REVIEW

The Nervous System of *C. elegans*: Its *in vivo* Visualization and Practical Applications

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In 1965, Sydney Brenner, now a Nobel laureate, began to use a nematode in his studies of animal and neural development. Forty years later, this worm, *Caenorhabditis elegans* (*C. elegans*), offers unsurpassed advantages as a multicellular model system with its easy maintenance, completely mapped cell lineages, and readily amenable genetics. Here, I review the nervous system of *C. elegans*, particularly its GABAergic system, and discuss the implications of nematode research to mammalian biology.

Introduction

One of the most common questions encountered by worm geneticists is: What is left in the worm to study? In the past forty years, the lineage and migration patterns of every single cell in the adult worm from the fertilized zygote have been completely mapped (*1, 2*), and its genome has been sequenced (*3*). Many believe that we have learned all we can from this simple nematode and that the days of *C. elegans* as a model organism are numbered.

Nothing can be farther from the truth. The accumulated knowledge from the past forty years offers enormous leverage for the manipulation of the model system and allows for more complete interpretation of experimental results. If previous research can be designated as decipherment of the worm's biology, current research utilizes and exploits this knowledge to study complex biological processes and pathways of higher organisms.

In this review, a brief introduction to *C. elegans* will first be provided. Next, the *C. elegans* nervous system will be introduced with a focus on its inhibitory GABAergic system. Finally, implications of *C. elegans* nervous system research to mammalian biology and the use of the nematode as a model system for human diseases will be discussed.

Introduction to *C. elegans*

C. elegans is a soil nematode that feeds on bacteria (*4*). Measuring 1.5 – 2mm in length, this microscopic nematode has a transparent body and a digestive system consisting of a pharynx and an intestine that runs through the body. Bacteria are ingested by a six-lipped mouth on one end of the tubular body and excreted at the other (posterior) end.

While males do exist, the main mode of reproduction is via self-fertilization in the hermaphrodite *(4).* Sperm, generated and stored in the hermaphrodite, fertilizes oocytes, forming eggs within the worm. Each hermaphrodite lays over 300 eggs in its two- to three-week lifetime. When the eggs hatch, the worms proceed through four larval stages of development (**Figure 1**). This process takes about three days at 25°C, after which time the worm becomes fully mature and begins to reproduce. The life cycle of *C. elegans* is temperature-sensitive and proceeds more slowly at lower temperatures.

C. elegans is easy to maintain in the laboratory. Worms can be grown in liquid cultures or maintained on agar plates with *Escherichia coli* (*E. coli*) as a food source. Notably, one can readily prepare frozen stocks of worm strains for long-term storage.

Molecular characterization of the worm also has been done. The *C. elegans* genome has been sequenced, and a complete physical map is available (*3*). The genome is comprised of about 100 million basepairs (*3*) – 30 times smaller than the human or mouse genomes – and organized into six chromosomes: five somatic (I to V) and one X (*4*). While hermaphrodites have two X chromosomes, males have one. Over 19,000 genes have been identified, and the repository of the available information can be found at the WormBase

website (*5*). Over 40% of the predicted protein products display significant similarities to sequences found in other organisms (*3*).

In addition to easy maintenance and the availability of the complete genomic sequence, practical yet powerful genetic techniques in the worm make it a favourable model system. Various worm strains are prepared by crossing males of one strain to hermaphrodites of another strain, as hermaphrodites preferentially use the sperm from males over their own (*4*). In addition to this traditional method of strain generation, RNA interference (RNAi) can be performed by feeding the worm with *E. coli* that expresses double-stranded RNA of a target gene (*6*). Finally, transgenic worms can be generated by injecting DNA into the worm. When injected into the gonad of a hermaphrodite, the DNA is taken up by its oocytes, forms extrachromosomal arrays, and is transmitted through the subsequent cell divisions (*4*). By co-injecting a reporter construct (e.g., *gfp*) with the gene of interest, researchers can differentiate transgenics from non-transgenics under the fluorescent microscope. Combined with the large brood size and short generation time, *C. elegans* offers many advantages over other systems for conducting genetic manipulations.

Recently, a small scale high-throughput study was successfully conducted in *C. elegans* (*7*). Using some of the well-documented phenotypes of the animal, the authors identified small molecules, and later their targets, that induced a variety of the phenotypes (*7*). This work shows that, with the advances in the automation of several worm techniques (e.g., a worm dispenser that emits a single worm at a time) and easy cultivation in 96-well plates, large-scale chemical screens are now feasible.

C. elegans nervous system and GABAergic motor neurons

The organization of *C. elegans* nervous system is relatively simple. A wild-type adult hermaphrodite worm consists of about 1000 cells in total, of which 302 cells make up the nervous system (*4*). Similar to other organisms, the neurons serve sensory, interneuronal, or motor functions by forming specialized structures called synapses. Synapses allow for communication between neurons and their targets (e.g., other neurons or muscle cells) by releasing neurotransmitters. Most of the synapses formed by the neurons can be found in one of three synapse-rich regions: the nerve ring (the "brain" of the worm), the ventral nerve cord (VNC) that runs longitudinally along the ventral side of the body, and the dorsal nerve

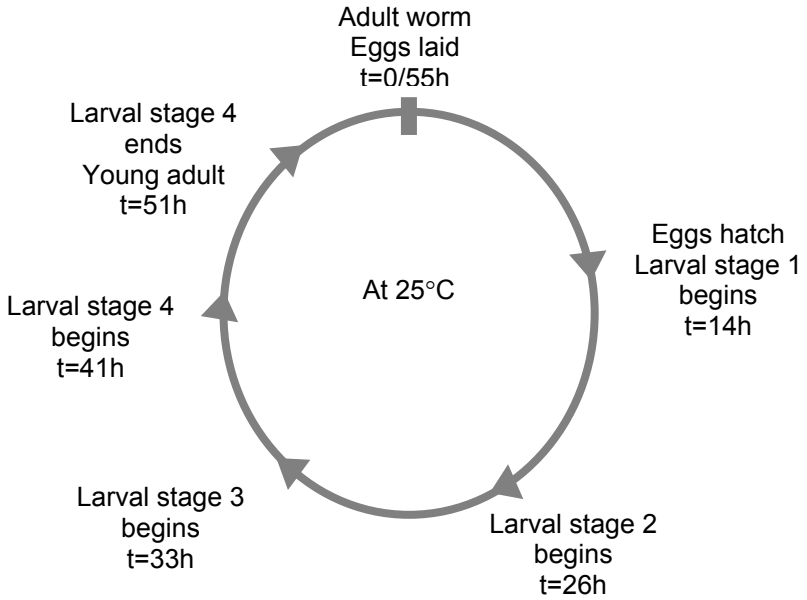

Figure 1: Life Cycle of *C. elegans* at 25°C. Eggs are laid five hours post-fertilization. After the eggs hatch, the worms proceed through four larval stages. The cycle takes about three days at 25°C and longer at lower temperatures. h, hours, t, time.

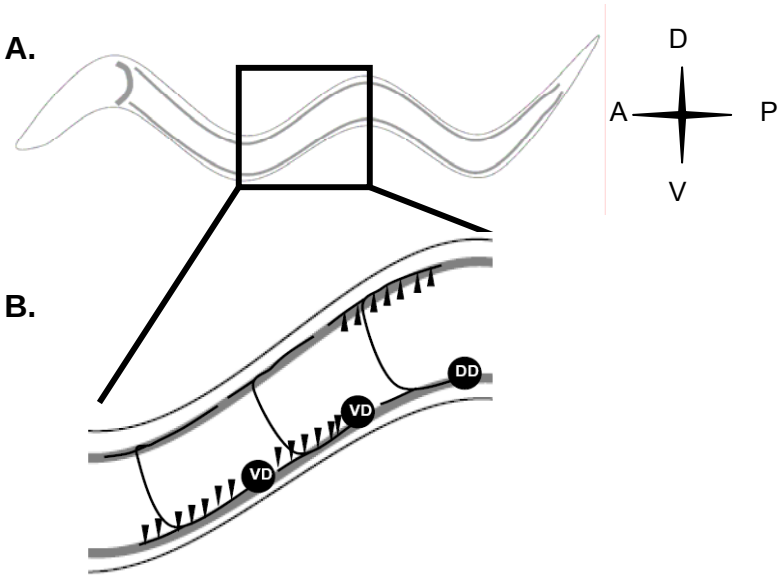

Figure 2: Nervous System and GABAergic Neurons in *C. elegans*. (**A**) Depicted in grey are the synapse-rich regions of the worm. Near the head is the nerve ring, equivalent to the "brain" of the worm. Running along the body on the dorsal and ventral sides are DNC and VNC, respectively. A, anterior, P, posterior, D, dorsal, and V, ventral. (**B**) Shown in the magnified section of the worm are GABAergic neurons of D-type motor neurons. While the VD and DD neurons – the two subtypes of the D-type – both have their cell bodies (•) on the VNC, their synapses (▲) exclusively form on the VNC or the DNC, respectively. The non-overlapping *en passant* synaptic circuitry in motor neurons can be observed.

cord (DNC) that runs along the dorsal side (**Figure 2A**).

Motor neurons in the worm make connections with the body muscles and control its locomotion. Most motor neurons have their cell body on the VNC and synapse-forming axon on one of the two nerve cords (*8*) (**Figure 2B**). Of the 302 neurons, 76 of them are motor neurons, and they are classified into one of five groups based on their morphology and synaptic connectivity (*8*, *9*).

The motor neurons of a single group form non-overlapping *en passant* synapses with each other in the worm (*9*) (**Figure 2B**). The synapses of a motor neuron linearly organize along the axon, instead of at the axon terminal. The synapse-containing axon forms a small stretch on one of the nerve cords in proximity to the endogenous cell body. The axons of two adjacent motor neurons of the same class do not overlap (**Figure 2B**).

One of the five types of motor neurons is the D-type, which comprises 19 of the 76 total motor neurons. D-type motor neurons can be sub-divided into ventral (VD) and dorsal (DD) neurons. While both VD and DD neurons have their cell bodies on the VNC, their axons form synapses on a distinct side – VD on the VNC and DD on the DNC (**Figure 2B**).

D-type motor neurons are GABAergic: they synthesize and release a neurotransmitter called γ-aminobutyric acid (GABA). D-type motor neurons receive inputs from excitatory motor neurons that release acetylcholine and cause muscle contraction. The release of GABA, triggered by cholinergic neurons, relaxes muscles on the side opposite to the contracted muscles in the worm (*10*). The excitatory and inhibitory neuronal circuitry results in the signature sinusoidal movement of the worm and hence regulates its locomotion.

GABAergic neurons in the study of *C. elegans* nervous system

The VDs and DDs are well-characterized and often used in studies of the nervous system. A distinct set of genes is expressed in the GABAergic neurons (*10*). One of these genes is *unc-25* (for uncoordinated), which encodes a glutamic acid decarboxylase (GAD) and is involved in the synthesis of GABA (*11*). GAD is exclusively expressed in all 26 GABAergic

neurons, 13 of which are VDs and six of which are DDs (*10*). Hence, driving the expression of a reporter gene under the *unc-25* promoter exclusively marks the GABAergic neurons. In fact, by tagging GFP to a synaptic protein (*12*) (e.g., synaptobrevin (SNB)) and expressing the fusion protein under the *unc-25* promoter in the transparent worm, one can visualize pools of synaptic vesicles at the individual synapses on the two nerve cords *in vivo* (*13*).

This SNB fusion protein serves as a sensitive marker for studying synapses. When the SNB fusion protein is visualized under the fluorescent microscope, the *en passant* synapses on the dorsal cord appear as beads on a string (**Figure 3A**): Each synapse appears as a round punctum separated from the adjacent puncta in an even fashion. These images, in black and white, appear as white dots on a black background (**Figure 3A**). As mentioned above, since the VNC contains cell bodies in addition to synapses, both of which show GFP fluorescence, studies involving reporter-marked synapse morphology often focus on the DNC (**Figure 3**).

In mutants with synaptic defects, abnormalities in the puncta morphology can be observed. For instance, in a screen using the GFP-SNB fusion protein, *syd-2* (for synapse-defective) was identified because of its abnormal puncta morphology (*13*). In other words, instead of the discrete round puncta, in *syd-2* mutants the puncta appeared fuzzy and expanded. Involved in the formation of active zones – a region on the synaptic membrane where vesicles dock and fuse for neurotransmitter release – SYD-2 localizes to the active zone of a synapse and can itself serve as a reporter of active zone formation (*14*).

From *C. elegans* to mammals

Another example of abnormal SNB morphology is *sad-1* (for synapses of amphids defective) (*15*). Initially identified as being involved in synapse formation (synaptogenesis) and cell polarity in sensory neurons, this serine/threonine kinase plays many roles in the nervous system development of the worm. In the GABAergic motor neurons, SAD-1 negatively regulates the size of the vesicle pool at the active zone (*15*). In the absence of SAD-1 activity, the SNB morphology in *sad-1* mutants is diffuse (**Figure 3B**).

Following the identification of SAD-1 in *C. elegans* (*15*), studies have reported the identification of mammalian SADs in the mouse (*16*) and the rat (*17*). In the mouse, two homologues of SAD-1, namely SAD-A and -B, were identified by sequence homology and have

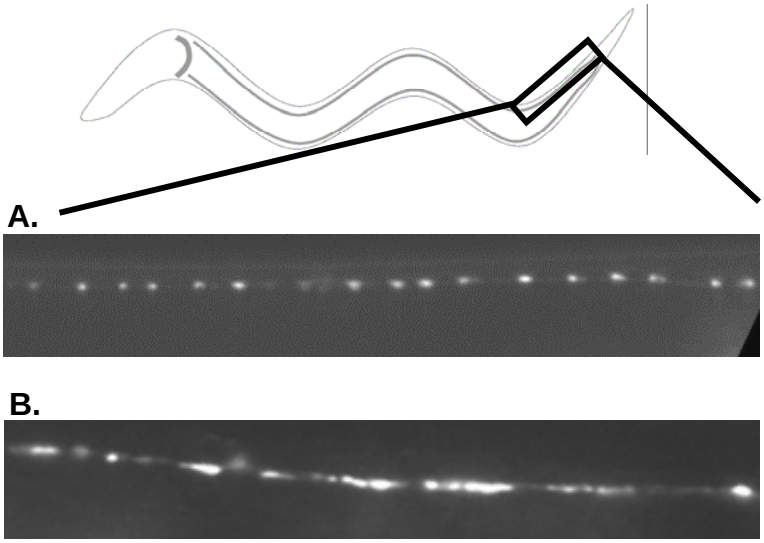

Figure 3: Wild-type and Abnormal Synapse Morphology on the DNC. (**A** and **B**) Images of the GFP-SNB fusion protein expressed in the GABAergic neurons are shown. Both focus on the DNC near the tail of the worm. (**A**) In wild-type, the GFP puncta marking synaptic vesicle pools at individual synapses are discrete, round, and evenly spaced. (**B**) In *sad-1* mutants, abnormal morphologies are observed. The puncta appear diffuse and unevenly spaced. Gaps are also observed, suggesting that some synapses are missing.

been shown to redundantly regulate axon/dendrite differentiation and development. Cultured hippocampal neurons from *sad-a/b* double knock-out mice fail to develop distinct dendrites and axons. Since the development of a single axon and multiple dendrites in a wild-type neuron is crucial for its function, this defect in the double knock-out has severe consequences. In fact, these mice die shortly after birth (*16*). While no observations were made in this study on the synaptic function of mammalian SAD-1, a recent study in the rat provides insight into this topic. One of the two rat SADs physically interacts with active zone proteins and regulates neurotransmitter release (*17*). Therefore, the identification and characterization of SAD-1 in *C. elegans* has helped in the recognition of some of the key players in mammalian nervous system development.

C. elegans as a model system for human diseases

Despite its simplicity and evolutionary distance from mammals, *C. elegans* is proving to be a useful model for human disease. One example is in the study of neurodegenerative diseases such as Huntington's disease and Amyotrophic Lateral Sclerosis (ALS). Both belong to a family of protein-misfolding diseases. In these diseases, proteinous aggregates of a disease-specific protein are found in specific cells/tissues. For instance, in ALS, aggregates of a radical scavenger protein called SOD1 (Cu/Zn superoxide dismutase) are found in dying motor neurons of the central nervous system (*18*). A *C. elegans* model expressing lengthy polyglutamine peptides, which are prone to aggregation, has been studied as a model for protein-misfolding diseases (*19*). The worm model exhibits protein aggregation *in vivo*, slowed motility, and selective neuronal toxicity, similar to the symptoms of the neurodegenerative diseases in humans. Combining this worm model with high-throughput methods now available in the worm (*7*), one can envision a screen to identify new therapeutic drugs for the protein-misfolding diseases.

The worm has also been proposed useful in studying multi-genic human diseases (*20*). Recently, a network of genetic interactions of some 1,750 *C. elegans* genes has been generated (*21*). The network validates previous yeast network studies (*22*). These data strongly support the idea that in addition to genes, genetic interactions are conserved among species. More importantly, novel genetic interactions were identified amongst signal transduction genes implicated in human diseases. The authors argue that a class of pleiotropic genes appears important in maintaining the integrity of the genome (*21*). In other words, this class of genes, when mutated, can render the host prone to various diseases. *C. elegans* offers more diverse phenotypic and behavioural observations than yeast does. This recent work is exemplary of the usefulness of *C. elegans* in studying complex biological processes that are disrupted in human diseases.

Conclusions

Over forty years of *C. elegans* research has established this simple nematode as a useful model system. Extensive knowledge of general worm biology allows manipulations of the system and detailed examination of complex pathways and biological processes. Information obtained from these studies is being applied to more complex organisms like humans.

In this review, I gave an overview of the *C. elegans* nervous system and demonstrated how the identification and characterization of a key player in worm synaptogenesis has helped in the successful characterization of its mammalian homologue. Further, *C. elegans* disease models have been developed for complex human diseases such as protein-misfolding neurodegenerative disorders.

C. elegans has provided insights to many biological processes and remains a powerful tool for future studies.

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