development abnormal, larval arrest, larval lethal, slow growth
14429.m00009	H19M22.2d	Embryonic and larval lethal, maternal sterile, adult lethal, paralyzed, molt defect
14975.m04377	T01E8.3	Intestinal physiology abnormal, maternal sterile, posterior body wall contraction abnormal, sterile progeny
13261.m00253	M176.5	lethal, embryonic lethal, morphology abnormal
13415.m00446	D2024.3	developmental delay postembryonic, transgene expression reduced, slow growth. In mass RNAi assays, elo-3(RNAi) animals grow abnormally slowly, though in individual RNAi assays, no obvious changes in fatty acid composition (or other obvious phenotypes) were observed in elo-3(RNAi) animals.
14965.m00428	F57C7.2b	Embryonic lethal, abnormal organism morphology, larval arrest/lethal, slow growth
14980.m02814	VW02B12L.1	Embryonic lethal. vha-6(RNAi) animals arrest as L1 larvae and remain alive (but do not grow) for one week

BMW00144.486	Y95B8A.10b	axon guidance abnormal, cord commissures fail to reach target, ventral cord patterning abnormal,
12621.m00167	F53H8.4	lethal, embryonic lethal, morphology abnormal

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