Profiling of gender-regulated gene transcripts in the filarial nematode *Brugia malayi* by cDNA oligonucleotide array analysis

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Abstract

Microarray technology permits high-throughput comparisons of gene expression in different parasite stages or sexes and has been used widely. We report the first use of this technology for analysis of gene expression in filarial male and female worms. The slide array (comprised of 65-mer oligos representing 3569 EST clusters) was spotted with sequences selected from the extensive *Brugia malayi* EST database ([http://zeldia.cap.ed.ac.uk/fgn/brugia.php](http://zeldia.cap.ed.ac.uk/fgn/brugia.php)). Arrays were hybridized with Cy dye labeled male and female cDNA. The experimental design included both biological and technical (dye-flip) replicates. The data were normalized for background and probe intensity, and the relative abundance of hybridized cDNA for each spot was determined. Genes showing two-fold or greater differences with \( P < 0.05 \) were considered gender-regulated candidates. One thousand one hundred and seventy of 2443 clusters (48%) with signals above threshold in at least one sex were considered as gender-regulated gene candidates. This included 520 and 650 clusters up-regulated in male and female worms, respectively. Fifty of 53 (94%) gender-regulated candidate genes identified by microarray analysis were confirmed by real-time RT-PCR. Approximately 61% of gender-regulated genes had significant similarity to known genes in other organisms such as *Caenorhabditis elegans*. Many *C. elegans* homologues of these genes have been reported to have reproductive phenotypes (sterility or abnormal embryo development) by RNA interference. This study has provided the first broad view of gender-regulated gene expression in *B. malayi*; this should lead to improved understanding of reproduction in filarial nematodes. More generally, this approach holds great promise as a means of studying stage-specific or tissue-specific gene expression in parasitic nematodes.

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Keywords: Microarray; *Brugia*; Filarial; Gene expression; Reproduction; Nematode

1. Introduction

Filarial nematodes cause important tropical diseases in humans such as onchocerciasis ("river blindness" caused by *Onchocerca volvulus*) and lymphatic filariasis ("elephantiasis" caused by *Brugia malayi*, *B. timor* and *Wuchereria bancrofti*). The parasites are dioecious, and they exhibit marked sexual dimorphism. Brugia adult worms live in lymphatic vessels. The ovoviviparous females release microfilariae (MF) that are ingested by insects; these are essentially equivalent to the L1 stage of other nematodes. Microfilariae molt twice in competent insect vectors to become infective stage larvae (L3) that are infective to humans. L3 molt twice in the human host and to become adult worms that are reproductively active for years. Obviously, parasite reproduction is necessary for transmission, and transmission is necessary for reproduction. Improved understanding of the reproductive biology of filarial worms may provide new insights into the epidemiology of filariasis and lead to new tools for controlling these diseases. Basic research may identify new...
targets for intervention that interfere with egg, microfilarial, or sperm production [1,2]. Filaria researchers stand to benefit from extensive functional genomic studies that have been performed on the free-living nematode Caenorhabditis elegans [3,4]. Since filarial parasites share many genes with C. elegans [5], information available on genes involved in reproduction of C. elegans is an important starting point for parallel studies in filarial worms [6]. However, there are important differences between filarial worms and C. elegans. For instance, while adult filarial worms exist as males (XY) and females (XX), C. elegans has males and hermaphrodites but no true females and no Y chromosome.

The large expressed sequence tag (EST) database and extensive genomic sequence information available for B. malayi [7–9] provide a solid foundation for studying the molecular biology of this organism using gene expression profiling and functional genomics approaches [10–13]. We have previously identified a small number of sex-regulated genes in B. malayi worms by differential display RT-PCR and real-time RT-PCR [11,12]. One of these genes (microfilarial sheath protein) was recently shown to be essential for normal production of microfilaria by RNA interference (RNAi) [13]. However, the pace of progress with this “molecule by molecule” approach is limited.

Microarrays can be used to rapidly assess and quantitate relative levels of expression of thousands of genes in parallel [14,15]. Early experience with small-scale arrays has demonstrated the potential value of this approach for studying gene expression in parasites [16–19]. We now report production of microfilaria by RNA interference (RNAi) male production of microfilaria by RNA interference (RNAi) male worms and a reciprocal dye-flip replicate. Since biological duplicates were tested, a total of four DNA microarrays were synthesized from two different male and female RNA samples (independently prepared as biological replicates).

2. Materials and methods

2.1. Parasite materials

Adult B. malayi worms were isolated from the peritoneal cavity of infected jirds (Meriones unguiculatus) obtained from the NIAID Filaria Repository (University of Georgia, Athens, GA). Male and female worms were carefully separated by size; broken worms were discarded. The worms were washed and immediately frozen at −80 °C.

2.2. RNA isolation and probe preparation

Worms (usually 30 female and 100 male adult worms per batch) were crushed under liquid nitrogen with a ceramic mortar and pestle and extracted in TRIzol reagent (Invitrogen, Carlsbad, CA) as previously described [12]. The accuracy of worm gender separation was assessed by RT-PCR with B. malayi major sperm protein primers (BmMSP) and B. malayi embryo-associated fatty acid-binding protein primers (BmEFAB-1) [11]. RNA quality was assessed with a model 2100 Bioanalyzer (Agilent, Palo Alto, CA). cDNA was synthesized from 5 to 7 μg each of male and female total RNA samples using 3DNA capture sequence primers (3DNA Array 350 Detection system, Genisphere, Hatfield, PA) and SuperScript II Reverse Transcriptase (Gibco BRL, Gaithersburg, MD) for each probe according to standard protocols. cDNA was concentrated by Microcon YM-100 filter (Millipore) and stored at −80 °C. cDNA was synthesized from two different male and female RNA samples (independently prepared as biological replicates).

2.3. Microarray fabrication

B. malayi clusters for arrays were selected from 8392 clusters generated by the Filarial Genome Project and posted at the website http://zeldia.cap.ed.ac.uk/fgn/brugia.php [7]. These clusters were derived from 15 cDNA libraries that represent the major B. malayi life cycle stages. They represent approximately 40% of the total number of predicted genes for B. malayi [8]. Clusters with multiple ESTs, or with detectable similarity to proteins in public databases using BLAST [20], and sequence permitting design of a unique 65-mer oligonucleotide were chosen for inclusion on the array. Oligonucleotides were synthesized from the consensus sequence of selected clusters (n = 3569) by standard methods by Illumina (San Diego, CA). The oligonucleotides (50 nM in 3 × SSC with 0.75 M betaine) were printed in duplicate on MWG Epoxide slides (MWG Biotech Inc, High Point, NC) by a locally constructed linear servo arrayer (after the DeRisi model, http://derisilabs.ucsf.edu/).

2.4. Data processing and analysis

Slides were scanned immediately after hybridization on a ScanArray Express HT Scanner (Perkin-Elmer, Boston, MA) to detect Cy3 and Cy5 fluorescence at 543 and 633 nm, respectively. Laser power was kept constant for Cy3/Cy5 scans, and photomultiplier tube values were 69 and 60 V, respectively. An additional scan was done for each slide with the PMT set for 54 and 46 V. The high PMT scan was done in order to maximize signal from low intensity spots. Likewise,
the low PMT scan was done in order to characterize differential hybridization for high intensity spots which appeared saturated in the high PMT scan. Gridding and analysis of images was performed with ScanArray software Express V2.0 (Perkin-Elmer, Boston, MA). Each spot was defined on a pixel-by-pixel basis, using a modified Mann-Whitney statistical test. The resultant values were background subtracted and Lowess [21] normalized by using GeneSpring 6.1 software (Silicon Genetics, Redwood City, CA). Twenty percent of the data were used to calculate the Lowess fit at each point. Oligonucleotide elements that received “present” intensity > 200 intensity unit or the signal/background > 2 called in all four microarrays and displayed > 700 or > 127 background subtracted intensity units (high or low PMT settings, respectively) in two of four channels for either Cy3 or Cy5 were identified, and all others were excluded from the analysis. The log2 ratio of median dye intensities for each remaining element was averaged across all four microarrays. Data were filtered using the Student’s T-test function in GeneSpring. Genes with differences with P-values < 0.05 in either the high or the low PMT scans and that had arithmetic ratios ≥ 2.0 were considered to be significantly gender-regulated. The best P-value obtained with high or low PMT scans and its accompanying arithmetic ratio were reported. Multiple testing corrections were not used in order to minimize type-2 error.

2.5. Real-time RT-PCR to confirm selected gender-regulated candidate genes identified by microarray analysis

Selected gender-regulated gene candidates from the microarray analysis were chosen for confirmation studies by real-time RT-PCR as previously described in detail [12]. Briefly, complementary DNA primers (primer sequence information is available upon request) were designed from EST sequences obtained from GenBank with Primer Express software (Version 1.0, PE Applied Biosystems, Foster City, CA). The PCR reactions were carried out in 96 well microtiter plate wells in a 25 μl reaction volume with SYBR Green Master Mix (PE Biosystems, Forster City, CA). The PCR reactions were carried out in 96 well microtiter plate wells in a 25 μl reaction volume with SYBR Green Master Mix (PE Applied Biosystems, Foster City, CA) with optimized concentrations of specific primers. Experimental design and relative quantification calculations were carried out according to RQ software (Relative Quantification (RQ) ABI Prism 7000 Sequence Detection System). Nicotinamide adenine dinucleotide dehydrogenase subunit 1 (BMC02280, NADH subunit 1) was used as a not gender-regulated internal control [12].

2.6. Homology search of B. malayi genes

A similarity search was performed using WU-BLASTX and WU-TBLASTX (https://blast.wustl.edu/); [20] with 1170 gender-regulated cluster consensus sequences as queries versus multiple databases including C. elegans proteome (Wormpep version 132) (Wellcome Trust Sanger Institute, unpublished), non-redundant protein GenBank (11/14/04) [22] and nematode nucleotide sequences [23] with B. malayi ESTs removed. Homologues were reported for E-values less than or equal to 1e − 20.

To identify cases in which C. elegans homologues of B. malayi clusters have been surveyed for phenotypes in C. elegans using RNAi the clusters with significant matches to C. elegans proteins were cross-referenced to a list of 17913 C. elegans genes for which information on RNAs-induced phenotypes was available (http://www.wormbase.org/) [24,25]. All matches with similarities with E-values less than or equal to 1e − 20 were reported.

3. Results

3.1. Gender-regulated gene transcripts were reproducibly detected by oligo arrays

As our study represents the first use of oligonucleotide microarrays to study gene expression in B. malayi, we took great care to ensure the validity of our results. Our first objective was to ensure that similar levels of high quality total RNA was isolated from each sexually mature parasite population. Bioanalyzer 2100 results showed that all RNA samples isolated from male and female worms had equivalent quality and displayed minimal degradation (data not shown). Additional evidence that equivalent quantities of male and female total RNA were used for each hybridization experiment was obtained by examining relative expression levels of internal controls [NADH subunit 1 (BMC02280) and histone H3 (BMC00764)] previously shown to be expressed equally in both sexes by real-time RT-PCR [12]. Expression of these control genes was equal in male and female worms by microarray analysis.

3.2. Expression profiling

A complete list of clusters for the arrays, oligonucleotide sequences, and hybridization data is posted at the NCBI GEO database [26] http://www.ncbi.nlm.nih.gov/projects/geo/query/acc.cgi?acc=GPL1483. Of 3569 clusters represented on the array, 2443 had hybridization signals above the threshold for at least one sex. One thousand one hundred and seventy (48% of these clusters and 33% of total clusters on the microarray) met our criteria for gender-regulated expression (two-fold or greater differences and P-values < 0.05) (Fig. 1). Of 1170 clusters, 455 clusters (39%) had no significant similarity to known proteins in other organisms. This is somewhat higher than the percentage of novel genes represented on the array (1160 of 3569 clusters or 32.5%). The differentially expressed clusters included 520 and 650 clusters up-regulated in male and female worms, respectively. The frequency distribution of higher/lower expression ratios is shown in Fig. 2. Although there were more female up-regulated gene candi-
Fig. 1. Expression profiling of clusters from *Brugia malayi*. This is scatter plot of the mean normalized fluorescence intensity values from the male versus female comparison derived from the low PMT scan. Expression values that are ≥2-fold (the flanking green lines) and *P* < 0.05 in male are blue (upper left) and those that are ≥2-fold higher and *P* < 0.05 in females are dark pink (lower right). White spots are less than two-fold unregulated. Spots outside the two-fold line that are not red or blue have a *P* > 0.05.

Fig. 2. Frequency distribution of expression ratios obtained from *B. malayi* clusters on a microarray probed with male- and female-specific cDNA. The signal intensity when probed with Cy5-labelled cDNA derived from female worms was divided by that of the Cy3-labelled cDNA derived from males or vice versa and then log2 transformed. Expression ratios were plotted on the abscissa and frequency was plotted on the ordinate.

Table 1

<table>
<thead>
<tr>
<th>Description</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytoskeleton</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>Muscle</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Major sperm protein</td>
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<td>0</td>
</tr>
<tr>
<td>Oxidation-reduction</td>
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<td>0</td>
</tr>
<tr>
<td>Protease</td>
<td>4</td>
<td>0</td>
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<tr>
<td>Actin-1</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Zinc-finger proteins</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>DNA synthesis</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>NADH dehydrogenase subunit 5</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>and 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein synthesis</td>
<td>6</td>
<td>27</td>
</tr>
<tr>
<td>Phosphatase/kinase</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>ATP synthase</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Citric acid cycle enzymes</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Histones</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Egg/larval development</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td><em>Brugia</em> serine protease</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>inhibitors (bio-gps-1 and 2)</td>
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<td>5</td>
</tr>
<tr>
<td>Chaperonin/heat shock protein</td>
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</tr>
<tr>
<td>GTP-binding/Ras-related</td>
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<td>3</td>
</tr>
<tr>
<td>Receptors (hormone, sperm and nuclear)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ion channels</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

3.3. Independent confirmation of gender-regulated genes identified by the oligo array with real-time RT-PCR

To verify the gender-regulated genes identified by the arrays, we examined gene expression in adult male and female worms by real-time RT-PCR analysis. Fifty-five gender-regulated gene candidates identified by microarray analysis were selected for real time RT-PCR analysis. Twenty-eight of these genes were up-regulated in female...
Male up-regulated clusters can be divided into four groups: (1) genes previously reported to be male up-regulated in filarial parasites, such as major sperm proteins (MSPs) [11,12]; (2) genes reported to be male up-regulated in other nematodes, such as a transcription factor (zinc finger, BMC07636 and BMC096240), a malate dehydrogenase (BMC04971), and a serine/threonine phosphatase (BMC10182) [18,27–29]; (3) genes that were newly identified as male up-regulated, such as genes with sequence similarity to muscle and cytoskeleton proteins (e.g. myosin, paramyosin, intermediate filament, troponin and actin) and genes related to cellular and extracellular extracellular metabolism (e.g. cuticular glutathione peroxidase (BMC02884, BMC06710 and BMC12436), cytochrome oxidases (BMC04127)); (4) novel genes (38% of the total). MSP proteins and MSP-like domain proteins, serine/threonine phosphatases and kinase were most abundant among highly male-biased expressed known genes (expression ratios \( \geq 10 \), Table 2); these accounted for 20 and 10% of the total, respectively. The *B. malayi* homologues (BMC06588 and BMC11125) of *C. elegans* gene coding for F34D7.3 protein (ortholog of the human gene gonadotropin release hormone receptor gene) were also highly up-regulated in males.

### 3.5. Female up-regulated clusters

Of the 650 candidate female-regulated clusters, 395 (61%) encoded proteins with homologues in other organisms (Suppl. Table 3). Of these, 303 clusters (77%) had a reproduction phenotype such as sterility. Male up-regulated clusters with significant similarity to known genes fell into four groups: (1) genes previously confirmed as being female up-regulated including microfilarial sheath protein, high mobility protein, fatty acid binding protein and caveolin in filarial nematodes [12,13]; (2) genes previously reported to be female up-regulated in other nematodes. These included genes involved in transcription and translation (e.g. nuclear hormone receptors, RNA polymerase, tRNA synthetase, ribosomal proteins and elongation factor), protein processing/trafficking (heat shock proteins and chaperonin), and genes involved in embryonic development and growth in *C. elegans* (e.g. protein F32B11.3-BMC07645, BO250.1-BMC00336 and F33B1.4-BMC09458); (3) genes newly identified as female-regulated including those involved in nematode reproduction (such as egg receptor for sperm, *B. malayi* serine proteinase inhibitor, collagen, multi-drug resistance factor, and tubulin); (4) novel genes (39% of the total). Known genes with expression ratios \( \geq 10 \) are listed by functional class in Table 2. Female up-regulated genes identified previously such as sheath proteins, caveolin and high mobility protein were also highly female up-regulated in this study.

### Table 3

<table>
<thead>
<tr>
<th>Identified by microarray</th>
<th>Confirmed by real-time RT-PCR</th>
<th>Identified by real-time RT-PCR</th>
<th>Confirmed by real-time RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>27</td>
<td>27</td>
<td>9</td>
</tr>
<tr>
<td>Male</td>
<td>26</td>
<td>23</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>50 (94%)</td>
<td>17</td>
</tr>
</tbody>
</table>

![Fig. 3](image-url)
4. Discussion

This paper presents the first use of oligonucleotide microarrays to profile gene expression in a filarial nematode. We focused on gender in this paper because of the relative ease of obtaining RNA from adult worms and the availability of pilot data on gender-biased genes from our prior work [11,12]. Our results show that gene expression profiles of ∼48% (1707/2443) of clusters that hybridized with cDNA from adult worms were gender-regulated. This is a higher percentage than that reported for C. elegans (∼12%) [27] and lower than that reported for Trichostongyulus vitrinus (72%) [18]. These differences may be due to selection bias of genes represented on the chip and may not reflect biological differences between these nematodes. The C. elegans microarray contained 17,871 genes and represented about 94% of the 18,967 genes annotated, while the T. vitrinus microarray contained only 716 ESTs from gender-selected cDNA libraries. There are also biological differences between these nematode species. For example, the uteri of mature Brugia female worms are often full of microfilariae, which are essentially equivalent to the L1 stage in C. elegans and T. vitrinus. Obviously, female up-regulated genes in B. malayi will include genes expressed by developing microfilariae.

Our experiments identified 520 male up-regulated genes and 650 female up-regulated genes with expression ratios ≥2 that were significant at the 95% confidence level. Considering that females produce eggs and embryos, we expected to find many more female up-regulated clusters than male up-regulated clusters. The finding that the mean expression ratio for male up-regulated clusters was higher than that for female up-regulated clusters was consistent with results reported for male up-regulated clusters in C. elegans [27].

Several lines of evidence support the validity of microarray results obtained in this study. First, reproducibility was good in biological replicate samples. Seventy-nine percent of the genes with gender-regulated expression in one biological sample also had gender-regulated expression in a second biological sample. Second, 94% of selected gender-regulated clusters identified by microarray were confirmed independently by real-time RT-PCR (Table 3). Third, all genes previously reported to have gender regulated expression by real-time RT-PCR [12] were confirmed to have gender regulated expression by microarray, and there was a strong correlation between fold-difference values obtained by these two methods. Fourth, many of the genes identified in our array experiment have reproductive phenotypes in C. elegans.

4.1. Gene expression in males

Thirty-eight percent (199/520) of the male-regulated B. malayi clusters were novel. Forty percent (210/520) of the male-regulated clusters had a C. elegans homologue, and many of these have been reported to be male up-regulated in C. elegans when compared with hermaphrodites [27]. Genes involved in reproductive processes (e.g. major sperm proteins), molecules in energy storage and supply, cytoskeleton and muscle-related molecules, and enzymes in cellular metabolism especially in oxidation-reduction (cysticidal glutathione peroxidase and cytochrome oxidase) were most notable amongst male up-regulated annotated clusters. The differential expression of these transcripts in male worms is likely to be related to their biological functions in reproduction.

Major sperm proteins (MSPs) are nematode-specific cytoskeleton proteins comprising ∼15% of total sperm protein [30,31]. These are among the most highly represented ESTs in B. malayi [8]. MSPs were also highly represented in male-enriched datasets for both T. vitrinus and C. elegans [3]. MSPs are involved in the movement of the nematode sperm [32]. Recent studies show that MSPs also have a signaling role in oocyte production and maturation in C. elegans [33,34]. The retention of this MSP signaling function in Ascaris suum [34] suggests that this may also be present in filarial nematodes. Thus, the finding that 20% of highly male-up-regulated clusters in our study were either MSPs or homologues of C. elegans genes that contain an MSP domain is impressive but not surprising (Table 2).

In addition to MSPs, clusters with significant similarity to C. elegans serine/threonine kinases and phosphatases were also common among highly male up-regulated transcripts (Table 2). These genes are also highly expressed in C. elegans germ line tissue [3] and in the T. vitrinus male-selected cDNA library [18]. These enzymes might play important roles in regulating sperm maturation by post-translational modification [3] and in signaling cascades or protein modification within the oocyte following fertilization [1,29]. Knockouts of the C. elegans homologue of a male-enriched serine/threonine protein phosphatase gene Od-nup1 from the parasitic nematode Oesophagostomum dentatum caused sterility of hermaphrodites [1,29] through impaired sperm function [35]. The B. malayi homologue of this serine/threonine phosphatase (BMC10182) was highly up-regulated in males with expression ratios of 37 by microarray and 101 by real-time RT-PCR.

Several other groups of male up-regulated clusters contained special domains thought to be related to reproductive processes. For instance, B. malayi homologues of the C. elegans PDZ-domain (C25G4.6, pfam00595) (clusters BMC11959 and BMC04055) were highly male up-regulated (expression ratios ≥20). This is consistent with the findings in the T. vitrinus male-selected cDNA library and in C. elegans microarray experiments [18,27]. The PDZ domain, a common modular protein-interaction domain, is believed to be involved in diverse signal transduction pathways [36]. RNAi of the C. elegans homologue with a PDZ domain (C25G4.6) caused sterility in the treated worms or their progeny, suggesting a role in gamete development [24,35]. In addition to the PDZ domain containing proteins, two other clusters highly expressed in B. malayi males had significant similarity to a PAZ domain (pfam02170) containing protein in C. elegans (ZK757.3B). This domain is found in the PIWI-domain gene
family that is essential for producing and maintaining germ-
line stem cells responsible for gametogenesis in Drosophila
[37]. PDZ domain gene family members are enriched in the
C. elegans germ-line-intrinsic group (genes expressed similarly
germ lines making only sperm or only oocytes) [3].

Several cytoskeleton and muscle-related molecules were
male up-regulated with ratios ranging from 2 to 7. These
certain 33 clusters with sequence significant similarity to
myosin, paramyosin, troponin and actin (Table 1). Genes
encoding tenden-associate molecules such as actin, tropomyosin and dynein light chain have been previously
been shown to be differentially expressed in adult Schistosoma
mansoni male [38,39]. The explanation suggested for this
was that muscles are larger (with more tenden and muscle)
more active in copulation than females [40,41]. How-
ever, this may not apply to B. malayi, since Brugia females
seem to be as active as males in vitro, and there is no obvious
difference in relative muscle content.

Another possibility muscle-related male up-regulated B.
malayi cluster (BMC01860) is a homologue of C. elegans
gene Y1A5A.1, a male-enriched LIM domain (pfam00412)
containing protein with a homologue in T. virinutus [3,18,27].

Oncocerciasis patients and animals vaccinated with irradiat-
ed filarial L3 produce strong antibody responses to a related
protein cloned from the filarial worm Acanthocheilonema
viteae (AvL3-1) [42]. LIM domain-containing proteins are
involved in diverse biological processes including cytoskele-
ton organization and cell lineage specification by mediat-
ing protein-protein interactions. The reported localization of
AvL3-1 (the Oncocerca volvulus homologue of AvL3-1) in
muscle tissue [43] suggests that this LIM domain protein may
interact with muscle proteins.

Several genes encoding enzymes involved in energy pro-
duction (NADH dehydrogenase subunit 5 and 6, BMC12393,
BMC5921) and oxdation of fatty acid oxidation (3-keto-
acyl-CoA thiolase, BMC07758) were highly expressed in
male worms. Enzymes involved in glycolysis and the cit-
tric acid cycle were also male up-regulated in C. elegans
and T. virinutus [3,18]. These findings may reflect the high
energy required for sperm motility, since fatty acids provide
an efficient energy source. In addition to these enzymes, two
Brugia clusters with high male expression ratios were homol-
ogous to C. elegans trehalases. The disaccharide trehalose
has been proposed to have essential physiological functions
in nematodes as a source of energy and as a protectant against
environmental stress [44]. Increased trehalose transcription
may reflect an increased energy requirement in male worms
or their sperm.

4.2. Gene expression in females

Thirty-nine percent (255/650) of female up-regulated B.
malayi clusters were novel. Forty-seven percent (303/650)
of the female up-regulated clusters had significant similarity
to genes from C. elegans. Many of these C. elegans genes
were highly expressed in embryos by microarray experi-
ments [4], and 47% (112/237) of those studied by RNAi
had phenotypes such as embryo lethality, growth defects and
post-embryonic defects. Many of the annotated female up-
regulated clusters in our study were associated with embry-
onic development (fatty acid binding protein, chaperonin-like
protein), eggshell formation (microfilaria sheath protein),
facets involved in transcription and translation (RNA poly-
merase, rRNA-synthetase, ribosomal proteins), and energy
supply (ATP synthase and enzymes in the citric acid cycle).
A few of these genes deserve more comment. A gene cod-
ing for a lipid-binding protein (Bm-1FA-2), has been previ-
osely shown to have female up-regulated expression [11,12].
Its localization in perivitelline fluid of developing embryos
within the uterus of adult female worms supports the hypoth-
esis that this protein is involved in transfer of fatty acids to
developing embryos [45]. The gene coding for microfilaria
sheath protein (Bm-SHP-1), an abundant eggshell protein, has
previously been shown to be female up-regulated in filaria-
lar parasites [12], and the inactivation of MF sheath protein by
RNAi in adult female B. malayi has been reported to inhibit
MF release from female worms [13]. Chaperonins such as
heat shock protein 70 (Bm-HSP-70) have been reported to
have female up-regulated expression in other nematodes [18].
Chaperonins are large, multi-subunit proteins that facilitate
the protein-folding process by providing a protected environ-
ment. Recent studies show that these molecules play a role
in transduction and regulation of cGMP levels [46], and that
they are essential for embryonic development in C. elegans
[47,48]. The notable female up-regulated expression of a heat
shock gene (Bm-shp-70) and chaperonins in our study sug-
gests that these molecules may play a parallel role in B. malayi
reproduction and perhaps also in protein synthesis. The fact
that other genes required for protein synthesis had female up-
regulated expression is consistent with a high requirement for
protein synthesis for egg production and larval development
in female worms.

Several clusters with significant similarity to genes encod-
ing histones 2 and 4 were expressed in a female up-regulated
manner in B. malayi. Histones comprise a family of closely
related, basic DNA-binding proteins that play a role in gene
regulation. Several histones and histone-like proteins are dif-
ferentially expressed between males and hermaphrodites in
C. elegans [20]. RNAi of his-71, a histone-like gene with a
female-enriched homologue in T. virinutus, resulted in embry-
onic lethality in C. elegans [24,25].

Interestingly, genes coding for two B. malayi serine pro-
tenase inhibitors (Bm-spn-1 and Bm-spn-2) had female
up-regulated expression by microarray. Bm-spn-1 has been
reported to be enriched in filarial L3 [49,50], and Bm-spn-
2 has been reported to be expressed only by microfilariae
[51]. Increased expression of Bm-spn-2 in female worms
suggests that this gene is also expressed in developing lar-
vae and microfilariae within female worms. Bm-spn-2 has
been suggested to have a role in immune evasion in filar-
ial parasites [52]. Related mammalian proteins have been
reported to be involved in remodeling of germ line tissues and
migration of germ cells [53]. Additional studies are needed to establish whether these proteins are necessary for filarial reproduction.

Several clusters with significant similarity to transcription regulatory elements, (including high motility group proteins and Y-box factor), had female up-regulated expression, while clusters encoding transcription factors such as zinc finger proteins (BMC07636, BMC06355) were up-regulated in male worms. These elements may be involved in coordinated expression of gender-regulated transcripts in Brugia.

In conclusion, microarray analysis has provided the first broad view of gender-regulated gene expression in B. malayi. It is likely that many of these genes are involved in reproduction (gamete production, gamete function, and embryo development). Much more work will be required to understand how the products of these genes function and interact and to explore their potential as new drug targets. However, we believe this work represents an important step toward a deeper understanding of reproduction in filarial worms. More generally, this approach holds great promise as a means of studying stage-specific or tissue-specific gene expression in parasitic nematodes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molbiopara.2005.05.005.

References


