Parasitology

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Caenorhabditis elegans: nature and nurture gift to nematode parasitologists

Gustavo Salinas[1,2] and Gastón Risi[1,2]

[1]Worm Biology Laboratory, Institut Pasteur de Montevideo, Mataojo 2020, Montevideo 11400, Uruguay and [2]Departamento de Biociencias, Facultad de Química, Universidad de la República, Avda. gral Flores 2124, Montevideo 11300, Uruguay

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Author for correspondence:
Gustavo Salinas, E-mail: gsalin@fq.edu.uy

Abstract

The free-living nematode *Caenorhabditis elegans* is the simplest animal model organism to work with. Substantial knowledge and tools have accumulated over 50 years of *C. elegans* research. The use of *C. elegans* relating to parasitic nematodes from a basic biology standpoint or an applied perspective has increased in recent years. The wealth of information gained on the model organism, the use of the powerful approaches and technologies that have advanced *C. elegans* research to parasitic nematodes and the enormous success of the omics fields have contributed to bridge the divide between *C. elegans* and parasite nematode researchers. We review key fields, such as genomics, drug discovery and genetics, where *C. elegans* and nematode parasite research have convened. We advocate the use of *C. elegans* as a model to study helminth metabolism, a neglected area ready to advance. How emerging technologies being used in *C. elegans* can pave the way for parasitic nematode research is discussed.

Introduction

The free-living nematode *Caenorhabditis elegans* was introduced as a model organism more than half a century ago by Sidney Brenner (Brenner, 2002). Since then, this 1 mm free-living soil nematode has been extensively studied in the laboratory, and *C. elegans* research has contributed to the discovery and understanding of many biological processes relevant to other multicellular organisms. A milestone discovery was the elucidation of the origin and fate of each of the 959 somatic cells of this organism (Sulston and Horvitz, 1977). This allowed development from the perspective of the individual cell to be understood. Other landmarks were the reconstruction of the complete set of synaptic connections in its nervous system (White *et al.* 1986) and the discovery of apoptosis genes (Hedgecock *et al.* 1983). Equally important, *C. elegans*-driven research has allowed the development of new techniques and tools for broad applications. The introduction of GFP as an *in vivo* reporter brilliantly illustrates this concept (Chalfie *et al.* 1994). Another key discovery made in *C. elegans* is gene silencing by RNA interference (RNAi) (Fire *et al.* 1998). A timeline of significant discoveries made in *C. elegans* can be found in (Corsi *et al.* 2015). The success of *C. elegans* as a metazoan model lies in its biological simplicity combined with its easiness to grow, manipulate and perform genetics studies. Key attributes are its rapid life cycle (~3 days) (Fig. 1A), large progeny (~300 eggs per generation), low maintenance cost, long-term cryopreservation, transparency and its invariant cell number and development (Hall and Altun, 2008). The intensive study of this organism has led to the accumulation of knowledge and to the development of a large repertoire of tools and resources over time. A list of high-quality *C. elegans* resources freely available is provided in Table 1. Importantly, since its inception the *C. elegans* research community promotes sharing of reagents and ideas.

For helminth parasitologists, and particularly for those who work in parasitic nematodes, *C. elegans* provides additional advantages. In contrast to *C. elegans*, nematode parasites are inherently difficult to grow, maintain and manipulate, and only in rare cases their lifecycle can be maintained in the laboratory. Indeed, none of the medically relevant parasitic nematodes can be maintained through the entire lifecycle without passing through a host. Nematodes are monophyletic (Blaxter and Koutsovoulos, 2015) and have a remarkably similar anatomy, development plan and life cycle through four larval stages (Fig. 1B). It is worth mentioning that *C. elegans* resistant and dispersal larval stage, the dauer larval stage (Fig. 1A), closely resembles free-living L3 stage of parasitic nematodes (Crook, 2014). The dauer stage is highly resistant to stress. It acts as a dispersal stage, either through its own locomotion or by hitchhiking on a larger invertebrate to resume development as L4 in a propitious environment (Felix and Braendle, 2010). It is thought that this resistant larval stage may have been the evolutionary precursor of the L3 infective stage, and important for the recurrent emergence of parasitism from free-living species in the nematode lineage (Crook, 2014).

How this amenable free-living relative that naturally lives in the soil can serve as a powerful tool for many studies in which nematode parasitologists are interested will be reviewed in following sections.

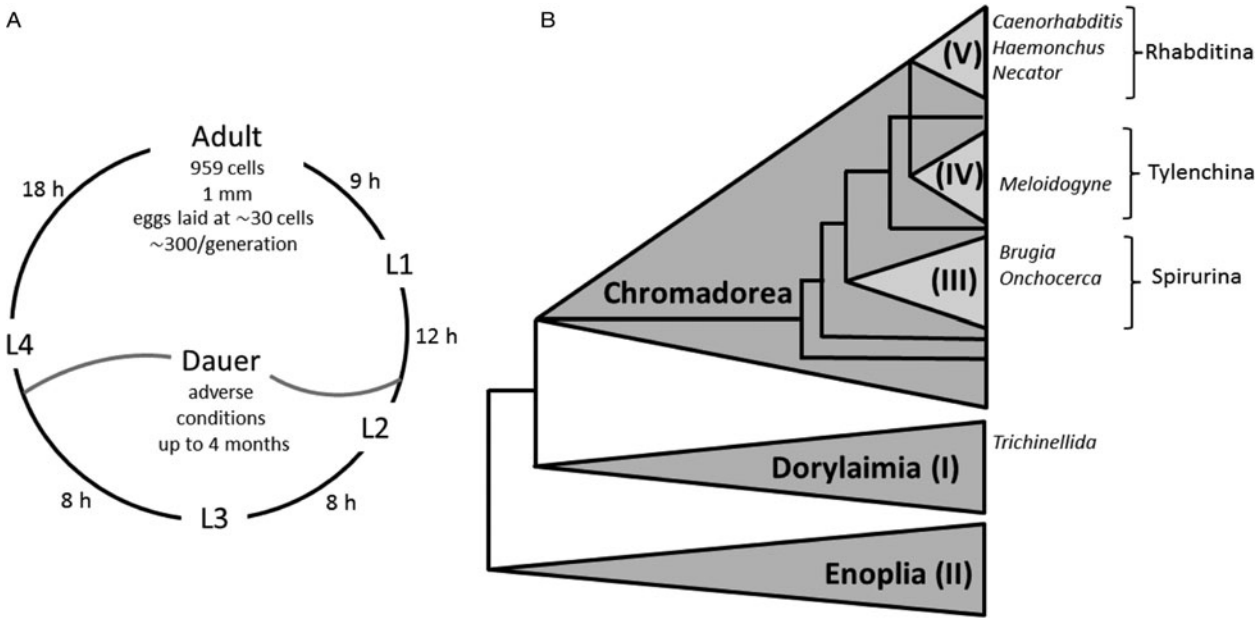

Fig. 1. *Caenorhabditis elegans* is a nematode (A) Life cycle of *C. elegans*. The length of time the animal spends at certain stages at 22 °C is indicated in hours (h). Eggs of approximately 30 cells are laid outside at about 40 min post-fertilization. Embryonic development continues *ex utero* during 9 h. After hatching as L1 (558 cells) development, continues under favourable conditions, through four stages interrupted by moulting. Approximately 300 eggs are laid per generation per adult worm. Under adverse conditions (e.g. starvation, crowding, high temperature), *C. elegans* enters the diapause cycle (dark grey) and gives rise to dauer larva (an enduring larval form), which can survive for up to 4 months. There are two *C. elegans* sexes, a self-fertilizing hermaphrodite (XX, 959 somatic cells) and a male (XO, 1031 somatic cells). Males arise infrequently (0·1%) by spontaneous non-disjunction in the hermaphrodite germ line and at a higher frequency (up to 50%) through mating (hermaphrodites can also be induced to generate male progeny spontaneously at a higher rate by treatment at high temperature) (Hall and Altun, 2008). (B) Simplified phylogeny of the Phylum *Nematoda*. In all groups, there are free-living and parasitic species. Traditional clades (I–V) are indicated. *Caenorhabditis elegans* belongs to clade V, which includes several vertebrate parasitic species such as *Necator* spp. and *Haemonchus* spp. The phylogeny is based on (Blaxter and Koutsovoulos, 2015), *Enoplia* is a now recognized as a basal lineage (Mark Blaxter, personal communication).

WormBase and WormBase parasite: key resources in an information ERA

There is a wealth of open access resources for those working in *C. elegans* and nematodes that provide information on a range of topics (Table 1). WormBase (http://www.wormbase.org) is a central repository for research data on the biology, genetics and genomics of *C. elegans* and other nematodes. WormBase integrates several online resources (see Table 1), such as Textpresso, an information extracting and processing package for the biological literature. WormBook, the online text companion to WormBase, is a comprehensive and open-access resource covering topics related to the biology of *C. elegans* and other nematodes. Indeed, several chapters dedicated to parasitic nematodes help to bridge the *C. elegans* and the parasitic nematode research communities. In this review, we will emphasize the genomic and gene information currently available through Wormbase and its recent relative WormBase ParaSite (Howe *et al.* 2016, 2017).

Caenorhabditis elegans was the first animal genome to be sequenced, curated and annotated and became the animal reference genome (Consortium, 1998). This contributed to accelerating genome sequencing and annotation of the human and other animal genomes, notably those of nematode species. *Caenorhabditis elegans* genome is manually revised and refined incorporating literature information and RNAseq data (mRNA and non-coding RNA) from experiments, and updated regularly by WormBase curators. RNA-seq is important for transcriptomic profiling and to identify novel transcripts and alternative splicing events. In turn, this provides accurate gene models and genome annotation for further studies. Importantly, WormBase contains information on resources for each gene (e.g. gene model, clones, mutant strains), functional information of genes and gene products (e.g. phenotypes), and its interface allows easy exploration of data. Recently, FPKM (fragment per kilobase of exon per million reads mapped) expression values of cDNA libraries across all stages of *C. elegans* life cycle have been included in Wormbase. This transcript profiling data includes, in addition to the classical stages an embryonic time series at 30- min intervals (Hashimshony *et al.* 2015). This provides a correlation between developmental stages and their underlying molecular activity. For genes that have parasitic nematode orthologues this transcriptomic profiling may serve as a proxy and to generate and test-specific hypothesis.

Fortunately, the number of both free-living and parasitic worm genomes sequenced has sharply increased in the last years due to independent and combined efforts such as the 959 nematode genome initiative (http://www.nematodes.org/nematodegenomes/index.php/Main_Page). In response to the challenge of having good quality genomes annotated a new sub-portal from WormBase, WormBase ParaSite, has recently been launched as a collective effort (Howe *et al.* 2016, 2017). Both databases include three parasitic genomes (*Brugia malayi*, *Onchocerca volvulus* and *Strongyloides ratti*) for which physical maps, careful automatic and manual annotation are continuously being refined (Howe *et al.* 2016). WormBase ParaSite is publicly updated three times a year and its release 7 (April 2017) includes 84 nematode species (101 genomes) as well as 30 platyhelminthes species (33 genomes). RNAseq expression data from experiments in eight helminth species has also been included in WormBase ParaSite, helping to improve gene models. The database allows comparative genomics to be performed easily and provides foundations for the application of modern functional genomics approaches to this group of pathogens. Importantly, the WormBase community forum has approximately 2000 members that discuss general nematode biology issues and Wormbase curators provide help to users.

Similar to WormBase, genes can be searched in WormBase ParaSite by a variety of criteria (including gene name, blast

Table 1. Open-access informational and experimental resources

Wormbase: A comprehensive database to explore worm biology (see also text) www.wormbase.org. Integrates several online resources. For example Textpresso, an information extracting and processing package for biological literature http://www.textpresso.org/celegans
Wormbase Parasite: Designed specifically to support helminth genomic research, it contains all publicly available nematode and platyhelminth annotated genome sequences parasite.wormbase.org
***C. elegans* II**: Historic but still useful source of information. http://www.ncbi.nlm.nih.gov/books/NBK19997
Wormbook: A comprehensive collection of updated reviews covering most topics of *C. elegans* biology. It also contains WormMethods, a collection of protocols for nematode researchers http://www.wormbook.org
Wormatlas: A database featuring behavioral anatomical and structural anatomy of *C. elegans.* It also contains an extensive guide to cell-identification methods http://www.wormatlas.org
Wormwiring: Hosts the nervous system synaptic connectivity data of individual nematode worms, determined by serial section electron microscopy wormwiring.org
WormClassroom: An education portal for the research model organism http://www.wormclassroom.org/
Openworm: Aims to build the first comprehensive computational model of *C. elegans* http://www.openworm.org
Nembase4: A comprehensive Nematode Transcriptome Database including 63 nematode species, over 600 000 ESTs and over 250 000 proteins from http://www.nematodes.org/nembase4
959 nematode genomes: A resource that provides an overview over a complete and ongoing nematode sequencing projects. http://http://www.nematodes.org/nematodegenomes/index.php/Main_Page
NextDB: The Nematode Expression Pattern DataBase. Through NextDB an extensive collection of cDNA clones generated by the Kohara laboratory at the National Institute of Genetics of Japan is available
***Caenorhabditis* Genetics Center (CGC)**: Collects, maintain and distributes *C. elegans* strains. It hosts the collection of mutants generated by the *C. elegans* Gene Knockout Consortium cbs.umn.edu/cgc
National Bioresource Project: Generates deletion alleles at specified gene targets. It also hosts a *C. elegans* Promoter/Marker Database. shigen.nig.ac.jp/c.elegans
Fire Lab *C. elegans* Vector Kit: A set of vectors for *C. elegans* research containing 288 vectors available from addgene http://https://www.addgene.org/kits/firelab/

tools, text mining) through an amenable browser. For a given gene different information can be easily explored (e.g. transcript models, functional annotations, cross-references to other resources, genomic context). The gene page is the main entrance to explore helminth comparative genomics and infer the history of genes and gene families (Fig. 2A). The pipeline organizes worm and other reference species genes in homologous clusters, constructs protein multiple alignments for each cluster, and produces a gene tree which takes into account sequence-based phylogeny and the species phylogeny, which can be used to understand gene history by speciation and gene duplication (Fig. 2B). Advanced search tools and export data is available through the Biomart tool available on the site. This advanced searcher allows the generation of lists of genes of interest (e.g. phosphatases) and to retrieve specific information about them. Future plans for WormBase ParaSite include the identification of putative targets for anthelmintic drugs and linking gene products to the ChEMBL database of medicinal chemistry (Davies *et al.* 2015). This is important for parasitologists whose primary goal is to identify ways of controlling parasites. Finally, a Worm Community Forum and Blog allow researchers to stay connected and updated with new features and data.

Caenorhabditis elegans genetic tools and knowledge can serve nematode parasitologists in several ways

Caenorhabditis elegans has key attributes to understand biological processes from a genetic viewpoint, leading to a continuous development of powerful genetics tools (Corsi *et al.* 2015). There are two *C. elegans* sexes: a self-fertilizing hermaphrodite (XX) and a male (XO). Males arise infrequently (0·1%) by spontaneous nondisjunction in the hermaphrodite germ line and at a higher frequency (up to 50%) through mating. Self-fertilization of the hermaphrodite allows for homozygous worms to generate genetically identical progeny, and male mating facilitates the isolation and maintenance of mutant strains as well as moving mutations between strains (http://www.wormatlas.org/hermaphrodite/introduction/Introframeset.html). The transparency of the organism and the ease at which this organism can be mutagenized and genetically manipulated by transgenesis has greatly helped to consolidate this model organism. Microinjection of DNA into the gonads leads to stable extrachromosomal arrays that are expressed and inherited during several generations after transformation. This has been extremely informative for studying the spatial and temporal expression of genes in a live organism, to dissect gene regulatory elements and to directly interrogate gene function. RNAi by feeding is also easy and convenient for interfering with gene expression.

New technologies are accelerating the pace: CRISPR/Cas9 is revolutionizing genetics and genome editing in *C. elegans* (Dickinson and Goldstein, 2016) and Next-Generation Sequencing-based approaches being used for rapid mutation mapping and identification (Doitsidou *et al.* 2016). The resources available to researchers are unparalleled (Table 1). There is a wide and ever-increasing repertoire of vectors for different genetic studies. The existence of comprehensive RNAi libraries is another example. Worm strains can be requested at low cost from the *Caenorhabditis Genetics Center*, which is dedicated to collect, maintain and distribute *C. elegans* strains and host an enormous repository of mutant and reporter strains.

In contrast to the advances made in *C. elegans* genetics, progress in genetics studies in parasitic nematodes has lagged behind (Ward, 2015; Zamanian and Andersen, 2016). A major difficulty has been the impossibility to maintain parasite life cycles outside a host. The most advanced genetic studies in nematode parasites have been achieved in *Strongyloides* spp. and *Parastrongyloides* spp., which alternate parasitic and free-living generations; a thorough recent review on the genetics of these organisms is presented in (Streit, 2017). It was soon realized that the toolkit of *C. elegans*

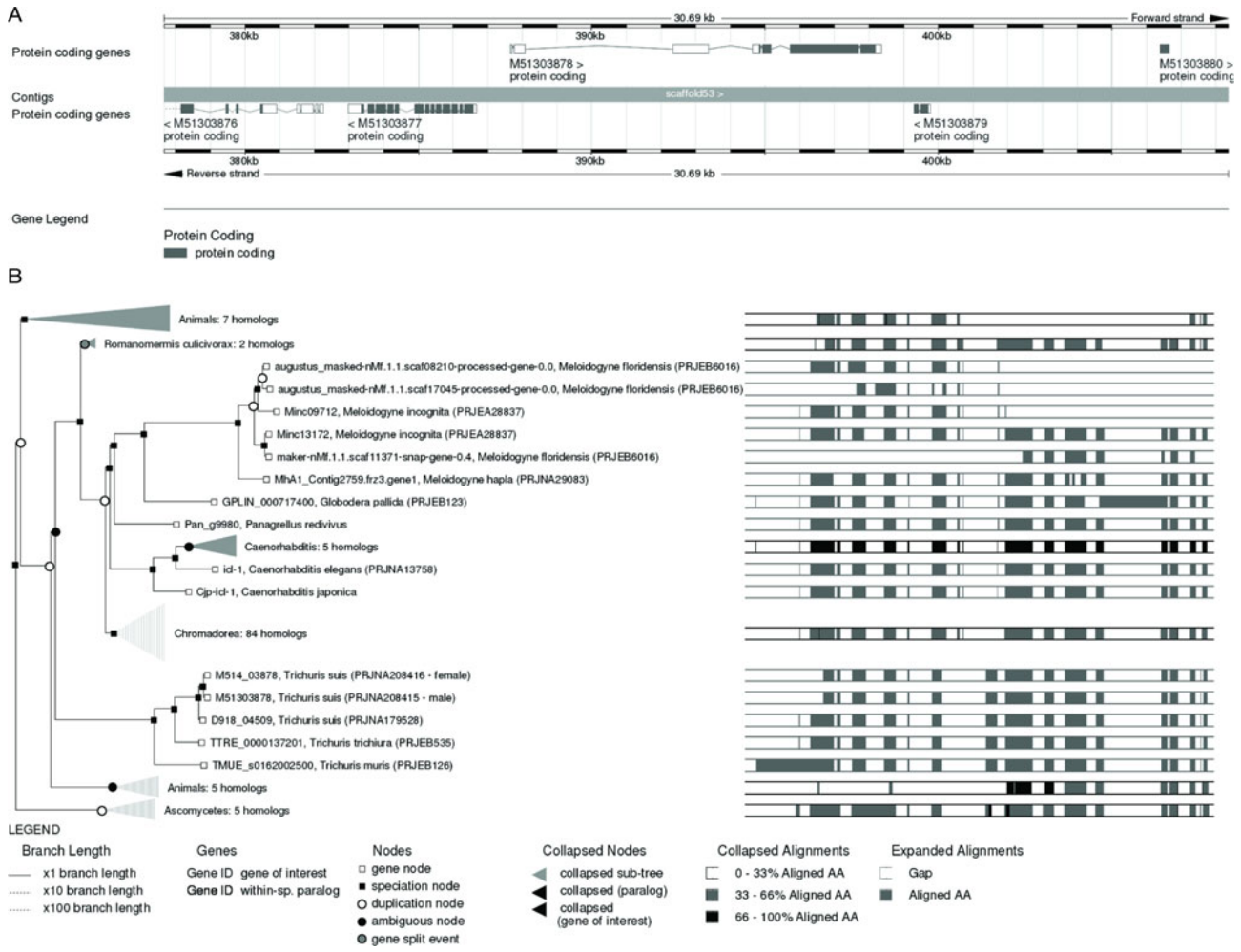

Fig. 2. WormBase and WormBase ParaSite: useful tools for nematode research. (A) Typical gene model provided by WormBase ParaSite. Gene model for bifunctional glyoxylate cycle protein isocitrate lyase-malate synthase from *Trichuris suis* (Gene ID: M51303878), projected from *C. elegans* orthologue *icl-1*. Detailed information about the gene is available at the gene page; it is possible to inspect the region for additional features or detailed information of the gene through friendly navigation options (e.g. zooming to see the nucleotide and protein sequences). (B) Gene tree provided by WormBase ParaSite. For any given gene WormBase ParaSite provides information on transcript variants (gene node, open square), orthologue genes (speciation node, black square) and paralogue genes (duplication nodes, open circle). Specific nodes can be collapsed or expanded according to particular needs. The information provides the gene models for the species selected. The gene tree for isocitrate lyase-malate synthase (*icl-1*) is shown.

required adjustments for performing genetics in these species (e.g. the use of specific 5′ and 3′ UTR (untranslated region) sequences to obtain effective transient transgene expression). Microinjection of DNA into the gonad of free-living *Strongyloides* females was achieved and expression observed after infection in an experimental host; this constituted a milestone that highlighted the potential of transgenesis for studying host–parasite interactions (Li *et al.* 2011). Although the arrays were inherited by several generations, silencing was observed after the F_1 generation. The following key advance was the heritable and stable expression of an *S. ratti* transgene (*Ss-act-2*) integrated into the chromosome by means of the *piggyBac* system (Shao *et al.* 2012). Through alternation of host and culture passage with GFP selection, a stable transgenic line of *S. ratti* was established. The high efficiency of infection of this rat parasite was instrumental to this achievement. Recently, another milestone was achieved: a proof of principle of target-specific insertional mutagenesis *via* CRISPR/Cas9 was reported in *Strongyloides stercoralis* (Lok *et al.* 2017). Lok and coworkers succeed using this methodology inserting a 24 bp sequence containing stop codons in all frames of exon 5 of the insulin-transcription factor *Ss-daf-16*, resulting in a null mutation. In a recent study, heritable *Ss-unc-22* mutations generated by CRISPR/Cas9 succeeded in passing a mutant F_1 progeny through a laboratory host and collected F_2/F_3 nematodes with *Ss-unc-22* phenotypes (Gang *et al.* 2017). This mutant provides the same uncoordinated twitching phenotype as the *C. elegans* ortholog and this can be used as a marker to select for transgenic worms. Importantly, in this study the CRISPR/Cas9-induced double-strand breaks and the presence of a homology-directed repair template improved targeting efficiency and produced *Ss-unc*-22 homozygous knockouts (KO) in the F_1 generation. This suggests a practical method to render the mutation homozygous, which *a priori* appeared as another bottleneck.

Strongyloides spp. with their key advantages and the most advanced toolkit still illustrates the difficulties in performing genetic studies in parasitic nematodes. The challenge ahead for most parasitic nematodes is enormous, and the techniques that have been established for *C. elegans* are not easily transferable to the research on other parasitic worms. For most parasites, a key problem is the inaccessibility to the gonad of the adult worms. Particle bombardment has been explored as an alternative, but in practice led to high mortality (Ward, 2015). The thick cuticle of nematodes is a barrier for electroporation and chemical-mediated DNA transformation. The moulting of the cuticle can provide a window at which the organism may be more susceptible to the entrance of foreign DNA. The discovery of viruses that infect *Caenorhabditis* spp. (Felix and Braendle, 2010) may eventually offer a vehicle for gene delivery, but this has not been tried in

the model organism, and remains speculative. Recent reviews have addressed in detail the limited advances in functional genomics and genome-editing techniques made in *Brugia* spp. and *Haemonchus contortus* (Ward, 2015; Britton *et al.* 2016; Zamanian and Andersen, 2016). These two relevant human and livestock nematode parasites are among those in which research in genetics approaches have concentrated. RNAi has found limited application in these parasites, the translation of CRISPR/Cas9 technology to these parasites still appears distant. Some critical needs are: (i) the development of reliable and robust methods of transformation, (ii) appropriate vectors and markers that allow stable expression and easy selection as well as vectors that allow conditional or regulatable transgene expression for essential genes (Ward, 2015).

Caenorhabditis elegans may provide a surrogate system to study parasite gene function. The characterization of parasite genes by heterologous expression in *C. elegans* provides a tool for studying the cellular functions of specific genes. If a parasite protein is similar enough to the *C. elegans* protein, it could be used to test recovery of a phenotype in a *C. elegans* KO strain. *Caenorhabditis elegans* mutant libraries can also be exploited by nematode parasitologists. The Million Mutation Project, a library of more than 2000 *C. elegans* strains with an average of eight non-synonymous mutations for all 20 000 *C. elegans* protein-coding genes (Thompson *et al.* 2013). The Gene Knockout Project has generated over the years another useful library containing strains with loss of function mutations in more than 14 000 of the 20 000 worm's protein-coding genes (Hutter and Moerman, 2015). The National Bioresource Project also stores and distributes its own *C. elegans* deletion mutants. These libraries can be readily exploited for many uses such as reverse genetic approaches to explore specific ligand (e.g. drug) binding sites (Mathew *et al.* 2016) and forward genetics (i.e. random mutagenesis followed by screening selection).

Despite the advantages of *C. elegans* as a model, there are limitations. There is a large number of species-specific genes in parasitic nematodes with no homologues in *C. elegans* (and vice versa), precluding the use of *C. elegans* as a surrogate system for those cases. Approximately 25% of genes are unique to each species within the phylum *Nematoda* (Parkinson *et al.* 2004). Furthermore, other genes have undergone lineage-specific duplications (Blaxter and Koutsovoulos, 2015). There are also inherent limitations of heterologous expression studies. *Caenorhabditis elegans* may not reproduce the native expression pattern of a parasite gene. Differences in regulatory elements may vary between species and lead to differences in tissue-specific expression or temporal regulation. These limitations illustrate the necessity for working with *C. elegans* and the parasite of interest in parallel.

Caenorhabditis elegans as a model in anthelmintic research

About one-quarter of the world population is estimated to be infected with parasitic nematodes, mainly in poor developing countries. In addition, food production is greatly reduced by animal and crop nematode infections causing large economic losses across the globe (Keiser and Utzinger, 2010; Lo *et al.* 2016). There is widespread resistance to currently used drugs in the veterinary field and concern that resistance may arise in humans parasitic nematodes (Besier, 2007; Sutherland and Leathwick, 2011). Thus, new anthelmintics and drug discovery projects are essential.

In this regard, *C. elegans* drug screenings present a valuable choice. However, there are some considerations to be made. As a free-living nematode, *C. elegans* has complex and developed detoxification mechanisms as well as a thick cuticle to prevent damage from the wide range of substances that might be present in changing environments as the soil. In order to circumvent this issue, there is a broad spectrum of screening design adjustments available. Some examples are to increase the concentration of the tested compounds, to improve the target accessibility by adding a mild detergent such as Triton X-100 or Tween-20, to sensitize worms with a low dose of a known anthelmintic or to use mutant worms, for example with modified xenobiotic defences or altered cuticle integrity (Burns and Roy, 2012). Another issue is that as *C. elegans* lacks unique parasitic pathways, a considerable number of active molecules against parasites may be lost. Nonetheless, the advantages of using *C. elegans* as a primary screening filter (high-throughput capabilities, cost and ease of use) far outweigh its disadvantages.

In the past years, multiple approaches have been carried out; some recent key studies are reviewed in this section. A notable work in this regard was the performance of an extensive *C. elegans* screen of chemical compounds and re-screen of the active compounds against *Cooperia oncophora* and *H. contortus* (Burns *et al.* 2015). Molecules that killed *C. elegans* were 15 times more likely to also kill these parasitic nematodes compared with randomly chosen molecules. In addition, almost 40% of the *C. elegans* lethal molecules were also lethal to both of the other nematodes tested. This confirms the usefulness of *C. elegans* for anthelmintic drug discovery. Moreover, the authors proposed a pipeline for the primary screening process, starting with *C. elegans* as a primary filter, continuing with parasitic nematodes (e.g. *C. oncophora*, *H. contortus*) to confirm the nematocidal activity and ending with vertebrate models (e.g. HEK293 cells, zebrafish) to address possible host toxicity.

Several different *C. elegans* assays have been developed. Changes in worm behaviour and metabolism are the most used readouts, which are usually automated. In the case of metabolism analysis, colorimetric and even fluorescence-based assays have been reported (James and Davey, 2007; Ferreira *et al.* 2015). Regarding behaviour, the range of approaches is broader. Mathew *et al.* reported the usage of a scanner to identify decreased motility (Mathew *et al.* 2016). The light intensity produced by the scanner was enough to produce negative phototaxis, which allowed worm paralysis to be detected. Recently, Weaver *et al.* proposed the implementation of a health-rating system to prevent the oversight of possible active drugs in binary dead/alive approximations (Weaver *et al.* 2017). There are numerous automatic systems to analyse behaviour changes, including worm tracking by beam interruption or camera displays, targeting specific or general behaviour, microfluidics, among others (Buckingham *et al.* 2014).

Although *C. elegans* is a good model organism for anthelmintic screening, the selection of what to screen is equally important. In this regard, there are several options: natural products, chemically synthesized libraries, Food and Drug Administration (FDA)-approved drug libraries, among others. The complexity and molecular diversity that can be obtained from natural sources are vast. Moreover, the most effective anthelmintic family, the avermectins, has arisen from this origin (Cragg and Newman, 2013). Chemically synthesized libraries, on the other hand, can easily reach huge sizes, allowing the study of more molecules. Besides, active compounds usually can be readily identified and synthesized in great quantities. FDA-approved drug libraries have the advantage that their compounds are known in detail, accelerating future regulatory studies (Panic *et al.* 2014).

In addition to being a model for drug discovery, *C. elegans* has also been important in defining molecular targets of several nematocidal drugs and understanding the complex phenomena of resistance to anthelmintics. The ability to generate random mutants in the laboratory followed by selection with anthelmintics simulates the natural process that gives rise to the emergence of resistance. The identification of the mutated gene (or genes)

that generates resistance creates a link, either direct or indirect, between the gene and the resistance mechanism. This approach used in *C. elegans* has been important in identifying anthelmintic targets, downstream signalling, metabolization and detoxification (Sloan *et al.* 2015; Duguet *et al.* 2016). A review of the contributions that *C. elegans* research has made to this specific field has recently been provided in (Holden-Day and Walker, 2014). For instance, *C. elegans* was instrumental in identifying the target and mutations conferring resistance to monepantel, one of the most recent nematocidal drugs (Kaminsky *et al.* 2008). *Caenorhabditis elegans* is also useful to uncover new drug targets. Recently, nematode-specific acetylcholine-gated chloride channels have been validated as anthelmintic targets in a *C. elegans*-based study (Wever *et al.* 2015). This was determined by ectopically expressing AVR-15, an ivermectin-gated chloride channel, in tissues that endogenously express acetylcholine-gated chloride channels and using ivermectin to predict the effect of an agonist drug on them.

Caenorhabditis elegans as a model to study helminth metabolism: a neglected area ready to advance

Caenorhabditis elegans offers an excellent model to understand nematode biochemistry at different organizational levels (cell, tissue, organ and organism). A limitation has been the isolation of large quantities of particular tissues. The development of sensitive metabolomics techniques for analysing small-volume samples [mass spectrometry (MS), gas–liquid chromatography–MS, capillary electrophoresis-MS and nuclear magnetic resonance spectroscopy] should help to reduce this problem. *Caenorhabditis elegans* reporters for specific genes and pathways would be particularly useful to understand cell/tissue/organ metabolic specializations and contribute to the understanding of this unexplored area. For nematode parasitologists to understand metabolism is also important from a practical viewpoint. Knowing how parasites make a living in the host can provide pharmacological targets to treat infections. In this section, we will focus on key intermediary metabolic and other unique aspects of nematode metabolism that *C. elegans* shares with parasitic nematodes, but not with the host.

Malate dismutation is a pathway that serves parasitic nematodes (and also platyhelminths) to harvest energy under hypoxic conditions, such as those found in the gastrointestinal tract (Tielens and Van Hellemond, 1998). This mitochondrial pathway uses an alternative electron transport chain (ETC) in which fumarate instead of oxygen is the final electron acceptor, and rhodoquinone instead of ubiquinone is an electron carrier (Fig. 3A) (Van Hellemond *et al.* 1995; Kita and Takamiya, 2002). Although ubiquinone and rhodoquinone share some steps in their biosynthetic route, rhodoquinone synthesis has not been completely elucidated (Lonjers *et al.* 2012; Mentel *et al.* 2014). Due to its size, *Ascaris suum* has been a model nematode for biochemistry. Studies in *A. suum* have shown that exchange of complex II subunits occurs from the L3 free-living stage to the adult worm, which resides in the pig intestine where there is low oxygen tension (Kita and Takamiya, 2002; Iwata *et al.* 2008). This subunit exchange appears to be responsible for allowing complex II to work in reverse direction to the conventional ETC (Fig. 3A). The fact that adjustments in ligand recognition (rhodoquinone *vs* ubiquinone) and/or redox potentials may be required in complexes I and II might explain that some identified nematocides appear to target these complexes without affecting their mammalian counterpart (Kita, 2016). The key metabolite rhodoquinone

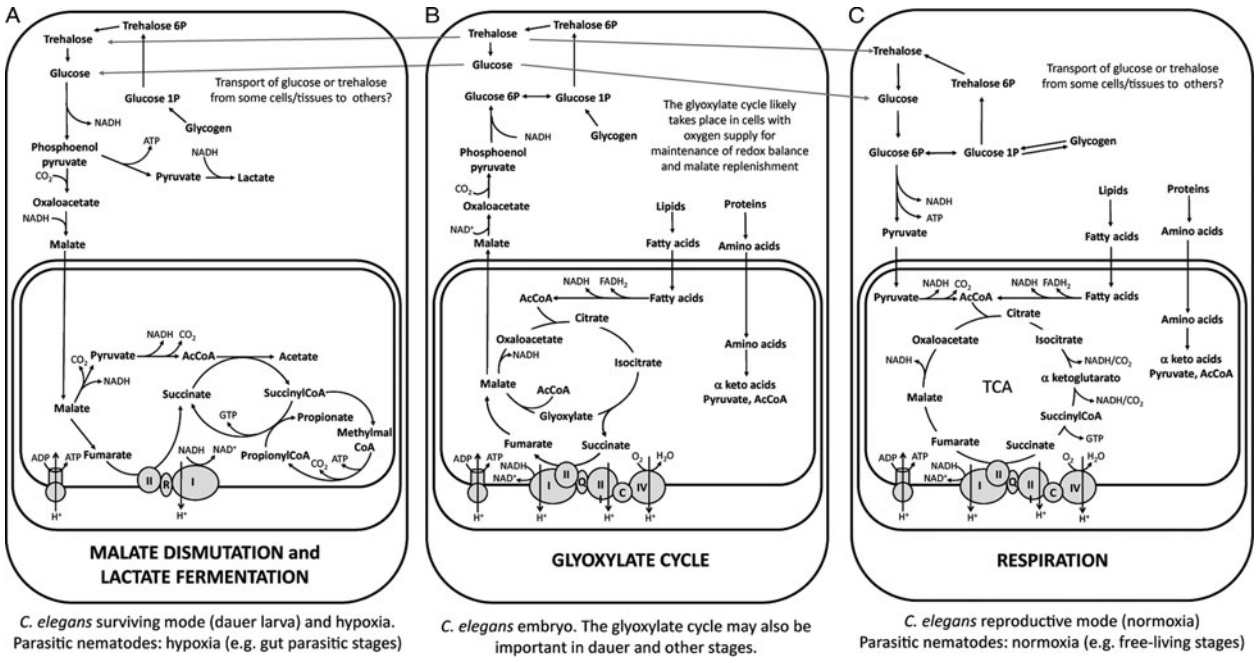

Fig. 3. Nematode metabolic pathways for harvesting energy. (A) Schematic cell depicting the malate dismutation pathway. In this pathway, part of the malate is oxidized to acetate and part is reduced to succinate, using an alternative electron transport chain that involves rhodoquinone (R) instead of ubiquinone (Q). This pathway harvests energy (5 ATP/glucose) during dauer larval stage (a resistance larval form) and it is also thought to be important under hypoxic conditions and possibly in certain tissues or cells under normoxic conditions. Lactic and ethanol fermentation (mainly lactic) occur concomitantly to malate dismutation with a lower energy yield (2ATP/glucose). (B) Schematic cell depicting the glyoxylate shunt. This shunt is a shortcut to TCA that produces succinate from 2 AcCoA, allowing fatty acids to be oxidized and to harvest energy and support gluconeogenesis. It is thought to be important during embryonic development, and it has also been reported to be active in dauer metabolism. In parasitic nematodes, this pathway has not been studied in detail, but it is known to be present in several nematodes. C. Schematic cell depicting aerobic respiration. This pathway is functional in normoxia conditions, during the reproductive cycle of *C. elegans*. The rectangles within the cells represent the inner and outer mitochondrial membranes. In parasitic nematodes, conventional respiration occurs in normoxia (free-living stages) and malate dismutation under hypoxia. In all cells, the trehalose shunt is shown. Trehalose is important for a variety of environmental stresses (osmotic, anoxic, temperature), as an energy reserve and connects carbohydrate and lipid reserves. (TCA, tricarboxylic acid cycle; R, rhodoquinone; U, ubiquinone; C, cytochrome C).

and the malate dismutation pathway are present in *C. elegans* where it is used, at least in the dauer stage, providing a model to elucidate this partially understood pathway.

Another key pathway that is absent in mammalian hosts, but present in *C. elegans* and parasitic nematodes is the glyoxylate cycle that consumes Acetyl-CoA derived from fatty acid oxidation and produces succinate (Fig. 3B) (Braeckman *et al.* 2009). This cycle, when functioning together with TCA (tricarboxylic acid cycle) enzymes generates reducing power and ATP, and fuels gluconeogenesis and trehalose (a glucose disaccharide) synthesis (see below). In *C. elegans* the glyoxylate cycle is important during embryogenesis and in the dauer stage (Braeckman *et al.* 2009; Erkut *et al.* 2016). These dissimilar scenarios (development and arrested development, respectively) have no access to food, and thus depend on lipid and carbohydrate reserves. This pathway has not been much studied in parasitic nematodes, but its gene signature is present in several nematodes (Fig. 2). How the glyoxylate cycle can function linked to malate dismutation in dauer larva (or other conditions) is not clear, particularly considering that they function in opposite directions (i.e. one generates glucose, while the other consumes glucose). One possibility is that these two pathways are separated in space, with the glyoxylate cycle generating glucose in some cells (likely with some oxygen supply), and malate dismutation catabolizing glucose in other cells (without reserves or oxygen supply). There is a need to fully characterize these pathways in nematodes, particularly regarding cell/tissue metabolic specializations.

The trehalose shunt is another difference between parasitic nematodes and their hosts (Fig. 3). This shunt allows production, storage and use of trehalose (Braeckman *et al.* 2009). Trehalose is essential as a protective metabolite against different environmental stresses such as desiccation, osmotic stress, anoxia, heat, cold, freezing and as a ready to use saccharide reserve (Braeckman *et al.* 2009; Farelli *et al.* 2014; Erkut *et al.* 2016). Nematodes lack glucose 6-phosphatase (Farelli *et al.* 2014), a key enzyme in conventional gluconeogenesis and glycogen→glucose conversion. Thus, the trehalose shunt is a key pathway to obtain glucose from carbohydrate reserves or lipid reserves by gluconeogenesis (Fig. 3B). Trehalose-6-phosphate phosphatase is essential in *C. elegans*, since its absence leads to toxic accumulation of trehalose 6-phosphate (Kormish and McGhee, 2005; Erkut *et al.* 2016), and is a promising pharmacological target for some nematodes (Farelli *et al.* 2014).

Caenorhabditis elegans can also serve as a model to study certain pathways that are present in nematodes, but not in their mammalian hosts. Unlike vertebrates, nematodes lack the enzymes needed for haeme synthesis, yet haeme is essential and therefore must be acquired from the diet (Rao *et al.* 2005; Luck *et al.* 2016). Furthermore, nematodes possess an unusually high number of haeme-containing globins, and require haeme for several protein families such as guanylate cyclases, adenylate cyclases and cytochromes. This auxotrophy may be exploited to develop drugs that interfere with haeme uptake and homeostasis in parasites. Haeme transporters and several haeme-responsive genes have been characterized in *C. elegans*, and a recent study has shown that at least some of them are expressed in *B. malayi* (Luck *et al.* 2016). Another auxotrophy of nematodes is cholesterol. Specific cholesterol-modifying enzymes (absent in vertebrates) have been reported to be important in *C. elegans* development (Chitwood, 1999).

A number of small molecules such as ascarosides, dafachronic acids, and nemamides act as pheromones and hormones that control nematode development. Research in *C. elegans* and the advances in metabolomics have been important in elucidating the chemical nature and biosynthesis of these signals in recent years. The advances made in nematode small-molecule identification and biosynthesis is fully discussed in a recent perspective article (Butcher, 2017). The biosynthesis of these molecules offers potential drug targets, and analogues of these metabolites may function as potential anthelmintics. Another specific metabolic activity of nematodes, absent in their mammalian hosts, is the synthesis and turnover of the cuticle. Much of what is known about the nematode cuticle is due to research in *C. elegans* and this has recently been reviewed (Lazetic and Fay, 2017).

The development of more amenable axenic culture is a pending issue for *C. elegans* biochemical studies. Nevertheless, *Escherichia coli* (the diet of the worm in the lab) can be genetically modified, which may be useful for some specific metabolic studies.

Conclusions and perspectives

Although the phenomenon of parasitism cannot be fully understood with a free-living nematode, *C. elegans* is a powerful model to address many biological questions of parasitic nematodes. The wealth of knowledge on *C. elegans* that has accumulated over the years provides key information on numerous genes, gene products and biological processes. This information is extremely well organized and is being exploited by nematode parasitologists.

As stated by Sidney Brenner, progress in science depends on new techniques, new discoveries and new ideas, probably in that order. RNAi and the use of GFP as an *in vivo* gene reporter, pioneered in *C. elegans*, clearly illustrate this concept. The powerful genetic tools developed for *C. elegans* research are helping helminth research in different ways, notably as a surrogate system. Yet, there is a need to invest efforts in appropriate genetics techniques and tools to advance the helminth parasitology field. Microfluidics is another technology in which *C. elegans* advances can fuel helminth research. This technology enables experiments that are otherwise impossible with conventional methods. Indeed, microfluidics is becoming very useful in some *C. elegans* research areas where precise environmental conditions and worm handling is needed (e.g. imaging, drug studies, behavioural studies, signalling and sorting screening) (San-Miguel and Lu, 2013). Fostering interactions with researchers at the intersection of *C. elegans* biology and parasitology is important for cross-fertilization of both fields.

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References

Besier B (2007) New anthelmintics for livestock: the time is right. *Trends in Parasitology* **23**, 21–24.

Blaxter M and Koutsovoulos G (2015) The evolution of parasitism in Nematoda. *Parasitology* **142**(suppl. 1), S26–S39.

Braeckman BP, Houthoofd K and Vanfleteren JR (2009) Intermediary metabolism. In *Wormbook* The *C. elegans* Research Community, pp. 1–24. doi: 10.1895/wormbook.1.146.1.

Brenner S. (2002). Nature's Gift to Science. *Nobel Lecture, December* 8, 2002. Available at http://www.nobelprize.org/nobel_prizes/medicine/laureates/20.

Britton C, Roberts B and Marks ND (2016) Functional genomics tools for *Haemonchus contortus* and lessons from other helminths. *Advances in Parasitology* **93**, 599–623.

Buckingham SD, Partridge FA and Sattelle DB (2014) Automated, high-throughput, motility analysis in *Caenorhabditis elegans* and parasitic

nematodes: applications in the search for new anthelmintics. *International Journal for Parasitology: Drugs and Drug Resistance* **4**, 226–232.

Burns AR. and Roy PJ. (2012). To kill a mocking worm: strategies to improve *Caenorhabditis elegans* as a model system for use in anthelmintic discovery. In Caffrey CR (ed). *Parasitic Helminths: Targets, Screens, Drugs and Vaccines*. Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA, pp. 201–216.

Burns AR, Luciani GM, Musso G, Bagg R, Yeo M, Zhang Y, Rajendran L, Glavin J, Hunter R, Redman E, Stasiuk S, Schertzberg M, Angus McQuibban G, Caffrey CR, Cutler SR, Tyers M, Giaever G, Nislow C, Fraser AG, MacRae CA, Gilleard J and Roy PJ (2015) *Caenorhabditis elegans* is a useful model for anthelmintic discovery. *Nature Communications* **6**, 7485.

Butcher RA (2017) Small-molecule pheromones and hormones controlling nematode development. *Nature Chemical Biology* **13**, 577–586.

Consortium CES (1998) Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* **282**, 2012–2018.

Corsi AK, Wightman B and Chalfie M (2015) A transparent window into biology: a primer on *Caenorhabditis elegans*. *Genetics* **200**, 387–407.

Chalfie M, Tu Y, Euskirchen G, Ward WW and Prasher DC (1994) Green fluorescent protein as a marker for gene expression. *Science* **263**, 802–805.

Chitwood DJ (1999) Biochemistry and function of nematode steroids. *Critical Reviews in Biochemistry and Molecular Biology* **34**, 273–284.

Cragg GM and Newman DJ (2013) Natural products: a continuing source of novel drug leads. *Biochimica et Biophysica Acta* **1830**, 3670–3695.

Crook M (2014) The dauer hypothesis and the evolution of parasitism: 20 years on and still going strong. *International Journal for Parasitology* **44**, 1–8.

Davies M, Nowotka M, Papadatos G, Dedman N, Gaulton A, Atkinson F, Bellis L and Overington JP (2015) ChEMBL web services: streamlining access to drug discovery data and utilities. *Nucleic Acids Research* **43**, W612–W620.

Dickinson DJ and Goldstein B (2016) CRISPR-Based Methods for *Caenorhabditis elegans* genome engineering. *Genetics* **202**, 885–901.

Doitsidou M, Jarriault S and Poole RJ (2016) Next-Generation sequencing-based approaches for mutation mapping and identification in *Caenorhabditis elegans*. *Genetics* **204**, 451–474.

Duguet TB, Charvet CL, Forrester SG, Wever CM, Dent JA, Neveu C and Beech RN (2016) Recent duplication and functional divergence in parasitic nematode levamisole-sensitive acetylcholine receptors. *PLoS Neglected Tropical Diseases* **10**, e0004826.

Erkut C., Gade V. R., Laxman S. and Kurzchalia T. V. (2016). The glyoxylate shunt is essential for desiccation tolerance in *C. Elegans* and budding yeast. *Elife* **5**.

Farelli JD, Galvin BD, Li Z, Liu C, Aono M, Garland M, Hallett OE, Causey TB, Ali-Reynolds A, Saltzberg DJ, Carlow CK, Dunaway-Mariano D and Allen KN (2014) Structure of the trehalose-6-phosphate phosphatase from *Brugia malayi* reveals key design principles for anthelmintic drugs. *PLoS Pathogens* **10**, e1004245.

Felix MA and Braendle C (2010) The natural history of *Caenorhabditis elegans*. *Current Biology* **20**, R965–R969.

Ferreira SR, Mendes TA, Bueno LL, de Araujo JV, Bartholomeu DC and Fujiwara RT (2015) A new methodology for evaluation of nematode viability. *BioMed Research International* **2015**, 879263.

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE and Mello CC (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**, 806–811.

Gang SS, Castelletto ML, Bryant AS, Yang E, Mancuso N, Lopez JB, Pellegrini M and Hallem EA (2017) Targeted mutagenesis in a human-parasitic nematode. *PLoS Pathogens* **13**, e1006675.

Hall DH and Altun ZF (2008) *C. elegans Atlas*, 1st edn., Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Hashimshony T, Feder M, Levin M, Hall BK and Yanai I (2015) Spatiotemporal transcriptomics reveals the evolutionary history of the endoderm germ layer. *Nature* **519**, 219–222.

Hedgecock EM, Sulston JE and Thomson JN (1983) Mutations affecting programmed cell deaths in the nematode *Caenorhabditis elegans*. *Science* **220**, 1277–1279.

Holden-Day L. and Walker R. J. (2014). Anthelmintic drugs and nematicides: studies in Caenorhabditis elegans. In *Wormbook* The *C. elegans* Research Community, pp. 1–13. doi: 10.1895/wormbook.1.143.2.

Howe KL, Bolt BJ, Cain S, Chan J, Chen WJ, Davis P, Done J, Down T, Gao S, Grove C, Harris TW, Kishore R, Lee R, Lomax J, Li Y, Muller HM, Nakamura C, Nuin P, Paulini M, Raciti D, Schindelman G, Stanley E, Tuli MA, Van Auken K, Wang D, Wang X, Williams G, Wright A, Yook K, Berriman M, Kersey P, Schedl T, Stein L and Sternberg PW (2016) Wormbase 2016: expanding to enable helminth genomic research. *Nucleic Acids Research* **44**, D774–D780.

Howe KL, Bolt BJ, Shafie M, Kersey P and Berriman M (2017) Wormbase ParaSite – a comprehensive resource for helminth genomics. *Molecular & Biochemical Parasitology* **215**, 2–10.

Hutter H and Moerman D (2015) Big data in *Caenorhabditis elegans*: quo vadis? *Molecular Biology of the Cell* **26**, 3909–3914.

Iwata F, Shinjyo N, Amino H, Sakamoto K, Islam MK, Tsuji N and Kita K (2008) Change of subunit composition of mitochondrial complex II (succinate-ubiquinone reductase/quinol-fumarate reductase) in *Ascaris suum* during the migration in the experimental host. *Parasitology International* **57**, 54–61.

James CE and Davey MW (2007) A rapid colorimetric assay for the quantitation of the viability of free-living larvae of nematodes in vitro. *Parasitology Research* **101**, 975–980.

Kaminsky R, Ducray P, Jung M, Clover R, Rufener L, Bouvier J, Weber SS, Wenger A, Wieland-Berghausen S, Goebel T, Gauvry N, Pautrat F, Skripsky T, Froelich O, Komoin-Oka C, Westlund B, Sluder A and Mäser P (2008) A new class of anthelmintics effective against drug-resistant nematodes. *Nature* **452**, 176–180.

Keiser J and Utzinger J (2010) The drugs we have and the drugs we need against major helminth infections. *Advances in Parasitology* **73**, 197–230.

Kita K (2016) Current trend of drug development for neglected tropical diseases (NTDs). *Yakugaku Zasshi* **136**, 205–211.

Kita K and Takamiya S (2002) Electron-transfer complexes in *Ascaris* mitochondria. *Advances in Parasitology* **51**, 95–131.

Kormish JD and McGhee JD (2005) The *C. elegans* lethal gut-obstructed gob-1 gene is trehalose-6-phosphate phosphatase. *Developmental Biology* **287**, 35–47.

Lazetic V and Fay DS (2017) Molting in *C. elegans*. *Worm* **6**, e1330246.

Li X, Shao H, Junio A, Nolan TJ, Massey HC Jr, Pearce EJ, Viney ME and Lok JB (2011) Transgenesis in the parasitic nematode *Strongyloides ratti*. *Molecular & Biochemical Parasitology* **179**, 114–119.

Lo NC, Addiss DG, Hotez PJ, King CH, Stothard JR, Evans DS, Colley DG, Lin W, Coulibaly JT, Bustinduy AL, Raso G, Bendavid E, Bogoch II, Fenwick A, Savioli L, Molyneux D, Utzinger J and Andrews JR (2016) A call to strengthen the global strategy against schistosomiasis and soil-transmitted helminthiasis: the time is now. *The Lancet Infectious Diseases*. doi: 10.1016/S1473-3099(16)30535-7.

Lok JB, Shao H, Massey HC and Li X (2017) Transgenesis in *Strongyloides* and related parasitic nematodes: historical perspectives, current functional genomic applications and progress towards gene disruption and editing. *Parasitology* **144**, 327–342.

Lonjers ZT, Dickson EL, Chu TP, Kreutz JE, Neacsu FA, Anders KR and Shepherd JN (2012) Identification of a new gene required for the biosynthesis of rhodoquinone in *Rhodospirillum rubrum*. *Journal of Bacteriology* **194**, 965–971.

Luck AN, Yuan X, Voronin D, Slatko BE, Hamza I and Foster JM (2016) Heme acquisition in the parasitic filarial nematode *Brugia malayi*. *FASEB Journal* **30**, 3501–3514.

Mathew MD, Mathew ND, Miller A, Simpson M, Au V, Garland S, Gestin M, Edgley ML, Flibotte S, Balgi A, Chiang J, Giaever G, Dean P, Tung A, Roberge M, Roskelley C, Forge T, Nislow C and Moerman D (2016) Using *C. elegans* forward and reverse genetics to identify New compounds with anthelmintic activity. *PLoS Neglected Tropical Diseases* **10**, e0005058. 10.1371/journal.pntd.0005058.

Mentel M, Rottger M, Leys S, Tielens AG and Martin WF (2014) Of early animals, anaerobic mitochondria, and a modern sponge. *BioEssays* **36**, 924–932.

Panic G, Duthaler U, Speich B and Keiser J (2014) Repurposing drugs for the treatment and control of helminth infections. *International Journal for Parasitology: Drugs and Drug Resistance* **4**, 185–200.

Parkinson J, Mitreva M, Whitton C, Thomson M, Daub J, Martin J, Schmid R, Hall N, Barrell B, Waterston RH, McCarter JP and Blaxter ML (2004) A transcriptomic analysis of the phylum Nematoda. *Nature Genetics* **36**, 1259–1267.

Rao AU, Carta LK, Lesuisse E and Hamza I (2005) Lack of heme synthesis in a free-living eukaryote. *Proceedings of the National Academy of Sciences USA* **102**, 4270–4275.

San-Miguel A and Lu H (2013) Microfluidics as a tool for *C. elegans* research. In *Wormbook* The *C. elegans* Research Community, pp. 1–19. doi: 10.1895/wormbook.1.162.1.

Shao H, Li X, Nolan TJ, Massey HC Jr, Pearce EJ and Lok JB (2012) Transposon-mediated chromosomal integration of transgenes in the

parasitic nematode *Strongyloides ratti* and establishment of stable transgenic lines. *PLoS Pathogens* **8**, e1002871.

Sloan MA, Reaves BJ, Maclean MJ, Storey BE and Wolstenholme AJ (2015) Expression of nicotinic acetylcholine receptor subunits from parasitic nematodes in *Caenorhabditis elegans*. *Molecular & Biochemical Parasitology* **204**, 44–50.

Streit A (2017) Genetics: modes of reproduction and genetic analysis. *Parasitology* **144**, 316–326.

Sulston JE and Horvitz HR (1977) Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. *Developmental Biology* **56**, 110–156.

Sutherland IA and Leathwick DM (2011) Anthelmintic resistance in nematode parasites of cattle: a global issue? *Trends in Parasitology* **27**, 176–181.

Thompson O, Edgley M, Strasbourger P, Flibotte S, Ewing B, Adair R, Au V, Chaudhry I, Fernando L, Hutter H, Kieffer A, Lau J, Lee N, Miller A, Raymant G, Shen B, Shendure J, Taylor J, Turner EH, Hillier LW, Moerman DG and Waterston RH (2013) The million mutation project: a new approach to genetics in *Caenorhabditis elegans*. *Genome Research* **23**, 1749–1762.

Tielens AG and Van Hellemond JJ (1998) The electron transport chain in anaerobically functioning eukaryotes. *Biochimica et Biophysica Acta* **1365**, 71–78.

Van Hellemond JJ, Klockiewicz M, Gaasenbeek CP, Roos MH and Tielens AG (1995) Rhodoquinone and complex II of the electron transport chain in anaerobically functioning eukaryotes. *Journal of Biological Chemistry* **270**, 31065–31070.

Ward JD (2015) Rendering the intractable more tractable: tools from *Caenorhabditis elegans* ripe for import into parasitic nematodes. *Genetics* **201**, 1279–1294.

Weaver KJ, May CJ and Ellis BL (2017) Using a health-rating system to evaluate the usefulness of *Caenorhabditis elegans* as a model for anthelmintic study. *PLoS ONE* **12**, e0179376.

Wever CM, Farrington D and Dent JA (2015) The validation of nematode-specific acetylcholine-gated chloride channels as potential anthelmintic drug targets. *PLoS ONE* **10**, e0138804.

White JG, Southgate E, Thomson JN and Brenner S (1986) The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philosophical Transactions of the Royal Society of London B, Biological Sciences* **314**, 1–340.

Zamanian M and Andersen EC (2016) Prospects and challenges of CRISPR/Cas genome editing for the study and control of neglected vector-borne nematode diseases. *FEBS Journal* **283**, 3204–3221.