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Watching Worms Whither: Modeling Neurodegeneration in *C. elegans*

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Abstract

C. elegans is increasingly being used to study neurodegenerative diseases. Nematodes are translucent, which facilitates study of particular neurons in the living animal, and easy to manipulate genetically. Despite vast evolutionary divergence, human proteins are functionally active when expressed in *C. elegans*, and disease-linked mutations in these proteins also cause phenotypic changes in the nematode. In this manuscript, we review use of *C. elegans* to investigate the pathophysiology of Alzheimer's disease, Parkinson's disease and axonal degeneration. Studies of presenilin, β -amyloid, tau, α -synuclein and LRRK2 all produce strong phenotypic effects in *C. elegans*, and in many cases reproduces selective neuronal vulnerability observed in humans. Disease-linked mutations enhance degeneration in the *C. elegans* models. These studies are increasingly leading to high throughput screens that identify novel genes and novel pharmaceuticals that modify the disease course.

Keywords

Beta-amyloid; Microtubule associated protein tau; presenilin; α -synuclein; Leucine Rich Repeat Kinase 2; Green fluorescent protein; dopamine; axonal dystrophy; laser ablation

Introduction

C. elegans is a small roundworm with a precise, predetermined anatomy, a rapid reproductive cycle, a short lifespan, and a translucent body (<http://www.wormbook.org/>) [1]. Each of these features contributes to the power of *C. elegans* as a valuable tool for understanding the functional genomics of disease. Gene expression can be readily modulated by promoter-driven expression, knock down or gene deletion. Transgenes can be directed to particular neuronal subtypes, such as dopaminergic neurons or touch neurons. The integrity of the neurons can be readily examined in live animals using fluorescent indicators, and over three weeks these neurons can be followed through the nematode life cycle from the larval stage until the animal dies from senescence (Figure 1). These features combine together to make *C. elegans* a powerful system for studying diseases of aging

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where particular genes are typically expressed in a manner that gradually induces pathological changes as the animal senesces.

Human transgenes show a remarkable ability to function appropriately within the context of *C. elegans*. At the simplest level, poly-glutamine peptides aggregate in similar manners in human and nematode cells, and these aggregation properties have been elegantly used to elucidate mechanisms of proteostasis that smoothly translate from *C. elegans* to eukaryotic cells [2]. Functional equivalency across species extends beyond stretches of homomeric peptides, such as poly-glutamines. Human proteins, such as presenilin, LRRK2 and TDP-43, exhibit functional activities that are shared between *C. elegans* and humans despite sequence homologies that are often 30 – 45% [3–5]. Genetic mutations that cause dysfunction and disease in humans, frequently exhibit similar types of dysfunction in *C. elegans*. The cross-species translation of function might derive from two factors: functional domains and conserved structure. Seemingly low levels of total protein homology frequently hide much higher levels of cross-species homology present in functional domains that are shared between humans and nematodes. For instance, presenilins regulate notch in both human and *C. elegans* [6]. Mutations linked to Alzheimer's disease cause a loss of presenilin mediated notch cleavage in human, and also cause a phenotype associated with the loss of notch function nematode [6].

Applying results from *C. elegans* to understand diseases of the human central nervous system requires a nuanced view of anatomy. Nematodes models provide outstanding systems for understanding the behavior of individual cell types in vivo because key elements of the biology of neurons are conserved between nematode and man. These similarities mean that studies of neurodegeneration in the nematode provide strong insight into mechanisms of degeneration that apply across species. For instance, dopaminergic neurons show vulnerabilities to toxins and aggregating human proteins (e.g., α -synuclein) that are largely similar to that of dopaminergic neurons in the human brain [7–9]. On the other hand, the synaptic connectivity in nematodes is entirely different than that of the human brain. Nematodes have no substantia nigra, striatum or cortex for modeling circuits present in the human brain. These differences preclude using the nematode to understand how disease processes affect nerve circuits characteristic of the human brain. For instance, in humans dopamine neurons are required to initiate movement, and loss of dopaminergic neurons leads to motor impairment. Loss of dopaminergic neurons in nematodes, though, increases motor activity, removing the ability of hungry nematodes to slow down and feed when encountering a new food supply [10].

Modeling the pathophysiology of Alzheimer's disease: β -amyloid, tau and presenilin

Tau, β -amyloid and presenilin are three major proteins that have been implicated in AD. The amyloid cascade hypothesis describes the pathophysiological relationship between each of these proteins. β -Amyloid is generated from its precursor protein, amyloid precursor protein (APP), by cleavage at two sites, termed the β and γ -cleavage sites, which occur just outside the membrane (the β -site) and within the membrane (the γ -site) [11]. The enzymes that generate these cleavages are termed β -secretase and γ -secretase, respectively. Presenilin is the proteolytic subunit of γ -secretase. Cleavage of APP generates $A\beta$, which is secreted as a mixture of monomers and oligomers, and the oligomers are thought to be toxic to neurons. $A\beta$ toxicity leads to phosphorylation and aggregation of tau in neurons with subsequent formation of neurofibrillary tangles. Tau accumulates in neurons as an intracellular aggregate, and over-expressing tau in neurons in *C. elegans* produces neuronal dysfunction that is functionally apparent, and biochemical changes similar to those seen in the brains of subjects with AD or frontotemporal dementia [12]. β -amyloid is produced in neurons, can accumulate in neurons, but predominantly accumulates as an extracellular deposit. β -amyloid is generated from its precursor, amyloid precursor protein, by cleavage at the β and

γ sites. *C. elegans* naturally expresses the homologous protein that generates the γ cleavage, termed presenilin (sel-12), but lacks the homologue of β -secretase, which cleaves at the β -site [13–15]. Consequently, β -amyloid accumulation has been modeled in *C. elegans* by directly producing the β -amyloid peptide, and the peptide remains intracellular. The effects of intracellular accumulation of β -amyloid, though, are dramatic, with marked growth stunting and movement deficits.

Analysis of the biology of the *C. elegans* homologue of presenilin 1, Sel-12, represents one of the most striking examples of the utility of *C. elegans* functional genomics. Presenilin is an aspartyl protease that makes up one of four proteins of the γ -secretase complex, which carries out intra-membranous cleavages of transmembrane proteins (via presenilin-mediated cleavage). When presenilin was first discovered its function and substrates were unknown. Functional insights rapidly evolved based on homologies between human presenilin 1, and *C. elegans* Sel-12, which functions in a pathway that regulates notch activity [13–15]. Loss of Sel-12 leads to deficits in Notch function, which cause reduced egg-laying. This nematode assay provided the first link between presenilin and notch function, and set the stage for future papers defining the biochemical role of presenilin in γ -secretase in regulating Notch by intra-membranous cleavage. Transgenic expression of human wildtype and mutant presenilin in *C. elegans* confirmed the relationship by showing that wildtype human presenilin rescued *C. elegans* lines defective for Sel-12 activity. These same assays indicated that disease-linked mutations in presenilins represent a loss of function. The hypothesis that disease-linked mutations represented a loss of function was difficult to reconcile with the dominant mode of action of these mutant genes and their tendency to increase production of A β 42 in humans. However, advances in our understanding of presenilin biology have validated the *C. elegans* work. Presenilin 1 cleaves amyloid precursor protein by a process of multiple cleavages that are progressively closer to the classic γ -secretase cleavage site generating A β . Reduced presenilin activity slows down the cleavage progression leading to production of longer A β peptides. Disease-linked mutations in presenilin 1 appear to reduce proteolytic activity, which increases production of A β 42 and reduces production of A β 40. This story exemplifies the value of *C. elegans* in analyzing structure and function.

C. elegans has also been used to analyze factors regulating aggregation of A β and tau proteins, *in vivo*. Work on β -amyloid toxicity has been elegantly pioneered by Christopher Link. Modeling β -amyloidosis in nematodes requires a different approach than in mammalian cells because *C. elegans* lacks β -secretase, which is required to generate β -amyloid from its precursor, APP. Link and colleagues circumvented this issue by expressing constructs that directly produce A β in muscle via the myo-3 promoter [16]. These nematode lines exhibit many functional deficits, including movement dysfunction, growth retardation, reduced movement and premature death. Link's group used the *C. elegans* system to analyze the molecular changes associated with expressing A β . Transcriptome analysis indicated that expressing A β increased expression of the small heat shock protein, hsp-16, which is the *C. elegans* homologue of α / β -crystallin. They also observed increased expression of a number of pro-apoptotic proteins including a protein homologous to TNFAIP1, which is induced by tumor necrosis factor [17]. Proteomic analysis showed strong evidence of mitochondrial dysfunction induced by A β expression, with increased levels of oxidized mitochondrial proteins evident by 2-D gel analysis [18]. In each case, similar changes are observed in brains from subjects who died with Alzheimer's disease, which indicates that the results observed in *C. elegans* translate back to human pathophysiology [17, 18]. Work in *C. elegans* also suggests potential avenues of therapy, because cellular responses that protect against oxidative stress, such as inhibiting the insulin receptor daf-2 signaling cascade, protect against A β toxicity in these models [19]. These studies demonstrate the utility of

using *C. elegans* to study A β toxicity, and also convey the ability of results from *C. elegans* to translate to the human context.

Studies of investigating the effects of transgenic expression of human tau protein in *C. elegans* provide elegant examples of cross-platform translation. Brian Kraemer's group expressed M337V or P301L tau driven by the neuronal specific *aex-3* promoter in *C. elegans* neurons [20]. The nematodes showed strong evidence of tau toxicity, with motor dysfunction, loss of GABAergic neurons and premature death. The biochemical changes in tau protein were associated with the motor dysfunction paralleled biochemical changes observed in the brains of subjects with Alzheimer's disease. Tau from the transgenic nematode lines accumulated in an insoluble fraction and showed increased phosphorylation at sites classically hyper-phosphorylated in the Alzheimer brain, including phosphorylation of sites identified by the antibodies CP13 (phospho-S202) and PHF1 or AT8 (phospho-394/404) [20]. These data suggest that the signaling changes impinging on tau were similar in both nematode and human. Tau pathology in the nematodes did not fully reflect that of the human AD brain because there was no evidence of paired helical filament formation. The presence of a strong phenotype and clear biochemical changes provides a good platform for genetic studies, which is one of the strengths of the nematode system. Kraemer's group used the tau phenotype in a forward genetic screen to identify genetic modifiers of tau pathology. These screens highlighted putative roles of the enabled, a cytoskeletal regulatory protein, and HOOK1-3, a family of proteins with no known function, in regulating tau toxicity [21, 22]. These data provide solid evidence that proteins associated with disease in humans can elicit pathological reactions in nematodes that share many similarities with the events occurring in humans, and that nematode models can identify novel putative modifiers of disease function.

Modeling dopaminergic dysfunction

C. elegans is a readily used model for evaluating the effect of toxins and/or genetic factors on the survival of dopamine (DA) neurons. The hermaphrodite has 8 dopamine neurons, while the male worm has 12 [1]. The additional neurons in the male are utilized in the distinctive mating behavior of the male. 6 neurons are found in the head of the animal, 4 CEP and 2 ADE neurons, which mediate locomotion behavior in response to the presence of food (Figure 2). The survival of DA neurons can be evaluated by the expression of GFP driven by the dopamine transporter gene, *dat-1*. GFP fluorescence has been shown by Nass et. al. to correlate with neuron survival as measured by EM [23]. A number of studies have shown that the DA neurons are specifically sensitive to toxins used to model aspects of PD, including: 6-OHDA, MPP+, and rotenone [8, 23] (Table 1). In addition, the modulation of DA survival by the expression of proteins involved in PD etiology such as alpha synuclein has been evaluated [9, 24, 25] (Table 1).

Models of Parkinson's disease: Neurotoxins, α -Synuclein and LRRK2

Multiple laboratories have used *C. elegans* to query molecular and genetic mechanisms contributing to Parkinson's disease (PD). The translucence of *C. elegans* provides an excellent for monitoring the physiology and pathophysiology of dopaminergic neurons *in vivo*, by selective expression of green fluorescent protein driven by the dopamine transporter (*dat-1*) promoter [8, 9, 23–29]. The well-characterized dopaminergic neurons of *C. elegans* are highly sensitive to many classic neurotoxins used to model PD in higher organisms, such as 6-hydroxydopamine, MPP+, and rotenone [25, 27, 28, 30]. In the case of rotenone, we observe that dopaminergic neurons are 50 – 100-fold more sensitive than the entire organism, which allows one to screen for effects of rotenone on dopaminergic neuronal death under conditions in which nematode survival is not impaired (Wolozin, personal communication).

Transgenic approaches have also been used to model PD in *C. elegans*. Orthologues of many genes linked to PD exist in the nematode including, parkin (*pdr-1*), PINK1 (*pink-1*), DJ-1 (*djr-1.1, 1.2*), LRRK2 (*lrk-1*), ATP13A2 (*capt-6*), and GBA (*F11E6.1, TO4A8.7*). Knockout models of these orthologues have produced some important insights into their normal function [25, 30–33]. In each case mutations that cause PD either cause direct degeneration of dopaminergic neurons, or sensitize the nematodes to mitochondrial toxins [25, 30–32, 34], (Gilter et al., 2009). The phenotype observed depends can vary, depending on the model.

An example of the types of phenotypes observed is shown in figure 3, where age synchronized lines of *C. elegans* expressing GFP driven by the dopamine transporter promoter were subjected knockdown by empty vector (Control) or two different RNAi vectors. The combination of fluorescence intensity and morphology of the dopaminergic neurons have been shown by us and others to strongly correlate with survival of dopaminergic neurons [25]. L1 nematodes were placed on plates, and allowed to feed on bacteria containing RNAi for two different constructs or a vector control. The nematodes were aged at 20°C for three days, until approximately day 1 of adulthood, and then exposed to 6-hydroxydopamine (1 hr, 50 mM 6-OHDA, plus 10 mM ascorbic acid; followed by neutralization). After replating, the nematodes were aged for 4 days, and survival of the dopaminergic neurons was assessed by microscopy. As shown in figure 3, one RNAi construct (4) greatly reduced survival of the dopaminergic neurons, while the other construct (16) was actually trophic for the neurons.

Work from our laboratory showed increased sensitivity to rotenone, which is a pattern that we have observed with multiple genes linked to PD [30]. Multiple groups have also observed age dependent degeneration of dopaminergic neurons in nematode lines expressing α -synuclein [7, 9, 24, 35]. Degeneration of the dopaminergic neurons presents as a combination of three different outcomes, which are present to differing degrees in each nematode: loss of neuronal bodies, loss of neuronal processes, and reduces expression of dopaminergic markers. One issue that arises is marked variability in the time to degeneration among lines, with some groups observing loss of dopaminergic neurons in as little as 5 days and other groups observing survival up to 14 days [9, 24]. Many of these studies would benefit from use of a comparison to the parent line expressing the reporter but not α -synuclein. Despite some experimental issues, the cumulative results consistently indicate that expressing α -synuclein renders dopaminergic neurons susceptible to degeneration.

Three findings are emerging from the models of PD in *C. elegans*. The first finding is the tendency of different PD-linked genes to produce a similar phenotype of dopaminergic vulnerability and degeneration. A second finding is the ability of different PD-linked genes to complement each other in genetic studies, and the third finding is linkage of the degeneration to dysfunction of membrane bound organelles, such as the mitochondria and autophagosomes. Work from our lab provided one of the first examples of different PD-linked genes producing similar phenotypes. We observed that three different models of PD in *C. elegans* all showed enhanced vulnerability to mitochondrial toxins, including lines expressing A53T α -synuclein, a line lacking *pdr-1* (the *C. elegans* homologue of parkin) and a line subject to knockdown for DJ-1 [30]. In more recent studies, we observed that lines of *C. elegans* expressing mutant forms of LRRK2 (G2019S or R1441C) are more sensitive than lines of *C. elegans* expressing WT LRRK2 [25]. These data raise the possibility that enhanced vulnerability to mitochondrial dysfunction is a general feature of genes linked to PD. Complementation is observed in studies of parkin, *pink-1*, DJ-1 and *lrk-1*. LRRK2 has not been formally linked to *pink-1* yet, but using a *lrk-1* knockout line, Samann and colleagues demonstrated genetic complementation by *pink1* of sensitivity to the endoplasmic reticulum toxin, tunicamycin [32]. Two different studies noted that expressing α -synuclein

alters levels of parkin [35, 36]. Genetic complementation between PINK1 and parkin has also been shown in *drosophila* and in mammalian cells, and interaction between LRRK2 and parkin/PINK1 is also evident in *drosophila* [37–41]. These data contribute to an emerging view that many of the PD-linked genes share mechanisms and biochemical pathways in common.

Groups have made particular use of α -synuclein expressing nematode lines for forward genetic screens [7, 24, 42] and to monitor changes gene expression associated with α -synuclein expression and/or toxicity [33, 35, 43, 44]. A unifying theme that has arisen from these synuclein experiments is a strong connection with mitochondrial function, proteostasis and vesicle/vacuolar biology. Mitochondrial genes, such as *ymel-1* (a mitochondrial protease), F59F4.1 (acyl Co-A oxidase), ubiquitin/proteasomal proteins, such as *hrdl-1* and HIP (a ubiquitin ligase and transcription factor, respectively), and vacuolar proteins, such as *vps41* and *yptlp* (Rab1, a vesicle linked GTPase) all suppress α -synuclein toxicity [24, 42, 45, 46]. In addition, over-expressing the lysosomal protease cathepsin D also strongly protects against α -synuclein toxicity [47]. These forward screens also contribute to the growing consensus placing all PD genes in convergent pathways, because in most screens other PD-linked genes modify α -synuclein toxicity. PINK1, *pdr-1* (parkin), *djr-1.1* (*dj-1*) and *capt-6* (ATP13A2, PARK9) consistently appear as protective factors in screens of α -synuclein toxicity [24, 33, 42]. Finally, chemical screen also suggest that chemicals that protect mitochondria or increase autophagy (Wolozin, personal communication) protect against α -synuclein toxicity [30]. Thus, genes linked to PD appear to act through common pathways that involve the mitochondria and proteostasis. An emerging concept is the hypothesis that deficits in autophagy might unify each of these disparate areas of biology. In this context, the genes mentioned above that are linked to vacuolar function might act by enhancing the autophagic cascade.

Models of Axonal Degeneration: Laser ablation

C. elegans has recently emerged as a powerful model system for the study of neuronal damage and regeneration. Using advanced laser ablation techniques and time-lapse fluorescence imaging, researchers can selectively damage target neurons and observe subsequent re-growth *in vivo*. The advent of femtosecond laser surgery allows submicron ablation of biological tissue deep within a sample [48]. Applied to the *C. elegans* nervous system, this enables the precise cutting of individual nerve fibers within an adult animal, with minimal collateral damage to the surrounding tissue. Remarkably, severed *C. elegans*' neurons display robust regeneration within a matter of hours following laser damage (Figure 4). The simple stereotyped pattern of the *C. elegans* nervous system and the precision of laser ablation allows for exquisite single cell experimental repeatability and quantitative cell specific comparison across genetic backgrounds. Coupled with the unprecedented power of *C. elegans* genetics these techniques are opening the door for the study of nerve damage and recovery on a genetic and molecular basis, at a level unattainable in higher systems.

Regeneration of *C. elegans* neurons was first observed by Yakin et al. 2004 [49]. Using a femtosecond laser they snipped motor neurons within the body of *C. elegans* and observed the subsequent re-growth and apparent restoration of nerve function. Numerous studies have reported the remarkable ability of *C. elegans*' neurons to reconnect with the severed distal fragment of the damage process [50, 51]. In such cases, the cell appears to effectively repair itself without re-growing the entire nerve process. Regenerative ability in *C. elegans* neurons also appears to vary with neuron type. Motor neurons in the body of the worm, controlling body movement and egg-laying, readily re-grow as do mechanosensory and some chemosensory neurons. In contrast, many sensory neurons in the head and tail of the animal do not show significant re-growth [49, 52, 53].

The simplicity and stereotype morphology of the *C. elegans* nervous system as well as the submicron precision of femtosecond laser surgery allows targeting not only of a single neuron but a particular location along a specific nerve fiber as well. The cellular response to damage depends in part on the location of the ablation point. For example, cut points close to the cell soma tend to result in new collateral branches extending from the cell body. In contrast, cuts farther along the same neuron result in regenerative outgrowth from the severed end of the original fiber [53]. Branch points within a single nerve process can also delineate regions of regenerative ability. For example, beyond a particular branch point of the PLM neuron (a posterior mechanosensory neuron in the worm's body) regeneration does not readily occur, while cuts proximal to this point show substantial re-growth [50]. These findings, as with cell typed characteristics, are striking as similar variability in regeneration is also seen in the vertebrate nervous system. The peripheral nervous system of higher animals displays substantial re-growth following traumatic damage while the central nervous system tragically does not [54].

Cell specific comparison of neuronal recovery and regeneration after laser surgery across genetic backgrounds is fueling an array of new discoveries on the genetic and molecular level. For example, the *vab-1* gene, which encodes an enhrin protein and has been shown to be a regenerative deterrent in mammals, appears to detrimentally effect axon guidance during regeneration. *C. elegans* lacking the *vab-1* gene display substantially more accurate neuronal regeneration following laser damage [50]. Likewise, researchers have found that many of the genes involved in developmental axon outgrowth and guidance play similar roles in adult stage axon regeneration [53]. Reports have also demonstrated the critical nature of cAMP signaling in damaged neurons for efficient regeneration [51].

The *C. elegans* system greatly facilitates the direct discovery of novel factors specifically relevant to cell damage and regeneration. A prominent example is the demonstration of the DLK-1 MAP kinase pathway as an essential element of neural regeneration [55]. Components of this pathway, and specifically the DLK-1 protein, are required for growth cone formation after neuron damage in *C. elegans* but are dispensable during initial developmental axon outgrowth. Moreover, over expression of DLK-1 resulted in improved regeneration both in speed of growth cone formation and accuracy of the re-growth. Further studies along these lines have identified additional factors in this signaling pathway [56], showing that the DLK-1 pathway triggers local synthesis of key proteins within the severed axon. While large-scale screening for neural regenerative factors using single cell laser surgery presents numerous technical challenges, several systems are in development that will automate much of the process. In particular, microfluidic devices designed to automatically manipulate and restrain *C. elegans* for laser surgery and imaging show great potential in increasing experimental throughput [57–59]. These advances will bring the full power of *C. elegans* genetics to bear on the study of neural regeneration, allowing genome wide screening and analysis.

Conclusion

C. elegans is clearly a versatile organism that has strong utility for investigations of the neurobiology of disease. From a conceptual standpoint, study of *C. elegans* has the greatest strength for studies of human neurodegenerative disease aimed at understanding the biology of neuron in vivo, but is less applicable to studies whose goal is to understand neuronal connectivity. Despite a vast evolutionary distance between nematodes and humans, human proteins appear to function appropriately when expressed in nematodes. Surprisingly, mutations that cause disease in humans also cause phenotypic abnormalities in nematodes. This cross-species translation of proteins greatly facilitates study of human disease in such simple organisms as *C. elegans*.

Nematodes will continue to serve as an important experimental system as technology advances. Nematodes often represent a superb platform for preliminary screening of putative therapeutic medications before moving into transgenic rodents. New techniques such as optogenetics are readily applied to *C. elegans*, which is translucent and very amenable to photo-activation of compounds. Nematodes represent an outstanding platform for testing advanced physical techniques, such as femtosecond laser ablation (described above) under conditions that can be tightly controlled. Complex fields, such as systems biology, are increasingly applied to eukaryotic systems. The simplicity of the nematode and the ability to genetically manipulate the organisms makes it a valuable stepping-stone bridging the gap between the simplicity of in vitro studies and the complexity of the human brain.

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Figure 1.

A. *C. elegans* life cycle. *C. elegans* progress through four larval stages until it reaches adulthood, whereupon it lays eggs. Larval development takes 2–3 days, depending on the temperature at which the nematode is grown. Adult nematodes can live for 2–3 weeks. Nematodes that encounter food deprivation during early development enter an alternate stage, termed dauer. The dauers can survive for extended periods of time on very little food, and don't continue through development until nutrient resources are restored. B. Neurons in the nematode are mainly present in a region in the head termed the nerve ring. Panel B shows the head region in a line of *C. elegans* the human protein, Tar DNA Binding Protein – 43 (TDP-43, wild type) linked to GFP driven by the neuronal selective synaptobrevin promoter. TDP-43 localizes exclusively to neuronal nuclei. The mouth and pharynx are observed in the background, with the pharynx surrounded by GFP-positive neurons.

Figure 2. Localization of dopaminergic neurons in *C. elegans* shown using GFP driven by the dopamine transporter promoter. *C. elegans* has 6 dopaminergic neurons in the head; 4 neurons are termed CEP and 2 are termed ADE (arrows).

Figure 3.

Fluorescence of dopaminergic neurons following treatment with three different RNAi constructs (empty vector control, RNAi #4 and RNAi #16), followed by exposure to 6-hydroxydopamine (50 mM) and subsequent aging for 4 more days with continued exposure to RNAi. RNAi #4 sensitized the dopaminergic neurons to 6-hydroxydopamine, while RNAi #16 protected the dopaminergic neurons against 6-hydroxydopamine.

Figure 4.

In vivo neuronal regeneration in *C. elegans* after femtosecond laser surgery. Top: An intact mechanosensory neuron imaged *in vivo* using transgenically expressed fluorescent markers. Middle: Immediately following laser exposure, a clean break is seen at the target point (arrow). Femtosecond laser surgery allows *in vivo* ablation on the submicron scale and snipping of single nerve fibers within an intact adult *C. elegans*. Bottom: 24 h after laser surgery substantial regenerative growth is observed. In this example growth is seen both at the original cut point as well as sprouting directly from the cell soma.

Table 1

Table 1 illustrates the diverse methodologies used to evaluate DA neurons in Parkinson's disease related *C. elegans* models. The CEP and ADE neurons in the head of the worm have been shown to be sensitive to both PD related gene expression and toxins. These studies utilized the expression of GFP driven by the *dat-1* promoter as a marker of DA neuron survival. A variety of methodologies have been used including counting cell bodies, evaluating degeneration of dendrites, and analyzing fluorescence intensity levels. Both Levamisole and Na-azide have been utilized to paralyze the worms to facilitate the assay.

Parkinson's disease related <i>C. elegans</i> Models: DA Neuron Survival Assays			
Reference	Neurons	Method of Evaluation	Treatment
Saha 2009 ²⁵	2 CEP	3mM Levamisole Flourescence Intensity	20 uM Rotenone hLRRK2
Ruan 2009 ⁶⁰	4CEP, 2ADE	3mM Levamisole Wild type Vs. Degenerating	10mM 6-OHDA
Pu 2008 ⁶¹	4CEP, 2ADE	% Worms with intact neurons	.5mM MPP+
Hamamachi 2008 ²⁴	4 CEP, 2ADE	2mM Levamisole Rescued if all neurons intact	alpha synuclein
Cao 2005 ⁶²	4CEP	3mM Levamisole	10mM 6-OHDA
Lakso 2003 ⁷	2CEP,4ADE,2PDE	Cell bodies and Dendrites	h alpha synuclein
Nass 2002 ²³	4 CEP	2% NaAzide Wild type If dendrites were intact	10 or 50 mM 6OHDA