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Deep insights into *Dictyocaulus viviparus* transcriptomes provides unique prospects for new drug targets and disease intervention

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Abstract

The lungworm, *Dictyocaulus viviparus*, causes parasitic bronchitis in cattle, and is responsible for substantial economic losses in temperate regions of the world. Here, we undertake the first large-scale exploration of available transcriptomic data for this lungworm, examine differences in transcription between different stages/both genders and identify and prioritize essential molecules linked to fundamental metabolic pathways, which could represent novel drug targets. Approximately 3 million expressed sequence tags (ESTs), generated by 454 sequencing from third-stage larvae (L3) as well as adult females and males of *D. viviparus*, were assembled and annotated. The assembly of these sequences yielded ~61,000 contigs, of which relatively large proportions encoded collagens (4.3%), ubiquitins (2.1%) and serine/threonine protein kinases (1.9%). Subtractive analysis in silico identified 6,928 nucleotide sequences as being uniquely transcribed in L3, and 5,203 and 7,889 transcripts as being exclusive to the adult female and male, respectively. Most peptides predicted from the conceptual translations were nucleoplasmins (L3), serine/threonine protein kinases (female) and major sperm proteins (male). Additional analyses allowed the prediction of three drug target candidates, whose *Caenorhabditis elegans* homologues were linked to a lethal RNA interference phenotype. This detailed exploration, combined with future transcriptomic sequencing of all developmental stages of *D. viviparus*, will facilitate future investigations of the molecular biology of this parasitic nematode as well as genomic sequencing. These advances will underpin the discovery of new drug and/or vaccine targets, focused on biotechnological outcomes.

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Keywords

Dictyocaulus viviparous; Bovine lungworm; Next-generation sequencing; Bioinformatics; Transcriptome; *Ancylostoma*-secreted proteins; Drug target prediction

1. Introduction

Parasitic nematodes of livestock are responsible for substantial economic losses due to poor productivity, failure to thrive and deaths (Coles, 2001; Panuska, 2006). Diseases caused by these nematodes cost the meat and livestock industries billions of dollars per annum (Jackson et al., 2007). Lungworms of the genus *Dictyocaulus* (Strongylida: Dictyocaulidae) cause parasitic bronchitis (= dictyocaulosis) in different ruminant hosts, particularly in young animals (Eysker and van Miltenburg, 1988; David 1997; reviewed by Panuska, 2006). The clinical manifestation of dictyocaulosis varies from mild respiratory signs to emphysema and pneumonia, and can result in death in severely affected animals (Coles, 2001; Schnieder et al., 1991; Panuska, 2006). The life cycle of *Dictyocaulus viviparus*, the bovine lungworm, is direct (Anderson, 2001). The adult worms live in the bronchi and bronchioles of cattle; the ovoviviparous females produce eggs from which first-stage larvae (L1) hatch within the lungs or the intestinal tract. L1s are excreted in the faeces and develop, under favourable environmental conditions, into third-stage larvae (L3) within ~4–6 days. The cuticle from the second-stage larva (L2) is retained as a sheath around the L3 and protects this stage from adverse environmental conditions. L3s are ingested by grazing cattle, exsheath, penetrate the intestinal wall and migrate to the mesenteric lymph nodes, where they moult to fourth-stage larvae (L4); L4s are then transported to the lungs, where they penetrate the alveoli and develop into adults (within 3–4 weeks). However, larval stages can undergo arrested development in the lungs of the vertebrate host for up to 5 months (von Samson-Himmelstjerna and Schnieder, 1999; Anderson, 2001)..

Traditionally, the control of *Dictyocaulus* infection and dictyocaulosis has been based on the prophylactic administration of an irradiated larval vaccine (Jarrett et al., 1960) or anthelmintic drugs, such as macrocyclic lactones (Schnieder et al., 1996; Ploeger, 2002). However, the disadvantages of the current (live) vaccination strategies (i.e., instability of the irradiated larvae and inability to confer a sterile immunity and a life-long protection; McKeand, 2000), the unsuccessful attempts to develop a recombinant vaccine and recent reports of emerging anthelmintic resistance (Matthews et al., 2001; Ploeger, 2002; Molento et al., 2006) are driving the search for new intervention targets. The genomic-bioinformatic explorations of fundamental aspects of the molecular biology of *D. viviparus* could provide a basis for a detailed understanding of mechanisms linked to parasite development, survival, reproduction and interaction/s with the bovine host; such advances in knowledge could represent a platform for the identification of essential genes and/or gene products and the subsequent validation in vitro and in vivo of rationally designed nematocides.

Although knowledge of the genomics and cellular biology of lungworms is limited, recent molecular studies used quantitative real-time PCR to elucidate patterns of transcription of individual genes for different developmental stages of *D. viviparus* (see Strube et al., 2007a, 2009a, b) and a suppressive-subtractive hybridization approach to explore differential transcription in hypobiosis-induced versus non-induced L3s (Strube et al., 2007b). Another study (Ranganathan et al., 2007) utilised a conventional (Sanger) sequencing approach to determine and analyse ~4,500 expressed sequence tags (ESTs) from adult *D. viviparus* using a semi-automated bioinformatic pipeline (ESTExplorer; Nagaraj et al., 2007); these authors identified conserved protein domains and linked them to known biological pathways, based on comparative analyses with the free-living nematode *Caenorhabditis elegans* and/or other

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organisms, employing data available in public databases. However, in the latter study, differences in transcription among stages and between sexes of *D. viviparus* were not investigated on a large scale.

Advances in sequencing techniques and computational approaches for the pre-processing, assembly and annotation of sequence data (Morozova and Marra, 2008; Metzker, 2010) are leading to a much better understanding of the transcriptomes of parasitic helminths (e.g., Cantacessi et al., 2010a, b; Wang et al., 2010; Young et al., 2010a, b). In particular, next-generation sequencing technologies (NGS), such as 454-Roche (www.454.com; Margulies et al., 2005) and Illumina-Solexa (www.illumina.com; Bentley et al., 2008), are enhancing our understanding of the molecular processes involved in parasite development, reproduction and interactions with their hosts (see Cantacessi et al., 2010b; Wang et al., 2010). In addition, given that the data files generated by these technologies are often gigabytes (1×10^9) to terabytes (1×10^{12}) in size, such that many web-interfaces are no longer able to cope with large-scale analyses, we recently developed a semi-automated, custom-built bioinformatic workflow system for the detailed analysis and annotation of NGS data (Cantacessi et al., 2010c). In the present article, through a detailed exploration of available NGS data using this integrated system, we have reviewed and substantially expanded the knowledge of the transcriptome of *D. viviparus*, and mined for essential genes in each stage/gender in a first effort to predict new drug targets in this parasite.

2. Next-generation technological approaches

Transcriptomic datasets for the L3, adult female and adult male stages of *D. viviparus* (**SRA_XXXXXXX**), determined using a massively parallel sequencing approach (see Cantacessi et al., 2010a), were annotated and analysed using a custom-built bioinformatic workflow system (Cantacessi et al., 2010c). FASTA and associated files of sequence quality scores for each dataset were extracted from each SFF-file; sequence adaptors were clipped using the ‘sff_extract’ software (available at http://bioinf.comav.upv.es/sff_extract/index.html). For each stage/sex, sequences were assembled de novo with sequence quality scores using the Contig Assembly Program v.3 (CAP3; Huang and Madan, 1999), employing a minimum sequence overlap length of 40 nucleotides and an identity threshold of 90%. ESTs (and associated sequence quality scores) from all datasets were then combined and assembled using the same parameters as described above. Sequences from adult male, female and L3 of *D. viviparus* (n = 4,463; www.ncbi.nlm.nih.gov) available from previous studies (Ranganathan et al., 2007; Strube et al., 2007b) were included for comparison. A small number of sequences in the present data (n = 103; i.e., 0.1% of 61,134 contigs) with a perfect match to those available for *Bos taurus* (GenBank accession numbers T25280-GW425382; e-value cut-off: < 1e-15) were excluded. Contigs and singletons in each of the assemblies were then compared (using BLASTn and BLASTx algorithms; Altschul et al., 1997) with sequences available in public databases, including NCBI (www.ncbi.nlm.nih.gov) and the EMBL-EBI Parasite Genome Blast Server (www.ebi.ac.uk), in order to identify putative homologues (cf. Koonin, 2010) in *C. elegans*, other nematodes and organisms other than nematodes (e-value cut-off: < 1e-05). WormBase (release WS200; www.wormbase.org) was interrogated extensively for relevant information on *C. elegans* homologues, including transcriptomic, proteomic, RNAi phenotypic and interactomic data.

Peptides were conceptually translated from contigs and singletons using ESTScan (Iseli et al., 1999). Predicted peptides were classified functionally by InterPro and/or Pfam terms using InterProScan (http://www.ebi.ac.uk/InterProScan/; Hunter et al., 2009) and HMMR (http://hmmer.janelia.org/; Eddy, 1999), respectively. Predicted peptides were then assigned gene ontology (GO) terms (Ashburner et al., 2000) based on their homology to conserved

domains and protein families. Peptides were mapped to respective pathways in *C. elegans* using the KEGG Orthology-Based Annotation System (KOBAS; Wu et al., 2006); conserved metabolic pathways were displayed using the iPath tool (Letunic et al., 2008).

All sequences (i.e., contigs and singletons) in each of the three assemblies were subtracted from one another (in both directions) by in silico-subtraction using a BLASTn algorithm (Altschul et al., 1997), set at a stringent cut-off (e-value cut-off: < 1e-15; see Cantacessi et al., 2010b). Peptides corresponding to transcripts that were unique to a particular dataset were assigned parental (= level 1) InterPro terms and compared, using a BLASTp algorithm (Altschul et al., 1997; e-value cut-off: < 1e-15), with peptides inferred from the assembly of sequences from the combined dataset.

Interaction networks for *C. elegans* homologues of differentially transcribed molecules were predicted using an established method (Zhong and Sternberg, 2006), and the druggability of *C. elegans* homologues unique to a particular *D. viviparus* dataset or common to all datasets was predicted (Cantacessi et al., 2010a). Briefly, InterPro domains of predicted proteins were compared with those linked to known, small molecular drugs, which follow the 'Lipinski rule of 5' regarding bioavailability (Lipinski et al., 1997; Hopkins and Groom, 2001). GO terms were mapped to Enzyme Commission (EC) numbers, and a list of enzyme-targeting drugs was compiled based on all data available in the BRENDA database (www.brenda-enzymes.info; [Robertson, 2005; Chang et al., 2009]). Each predicted drug target was selected based on: (i) the presence of homologues linked to gene peturbation phenotypes in *C. elegans*, *Saccharomyces cerevisiae*, *Drosophila melanogaster* and *Mus musculus* and (ii) their absence from the bovine host. The *C. elegans* homologues identified were ranked according to the 'severity' of non-wildtype RNAi phenotypes (including lethality or sterility at different life cycle stages; see www.wormbase.org; release WS200).

3. Integrated bioinformatic exploration of available transcriptomic datasets, and mining for potential drug targets

3.1. The transcriptomes representing D. viviparus

A total of 2,952,411 ESTs (454 bases ± 86 in length; i.e., average length ± standard deviation) generated by 454 sequencing from L3, and adult female and male *D. viviparus* were subjected to detailed annotation and analyses using our custom-built workflow system. All previously published ESTs (available via www.ncbi.nlm.nih.gov/; Ranganathan et al., 2007; Strube et al., 2007b) represented ~0.1% of this total number. Following the clipping of adapter sequence residues from all ESTs, only reads of >100 bases (n = 2,943,357; 99.7%) were considered for further analysis. The total numbers of contigs assembled for the three individual datasets are given in Table 1. Briefly, ESTs representing the L3 were assembled into 7,661 contigs and 27,455 singletons, while sequences representing adult female and male were assembled into 9,946 contigs and 28,840 singletons and 9,226 contigs and 28,784 singletons, respectively. The assembly of sequences from all three datasets yielded a total of 6,119 contigs and 55,015 singletons. The mean length (= number of bases) and standard deviation (SD) of assembled contigs included in each of the individual and the combined datasets are given in Table 1. The female dataset had the largest number of sequence clusters with homologues in *C. elegans* (n = 17,979), in other parasitic nematodes (n = 16,100) and in organisms other than nematodes (n = 19,566) (Table 1).

In total, 59,606 peptides were predicted for all sequences from the combined assembly of the three datasets (Table 1); 20,974 (34%) of them could be mapped to known proteins defined by 1,460 different domains, the most represented being 'collagen triple helix repeat' (Pfam code: PF01391; 4.3% of the peptides mapping to a conserved protein motif), 'ubiquitin' (PF00240; 2.1%) and 'serine/threonine protein kinase domain' (PF00069; 1.9%)

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(Table 2). GO annotation allowed 12,151 (20.3%) inferred proteins to be assigned to 678 'biological process', 231 'cellular component' and 770 'molecular function' terms (Table 1). The predominant terms were 'translation' (GO:0006412; 4.7% of the predicted peptides assigned GO terms), 'oxidation reduction' (GO:0055114; 4.3%) and 'proteolysis' (GO:0006508; 4.2%) for 'biological process'; 'membrane' (GO:0016020; 8%), 'nucleus' (GO:0005634; 7.5%) and 'intracellular' (GO:0005622; 7.4%) for 'cellular component' and 'ATP binding' (GO:0005524; 12%); 'DNA binding' (GO:0003677; 66.8%) and 'protein binding' (GO:0005515; 5.7%) for 'molecular function' (Table 3). Proteins inferred from the combined assembly were predicted to be involved in 245 different biological pathways, defined by 1,889 unique KEGG terms, of which 'peptidases', 'protein kinases' and 'chaperones and folding catalysts' predominated (see Appendix A). Metabolic pathways, defined by KEGG terms inferred from predicted peptides and mapped to the complement of known pathways in *C. elegans*, are shown in Fig. 1.

The numbers of peptides predicted from EST clusters in each of the three datasets (i.e., L3, female and male), together with the corresponding number of peptides mapped to known protein domains/motifs and/or assigned GO terms, are reported in Table 1. For both L3 and adult female, the 'collagen triple helix repeat' (PF01391; 5.8% and 8.2% of 5,471 and 5,768 protein domains identified in the L3 and female, respectively) and 'ubiquitin' (PF00240; 4.5% and 3.7%, respectively) were most commonly detected. Amongst the predicted protein domains identified in the male dataset, 'major sperm protein' (PF00635; 43.3% of 5,565 protein domains) and 'SCP-like extracellular' (PF00188; 13.2%) were mostly represented (Appendix B). The descriptions of GO terms linked to predicted peptides in each of the three datasets (i.e., L3, female and male), according to the categories 'biological process', 'cellular component' and 'molecular function' are given in Appendix C. For L3, pathway mapping using KOBAS predicted 193 distinct pathways, of which the most represented were 'ribosome', and 'receptors and channels' (see Appendix A). The predicted peptides in the female dataset could be linked to a total of 164 biological pathways, of which 'receptors and channel' and 'protein kinases' were most commonly represented (Appendix A). For the male, pathway mapping predicted 138 biological pathways, of which 'glycolysis/gluconeogenesis' and 'fructose and mannose metabolism' were most common (see Appendix A).

3.2. Molecules uniquely transcribed in different stages/sexes, and the inference of drug targets

Subtractive analysis in silico identified 6,928 nucleotide sequences as being uniquely transcribed in the L3 stage, and 5,203 and 7,889 transcripts as exclusive to the female and male dataset, respectively. The specificity of this in silico-subtraction approach has been demonstrated elsewhere (Cantacessi et al., 2010b). Of the 2,050 functional domains inferred for predicted peptides unique to the L3 dataset, 'nucleoplasmin' (PF03066; n = 33), and 'Nop-14-like protein (PF04147; n = 22) were most commonly represented (Table 2). Among the 1,741 functional domains mapped to predicted peptides unique to the female dataset, the 'von Willebrand factor' (PF00094; n = 77) and 'serine/threonine protein kinase' (PF00069; n = 25) domains had the greatest representation (Table 2). Of the 2,945 protein motifs linked to predicted peptides unique to the male dataset, 'major sperm protein' (PF00635; n = 380) and 'SCP-like extracellular' (PF00188; n = 142) had the highest representation (Table 2). The descriptions of the most commonly detected GO terms linked to predicted peptides unique to each of the three datasets are given in Table 3. A schematic representation of the biological pathways inferred by KOBAS for predicted peptides unique to each of the three datasets, mapped to the complement of known metabolic pathways in *C. elegans*, is given in Fig. 2.

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Probabilistic genetic interaction networking predicted 5,196 *C. elegans* homologues, representing sequence clusters unique to *D. viviparus* L3, to interact directly with a total of 2,435 other genes (range: 1–271; not shown), including some (e.g., *dbl-1*; Fig. 3) that are essential to the regulation of body length and size (see www.wormbase.org). The 5,093 *C. elegans* homologues of contigs and singletons unique to the adult female of *D. viviparus* were predicted to interact directly with a total of 2,394 other genes (range: 1–260). Amongst them were genes involved in embryonic, larval and vulval development (i.e., *glp-1*, *lin-12*, *let-92* and *mig-2*) and behaviour and egg-laying (i.e., *goa-1*) (Fig. 3; www.wormbase.org). A total number of 7,396 *C. elegans* homologues of male-unique molecules, were predicted to interact with 2,727 (range: 1–122) other genes, respectively, including some involved in sperm maturation and development (i.e., *ima-3* and *air-2*) (Fig. 3; www.wormbase.org).

Of peptides inferred to be unique to L3 (n = 4,902), adult female (4,947) and adult male (6,003) of *D. viviparus*, 609, 245 and 526 were associated with 'druggable' InterPro domains and/or EC numbers, respectively (Table 4). For individual stages/sexes, these 'druggable' molecules had significant homologies (e-value cut-off: >1e-05) to ~1,200–2,300 *C. elegans* homologues linked to RNAi phenotypes, of which ~56–60% were unequivocally linked to lethal or sterile phenotypes (Table 4). Of peptides predicted from the combined assembly of all three sequence datasets (i.e., *D. viviparus* L3, adult female and adult male), 5,931 were linked to druggable InterPro domains and/or EC numbers (Table 4). These molecules had significant homologies (e-value cut-off: > 1e-05) to ~2,300 *C. elegans* genes, of which ~73% were linked to lethal or sterile RNAi phenotypes (Table 4). Druggable molecules, linked to a lethal RNAi phenotype, predicted for each of the datasets or common to all three datasets and examples of compounds (from the BRENDA database) are listed in Table 5. For instance, the compounds oligomycin and paclitaxel were inferred to specifically target an homologue represented by gene F09F9.4, whereas tetracyclin and vanadate as well as streptovaricin and tagetotoxin targeted molecules represented by the homologues H27M09.1 and *DCR-1*, respectively (see Table 5).

4. Conclusions – exciting prospects for biological insights, disease intervention and biotechnological outcomes

The present review article substantially enhances our knowledge of the complement of molecules transcribed in different stages and both sexes of *D. viviparus*. Indeed, contigs assembled from combined sequence data for L3, adult female and male could be mapped with high confidence to ~13,000 unique *C. elegans* homologues (Table 1), thus increasing the number of newly discovered *D. viviparus* genes by ~8-fold (cf. Ranganathan et al., 2007). In addition, this result supports those of previous comparative analyses of genetic data sets, showing that strongylid nematodes share at least ~60% of genes with *C. elegans* (see Blaxter, 1998; Ruvkun and Hobert, 1998; Parkinson et al., 2004). This information has indicated that this free-living nematode provides a surrogate for investigating the function/s of some key genes/gene products in strongylids, further supported by successful genetic complementation studies (Britton and Murray, 2002; Lok, 2009; Hu et al., 2010; Stepek et al., 2010).

Of the ~60,000 peptides predicted from EST clusters assembled from all sequence data for all stages/sexes included (L3, and adult female and male), ~30% could be mapped to known peptides defined according to their conserved protein domains (see section 3.1). Of the 1,460 unique domains identified, the most represented were 'collagen triple helix repeat' (PF01391), 'ubiquitin' (PF00240) and 'serine/threonine protein kinase' (PF00069). Collagens are a large family of structural proteins representing the major component of the extracellular matrix (ECM) of all animals and, in particular, of the external, protective cuticle of nematodes (Page and Winter, 2003). Nematode collagens have been shown to be

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most similar, although smaller in size, to vertebrate 'fibril-associated collagens with interrupted triple helices' collagens (Shaw and Olsen, 1991) and act as molecular bridges required for the organization and stability of ECM (Page and Winter, 2003). Genes encoding collagens have been well characterised in *C. elegans*, in which they represent almost 1% of all genes (Johnstone, 2000). In this nematode, the biogenesis of the cuticle is known to be dependent upon the function of a group of astacin metalloproteases that are responsible for the cleavage of the C-terminal region of the collagens, thus catalysing the correct formation of ECM (Stepek et al., 2010). In particular, loss of function via gene peturbation of the astacin metalloprotease gene *dpy-31* of *C. elegans* has been shown to result in cuticle defects, abnormal morphology and embryonic lethality, thus indicating that this gene is essential for the formation of the collagenous exoskeleton (Novelli et al., 2004). Recently, the homologues of *C. elegans dpy-31* have been isolated and characterised in *Brugia malayi*, a filarial nematode of humans, and in *Haemonchus contortus*, a trichostrongylid nematode of small ruminants (Stepek et al., 2010). In the latter study, the expression of the *H. contortus* homologue in a *C. elegans dpy-31* mutant strain resulted in the rescue of the mutant body form, suggesting that the function of DPY-31 is conserved between these two nematodes (Stepek et al., 2010). In *H. contortus* L3s, astacin metalloproteases are known to mediate the ecdysis from the L2 cuticle through the formation of an anterior refractile ring and removable cap structure (Gamble et al., 1989, 1996). Interestingly, the 'peptidase M12A, astacin' (PF01400) was amongst the protein domains most commonly identified in the group of EST clusters unique to the L3 stage of *D. viviparus* (see section 3). Therefore, considering the results of previous studies, showing that peptidases are essential to the exsheathment process of the *D. viviparus* L3 in vitro (Silverman and Podger, 1964), it is tempting to speculate a similar role of astacin metalloproteases in the transition from the free-living to the parasitic stage of this nematode in vivo, an hypothesis which requires rigorous testing.

'Serine/threonine protein kinases' (PF00069) were also commonly represented in transcriptome of *D. viviparus* (see section 3). Such PAR-1/MARK serine/threonine protein kinase (STK) subfamily members have been implicated in a number of fundamental pathways of the biology of the cell, such as the establishment and maintenance of cell polarity (i.e., through the activity of the cytoskeleton; Drewes et al., 1998). Particularly in *C. elegans* and *D. melanogaster*, a gene (*par-1*) encoding an STK is known to be required for cytoplasmic partitioning and asymmetric cell division in early embryogenesis (Guo and Kemphues, 1995) and oocyte specification and determination of the embryonic axis, respectively (Cox et al., 2001). An additional role of *C. elegans par-1* in the morphogenesis of the vulva has also been implicated, based on the results of a study showing that post-embryonic gene silencing of *par-1* causes a 'protruding vulva' phenotype, which results from the failure of the two mirror-symmetric halves of the vulva to join into a single organ (Hurd and Kemphues, 2003). Indeed, the in silico-subtraction approach employed in the present study identified the serine/threonine protein kinase' (PF00069) domain as highly represented amongst the functional domains specific to the adult female of *D. viviparus* (see section 3). Although the role of STKs in fundamental biological processes of parasitic nematodes is unclear, these results suggest an active involvement into molecular mechanisms linked to the reproductive activity of the adult female. This hypothesis is supported by the localisation of an homologue (i.e., HcSTK) of *C. elegans* PAR-1 to a cytoplasmic compartment around the nuclei of intestinal and ovarian tissues of the adult stage of *H. contortus*, a strongylid nematode which is related to *D. viviparus* (see Nikolaou et al., 2006; Nikolaou and Gasser, 2007).

Other groups of molecules inferred to play fundamental biological roles in reproductive mechanisms in *C. elegans* were commonly represented in the transcriptome of *D. viviparus*. For instance, the 'major sperm protein (MSP)' domain (PF00635) was commonly identified

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within peptides inferred from both the combined assembly of data from the L3, adult female and male and specific to the male (cf. section 3). MSPs belong to a large protein family whose members have been recognised to act as bipartite signalling molecules, activating pathways linked to oocyte production and maturation in *C. elegans* (see Miller et al., 2001, 2003). However, MSPs are known to be predominantly involved in nematode sperm motility through their polymerisation into dense filaments that are required for the movement of the plasma membrane of the amoeboid sperm cells (Roberts and Stewart, 2000; Italiano et al., 2001; Buttery et al., 2003). The pattern of transcription of genes encoding *msp*s throughout different developmental stages has already been investigated in *D. viviparus* (Strube et al., 2009b). Using quantitative real-time PCR, an up-regulation of genes encoding *msp*s was observed in hypobiotic L5 and adult male of this lungworm (Strube et al., 2009b). Interestingly, in adult males, the authors observed an ~200,000-fold increase in *msp* transcription levels compared with adult females (Strube et al., 2009b). This difference contrasts the results of other transcriptional analyses of gender-enriched molecules in other species of parasitic nematodes. For instance, a 2-fold increase in *msp* transcriptional rates were observed in the males of intestinal nematodes of the pig, including *Oesophagostomum dentatum* and *Ascaris suum* (see Cottee et al., 2004; Cantacessi et al., 2009a), whereas an ~480-fold increase was detected in males of the filarial nematode *B. malayi* (see Li et al., 2004). The high transcriptional levels of *msp*s in the adult male of *D. viviparus* could be indicative of high sperm production or a distinct biological function of this group of molecules in this lungworm, besides the activity in reproductive processes. This question remains to be explored. In addition, a MSP (encoded by a gene designated Dv3–14; Schnieder, 1993) was identified as an immunodominant antigen by immunoblotting using sera from cattle experimentally infected with *D. viviparus* (see Schnieder, 1992); therefore, given their abundance in the excretory/secretory (ES) products of the adult worm, MSPs (expressed in recombinant form) have been proposed as vaccine candidates (Strube et al., 2009b).

Of the groups of molecules identified herein as being specific to the adult male of *D. viviparus*, the SCP/Tpx-1/Ag5/PR-1/Sc7 proteins (designated SCP/TAPS; PF00188) are also under investigation for their potential as vaccine targets for other parasitic nematodes (i.e., the human hookworm *Necator americanus*; Loukas et al., 2006; Bethony et al., 2008; Mendez et al., 2008; Xiao et al., 2008). Such SCP/TAPS are also commonly referred to as *Ancylostoma* secreted proteins or activation-associated proteins (ASPs), mainly because they were originally discovered in hookworms (Hawdon et al., 1996; Datu et al., 2008; Cantacessi et al., 2009b). Given their abundance in the ES products from serum-activated third-stage larvae (aL3s) of *A. caninum* and high transcriptional levels in aL3s compared with non-activated L3s, ASPs/*asp*s have been hypothesized to play a major role in the transition from the free-living to the parasitic stage of hookworms (Datu et al., 2008). Other ASP homologues have been characterized in the adult stage of hookworms, where they might play a role in the initiation, establishment and/or maintenance of the host-parasite relationship (Zhan et al., 2003; Cantacessi et al., 2009b; Mulvenna et al., 2009). A male-biased transcription of genes encoding ASPs had been reported for other species of parasitic nematodes, including *A. suum*, *O. dentatum* and *Trichostrongylus vitrinus* (intestinal nematode of small ruminants) (Nisbet and Gasser, 2004; Cottee et al., 2006; Cantacessi et al., 2009a). However, because the sequence datasets analysed here were generated from normalized cDNA libraries, the transcriptional levels of genes encoding ASPs in the adult male of *D. viviparus* could not be determined. Future work could explore developmentally regulated transcription of these molecules in all developmental stages of *D. viviparus*, employing, for example, digital gene expression profiling or non-normalized Illumina sequencing (Morrissy et al., 2009, 2010), to assist in understanding their role(s) in the parasite and/or its interplay with the host.

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Knowledge of the function of key molecules in a range of organisms (e.g., *C. elegans*, *S. cerevisiae*, *D. melanogaster* and *M. musculus*) assists in the prediction novel drug targets in parasitic helminths (see Geary et al., 1999; McCarter, 2004; Krasky et al., 2007; Caffrey et al., 2009; Doyle et al., 2010). Such knowledge, combined with the InterPro and/or GO classifications of inferred peptides from the combined assembly of sequence data from L3, adult female and male of *D. viviparus* as well as peptides unique to each stage/sex, allowed the prediction and prioritisation of molecules considered to be essential in the life cycle of this lungworm and, by inference, other parasitic nematodes (see Table 5). In particular, the *C. elegans* homologues F09F9.4, H27M09.1 and *DCR-1*, linked to druggable InterPro domains and/or EC numbers, were associated with an 'adult lethal' phenotype for all of the organisms investigated (see section 3.2 and Table 5). Specifically in *C. elegans*, these molecules are known to be involved in essential molecular processes linked to embryonic and larval development, locomotion and/or reproduction. For instance, the *C. elegans* gene H27M09.1 encodes an ATP-dependent helicase with a DEAD-box domain (see www.wormbase.org), whose corresponding homologues in *B. malayi* and *Plasmodium falciparum* (malaria parasite) have been proposed to represent putative drug targets in these parasites (Tuteja, 2007; Singh et al., 2009). This hypothesis is based on the knowledge that efficacious compounds targeting this group of molecules are available for the treatment of pathogens of humans, such as the Herpes simplex and Hepatitis C viruses (Frick, 2003). Future studies could focus on investigating the transcription levels of the *D. viviparus* homologue of *C. elegans* H27M09.1 throughout different life cycle stages and on assessing the specific biochemical activity of the encoded protein (cf. Singh et al., 2009). Insights through such studies could assist in the prediction and optimisation of the chemical structure of this group of molecules via chemoinformatic methods, such as homology modelling and ligand docking (Gasteiger, 2006; Krasky et al., 2007), and might lead to the definition of a target protein structure to assist the design of drugs for in vitro and in vivo testing.

In conclusion, the present study has provided the first large-scale exploration of key transcriptomes of *D. viviparus* and has predicted, using an integrated bioinformatic approach and knowledge of functional aspects of key genes/gene products in model organisms, molecules unique to and/or essential in different stages of this lungworm. This knowledge, combined with future sequencing efforts using, for instance, Illumina sequencing, should assist in the determination of the genome sequence of *D. viviparus*, which will constitute a foundation for proteomic and metabolomic studies of this parasite, leading to novel treatment and control strategies as well as major biotechnological outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A

Biological pathways assigned to peptides conceptually translated from individual contigs for *Dictyocaulus viviparus* (combined dataset for L3, adult female and adult male) and to predicted peptides unique to each developmental stage or sex.

Appendix B

Pfam and/or InterPro domains for predicted peptide sequences encoded in either L3, adult female and adult male of *Dictyocaulus viviparus*.

Appendix C

Gene ontology (GO) terms according to the categories 'biological process', 'cellular component' and 'molecular function' assigned to peptides conceptually translated from individual contigs for *Dictyocaulus viviparus* representing the combined dataset for L3, adult female and adult male, or individual stages/sexes.

References

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 1997; 25:3389–402. [PubMed: 9254694]

Anderson, RC. Nematode Parasites of Vertebrate: Their Development and Transmission. Wallingford: CABI Publishing; 2001.

Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet. 2000; 25:25–9. [PubMed: 10802651]

Bentley DR, Balasubramanian S, Swerdlow HP, Smith GP, Milton J, Brown CG, et al. Accurate whole human genome sequencing using reversible terminator chemistry. Nature. 2008; 456:53–9. [PubMed: 18987734]

Bethony JM, Simon G, Diemert DJ, Parenti D, Desrosiers A, Schuck S, et al. Randomized, placebo-controlled, double-blind trial of the *Na*-ASP-2 hookworm vaccine in unexposed adults. Vaccine. 2008; 26:2408–17. [PubMed: 18396361]

Blaxter M. *Caenorhabditis elegans* is a nematode. Science. 1998; 282:2041–6. [PubMed: 9851921]

Britton C, Murray L. A cathepsin L protease essential for *Caenorhabditis elegans* embryogenesis is functionally conserved in parasitic nematodes. Mol Biochem Parasitol. 2002; 122:21–33. [PubMed: 12076767]

Brock TJ, Browse J, Watts JL. Genetic regulation of unsaturated fatty acid composition in *C. elegans*. PLoS Genet. 2006; 2:e108. [PubMed: 16839188]

Buttery SM, Ekman GC, Seavy M, Stewart M, Roberts TM. Dissection of the *Ascaris* sperm motility machinery identifies key proteins involved in major sperm protein-based amoeboid locomotion. Mol Biol Cell. 2003; 14:5082–8. [PubMed: 14565983]

Caffrey CR, Rohwer A, Oellien F, Marhofer RJ, Braschi S, Oliveira G, et al. A comparative chemogenomics strategy to predict potential drug targets in the metazoan pathogen *Schistosoma mansoni*. PLoS One. 2009; 4:e4413. [PubMed: 19198654]

Cantacessi C, Zou FC, Hall RS, Zhong W, Jex AR, Campbell BE, et al. Bioinformatic analysis of abundant, gender-enriched transcripts of adult *Ascaris suum* (Nematoda) using a semi-automated workflow platform. Mol Cell Probes. 2009a; 23:205–17. [PubMed: 19361552]

Cantacessi C, Campbell BE, Visser A, Geldhof P, Nolan MJ, Nisbet AJ, et al. A portrait of the "SCP/TAPS" proteins of eukaryotes–developing a framework for fundamental research and biotechnological outcomes. Biotechnol Adv. 2009b; 27:376–88. [PubMed: 19239923]

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Cantacessi C, Mitreva M, Jex AR, Young ND, Campbell BE, Hall RS, et al. Massively parallel sequencing and analysis of the *Necator americanus* transcriptome. PLoS Negl Trop Dis. 2010a; 4:e684. [PubMed: 20485481]

Cantacessi C, Campbell BE, Young ND, Jex AR, Hall RS, Presidente PJA, et al. Differences in transcription between free-living and CO_2-activated third-stage larvae of *Haemonchus contortus*. BMC Genomics. 2010b; 11:266. [PubMed: 20420710]

Cantacessi, C.; Jex, AR.; Hall, RS.; Young, ND.; Campbell, BE.; Joachim, A., et al. A practical, bioinformatic workflow system for large datasets generated by next-generation sequencing. 2010c. Submitted for publication.

Ceron J, Rual JF, Chandra A, Dupuy D, Vidal M, et al. Large scale RNAi screens identify novel genes that interact with the *C. elegans* retinoblastoma pathway as well as splicing-related components with synMuv B activity. BMC Dev Biol. 2007; 7:30. [PubMed: 17417969]

Chang A, Scheer M, Grote A, Schomburg I, Schomburg D. BRENDA, AMENDA and FRENDA the enzyme information system: new content and tools in 2009. Nucleic Acids Res. 2009; 37:D588–92. [PubMed: 18984617]

Coles GC. The future of veterinary parasitology. Vet Parasitol. 2001; 98:31–9. [PubMed: 11516577]

Cottee PA, Nisbet AJ, Boag PR, Larsen M, Gasser RB. Characterization of major sperm protein genes and their expression in *Oesophagostomum dentatum* (Nematoda: Strongylida). Parasitology. 2004; 129:479–90. [PubMed: 15521637]

Cottee PA, Nisbet AJ, Abs El-Osta YG, Webster TL, Gasser RB. Construction of gender-enriched cDNA archives for adult *Oesophagostomum dentatum* by suppressive-subtractive hybridization and a microarray analysis of expressed sequence tags. Parasitology. 2006; 132:691–708. [PubMed: 16426483]

Cox DN, Lu B, Sun TQ, Williams LT, Jan YN. *Drosophila par-1* is required for oocyte differentiation and microtubule organization. Curr Biol. 2001; 11:75–87. [PubMed: 11231123]

David GP. Survey on lungworm in adult cattle. Vet Rec. 1997; 141:343–4. [PubMed: 9347626]

Datu BJ, Gasser RB, Nagaraj SH, Ong EK, O'Donoghue P, McInnes R, et al. Transcriptional changes in the hookworm, *Ancylostoma caninum*, during the transition from a free-living to a parasitic larva. PLoS Negl Trop Dis. 2008; 2:e130. [PubMed: 18235850]

Doyle MA, Gasser RB, Woodcroft BJ, Hall RS, Ralph SA. Drug target prediction and prioritization: using orthology to predict essentiality in parasite genomes. BMC Genomics. 2010; 11:222. [PubMed: 20361874]

Drewes G, Trinczek B, Illenberger S, Biernat J, Schmitt-Ulms G, Meyer HE, et al. Microtubule associated protein/microtubule affinity-regulating kinase ($p110^{mark}$). A novel protein kinase that regulates tau-microtubule interactions and dynamic instability by phosphorylation at the Alzheimer-specific site serine 262. J Biol Chem. 1995; 270:7679–88. [PubMed: 7706316]

Eddy SR. A new generation of homology search tools based on probabilistic inference. Genome Inform. 2009; 23:205–11. [PubMed: 20180275]

Eki T, Ishihara T, Katsura I, Hanaoka F. A genome-wide survey and systematic RNAi-based characterization of helicase-like genes in *Caenorhabditis elegans*. DNA Res. 2007; 14:183–99. [PubMed: 17921522]

Eysker M, van Miltenburg L. Epidemiological patterns of gastrointestinal and lung helminth infections in grazing calves in The Netherlands. Vet Parasitol. 1988; 29:29–39. [PubMed: 2972109]

Frick DN. Helicases as antiviral drug targets. Drug News Perspect. 2003; 16:355. [PubMed: 12973446]

Gamble HR, Lichtenfels JR, Purcell JP. Light and scanning electron microscopy of the ecdysis of *Haemonchus contortus* infective larvae. J Parasitol. 1989; 75:303–7. [PubMed: 2647956]

Gamble HR, Fetterer RH, Mansfield LS. Developmentally regulated zinc metalloproteinases from third- and fourth-stage larvae of the ovine nematode *Haemonchus contortus*. J Parasitol. 1996; 82:197–202. [PubMed: 8604083]

Gasteiger J. Chemoinformatics: a new field with a long tradition. Anal Bioanal Chem. 2006; 384:57–64. [PubMed: 16177914]

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Geary TG, Thompson DP, Klein RD. Mechanism-based screening: discovery of the next generation of anthelminthics depends upon more basic research. Int J Parasitol. 1999; 29:105–12. [PubMed: 10048823]

Guo S, Kemphues KJ. *par-1*, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. Cell. 1995; 81:611–20. [PubMed: 7758115]

Hawdon JM, Jones BF, Hoffman DR, Hotez PJ. Cloning and characterization of *Ancylostoma*-secreted protein. A novel protein associated with the transition to parasitism by infective hookworm larvae. J Biol Chem. 1996; 271:6672–8. [PubMed: 8636085]

Hopkins AL, Groom CR. The druggable genome. Nat Rev Drug Discov. 2002; 1:727–30. [PubMed: 12209152]

Hu M, Lok JB, Ranjit N, Massey HC Jr, Sternberg PW, et al. Structural and functional characterisation of the fork head transcription factor-encoding gene, *Hc-daf-16*, from the parasitic nematode *Haemonchus contortus* (Strongylida). Int J Parasitol. 2010; 40:405–15. [PubMed: 19796644]

Huang X, Madan A. CAP3: A DNA sequence assembly program. Genome Res. 1999; 9:868–77. [PubMed: 10508846]

Hunter S, Apweiler R, Attwood TK, Bairoch A, Bateman A, Binns D, et al. InterPro: the integrative protein signature database. Nucleic Acids Res. 2009; 37:D211–15. [PubMed: 18940856]

Hurd DD, Kemphues KJ. PAR-1 is required for the morphogenesis of the *Canorhabditis elegans* vulva. Develop Biol. 2003; 253:54–65. [PubMed: 12490197]

Iseli C, Jongeneel CV, Bucher P. ESTScan: a program for detecting, evaluating, and reconstructing potential coding regions in EST sequences. Proc Int Conf Intell Syst Mol Biol. 1999:138–48. [PubMed: 10786296]

Italiano JE Jr, Stewart M, Roberts TM. How the assembly dynamics of the nematode major sperm protein generate amoeboid cell motility. Int Rev Cytol. 2001; 202:1–34. [PubMed: 11061562]

Jackson F, Bartley D, Kenyon F, Sargison N. Dissemination of best practice to producers and advisors. Proc 21st Int Conf WAAVP. 2007:226.

Jarrett WF, Jennings FW, McIntyre WI, Mulligan W, Urquhart GM. Immunological studies on *Dictyocaulus viviparus* infection. Immunity produced by the administration of irradiated larvae. Immunology. 1960; 3:145–51. [PubMed: 14406846]

Johnstone IL. The cuticle of the nematode *Caenorhabditis elegans*. Trends Genetics. 2000; 16:21–7.

Kamath RS, Sohrmann M, Welchman DP, Ziperlen P, Ahringer J, et al. Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. Nature. 2003; 421:231–7. [PubMed: 12529635]

Kim JK, Kaplan JM, Vidal M, Ruvkun G, Gabel HW, et al. Functional genomic analysis of RNA interference in *C. elegans*. Science. 2005; 308:1164–7. [PubMed: 15790806]

Koonin, EV. The NCBI Handbook. The National Library of Medicine Press; 2010. The Clusters of Orthologous Groups (COGs) Database: Phylogenetic Classification of Proteins from Complete Genomes.

Krasky A, Rohwer A, Schroeder J, Selzer PM. A combined bioinformatics and chemoinformatics approach for the development of new antiparasitic drugs. Genomics. 2007; 89:36–43. [PubMed: 17070005]

Letunic I, Yamada T, Kanehisa M, Bork P. iPath: interactive exploration of biochemical pathways and networks. Trends Biochem Sci. 2008; 33:101–3. [PubMed: 18276143]

Li BW, Rush AC, Crosby SD, Warren WC, Williams SA, Mitreva M, et al. Profiling of gender-regulated gene transcripts in the filarial nematode *Brugia malayi* by cDNA oligonucleotide array analysis. Mol Biochem Parasitol. 2005; 143:49–57. [PubMed: 15992941]

Lipinski C, Lombardo F, Dominy B, Feeney P. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 1997; 23:3–25.

Lok JB. Transgenesis in parasitic nematodes: building a better array. Trends Parasitol. 2009; 25:345–7. [PubMed: 19617000]

Loukas A, Bethony J, Brooker S, Hotez P. Hookworm vaccines: past, present, and future. Lancet Infect Dis. 2006; 6:733–41. [PubMed: 17067922]

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Maeda I, Kohara Y, Yamamoto M, Sugimoto A. Large-scale analysis of gene function in *Caenorhabditis elegans* by high-throughput RNAi. Curr Biol. 2001; 11:171–6. [PubMed: 11231151]

Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, et al. Genome sequencing in microfabricated high-density picolitre reactors. Nature. 2005; 437:376–80. [PubMed: 16056220]

Matthews JB, Davidson AJ, Freeman KL, French NP. Immunisation of cattle with recombinant acetylcholinesterase from *Dictyocaulus viviparus* and with adult worm ES products. Int J Parasitol. 2001; 31:307–17. [PubMed: 11226458]

McCarter JP. Genomic filtering: an approach to discovering novel antiparasitics. Trends Parasitol. 2004; 20:462–8. [PubMed: 15363439]

McKeand JB. Vaccine development and diagnostics of *Dictyocaulus viviparus*. Parasitology. 2000; 120:S17–23. [PubMed: 10874707]

Mendez S, D' Samuel A, Antoine AD, Ahn S, Hotez P. Use of the air pouch model to investigate immune responses to a hookworm vaccine containing the *Na*-ASP-2 protein in rats. Parasite Immunol. 2008; 30:53–6. [PubMed: 18086017]

Metzker ML. Sequencing technologies – the next generation. Nat Rev Genet. 2010; 11:31–46. [PubMed: 19997069]

Miller MA, Nguyen VQ, Lee MH, Kosinski M, Schedl T, Caprioli RM, et al. A sperm cytoskeletal protein that signals oocyte meiotic maturation and ovulation. Science. 2001; 291:2144–7. Erratum in: Science 2001;292:639. [PubMed: 11251118]

Miller MA, Ruest PJ, Kosinski M, Hanks SK, Greenstein D. An Eph receptor sperm-sensing control mechanism for oocyte meiotic maturation in *Caenorhabditis elegans*. Genes Dev. 2003; 17:187–200. [PubMed: 12533508]

Molento MB, Depner RA, Mello MH. Suppressive treatment of abamectin against *Dictyocaulus viviparus* and the occurrence of resistance in first-grazing-season calves. Vet Parasitol. 2006; 141:373–6. [PubMed: 16790316]

Morozova O, Marra MA. Applications of next-generation sequencing technologies in functional genomics. Genomics. 2008; 92:255–64. [PubMed: 18703132]

Morrissy AS, Morin RD, Delaney A, Zeng T, McDonald H, et al. Next-generation tag sequencing for cancer gene expression profiling. Genome Res. 2009; 19:1825–35. [PubMed: 19541910]

Morrissy S, Zhao Y, Delaney A, Asano J, Dhalla N, et al. Digital gene expression by tag sequencing on the illumina genome analyzer. Curr Protoc Hum Genet. 2010; 11:1–36. Unit 11.11.

Mulvenna J, Hamilton B, Nagaraj S, Smyth D, Loukas A, Gorman J. Proteomic analysis of the excretory/secretory component of the blood-feeding stage of the hookworm, *Ancylostoma caninum*. Mol Cell Proteomics. 2009; 8:109–21. [PubMed: 18753127]

Nagaraj SH, Deshpande N, Gasser RB, Ranganathan S. ESTExplorer: an expressed sequence tag (EST) assembly and annotation platform. Nucleic Acids Res. 2007; 35:W143–7. [PubMed: 17545197]

Nikolaou S, Hartman D, Nisbet AJ, Gasser RB. *Haemonchus contortus*: prokaryotic expression and enzyme activity of recombinant HcSTK, a serine/threonine protein kinase. Exp Parasitol. 2006; 113:207–14. [PubMed: 16500645]

Nikolaou S, Gasser RB. Extending from PARs in *Caenorhabditis elegans* to homologues in *Haemonchus contortus* and other parasitic nematodes. Parasitology. 2007; 134:461–82. [PubMed: 17107637]

Nisbet AJ, Gasser RB. Profiling of gender-specific gene expression for *Trichostrongylus vitrinus* (Nematoda: Strongylida) by microarray analysis of expressed sequence tag libraries constructed by suppressive-subtractive hybridisation. Int J Parasitol. 2004; 34:633–43. [PubMed: 15064128]

Novelli J, Ahmed S, Hodgkin J. Gene interactions in *Caenorhabditis elegans* define DPY-31 as a candidate procollagen C-proteinase and SQT-3/ROL-4 as its predicted major target. Genetics. 2004; 168:1259–73. [PubMed: 15579684]

Page AP, Winter AD. Enzymes involved in the biogenesis of the nematode cuticle. Adv Parasitol. 2003; 53:85–148. [PubMed: 14587697]

Panuska C. Lungworms of rimunants. Vet Clin North Am Food Anim Pract. 2006; 22:583–93. [PubMed: 17071354]

Parkinson J, Whitton C, Schmid R, Thomson M, Blaxter M. NEMBASE: a resource for parasitic nematode ESTs. Nucleic Acids Res. 2004; 32:D427–30. [PubMed: 14681449]

Piano F, Schetter AJ, Morton DG, Gunsalus KC, Reinke V, et al. Gene clustering based on RNAi phenotypes of ovary-enriched genes in *C.* elegans Curr Biol. 2002; 12:1959–64.

Ploeger HW. *Dictyocaulus viviparus*: re-emerging or never been away? Trends Parasitol. 2002; 18:329–32. [PubMed: 12377272]

Ranganathan S, Nagaraj SH, Hu M, Strube C, Schnieder T, Gasser RB. A transcriptomic analysis of the adult stage of the bovine lungworm, *Dictyocaulus viviparus*. BMC Genomics. 2007; 8:311. [PubMed: 17784965]

Roberts TM, Stewart M. Acting like actin. The dynamics of the nematode major sperm protein (msp) cytoskeleton indicate a push-pull mechanism for amoeboid cell motility. J Cell Biol. 2000; 149:7–12. [PubMed: 10747081]

Robertson JG. Mechanistic basis of enzyme-targeted drugs. Biochemistry. 2005; 44:5561–71. [PubMed: 15823014]

Rual JF, van den Heuvel S, Vidal M, Ceron J, Koreth J, et al. Toward improving *Caenorhabditis elegans* phenome mapping with an ORFeome-based RNAi library. Genome Res. 2004; 14:2162–8. [PubMed: 15489339]

Ruvkun G, Hobert O. The taxonomy and developmental control in *Caenorhabditis elegans*. Science. 1998; 282:2033–41. [PubMed: 9851920]

Schnieder T, Kaup FJ, Drommer W. Morphological investigations on the pathology of *Dictyocaulus viviparus* infections in cattle. Parasitol Res. 1991; 77:260–5. [PubMed: 1828598]

Schnieder T. *Dictyocaulus viviparus*: isolation and characterization of a recombinant antigen with potential for immunodiagnosis. Int J Parasitol. 1992; 22:933–8. [PubMed: 1459787]

Schnieder T. The diagnostic antigen encoded by gene fragment Dv3–14: a major sperm protein of *Dictyocaulus viviparus*. Int J Parasitol. 1993; 23:383–9. [PubMed: 8359987]

Schnieder T, Epe C, von Samson-Himmelstjerna G, Kohlmetz C. The development of protective immunity against gastrointestinal nematode and lungworm infections after use of an ivermectin bolus in first-year grazing calves. Vet Parasitol. 1996; 64:239–50. [PubMed: 8888556]

Shaw LM, Olsen BR. FACIT collagens – diverse molecular bridges in extracellular matrices. Trends Biochem Sci. 1991; 16:191–4. [PubMed: 1882421]

Silverman PH, Podger KR. *In vitro* exsheathment of some nematode infective larvae. Exp Parasitol. 1964; 15:314–24. [PubMed: 14201362]

Singh M, Srivastava KK, Bhattacharya SM. Molecular cloning and characterization of a novel immunoreactive ATPase/RNA helicase in human filarial parasite *Brugia malayi*. Parasitol Res. 2009; 104:753–61. [PubMed: 19002498]

Sonnichsen B, Cassin E, Hewitson M, Holz C, Khan M, et al. Full-genome RNAi profiling of early embryogenesis in *Caenorhabditis elegans*. Nature. 2005; 434:462–9. [PubMed: 15791247]

Stepek G, McCormack G, Page AP. Collagen processing and cuticle formation is catalysed by the astacin metalloprotease DPY-31 in free-living and parasitic nematodes. Int J Parasitol. 2010; 40:533–42. [PubMed: 19883650]

Strube C, von Samson-Himmelstjerna G, Schnieder T. Genetic regulation of arrested development in nematodes: are *age-1* and *daf*-gene orthologs present in *Dictyocaulus viviparus*? Parasitol Res. 2007a; 101:1111–5. [PubMed: 17558520]

Strube C, Schnieder T, von Samson-Himmelstjerna G. Differential gene expression in hypobiosis-induced and non-induced third-stage larvae of the bovine lungworm *Dictyocaulus viviparus*. Int J Parasitol. 2007b; 37:221–31. [PubMed: 17112525]

Strube C, Buschbaum S, von Samson-Himmelstjerna G, Schnieder T. Stage-dependent transcriptional changes and characterization of paramyosin of the bovine lungworm *Dictyocaulus viviparus*. Parasitol Int. 2009a; 58:334–40. [PubMed: 19604498]

Strube C, Buschbaum S, Schnieder T. Molecular characterization and real-time PCR transcriptional analysis of *Dictyocaulus viviparus* major sperm proteins. Parasitol Res. 2009b; 104:543–51. [PubMed: 18853187]

Tuteja R. Helicases – feasible antimalarial drug target for *Plasmodium falciparum*. FEBS J. 2007; 274:4699–704. [PubMed: 17824956]

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von Samson-Himmelstjerna G, Schnieder T. Morphology of inhibited larvae of the bovine lungworm *Dictyocaulus viviparus*. J Helminthol. 1999; 73:79–83. [PubMed: 10431372]

Wang Z, Abubucker S, Martin J, Wilson RK, Hawdon J, Mitreva M. Characterizing *Ancylostoma caninum* transcriptome and exploring nematode parasitic adaptation. BMC Genomics. 2010; 11:307. [PubMed: 20470405]

Wood IB, Amaral NK, Bairden K, Duncan JL, Kassai T, et al. World Association for the Advacement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants. Vet Parasitol. 2005; 58:181–213. [PubMed: 7571325]

Wu J, Mao X, Cai T, Luo J, Wei L. KOBAS server: a web-based platform for automated annotation and pathway identification. Nucleic Acids Res. 2006; 34:W720–4. [PubMed: 16845106]

Xiao S, Zhan B, Xue J, Goud GN, Loukas A, Liu Y, et al. The evaluation of recombinant hookworm antigens as vaccines in hamsters (*Mesocricetus auratus*) challenged with human hookworm, *Necator americanus*. Exp Parasitol. 2008; 118:32–40. [PubMed: 17645877]

Young ND, Hall RS, Jex AR, Cantacessi C, Gasser RB. Elucidating the transcriptome of *Fasciola hepatica* – a key to fundamental and biotechnological discoveries for a neglected parasite. Biotechnol Adv. 2010a; 28:222–31. [PubMed: 20006979]

Young ND, Campbell BE, Hall RS, Jex AR, Cantacessi C, Laha T, et al. Unlocking the transcriptomes of two carcinogenic parasites, Clonorchis sinensis and Opisthorchis viverrini. PLoS Negl Trop Dis. 2010b In press.

Zhan B, Liu Y, Badamchian M, Williamson A, Feng J, Loukas A, et al. Molecular characterisation of the *Ancylostoma*-secreted protein family from the adult stage of *Ancylostoma caninum*. Int J Parasitol. 2003; 33:897–907. [PubMed: 12906874]

Zhong W, Sternberg PW. Genome-wide prediction of *C. elegans* genetic interactions. Science. 2006; 311:1481–4. [PubMed: 16527984]

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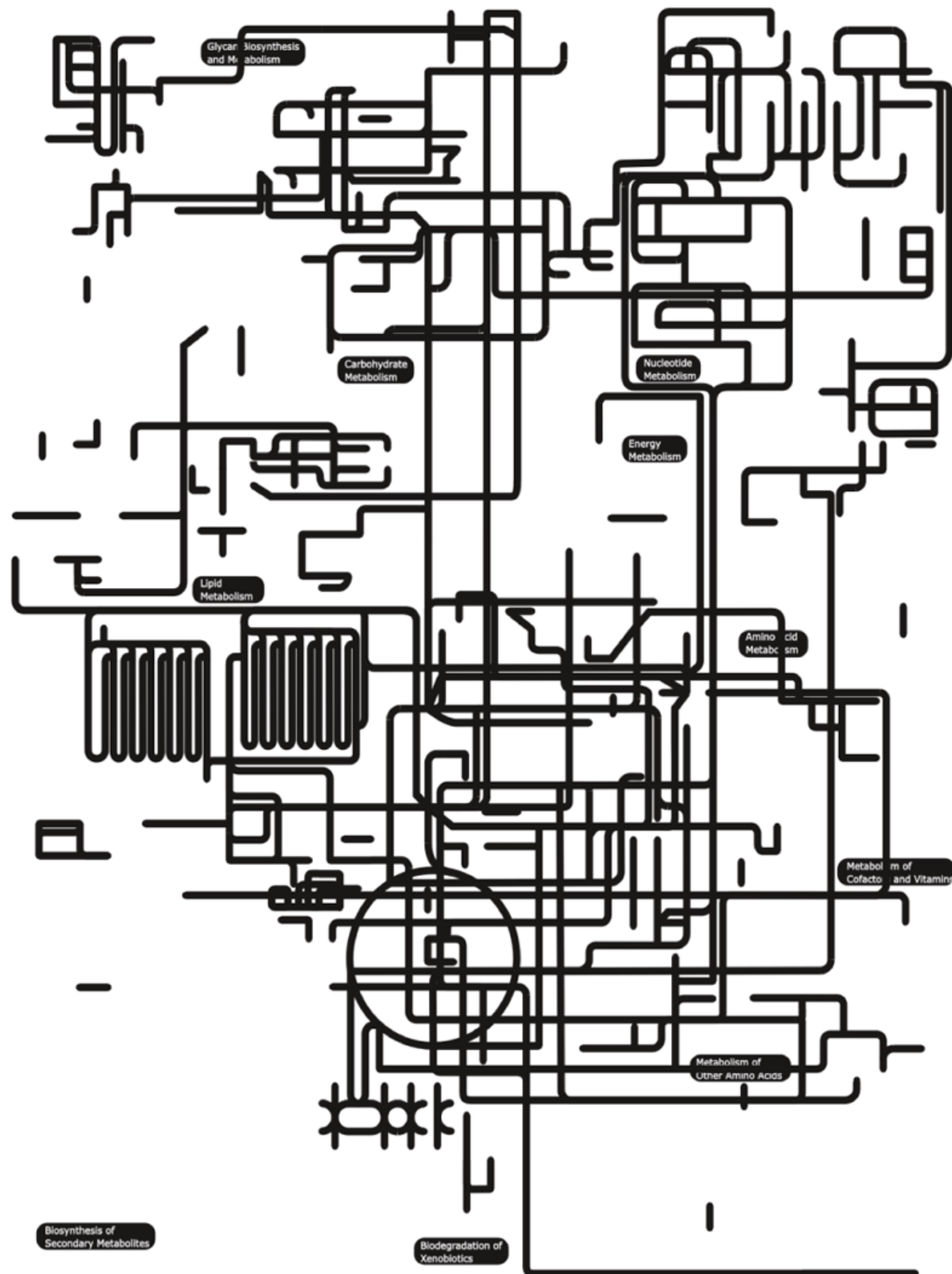

Fig. 1.

Mapping of peptides inferred from the transcriptome of *Dictyocaulus viviparus* (combined dataset for L3, adult female and adult male) to known metabolic pathways in *Caenorhabditis elegans*.

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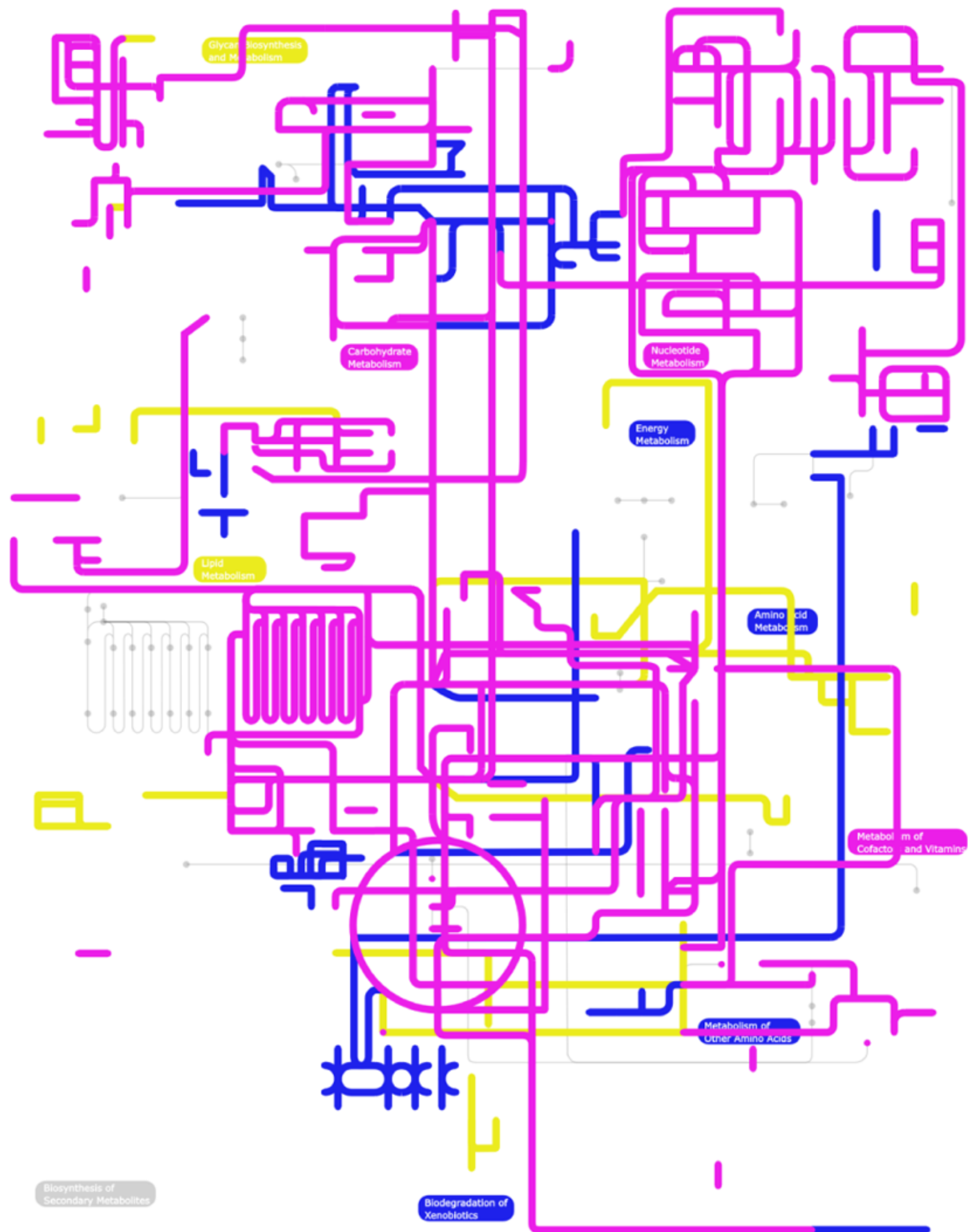

Fig. 2.

Mapping of predicted peptides unique to either the L3 (yellow), adult female (pink) and adult male (blue) of *Dictyocaulus viviparus* to known metabolic pathways in *Caenorhabditis elegans*.

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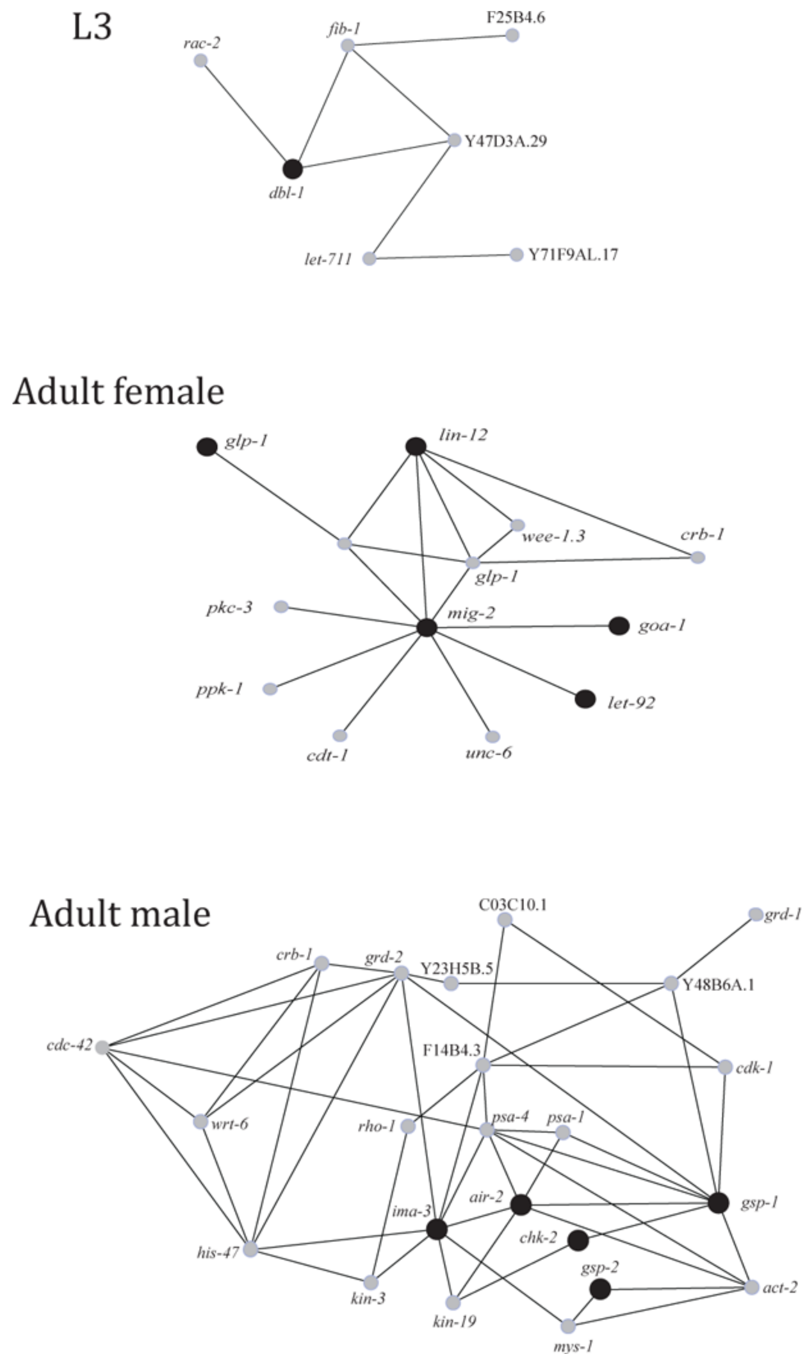

Fig. 3.

Examples of genetic interaction networks predicted for *Caenorhabditis elegans* homologues (grey dots) of expressed sequence tags (ESTs; black dots) unique to either L3, adult female and adult male of *Dictyocaulus viviparus*. Each of the represented *C. elegans* homologues was predicted to interact with a minimum of 100 other genes. Homologue *dbl-1* is linked to regulation of body length and size (L3); *glp-1*, *lin-12*, *let-92* and *mig-2* to embryonic, larval and vulval development and *goa-1* to behaviour and egg-laying (adult female); *ima-3* and *air-2* are associated to sperm maturation and development (adult male).

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Table 1

Summary of available nucleotide sequence data for L3, adult female and adult male of *Dictyocaulus viviparus* prior to and following in silico-subtraction as well as detailed bioinformatic annotation and analyses.

	L3	Female	Male	Combined
Expressed sequence tags (ESTs)				
Number of unassembled ESTs (average length ± SD)	1,095,063 (539 ± 84)	955,995 (521 ± 95)	889,873 (506 ± 99)	2,943,357 (454 ± 86)
Contigs (average length ± range)	7,661 (461 ± 238)	9,946 (616 ± 362)	9,226 (577 ± 307)	6,119 (214 ± 102)
Singletons	27,455 (317 ± 162)	28,840 (339 ± 193)	28,784 (360 ± 181)	55,015 (202 ± 142)
Total	35,116	38,786	38,010	61,134 (304 ± 141)
Containing an Open Reading frame (%)	31,288 (89)	34,338 (88.5)	34,931 (92)	59,606 (97)
Returning Pfam/InterPro results (%)	12,615 (40)	16,615 (48.3)	17,302 (49.5)	20,974 (37)
Gene Ontology (%)	8,306 (23.6)	10,514 (27)	11,822 (31)	12,151 (21)
Number of Biological process terms	635	644	656	678
Cellular component	208	220	214	231
Molecular function	723	752	736	770
With homologues in *C. elegans* (%)	13,918 (44)	17,979 (52)	17,103 (49)	18,877 (31.7)
other parasitic nematodes (%)	11,954 (38)	16,100 (46.8)	15,928 (45.5)	14,721 (24.7)
other organisms (%)	14,765 (47)	19,566 (57)	18,530 (53)	17,043 (28)
Number of unique *C. elegans* homologues	12,641	12,853	12,258	12,938
KOBAS (number of biological pathways predicted)	193	164	138	245
In silico subtracted datasets				
Number of EST clusters	6,928	5,203	7,889	
Containing an Open Reading frame (%)	4,902 (70)	4,947 (95)	6,003 (76)	
Predicted peptides				
Returning Pfam/InterProScan results (%)	1,932 (39)	1,586 (32)	3,898 (64)	
Gene Ontology (%)	1,246 (25)	1,027 (20)	2,724 (45)	
Number of Biological process terms	339	291	425	
Cellular component	114	106	164	
Molecular function	401	366	479	
With homologues in *C. elegans* (%)	1,608 (33)	1,816 (37)	3,890 (64)	
other parasitic nematodes (%)	1,313 (27)	1,345 (27)	3,305 (55)	
other organisms (%)	1,901 (39)	1,888 (38)	3,895 (65)	
KOBAS (number of unique KEGG terms)	331	176	131	

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Table 2

Top twenty Pfam protein domains inferred from peptides conceptually translated from individual contigs for *Dictyocaulus viviparus* (combined dataset for L3, adult female and adult male) and those assigned to predicted peptides unique to each stage following in silico-subtraction.

Pfam description	Pfam code	Number of predicted peptides
Combined assembly (1,460)[a]		
Collagen triple helix repeat	PF01391	63
Ubiquitin	PF00240	31
Serine/threonine protein kinase domain	PF00069	28
Major sperm protein	PF00635	16
Ankyrin	PF00023	15
WD40	PF00400	15
ATPase, AAA-type	PF00004	13
Viral-A type inclusion protein	PF04508	13
Septum formation initiator	PF04977	13
Kelch repeat type 1	PF01344	12
Leucine zipper, homeobox associated	PF02183	11
Major facilitator superfamily	PF07690	11
Actin	PF00022	10
Ras	PF00071	10
RNA recognition motif, RNP-1	PF00076	10
Peptidase C1A	PF00112	10
Carboxylesterase	PF00135	10
Chaperonin	PF00118	9
TGF-beta receptor	PF01064	9
IncA	PF04156	9
L3 (2,050)[a]		
Nucleoplasmin	PF03066	33
Nop-14-like protein	PF04147	29
Microtubule	PF10243	26
Mitochondrial substrate, solute carrier	PF00153	24
Meiotic nuclear division protein	PF03962	19
Nematode cuticle collagen	PF01484	17
Myosin tail	PF01576	15
Protein synthesis factor	PF00009	14
Aminoglycoside phosphotranferase	PF01636	14
Peptidase M12A, astacin	PF01400	13
Nucleoplasmin	PF03066	33
Female (1,741)[a]		
von Willebrand factor	PF00094	77
Serine/threonine protein kinase domain	PF00069	25
Transcription factor	PF03153	25

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Pfam description	Pfam code	Number of predicted peptides
IncA	PF04156	22
Collagen triple helix repeat	PF01391	18
CDC45-like protein	PF02724	18
RNA recognition motif, RNP-1	PF00076	16
WD40 repeat, subgroup	PF00400	16
Topoisomerase II	PF09770	15
Protein of unknown function	PF06156	14
Male (2,945)[a]		
Major sperm protein	PF00635	380
SCP-like extracellular	PF00188	142
Serine/threonine protein phosphatase	PF00102	115
Ryanodine receptor	PF06459	81
Neurotransmitter-gated-ion-channel transmembrane domain	PF02932	80
Metallophosphoesterase	PF00149	72
Nuclear RNA-splicing-associated protein	PF10500	63
Aminoglycoside phosphotransferase	PF01636	56
RNA polymerase II	PF03985	54
ATPase, V0/A0 complex	PF01496	51

[a]Number of unique Pfam domains assigned to predicted peptides in each dataset

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Table 3

Top five gene ontology (GO) terms, according to the categories 'biological process', 'cellular component' and 'molecular function', assigned to peptides conceptually translated from individual contigs for *Dictyocaulus viviparus* (combined dataset for L3, adult female and adult male).

GO description (GO code)	Number of predicted peptides
Combined assembly	
Biological process (678)[a]	
Translation (GO:0006412)	572
Oxidation reduction (GO:0055114)	523
Proteolysis (GO:0006508)	513
Regulation of transcription, DNA dependent	512
Metabolic process (GO:0008152)	503
Cellular component (231)[a]	
Membrane (GO:0016020)	977
Nucleus (GO:0005634)	921
Intracellular (GO:0005622)	903
Integral to membrane (GO:0016021)	708
Cytoplasm (GO:0005737)	503
Molecular function (770)[a]	
ATP binding (GO:0005524)	1,470
DNA binding (GO:0003677)	812
Protein binding (GO:0005515)	700
Zinc ion binding (GO:0008270)	470
Nucleic acid binding (GO:0003676)	446

[a]Total number of unique GO terms assigned to predicted peptides

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Table 4

Numbers of peptides conceptually translated from individual contigs for *Dictyocaulus viviparus* (combined dataset for L3, adult female and adult male) and of predicted peptides unique to each stage following in silico-subtraction mapped to druggable InterPro domain and/or Enzyme Commission (EC) number and corresponding homologues in *Caenorhabditis elegans*, ranked according to the 'severity' of known non-wildtype RNA interference (RNAi) phenotypes.

Descriptions	Datasets			
	L3	**Female**	**Male**	**Combined**
Numbers of peptides inferred from *D. viviparus* and linked to:				
a druggable InterPro domain	38	26	163	929
a druggable EC number	445	181	299	4,919
both a druggable InterPro domain *and* EC number	126	38	64	83
Totals	609	245	526	5,931
Numbers of druggable peptides inferred from *D. viviparus* with *C. elegans* homologues linked to RNAi phenotypes:				
adult lethality[a]	4	1	5	3
embryonic and/or larval lethality	456	387	768	635
sterility	230	233	423	1,076
other[b]	537	406	903	622
Totals[c]	1,227	1,027	2,099	2,336

[a] All *C. elegans* homologues were linked to multiple non-wildtype RNAi phenotypes.

[b] Other phenotypes (alphabetical): lifespan abnormal (Age), body morphology defect (Bmd), clear (Clr), cytokinesis abnormal (Cyt), dumpy (Dpy), egg laying defective (Egl), general pace of development abnormal early embryo (Emb), asymmetric cell division abnormal early embryo (Emb), pleiotropic defects severe early embryo (Emb), fewer germ cells (Fwr), slow growth (Gro), larval arrest (Lva), pathogen susceptibility increased, protruding vulva (Pvl), exploded through vulva (Rup), sick (Sck), sluggish (Slu), sterile progeny (Ste), uncoordinated (Unc).

[c] Some predicted peptides inferred from *D. viviparus* (i.e., from 17.7 [female dataset] to 20.1% [male dataset]) had more than one *C. elegans* homologue.

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Table 5

Caenorhabditis elegans homologues of peptides inferred from contigs unique to each *Dictyocaulus viviparus* L3, adult female and adult male following in silico-subtraction, and common to all datasets (linked to a 'lethal' RNA interference phenotype) and associated with druggable (InterPro) domain and/or Enzyme Commission (EC) number, as well as examples of candidate compounds linked to these domains, predicted using the BRENDA database. Strongylid and non-strongylid nematodes for which homologues are known are also listed.

***C. elegans* gene identification code**	**Gene name**	**Protein description**	**Non-wildtype RNAi phenotypes[a]**	**References**	**Presence of homologue/s in strongylid [non-strongylid nematodes][b]**	**Examples of BRENDA compounds**
L3						
WBGene00002845	LET-711	NOT1, core subunit of CCR4/NOT complex	Let, Emb, Lvl, Lva, Ste, embryonic defects	Kamath et al. (2003)	*Ace*[*Asu, Bma, Hgl. Mpa, Ovo, Ppa, Ptr. Tmu, Xin*]	Neamine; neomycin B; N-ethylmaleimide
WBGene00000408	CDK-7	cyclin-dependent kinase CDK7	Let, Emb, Lva, reduced brood size, small	Ceron et al. (2007)	*Aca, Hco*[*Bma, Gpa, Hgl, Mha, Min, Sra, Tsp, Xin*]	Guanidine hydrochloride; Iodoacetamide; Mitoxantrone
WBGene00004502	RPT-2	26S proteasome regulatory complex, ATPase RPT2	Let, Ste	Piano et al. (2002)	*Aca, Ace, Hco, Oos, Tci*[*Bma, Gro, Hgl, Mar, Mha, Mja, Ovo, Ppa, Sra, Sst*]	Guanidine hydrochloride; Iodoacetamide; Mitoxantrone
WBGene00004501	RPT-1	26S proteasome regulatory complex, ATPase RPT1	Let, Ste	Piano et al. (2002); Kamath et al. (2003)	*Ace, Tci*[*Asu, Bma, Hgl, Mha, Min, Mja, Mpa, Ppa, Sra, Sst, Wba*]	Guanidine hydrochloride; Iodoacetamide; Mitoxantrone
Female						
WBGene00003670	NHR-80	Hormone receptor	Let, Emb, Ste, thin, embryonic defects	Brock et al. (2006)	[*Asu*]	Ribostamycin; sparsomycin; streptogramin A
Male						
WBGene00021752	Y50D7A.2	RNA polymerase II transcription initiation/ nucleotide excision repair factor	Let, Emb, Lva	Eki et al. (2007)	*Aca, Nam*[*Gpa, Hsc, Mja, Ppa, Tsp*]	Pyrrole; Pyrrolidine; Tetrahydrofuran

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C. elegans gene identification code	Gene name	Protein description	Non-wildtype RNAi phenotypes[a]	References	Presence of homologue/s in strongylid [non-strongylid nematodes][b]	Examples of BRENDA compounds
WBGene00004189	PRS-1	Prolyl-tRNA synthetase region	Let, Lvl, Ste, embryonic defects	Kamath et al. (2003); Rual et al. (2004)	*Aca, Hco*[*Asu, Hgl, Mha, Min, Ppa, Ptr, Sra, Tvu*]	Tetracycline; vanadate; viomycin
WBGene00021857	IFFB-1	Initiation factor five B (eIF5B)	Let, Emb, Ste	Maeda et al. (2001); Sonnichsen et al. (2005)	*Aca, Ace*[*Asu, Gpa, Gro, Hsc, Min, Ppa, Sra, Wba*]	Imidodiphosphate; Chloramphenicol; dihydrostreptomycin
WBGene00001094	DRS-1	Aspartyl-tRNA synthetase	Let, Emb, Lva, Lvl, embryonic defects	Sonnichsen et al. (2005)	*Hco*[*Asu, Dim, Hgl, Mar, Mch, Mja, Ovo, Ppa, Sra, Tsp, Wba*]	Chloramphenicol; dihydrostreptomycin; EF-G GTPase inhibitor
WBGene00009012	F21D5.7	Signal recognition particle protein (SRP54)	Let, Emb, Lva, Lvl, Ste, embryonic defects	Kamath et al. (2003); Rual et al. (2004)	*Ace, Hco*[*Asu, Bma, Hgl, Mch, Ppa, Sra, Sst, Tsp, Tvu*]	Guanidine hydrochloride; Iodoacetamide; Mitoxantrone
Combined						
WBGene00017309	F09F9.4	Unnamed protein	Let, Emb, Lvl, Lva,	Ceron et al. (2007)		Oligomycin; Paclitaxel; PCMB
			Ste, Unc, embryonic defects			
WBGene00019245	H27M09.1	DEAD-box protein	Let, Emb, Lva, Ste	Ceron et al. (2007); Eki et al. (2007)	*Hco*[*Bma, Hgl, Gro, Mar, Mch, Mja, Mpa, Ovo, Ppa, Ptr, Sra, Sst, Tmu, Wba*]	Tetracyclin; vanadate; viomycin
WBGene00000939	DCR-1	dsRNA-specific nuclease Dicer and related ribonucleases	Let, Emb, Lvl, Ste, embryonic defects	Kim et al. (2005); Eki et al. (2007)	[*Mch, Min, Ppa, Sst*]	Streptovaricin; Tagetitoxin; ureidothiophene

[a]Abbreviations of RNAi phenotypes (alphabetical): Embryonic lethal (Emb), adult lethal (Let), larval arrest (Lva), larval lethal (Lvl), sterile (Ste), uncoordinated (Unc).

[b]Abbreviations of nematode species (alphabetical): *Ancylostoma caninum* (*Aca*), *Ancylostoma ceylanicum* (*Ace*), *Ascaris suum* (*Asu*), *Brugia malayi* (*Bma*), *Dirofilaria immitis* (*Dim*), *Globodera pallida* (*Gpa*), *Globodera rostochiensis* (*Gro*), *Haemonchus contortus* (*Hco*), *Heterodera glycines* (*Hgl*), *Heterodera schachtii* (*Hsc*), *Meloidogyne arenaria* (*Mar*), *Meloidogyne chitwoodi* (*Mch*), *Meloidogyne hapla*

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(*Mha*), *Meloidogyne incognita* (*Min*), *Meloidogyne javanica* (*Mja*), *Meloidogyne paranaensis* (*Mpa*), *Necator americanus* (*Nam*), *Onchocerca volvulus* (*Ovo*), *Ostertagia ostertagi* (*Oos*), *Parastrongyloides trichosuri* (*Ptr*), *Pristionchus pacificus* (*Ppa*), *Strongyloides ratti* (*Sra*), *Strongyloides stercoralis* (*Sst*), *Teladorsagia circumcincta* (*Tci*), *Trichinella spiralis* (*Tsp*), *Trichuris muris* (*Tmu*), *Trichuris vulpis* (*Tvu*), *Wuchereria bancrofti* (*Wba*), *Xiphinema index* (*Xin*).