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Draft Genome of the Filarial Nematode Parasite *Brugia malayi*

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Abstract

Parasitic nematodes that cause elephantiasis and river blindness threaten hundreds of millions of people in the developing world. We have sequenced the ~90 megabase (Mb) genome of the human filarial parasite *Brugia malayi* and predict ~11,500 protein coding genes in 71 Mb of robustly assembled sequence. Comparative analysis with the free-living, model nematode *Caenorhabditis elegans* revealed that, despite these genes having maintained little conservation of local synteny during ~350 million years of evolution, they largely remain in linkage on chromosomal units. More than 100 conserved operons were identified. Analysis of the predicted proteome provides evidence for adaptations of *B. malayi* to niches in its human and vector hosts and insights into the molecular basis of a mutualistic relationship with its *Wolbachia* endosymbiont. These findings offer a foundation for rational drug design.

The phylum Nematoda is speciose and abundant and, although most species are free-living, many are parasitic. Over one-third of all humans, mainly in the developing world, carry a nematode infection. Parasitic worms typically cause chronic, debilitating infections that are often difficult to treat and that, despite the high cost to human health, have been neglected in biomedical research. Current knowledge of nematode molecular genetics and developmental

biology is largely based on extensive studies of the free-living, bacteriovorous species *Caenorhabditis elegans*. Here, we present the initial analysis of the genome of the human filarial parasite *Brugia malayi*.

Brugia malayi is endemic in Southeast Asia and Indonesia. Like other filarial nematodes, *B. malayi* develops through four larval stages into an adult male or female (fig. S1), entirely within one of two host species—a mosquito vector (*Culex*, *Aedes*, and *Anopheles*) and humans, where adult worms can live for more than a decade. *B. malayi* was chosen for whole-genome sequencing (1) because it is the only major human filarial pathogen that can be maintained in small laboratory animals. Most filarial nematodes, including *B. malayi*, carry three genomes: nuclear, mitochondrial (available at GenBank, accession no. AF538716), and that of an alphaproteobacterial endosymbiont, *Wolbachia*. We present here the draft assembly and annotated genome of the TRS strain of *B. malayi*. We provide comparative analyses with *Caenorhabditis* and another well-annotated member of the superphylum Ecdysozoa, *Drosophila melanogaster*, to further illuminate the origins of novelty and loss of ancestral characters in the model species and the parasite. Comparative genome analysis reveals key features of Nematoda that define the scope of molecular diversity that has contributed to the success of the phylum. The analysis also uncovers adaptations that appear to have evolved in the *B. malayi* genome in response to the pressures of parasitism and to the presence of the parasite's *Wolbachia* endosymbiont, wBm.

The *B. malayi* nuclear genome is organized as five chromosomes (2), including an XY sex-determination pair, and has been estimated to be 80 to 100 megabases (Mb) (3,4). The sequence of the *B. malayi* nuclear genome was obtained to ~9× coverage with the use of whole-genome shotgun (WGS) sequencing (1,5). The sequences were assembled into scaffolds totaling ~71 Mb of data with a further ~17.5 Mb of contigs not integrated into any scaffold (orphan contigs). The repeat content of the *B. malayi* genome, estimated at ~15% (1), may have contributed significantly to assembly difficulties (5,6). From these sequence data, we estimate that the *B. malayi* genome is 90 to 95 Mb (Table 1) (5). In comparison, the *C. elegans* genome is 100 Mb and the *Caenorhabditis briggsae* genome 104 Mb. The overall G + C content (30.5%) is lower than that of *C. elegans* (35.4%) or *C. briggsae* (37.4%) (6).

The complement of protein-coding genes was derived by automated gene prediction from the ~71-Mb assembly and by manual annotation of selected gene families (table S1). The 11,515 robustly predicted gene-coding regions occupy ~32% of the sequence at an average density of 162 genes/Mb (Table 1).

After inclusion of genes estimated to be found in the unannotated portion of the genomic sequence (5), we infer that *B. malayi* has between 14,500 and 17,800 protein-coding genes, agreeing with previous estimates (7). Even the higher estimate is lower than the 19,762 (WormBase data release WS133) and 19,507 (6) genes reported for *C. elegans* and *C. briggsae*, respectively, which suggests that parasitic nematode genomes have fewer genes than their free-living counterparts, echoing a pattern observed in bacterial pathogens.

For the six scaffolds longer than 1 Mb, totaling ~25 Mb of the genome, the arrangement of *B. malayi* genes was compared with that of their *C. elegans* orthologs (Fig. 1). Linkage is in general conserved: For large regions of the *B. malayi* genome, orthologs map predominantly to one (or, in the case of scaffold 14972, two) *C. elegans* chromosome(s) (Fig. 1, A to C), which indicates maintenance of linkage of these genes despite ~350 million years of separation (8). However, local gene order is not conserved (Fig. 1D). The largest, 6.5-Mb scaffold contains interdigitating blocks of genes that map to chromosomes 4 and X of *C. elegans*, which suggests there were ancient breakage and fusion events between linkage groups. These data support a model where within-linkage group rearrangements have been many times more common than

between-linkage group translocations (7,9), a pattern that may be typical of nematode genomes (6,10).

Operons are a common form of gene organization in bacteria and some protozoa, but in Metazoa, operons have been identified only in nematodes, platyhelminths, and urochordates (11,12). Using 1000 base pairs (bp) as the upper limit of intergenic spacing, 838 potential operons (5), containing ~1800 genes (16% of the total; 2 to 5 genes per operon), were found in the assembled genome (Fig. 2 and fig. S2). Of these putative operons, only 10% of the gene pairs were also in operons in *C. elegans* (table S2).

To obtain an estimate of the core complement of proteins that defines the phylum Nematoda, we compared the proteomes of *B. malayi*, *C. elegans*, and *C. briggsae*. Comparisons with the arthropod *D. melanogaster* were also made to help define a list of lineage-restricted genes. We identified 3979 sets of orthologs with representatives in all four species and 1726 sets of orthologs limited to the three nematode species (fig. S3A; tables S3 to S8). The average pairwise identity of *B. malayi* proteins with orthologs from either caenorhabditid species is ~48%. The genes conserved in nematodes but absent from the fly include cathepsin Z-like cysteine proteases, major sperm proteins, and cuticle collagens, as well as several families of unknown function. In addition, these orthologs were significantly enriched (2.4- to 4.4-fold; $P > 0.0017$) for genes with RNA interference (RNAi) phenotypes in *C. elegans* (fig. S4), which is consistent with a gene set essential to the core of nematode biochemistry and cell biology. These lineage-restricted families may define a molecular “bauplan” of Nematoda.

As noted above, the *B. malayi* genome appears to have fewer genes than *C. elegans*. On examination, much of the disparity in gene numbers can be accounted for by the extent to which gene families in *Brugia* and *Caenorhabditis* have undergone lineage-specific expansion. More than 8% of the 5780 *B. malayi*-*C. elegans* ortholog clusters were expanded in *C. elegans* (fig. S3C).

Comparing the occurrence of protein domains in *B. malayi*, *C. elegans*, *C. briggsae*, and *D. melanogaster* (figs. S3B and S5 and table S9) revealed, to our surprise, that *B. malayi* is in some ways more similar to the fly than to the model worms. For example, *B. malayi* and *D. melanogaster* have similar numbers of genes of the most abundant domains, whereas several of the most abundant domains in the caenorhabditid nematodes rank much lower in or are absent from the filarial or fly genomes (fig. S5). For domains with high abundance in all four species, *C. elegans* tends to have 1.5- to 2-fold as many instances as do *B. malayi* or *D. melanogaster* (fig. S5).

The distinctive biology of *B. malayi* is likely to be underpinned by novel proteins with unique functions. After extensive comparative analyses, 20% of the predicted proteins were found to be *B. malayi*-specific (fig. S6 and table S10). More than one-third of the 1977 hypothetical proteins found only in *B. malayi* were confirmed by *B. malayi* expressed sequence tags. These genes constitute an interesting list of initial candidates for functional studies of putatively filaria-specific gene products.

The drugs used for treatment of filarial parasites, although effective in the short-term control of worm burden and transmission, require extended courses of treatment that have traditionally compromised their long-term effectiveness. Recently, issues of the emergence of drug resistance have become a concern (13,14). From the genome sequence we can identify several systems likely to be fruitful targets for the discovery of additional drug targets. (i) Molting: The *B. malayi* genome contains many homologs of genes that encode molecules required for molting in *C. elegans* (15) including proteases, protease inhibitors, nuclear hormone receptors (NRs), cuticular collagens, and chitinases (table S11). (ii) Nuclear receptors: Twenty-seven members of the NR family were identified in the *B. malayi* genome including orthologs of

Ecr (not present in the caenorhabditids) and other NRs acting in the *D. melanogaster* ecdysone-response cascade (table S12). (iii) Collagens and collagen processing: *B. malayi* has ~82 genes that encode for a collagen repeat (including cuticular collagens and basement membrane collagens) (table S13), which is less than half the number of collagens found in the *C. elegans* genome (~180). It also encodes enzymes important for cuticular collagen processing such as blisterase-like proteases, protease inhibitors, tyrosinases, mixed-function oxidases, and peptidyl-prolyl isomerase (table S1). (iv) Neuronal signaling: Seven putative biogenic amine heterotrimeric guanosine 5'-triphosphate-binding protein (G protein)-coupled receptors, 44 Cys-loop receptors, and 36 genes encoding potassium channels (table S14) were identified in *B. malayi*, a number of which are orthologs of *C. elegans* genes that can be mutated to give paralytic or uncoordinated phenotypes. (v) The *B. malayi* kinome: The *B. malayi* genome encodes ~205 conventional and ~10 atypical protein kinases (Table 2), of which 142 appear to be of fundamental importance based on the severity of their RNAi phenotypes in *C. elegans* (table S15). (vi) Reliance on host and endosymbiont metabolism: As 9 of 10 enzymes required for de novo purine synthesis, 6 of 7 genes required for heme biosynthesis, and all 5 enzymes required for de novo riboflavin biosynthesis are absent from the *B. malayi* genome, the worm may be forced to meet requirements for these key metabolic factors by active uptake of host-supplied molecules (16) or through reliance on wBm, which has complete purine, heme, and riboflavin synthesis pathways (17).

Effective RNAi by soaking worms in double-stranded RNA(dsRNA) has been demonstrated in *B. malayi* adults (18). We therefore expected to find components of the RNAi pathway in the genome (table S16). However, some genes necessary for systemic RNAi in *C. elegans* appear to be absent from *B. malayi*, including *sid-1*, a membrane channel that transfers dsRNA molecules from a source cell to neighboring cells (19); *sid-2*; *sid-3*; and *rsd-6*. The presence of a putative *drsh-1* ortholog suggests that *B. malayi* is also capable of microRNA processing. The effectiveness of RNAi in *B. malayi* implies either that these genes are rapidly evolving or are not required in *B. malayi* or that alternate pathways for siRNA transfer exist. Improvement of RNAi protocols for filarial nematodes would offer an attractive testing platform for verifying candidate drug targets.

Mapping *B. malayi* genes onto the *C. elegans* protein-protein interaction network (20) reveals an interesting pattern of evolutionarily conserved relations within the context of interconnected functional modules (figs. S7 and S8). Of 957 *B. malayi* genes that could be mapped, only 30 were found to be nematode-specific (supporting online text), revealing the overall conserved nature of the protein interaction network (21). Given the low level of sequence similarity between the two nematodes, the identification of conserved functional modules indicates that results from investigations of these complexes within *C. elegans* may be effectively translated to *B. malayi*.

B. malayi interacts with two hosts during its life cycle and is thought to have evolved mechanisms to suppress, subvert, or exploit host defense systems (22). Comparison of sequences of predicted proteins of *B. malayi* to that of interleukins, chemokines, and other signaling molecules from humans identified intriguing candidates including two genes encoding members of the macrophage migration inhibition (MIF) family of signaling molecules (23), transforming growth factor beta (TGF β) homologs (including *Bm-tgh-1*) (24), and a member of the PDZ domain/interleukin 16 family (table S17). These proteins may be immune modulators that promote parasite survival or growth and differentiation factors important in parasite development. In addition, members of the ALT (abundant larval transcript) family of proteins have been implicated as virulence factors through their ability to modulate macrophage function (25). The *B. malayi* genome sequence revealed an unexpected diversity of 13 ALT genes (table S17), most of which are expressed by adult parasites. Note

that the ALTs represent one of the few gene families that are expanded in *B. malayi* but not in *C. elegans* (which has only one member).

The innate immune systems encoded in the *B. malayi* genome have a complexity comparable to those of *C. elegans* (26) and include thioester proteins, scavenger receptors, C-type lectins, and galectins. However, both nematodes lack the peptidoglycan-recognition and lipopolysaccharide-binding proteins found in arthropods. Although there are orthologs of some components of the DAF-2, TGF β , and p38 mitogen-activated protein (MAP) kinase signaling cascades in *B. malayi* and *C. elegans*, there is no evidence for the nuclear factor κ B and Dif pathways. None of the small antibacterial peptides described from *C. elegans* and *Ascaris suum* (27) were identified in *B. malayi*, which suggests that the parasite might have a unique set of small peptide effectors or may lack this effector arm altogether. *B. malayi* gene products implicated in defense against and interaction with mammalian and insect immune systems were found, including seven genes encoding antioxidants deployed at the cuticle surface, where they may protect against oxyradical attack (28) (table S17).

Four representative organisms involved in the maintenance of Brugian filariasis have now been sequenced: the nematode parasite; its *Wolbachia* endosymbiont, wBm; mosquito vectors (*Aedes* and *Anopheles*); and the human host. Together these present opportunities for a systems-based approach to understanding the molecular basis of parasitism and for identification of targets for intervention. In addition, defining the molecular mechanisms that allow filarial worms to persist for decades in an immunologically competent host may yield new strategies for the control of autoimmunity and the management of transplanted tissues.

The differences in genome content and organization between *Caenorhabditis* and *B. malayi* underscore the importance of obtaining additional genome data from representative species from across the diversity of the Nematoda (29). The ability to carry out large-scale comparative genomics within Nematoda will be key in defining molecules and pathways unique to nematode development and parasitism that can serve as the targets for the next generation of antinematode drugs and vaccines.

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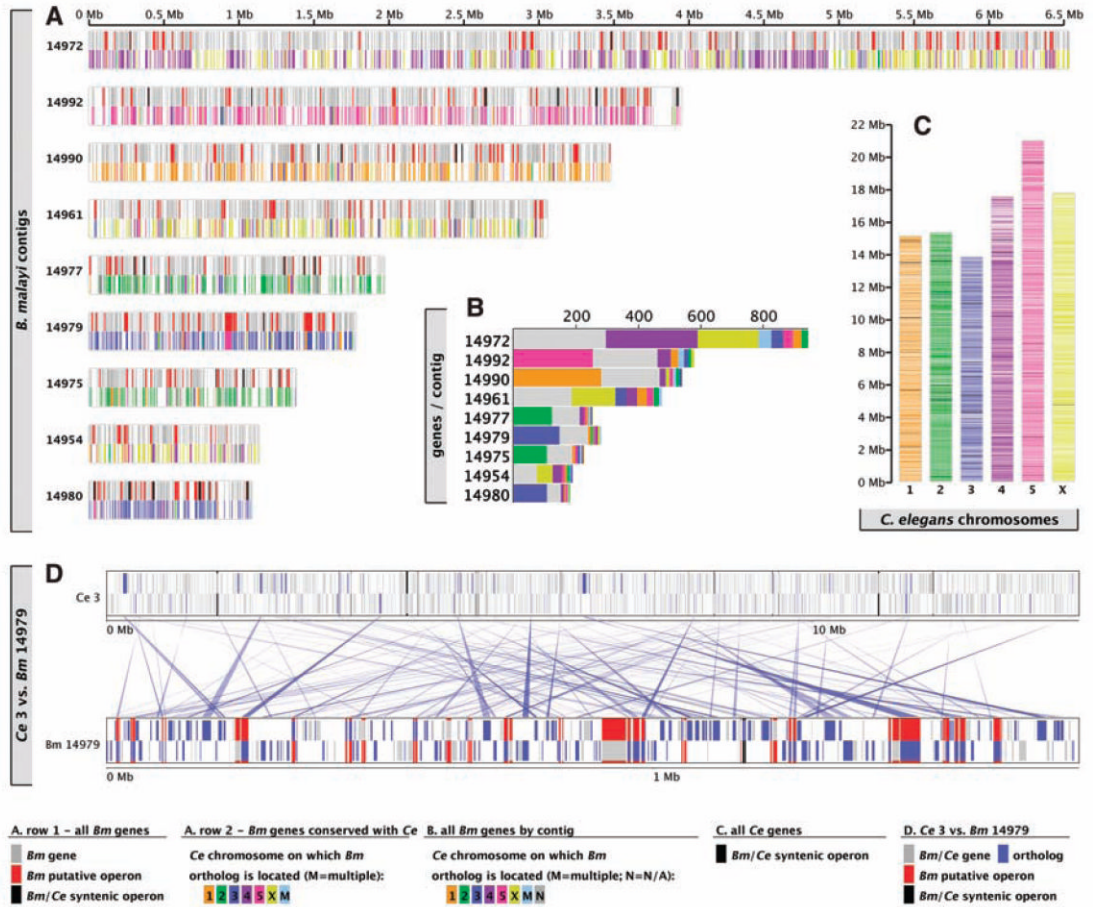
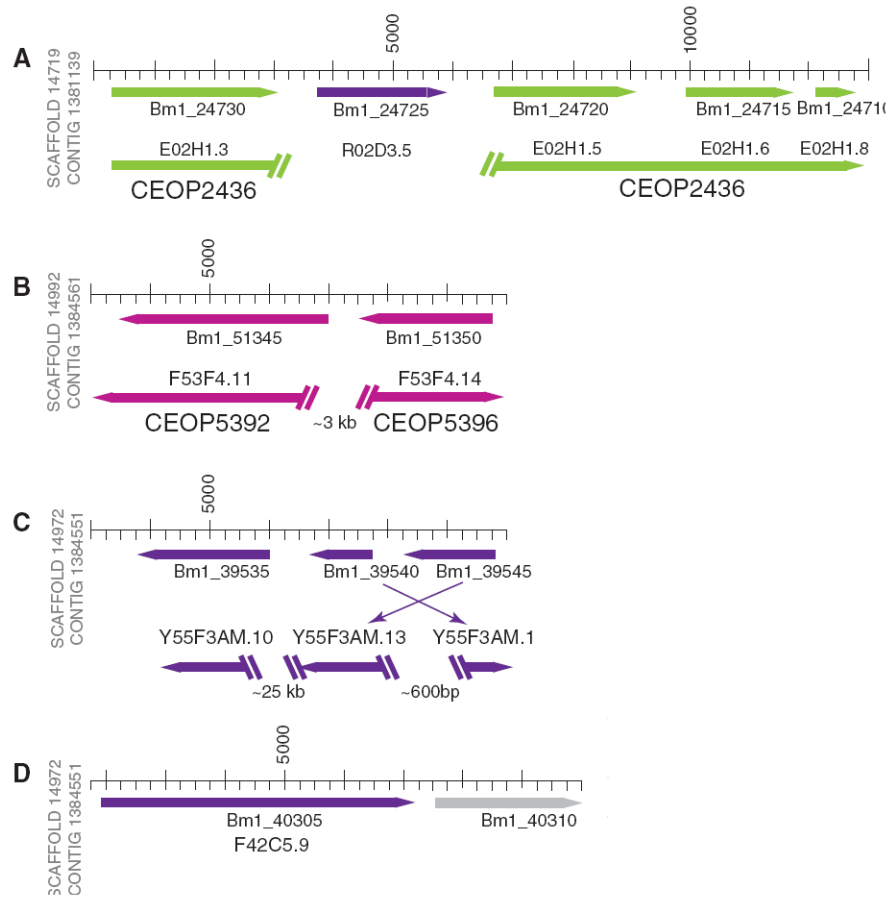


Fig. 1. Genome organization of *B. malayi* in comparison with *C. elegans*. **(A)** Genes on the eight largest *B. malayi* scaffolds. The upper part of each scaffold cartoon represents genes in conserved operons (black) or *B. malayi*-specific operons (red) or genes not in operons (gray). The lower part indicates genes with orthologs in *C. elegans*, colored to indicate the *C. elegans* chromosome on which the ortholog is located. **(B)** Numbers of genes on the longest *B. malayi* scaffolds that have orthologs on each of the six *C. elegans* chromosomes. **(C)** Distribution on the six chromosomes of *C. elegans* of orthologs of *B. malayi* genes. Orthologs map to both the conserved, central cores of the autosomes and to the less well conserved genes on the autosome arms. **(D)** Relative arrangements of the genes on *B. malayi* scaffold 14979 and their orthologs on *C. elegans* chromosome 3. Forward- and reverse-strand genes are distinguished (forward on top, reverse below). Red rectangles correspond to putative *B. malayi* operons, and black rectangles correspond to conserved operon structures.

**Fig. 2.**

Operon diversity in the genome of *Brugia malayi*. The cartoon shows the structure of four putative *B. malayi* operons (scale is shown in base pairs). Gene transcriptional orientations are shown with colored arrows, and the *C. elegans* chromosome on which the ortholog is contained is indicated (II, green; IV, dark purple; V, light purple, or none, grey), along with the peptide identification number (table S2). *C. elegans* orthologs are aligned below the *B. malayi* contigs, and their gene names and operon identifiers are shown. The angled double bars show gaps in the *C. elegans* genomic DNA; the relative size of the gap is indicated. **(A)** Four predicted *B. malayi* genes, annotated as “hypothetical,” have orthologs in a *C. elegans* operon. The *C. elegans* ortholog of the second gene is found on a different chromosome; the ortholog of E02H1.4 has not been identified within the *B. malayi* genome sequence. **(B)** In *C. elegans*, the orthologs of these genes are found in operons adjacent to each other but in opposing transcriptional orientations. **(C)** The *C. elegans* orthologs of genes found in this operon are relatively close to each other but are not in an operon. **(D)** The *C. elegans* ortholog of the first gene is not in the operon while the downstream gene (independently verified GenBank accession CAD22104) has no identifiable *C. elegans* ortholog.

Table 1

General features and genome content of *B. malayi*, with *C. elegans* values for comparison. Scaffolds are also called super-contigs, they are assembled into large regions of ordered sequence fragments separated by gaps. Orphan-contigs are sequence fragments (contigs) that do not have information allowing them to be linked to other fragments and into scaffolds. Singletons are sequences that cannot be assembled into sequence fragments (contigs). N50 is a length-weighted average of contig or scaffold size, such that the average nucleotide in an assembly will appear in a contig (or scaffold) of N50 size or greater.

Features	<i>B. malayi</i>	<i>C. elegans</i>
Overall		
Estimated size of genome (Mb)	90–95	
Total number of bp of assembled sequence (bp)	88,363,057	
Number of scaffolds	8,180	
N50 of scaffolds (bp)	93,771	
Maximum length of scaffold (bp)	6,534,162	
Number of bp assembled into scaffolds (bp)	70,837,048	
Number of orphan contigs	18,868	
Number of bp assembled into orphan contigs (bp)	17,526,009	
Number of singletons	176,099	
Number of bp in singletons (bp)	108,289,205	
Protein-coding regions		
Percent of genome containing protein-coding sequence (%)	17.84	
Number of gene models	11,515 [*]	
Number of proteins	11,508 (9,839) [†]	
Max/average protein length (amino acids)	9,445/371	18,563/440
Gene density (genes per Mb)	162	228
Number of exons	83,672	
Mean/median exon size (bp)	159/140	307/147
Mean/median number of exons per gene	7.27/5	6.38/6
Number of bp included in exons	13,282,846	
Number of introns	72,157	
Mean/median intron size (bp)	311/219	320/68
Number of bp included in introns	22,512,502	
Mean length of intergenic region (bp)	3,783	2218
Overall G + C content (%)	30.5	35.4
Exons, G + C content (%)	39.6	42.9
Introns, G + C content (%)	27.6	29.1
Intergenic regions, G + C content (%)	30.9	32.5
Non-protein coding genes		
Transfer RNA (tRNA) genes (+ tRNA pseudogenes)	~233 (+26)	
5S ribosomal RNA (found in scaffolds and orphan contigs)	~400	

* This number includes seven pseudogenes.

† The number of proteins 100 amino acids long or larger.

Table 2

The kinomes of the nematodes *B. malayi*, *C. elegans*, and *C. briggsae* in comparison with that of *Homo sapiens*. Eukaryotic protein kinases are mainly defined on the basis of sequence similarity of their catalytic domains, plus knowledge of accessory domains and any known modes of regulation. Conventional protein kinases (EPKs) include AGC [adenosine 3',5'-monophosphate–dependent protein kinase/protein kinase G/protein kinase C] kinases regulated by cyclic-nucleotide and calcium-phospholipid binding; casein kinase 1 and close relatives (CK1); calmodulin-dependent kinases (CaMK), cyclin-dependent kinases (CDK)/mitogen-activated protein kinases/ glycogen synthase kinases/CDK-like kinases (CMGC); receptor guanylate cyclase (RGC); a group including many kinases functioning in MAP kinase cascades (STE); tyrosine kinases (TK); tyrosine kinase-like kinases (TKL). Atypical protein kinases (APKs) include Alpha (exemplified by myosin heavy chain kinase of *Dicyostelium discoideum*); phosphatidylinositol 3-kinase–related kinases (PIKK); pyruvate dehydrogenase kinases (PDHK); “right open reading frame” (RIO) [named as such as it was one of two adjacent genes that were found to be transcribed divergently from the same intergenic region].

Protein kinases	Organism				Kinases shared by all 3 nematodes
	<i>H. sapiens</i>	<i>C. elegans</i>	<i>C. briggsae</i>	<i>B. malayi</i>	
EPKs					
AGC	84	35	46	22	19
CAMK	98	63	69	41	23
CK1	12	91	77	31	13
CMGC	70	56	60	33	28
RGC	5	27	24	4	4
STE	61	35	27	27	16
TK	93	96	73	35	21
TKL	55	22	21	12	9
Total	478	425	397	205	133
APKs					
PIKK	6	5	4	5	4
Alpha	6	1	1	1	1
PDHK	5	1	1	1	1
RIO	3	3	3	3	3
Total	20	10	9	10	9