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Review

Modeling Parkinson's Disease in *C. elegans*

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Abstract. Parkinson's disease (PD) is an adult onset neurodegenerative disease that is characterized by selective degeneration of neurons primarily in the substantia nigra. At present, the pathogenesis of PD is incompletely understood and there are no neuroprotective treatments available. Accurate animal models of PD provide the opportunity to elucidate disease mechanisms and identify therapeutic targets. This review focuses on *C. elegans* models of PD, including both genetic and toxicant models. This microscopic worm offers several advantages for the study of PD including ease of genetic manipulation, ability to complete experiments rapidly, low cost, and ability to perform large scale screens for disease modifiers. A number of *C. elegans* models of PD have been generated including transgenic worms that express α-synuclein or LRRK2, and worms with deletions in *PRKN/pdr-1, PINK1/pink-1, DJ-1/djr-1.1/djr-1.2* and *ATP13A2/catp-6.* These worms have been shown to exhibit multiple phenotypic deficits including the loss of dopamine neurons, disruption of dopamine-dependent behaviors, increased sensitivity to stress, age-dependent aggregation, and deficits in movement. As a result, these phenotypes can be used as outcome measures to gain insight into disease pathogenesis and to identify disease modifiers. In this way, *C. elegans* can be used as an experimental tool to elucidate mechanisms involved in PD and to find novel therapeutic targets that can subsequently be validated in other models.

Keywords: Parkinson's disease, *C. elegans*, animal model, genetics, neurodegeneration, α-synuclein, parkin, PINK1, DJ-1, ATP13A2

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting more than 10 million patients worldwide [1]. PD is a neurological movement disorder that is characterized by impaired balance, bradykinesia, rigidity, and the presence of resting tremors. In addition to deficits in movement, PD patients can also exhibit non-motor symptoms including depression, apathy, anxiety, dementia, constipation, disrupted sleep, and others. While rare cases of early onset PD have been described, PD is a disease of advanced age. The prevalence of PD is 0.3% among all ages, but increases to more than 3% in individuals over 80 years of age [2].

In the brain, PD patients exhibit progressive degeneration of dopaminergic neurons in the substantia nigra, although many other regions of the brain are also affected. Neuronal loss within the substantia nigra decreases dopamine signaling to the striatum thereby contributing to the motor symptoms of PD. At the cellular level, the disease is characterized by intracellular aggregation of a protein called α-synuclein into Lewy bodies, which are observed in the brains of patients with PD [3]. There are

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currently no neuroprotective treatments available for PD and the pathogenesis of the disease is incompletely understood.

While PD was traditionally thought of as an entirely idiopathic disorder, work in the past two decades has demonstrated that genetics make a significant contribution to the disease. Currently, it is estimated that 15% of all PD cases are familial. The first gene that was shown to cause monogenic PD is *SNCA/PARK1,* which encodes α-synuclein [4]. There are now 23 chromosomal regions that are defined as PARK to signify their connection to PD. These regions are numbered chronologically by the order of their discovery, either by linkage analysis of families or by genome wide association studies in large populations. Of the genes definitively linked to heritable PD, mutations in *SNCA/PARK1/PARK4* and *LRRK2/PARK8* [5, 6] are known to be the cause of autosomal-dominant PD, while mutations in *PRKN/PARK2* [7], *PINK1/PARK6* [8], *DJ-1/PARK7* [9], and *ATP13A2/PARK9* [10] are responsible for autosomal-recessive forms of disease. While monogenic forms of PD are relatively rare, the etiology of disease for the most common forms of genetic PD share many similarities with sporadic cases, suggesting that similar cellular pathways are involved in both cases. Thus, with each new gene that is implicated in PD comes the opportunity to learn more about the cellular pathways involved in disease pathogenesis.

C. ELEGANS AS AN ANIMAL MODEL OF PARKINSON'S DISEASE

The discovery of genes that cause monogenic forms of PD allowed for the generation of genetic models of PD in many different species, including *C. elegans.* Studying the biology of a particular gene implicated in PD in animal models can provide insight into the molecular pathways involved in the human disease. Animal models can also be used to identify biomarkers of disease and to test potential treatments. While models of PD have been generated in multiple species from single cellular yeast to monkeys, this review will focus on *C. elegans* models of PD.

The nematode *C. elegans* is a microscopic roundworm that grows to be 1-2 mm in length as adults. After hatching, these animals develop to adulthood in just 2 days under laboratory conditions at 20°C. Once these worms reach adulthood, their average lifespan is 2-3 weeks, making them useful for studies of aging. *C. elegans* exist primarily as a self-fertilizing hermaphrodite, in which all of the progeny are genetically identical. Males exist as a small fraction of the population (<0.1%) but their numbers can be greatly increased in the laboratory to facilitate genetic crosses. This animal is genetically tractable with robust tools for spatiotemporal control of gene expression and a highly annotated genome. Because *C. elegans* are transparent, fluorescent proteins can be readily visualized in a live worm to measure levels and location of gene products of interest. These animals have been utilized to address a variety of cellular and genetic questions [11] and specifically to gain insight into neurodegenerative disease [12].

C. elegans have a well-defined, invariant nervous system with exactly 302 neurons in each hermaphrodite out of a total of 959 cells in the organism. Unlike any other organism, all of the connections of all 302 neurons in *C. elegans* have been mapped using electron micrographs thereby providing the most complete nervous system connectome of any organism [13]. Importantly, these neurons encode complex behaviors, which, in several cases, have been described at the level of a single neuron [14–17]. Such behaviors include chemotaxis, thermotaxis, touch response, mating rituals, social and individual feeding, and scavenging as well as associative and non-associative learning [18–22].

EXPERIMENTAL TOOLS FOR *C. ELEGANS*

Genetics

One of the greatest advantages of *C. elegans* as a model organism is the ease of genetic manipulation and the wide array of genetic tools available. Transgenic animals can be easily generated in less than a month through microinjection or microparticle bombardment. Deletion and point mutants are readily available from the *Caenorhabditis* Genetics Center, which is a central repository for *C. elegans* mutant strains that currently has over 20,000 strains available. Importantly, generating double mutants to examine the effect of one gene on another is greatly facilitated by the fact that the worms are hermaphrodites because only one cross is required to combine two mutations in one worm, which can then be selfed to generate worms homozygous for both mutations. Once a homozygous double mutant animal is

produced, each hermaphrodite will produce up to 300 offspring.

C. elegans have 6 chromosomes and approximately 20,000 genes. The *C. elegans* genome was the first genome of a multi-cellular organism to be completely sequenced. About 40–50% of protein coding genes have orthologs in humans and it is estimated that 60–80% of human disease causing genes have orthologs in *C. elegans* [23–25]. Importantly for the study of PD, *C. elegans* has orthologs to many of the genes implicated in PD including *LRRK2/lrk-1, PINK1/pink-1, PRKN/pdr-1, DJ-1/djr-1.1/djr-1.2, and ATP13A2/catp-6*.

RNA interference

The precision and adaptability of RNAi in *C. elegans* makes it a particularly powerful tool. Unlike most animals, in *C. elegans* RNAi can be administered by simply feeding worms bacteria engineered to express a double stranded RNA targeting a gene of interest. Remarkably, in these animals, RNAi is heritable for up to three or more generations [26, 27]. In *C. elegans*, there are several methods that can be utilized in order to silence gene expression using RNAi: the dsRNA can be delivered via injection into any tissue [28]; by feeding bacteria expressing the dsRNA [29]; by soaking animals in a solution containing dsRNA [30]; or by production of dsRNA via a transgene for *in vivo* knock down that can be conditionally controlled [31].

While these methods are sufficient to mediate gene silencing in most tissues, it has been shown that specific tissues, such as neurons, can be less sensitive to RNAi, depending on the method of RNAi delivery [32–34]. Nonetheless, strains with enhanced sensitivity to RNAi, such as *rrf-3* or *eri-1*, have been identified to circumvent this limitation [35, 36]. Alternatively, it has been shown that the transgenic expression of the dsRNA transporter SID-1 in a tissue of interest can sensitize that tissue to RNAi [37]. Tissue-specific knockdown of a gene of interest can be accomplished by expressing a necessary component of the endogenous RNAi machinery in a mutant that is deficient in that component (e.g., the Argonaute protein RDE-1 can be expressed just in the intestine using an intestine specific *ges-1* promoter in *rde-1* mutant animals to achieve intestine-specific knockdown of a gene of interest when worms are exposed to an RNAi targeting that gene). Using this approach, strains have been constructed in which a gene of interest can be knocked down specifically in GABAergic neurons [38], serotonergic neurons [39], dopaminergic neurons [40], cholinergic neurons [40], glutamatergic neurons [40] or pan-neuronally [37].

Screening for disease modifiers

One of the biggest advantages of using a simple genetic model organism for the study of Parkinson's disease is the ability to perform large-scale screens. These screens are enabled by the small size, ease of maintenance, affordability, and the large brood size of *C. elegans*. Screening for disease modifiers can be done using a chemical/compound screen, a classical forward genetic screen [41], or an RNAi interference screen [42]. In fact, RNAi libraries covering nearly the entire genome are available commercially, including the Ahringer library which contains bacterial clones with 17,575 genes [43] and the Vidal RNAi library that includes open reading frame targeting clones of 11,800 genes [44]. A number of groups have performed screens for disease modifiers in *C. elegans* models of PD using various outcome measures including aggregation and neurodegeneration (Table 1).

OUTCOME MEASURES USED TO IDENTIFY DISEASE MODIFIERS

In order to screen for disease modifiers it is necessary to have quantifiable phenotypic deficits that can be used as outcome measures. Although *C. elegans* is a simple organism, there are a number of assays available to assess the toxicity of PD-related defects including very specific dopamine-dependent behaviors.

Survival of dopamine neurons

Because loss of dopamine neurons is a hallmark of human PD, the primary outcome measure in many *C. elegans* studies is the survival of dopamine neurons (Fig. 1). While staining for neurons is possible in fixed tissues, due to *C. elegan's* transparent cuticle, neuronal survival is normally assessed in live animals by expressing a fluorescent protein specifically in dopamine neurons using the promoter from the dat-1 dopamine transporter gene. In *C. elegans* hermaphrodites there are 8 dopamine neurons (out of 302 total neurons) including 6 anterior (four CEP neurons, two ADE neurons) and 2 posterior (PDE neurons). Male *C. elegans* possess 6 additional

Table 1
Summary of screens performed in *C. elegans* models of Parkinson's disease

Type of Screen	Model	Outcome measure	Comments	Reference
Compound	MPP+	Mobility	Proof-of-principle that MPP+ model could be used to screen for modifiers of mobility	Braungart et al., 2004 [100]
Compound	6-OHDA	Neuron loss	D2 receptor agonists bromocriptine and quinpirole are neuroprotective	Marvanova et al., 2007 [101]
Compound	*Pdat-1:α-syn*	Neuron loss	Combined screening with yeast and worms, genes responsible for intracellular trafficking and mitochondrial function were found to be neuroprotective	Su et al., 2010 [140]
Genetic	6-OHDA	Neuron loss	Mutations in *dat-1* dopamine transporter gene are neuroprotective	Nass et al., 2005 [141]
RNAi	*Punc-51:α-syn*	Uncoordinated movement, growth retardation	Knockdown of genes in endocytic pathway exacerbate α-syn toxicity	Kuwahara et al., 2008 [70]
RNAi	*Punc-54:α-syn:YFP*	α-synuclein aggregation	Identified genes that increase or decrease aggregation when knocked down	Van Ham et al., 2008 [58]
RNAi	*Punc-54:α-syn:GFP, Punc-54::tor-2*	α-synuclein aggregation	Homologs of PD-causing genes increase aggregation	Hamamichi et al., 2008 [73]
RNAi	*Punc-54:α-syn:YFP*	Uncoordinated movement	Kynurenine pathway and serotonin production play an important role in regulating protein homeostasis	Van der Goot et al., 2012 [74]
RNAi	*Punc-54:α-syn:GFP*	α-synuclein aggregation	Targeting upregulated genes in Insulin/IGF mutants that reduce α-syn aggregation	Knight et al., 2014 [138]
RNAi	*Punc-54:α-syn:YFP*	α-synuclein aggregation	Knockdown of neuroprotective genes also increased α-syn aggregation as well as causing changed motility, mitochondrial content, and ROS production	Jadiya et al., 2015 [142]

MPP+, 1-methyl-4-phenylpyridinium; 6-OHDA, 6-hydroxydopamine; RNAi, RNA interference.

dopaminergic neurons in the tail ray. In addition to the loss of neuron cell bodies, quantification of more subtle phenotypes is possible. This includes the disappearance of axons, broken neurites, retreat of dendritic terminals, and axonal and dendritic blebbing [45–49].

Dopamine-dependent behaviors

Basal slowing

The dopaminergic circuit has been found to directly affect several behaviors in *C. elegans.* Basal slowing is a feeding behavior in which the rate of locomotion changes depending upon whether the animal is in the presence or absence of the bacterial lawn that serves as its food source in the laboratory due to a mechanosensory response. Normally animals crawl more slowly in the presence of bacterial food than when there is no food present, but the disruption of dopamine signaling prevents the animal's ability to slow in the presence of food, rendering a higher crawling speed [22]. The basal slowing ratio can be calculated by determining the difference between crawling speed on and off food, then determining the ratio by dividing by speed off food:

basal slowing = ([rate of movement absence of food – rate of movement presence of food]) / rate of movement presence of food

Ethanol preference

C. elegans can sense environmental chemical cues that cause attractive or repulsive chemotaxis [50]. Chemotaxis assays are assessed by dividing an agar plate into quadrants and seeding two quadrants with the compound in question. Worms are then transferred to the test plate and allowed to freely explore. To assess the effect of the compound, the animals in the seeded and control quadrants are quantified and a preference index (PI) is calculated:

$$(PI) = ([\text{number of animals compound quadrants}] - [\text{number of animals control quadrants}]) / \text{total number of animals tested}$$

Under laboratory conditions *C. elegans* avoid ethanol [51, 52] and this is dependent on a functional dopamine signaling system [53]. A similar effect is observed using the compound nonanol, which induces a strong negative chemotaxis response that is dependent on functional dopaminergic circuity [54, 55].

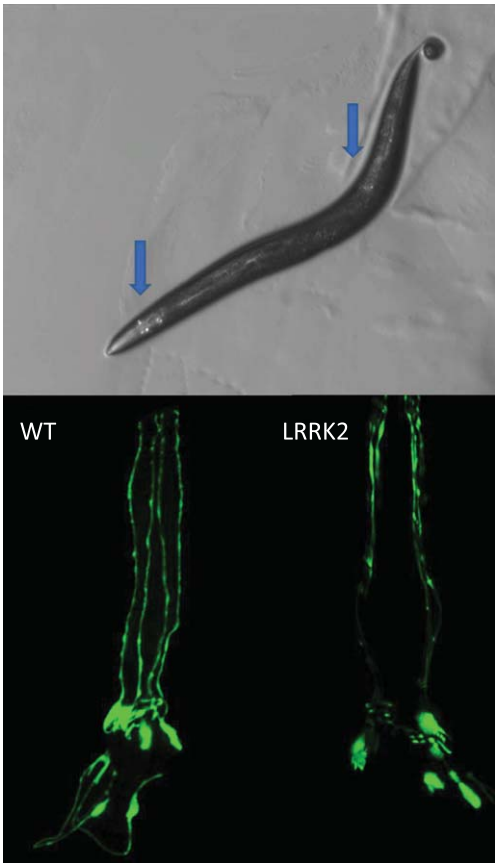

Fig. 1. Loss of dopamine neurons. *C. elegans* hermaphrodites have eight dopamine neurons that can be visualized in live worms by expressing a fluorescent protein such as GFP with a dopamine neuron specific promoter such as *dat-1*. There are six dopamine neurons in the head and two posterior, as indicated by the blue arrows (top). The progressive loss of dopamine neurons can be monitored throughout the lifespan of the worm. Expression of human mutant LRRK2 with G2019S mutation causes an accelerated loss of dopamine neurons (bottom). Bottom panels show only the head region of the worm with the tip of the nose facing the top of the page.

Area-restricted searching (ARS)

ARS is a foraging strategy in which *C. elegans* prioritize unexplored areas over those that have run out of food. When a worm is searching for food on an empty plate, they will turn frequently at sharp angles, containing their search to a confined area. As this behavior becomes unsuccessful at finding food, the search area is expanded, the number of turns decreases, and the scavenging behavior then consists of wide arcs that increase the efficiency of foraging. Dopamine signaling is involved in the successful switching of strategies [21]. ARS is evaluated by transferring animals to an agar plate without food. Video recordings are then taken 5 minutes after transfer and again after 30 minutes without food. Analysis of the videos then allow for the frequency of turns greater than 90° at each time point.

Swimming induced paralysis (SWIP)

While healthy, well fed animals initially thrash vigorously in liquid, after thrashing for about 6 minutes, *C. elegans* become paralyzed exhibiting a phenotype called SWIP. Animals with impaired dopaminergic function, such as tyrosine hydroxylase mutants, do not exhibit this behavior [56]. This suggests that endogenous dopamine is responsible for inactivation of the motor circuitry necessary for thrashing behavior. However, while SWIP is readily observed in animals at the L4 stage of development, it becomes less prominent with age.

Accumulation of α-synuclein

As the aggregation of α-synuclein is a key feature of PD, *C. elegans* models have been developed in which human SNCA is expressed in order to study α-synuclein accumulation. To visualize α-synuclein aggregation in live worms, α-synuclein has been linked to a fluorescent protein (e.g., GFP or YFP) and typically expressed in body wall muscle to facilitate visualization [57, 58] (Fig. 2). While neuronal α-synuclein aggregates can be visualized, due to the small size of nematode neurons, quantifiable intracellular inclusions can only be resolved in fixed tissue [59], which is not suitable large scale or screening analysis.

Movement: Thrashing and crawling

Because motor deficits are a major component of Parkinson's disease, multiple assays are utilized to measure movement [49, 60]. The rate of movement in liquid, also known as the thrashing rate, is counted as the number of body bends per unit time and can be assessed manually on individual worms or using video-tracking on entire populations [61]. Crawling speed on solid plates is measured as distance travelled in a given amount on time.

Mitochondrial morphology and function

As deficits in mitochondrial function have been implicated in the pathogenesis of PD, another important outcome measure that is examined in *C. elegans* models is mitochondrial morphology and function, especially since multiple genes that

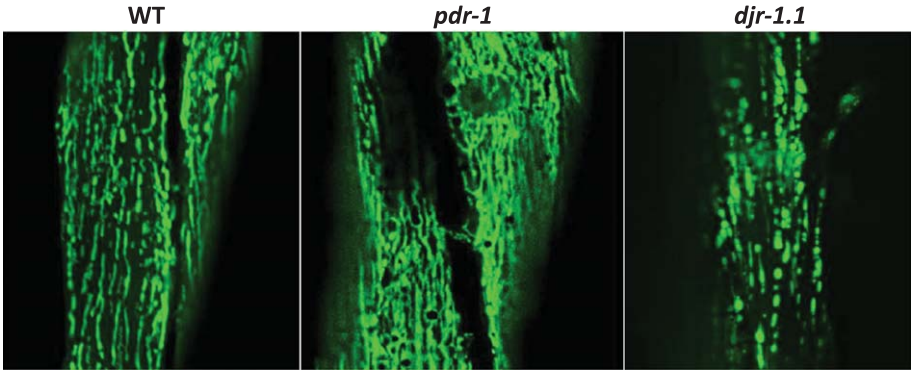

Fig. 2. Aggregation of α-synuclein. Although worms do not possess an ortholog of α-synuclein it is possible to study α-synuclein by expressing α-synuclein linked to a fluorescent proteion, such as YFP, with a body wall muscle specific promoter such as *unc-54*. While expression of YFP alone results in diffuse expression throughout the body wall muscle, the presence of α-synuclein results in the formation of aggregates.

cause PD are directly involved in mitochondrial function (*PRKN/pdr-1, PINK1/pink-1, DJ-1/djr-1.1*). Mitochondrial morphology can be visualized in live worms by utilizing transgenic animals that express a fusion protein of a mitochondrially-targeted protein (e.g., TOMM-20) and a fluorescent protein (e.g., GFP or YFP) [62–64] (Fig. 3). Using this technique, disruptions in mitochondrial morphology have been discovered in *pdr-1, pink-1* and *djr-1.1* mutants [53]. A basic assessment of mitochondrial function can be achieved through measurements of oxygen consumption and ATP levels. Oxygen consumption can be measured in individual or small numbers of samples using a Clark electrode or in multiple samples using a Seahorse Extracellular Flux Analyzer, while ATP can be measured from lysed worms using commercially available lucigenin based quantification kits.

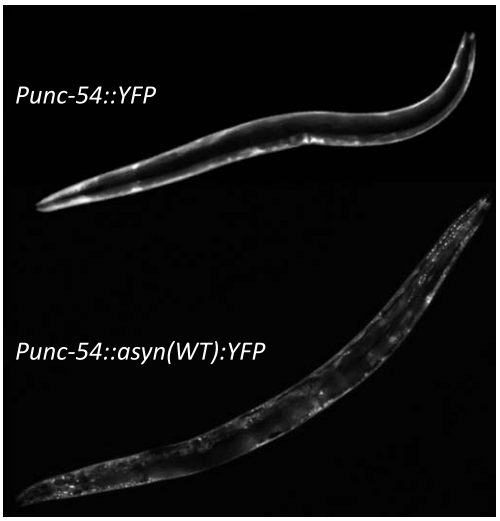

Fig. 3. Mitochondrial morphology. The morphology of the mitochondria can be monitored in live worms by expressing a mitochondrially-targeted fluorescent protein, such as GFP, under tissue-specific promoters, such as *myo-3* for body wall muscle. In wild-type worms, mitochondria in the body wall muscle exist as parallel tracks of elongated mitochondria. *pdr-1* mutants exhibit increased accumulation of mitochondria, while *djr-1.1* mutants exhibit mitochondrial fragmentation.

Resistance to stress

Multiple studies have examined resistance to stress in *C. elegans* models of PD and observed that the genetic defects that cause PD increase susceptibility to various stresses [53, 60, 65]. Interestingly, heat stress, oxidative stress and osmotic stress all act to increase protein aggregation (Cooper and Van Raamsdonk, unpublished data). The connection between increased sensitivity to stress, decreased proteostasis and the aggregation of α-synuclein has yet to be explored.

Overall, there are a number of different outcome measures that can be used to quantify phenotypic deficits in worm models of PD. Of all these measures, we think that loss of dopamine neurons is the most reliable and disease-relevant assay, and this is also the most frequently reported outcome in the literature. Note that it is important to combine this assay with tests of neuronal function to ensure that any increases in neuronal number that are associated with a therapeutic intervention also preserve neuronal function. For this purpose, we have found the basal slowing assay to be the most robust, and this is also the most commonly used assay in published studies. Finally, it should be noted that most of the tests of

dopamine-dependent behavior rely on crawling. As a result, it is important to test movement as deficits in movement can be a confounding factor in these assays.

C. ELEGANS MODELS OF PARKINSON'S DISEASE

SNCA

SNCA/PARK1/PARK4, the first gene to be definitively linked to familial PD [4], encodes α-synuclein, a protein involved in synaptic vesicle formation. Mutations in *SNCA* and duplications or triplications of the gene have been shown to cause autosomal dominant forms of PD [66, 67]. While *C. elegans* does not have a homolog to *SNCA*, a number of worm models have been generated by expressing human wild-type or mutant α-synuclein either ubiquitously or in specific tissues (Table 2). Expression of α-synuclein in all neurons or specific populations of neurons causes loss of dopamine neurons, deficits in dopamine dependent behavior, and decreased levels of dopamine [49, 59, 60, 68–72]. In order to study aggregation, α-synuclein has been linked to YFP or GFP and expressed in body wall muscle cells for ease of visualization [58, 73, 74]. In addition to aggregation, these worms show deficits in movement and increased sensitivity to stress (Cooper and Van Raamsdonk, unpublished data). Since worms do not normally express α-synuclein, worm models have also been generated that ubiquitously express wild-type α-synuclein from a single copy transgene (Cooper and Van Raamsdonk, unpublished data).

LRRK2/LRK-1

Mutations in the leucine rich repeat kinase 2 gene *LRRK2* have also been shown to cause autosomal dominant PD [6]. *lrk-1*, the *C. elegans* homolog of *LRRK2*, is expressed broadly in these animals, including neurons, in which it associates with the Golgi apparatus [65]. Expression of WT or mutant LRRK2 either pan-neuronally using the synaptobrevin promoter [75] or specifically in dopamine neurons using the dopamine transporter promoter [60, 76, 77] decreases dopamine levels, induces deficits in dopamine-dependent behaviors and causes a progressive loss of dopamine neurons (Table 2). Extrachromosomal arrays expressing *LRRK2* in dopamine neurons have also shown that kinase activity of LRRK2(G2019S) is important for the development of age-dependent neurodegeneration [78].

PRKN/PDR-1

Mutations in *PRKN/PARK2* cause an autosomal recessive form of PD with an early age of onset [7, 79]. Parkin, which is encoded by *pdr-1* in *C. elegans*, encodes an E3 ubiquitin ligase involved in protein degradation and mitophagy [80]. *pdr-1* mutants exhibit a loss of dopamine neurons [81], and deficits in the dopamine-dependent behaviors (Table 3) [53, 82]. In addition, *pdr-1* mutants have been shown to have increased sensitivity to various stresses [53, 64, 83]. At a cellular level, an in frame deletion in *pdr-1* causes the protein to aggregate and increases sensitivity to proteotoxic stress [71]. Finally, mutations in *pdr-1* have been shown to cause the accumulation of dysfunctional mitochondria [53, 64] and deficiencies in oxidative phosphorylation [53, 84], which are associated with activation of the mitochondrial unfolded protein response [53]. Perhaps as a means of jettisoning their accumulating mitochondria, *pdr-1* mutant animals also produce more exophers than wild-type worms [85].

PINK1/PINK-1

Mutations in *PINK1/PARK6* cause an autosomal recessive form of PD with early disease onset [8, 86]. PINK1 (PTEN-induced putative kinase 1) is a mitochondrial kinase that acts with Parkin in mitophagy [87, 88] and is also first identified ubiquitin kinase [89]. Mutations in the *PINK1* homolog *pink-1* result in increased sensitivity to multiple stresses [64, 65, 83]. While *pink-1* worms show a significant reduction in basal slowing, these mutants exhibit a wild-type survival of dopamine neurons [53]. The mitochondria of *pink-1* worms accumulate with age, show altered morphology and have deficiencies in oxidative phosphorylation [53, 65, 84]. As in *pdr-1* mutants, worms with dysfunctional *pink-1* also produce significantly more exophers in neurons expressing toxic proteins [85].

DJ-1/DJR-1.1,DJR-1.2

Mutations in the *DJ-1/PARK7* gene cause a recessive form of PD with an early age of onset [90]. DJ-1 is a deglycase that has been shown to protect against oxidative stress [91, 92]. There are two orthologs of *DJ-1* in *C. elegans*, *djr-1.1*, which is expressed primarily in the intestine, and *djr-1.2*, which is expressed primarily in neurons [93]. Deletion of either ortholog (or both together)

Table 2
Transgenic worm models of Parkinson's disease

Expression pattern	Transgene	Phenotypes	Reference
α-synuclein models			
Dopamine neurons	*Pdat-1::asyn*	↑DA neuron loss, ↓DA, asyn accumulation	Lakso et al., 2003 [49]; Cao et al., 2005 [68]; Kuwahara et al., 2006 [59]
	Pdat-1::asyn (A53T)	↑DA neuron loss, ↑DA neurite defects, ↓DA, asyn accumulation, movement deficit, ↓basal slowing, ↓ARS, ↓ethanol avoidance	Lakso et al., 2003 [49]; Kuwahara et al., 2006 [59]; Kuwahara et al., 2008 [70]; Cooper et al., 2015 [60]
	Pdat-1::asyn (A30P)	↑DA neuron loss, ↑DA neurite defects, ↓DA, asyn accumulation, ↓basal slowing	Kuwahara et al., 2006 [59]; Karpinar et al., 2009 [143]
	Pdat-1::asyn (A56P)	↑DA neurite defects, ↓basal slowing	Karpinar et al., 2009 [143]
Motor neurons	*Pacr-2::asyn*	Movement deficit	Lakso et al., 2003 [49]
	Pacr-2::asyn (A53T)	Movement deficit	Lakso et al., 2003 [49]
	Punc-30::asyn	Movement deficit	Lakso et al., 2003 [49]
	Punc-30::asyn (A53T)	Movement deficit	Lakso et al., 2003 [49]
Mechanosensory neurons	*Pmec-7::asyn*	Impaired touch response	Kuwahara et al., 2008 [70]
	Pmec-7::asyn (A53T)	Impaired touch response	Kuwahara et al., 2008 [70]
Pan-neuronal	*Paex-3::asyn*	↑DA neuron loss, movement deficit	Lakso et al., 2003 [49]
	Paex-3::asyn (A53T)	↑DA neuron loss, movement deficit	Lakso et al., 2003 [49]
	Punc-51::asyn	Movement deficit, growth defect, impaired touch response	Kuwahara et al., 2008 [70]
	Punc-51::asyn (A30P)	Movement deficit, growth defect, impaired touch response	Kuwahara et al., 2008 [70]
	Punc-51::asyn (A53T)	Movement deficit, growth defect, impaired touch response	Kuwahara et al., 2008 [70]
Body wall muscle	*Punc-54::asyn:GFP*	Movement deficit, asyn accumulation, asyn aggregation	Hamamichi et al., 2008 [73]
	Punc-54::asyn:YFP	Movement deficit, asyn accumulation, asyn aggregation, ↓stress resistance	van Ham et al., 2008 [58]; Cooper and Van Raamsdonk, unpublished data
Ubiquitous	*Peft-3::asyn :RFP*	asyn accumulation, axon blebbing, ↓basal slowing, ↓stress resistance	Cooper and Van Raamsdonk, unpublished data
LRRK2 models			
Dopamine neurons	*Pdat-1::LRRK2*	↑DA neuron loss, ↓DA, movement deficit	Yao et al., 2010 [76]; Cooper et al., 2015 [60]
	Pdat-1::LRRK2 (G2019S)	↑DA neuron loss, ↓DA, movement deficit, ↓basal slowing, ↓ARS, ↓ethanol avoidance	Yao et al., 2010 [76]; Liu et al., 2011 [78]; Yao et al., 2013 [77]; Cooper et al., 2015 [60]
	Pdat-1::LRRK2 (G2019S/D1994A)	↑DA neuron loss compared to WT, but ↓DA neuron loss compared to G2019S	Liu et al., 2011 [78]
	Pdat-1::LRRK2 (R1441C)	↑DA neuron loss, ↓DA, movement deficit, ↓basal slowing	Yao et al., 2010 [76]; Yao et al., 2013 [77]
Pan-neuronal	*Psnb-1::LRRK2*	↑DA neuron loss	Saha et al., 2009 [75]
	Psnb-1::LRRK2 (R1441)	↑DA neuron loss, ↓DA	Saha et al., 2009 [75]
	Psnb-1::LRRK2 (G2019S)	↑DA neuron loss, ↓DA	Saha et al., 2009 [75]

DA, dopamine; ARS, area-restricted searching; asyn, α-synuclein.

does not cause the loss of dopamine neurons or significantly impact dopamine-dependent behaviors [53]. Nonetheless, *djr-1.1* mutants show increased sensitivity to oxidative stress, mitochondrial fragmentation and a decreased ability to generate energy [53, 93].

ATP13A2/CATP-6

Mutations in ATP13A2/PARK9 cause an atypical form of early-onset parkinsonism called Kufor-Rakeb Syndrome [10, 94]. This gene encodes a lysosomal P-type ATPase transporter. Loss of

Table 3
Genetic loss of function *C. elegans* models of Parkinson's disease

Human gene	*C. elegans* gene	Phenotypes	Reference
PRKN	*pdr-1*	↑DA neuron loss, ↓basal slowing, ↓ethanol avoidance, ↓lifespan, mitochondrial accumulation, ↓stress resistance	Springer et al., 2005 [71]; Ved et al., 2005 [102]; Bornhorst et al., 2014 [81]; Chakraborty et al., 2015 [82]; Kirienko et al., 2015 [83]; Palikaras et al., 2015 [64]; Luz et al., 2015 [84]; Cooper et al., 2017 [53]
PINK1	*pink-1*	↓basal slowing, mitochondrial accumulation, ↓stress resistance	Samann et al., 2009 [65]; Kirienko et al., 2015 [83]; Palikaras et al., 2015 [64]; Luz et al., 2015 [84]; Cooper et al., 2017 [53]
DJ-1	*djr-1.1*	↓stress resistance, mitochondrial fragmentation	Lee et al., 2012 [93]; Cooper et al., 2017 [53]
	djr-1.2	↓stress resistance	Lee et al., 2012 [93]
ATP13A2	*catp-6*	↑DA neuron loss, ↓basal slowing, movement deficit, ↓stress resistance	Cooper and Van Raamsdonk, unpublished data

DA, dopamine.

ATP13A2 function leads to enhanced oxidative stress, protein misfolding, and aggregation due to deficiencies within the lysosome [94]. Mutations in *catp-6* causes a number of deficits including accelerated loss of dopamine neurons, deficits in dopamine-dependent behavior, decreased rate of movement and increased sensitivity to multiple stresses (Cooper and Van Raamsdonk, unpublished data). Levels of CATP-6 have been shown to influence the accumulation of α-synuclein [95].

TOXICANT MODELS

In addition to genetic models, a number of groups have used neurotoxins to induce dopaminergic cell death as a model of PD [45, 96, 97]. Treating worms with MPP+ (1-Methyl-4-phenylpyridinium), the active metabolite of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), has been shown to cause deficits in movement and dopamine neuron loss [98–100]. Similarly, treating worms with the dopamine analog 6-hydroxydopamine (6-OHDA), Manganese (Mn), or methylmercury also causes degeneration of dopamine neurons [45, 48, 101]. The insecticide rotenone and the herbicide paraquat have also been used to induce behavioral deficits and neurodegeneration in *C. elegans* [15, 102–104]. These compounds cause toxicity by increasing ROS leading to cellular toxicity, damage and disruption of neuronal morphology [45, 46, 97, 105–107].

Overall, there are a large variety of *C. elegans* models of PD to choose from when designing an experiment. The best model to utilize depends on the experimental question of interest. Of the genetic models, those expressing mutant α-synuclein or LRRK2 in dopamine neurons provide the most robust loss of dopamine neurons and dopamine-dependent behaviors. These models would be most appropriate for the development of neuroprotective strategies. *pdr-1*, *pink-1* and *djr-1.1* mutants have subtler phenotypic deficits making them less ideal for therapeutic studies, but can be used to identify factors contributing to disease (e.g., identification of synthetic lethal interactions).

CONSERVATION ACROSS SPECIES

An important premise for studying PD in animal models is that the findings obtained in the animal model are also observed in PD. There have been multiple examples in which findings from *C. elegans* have been shown to be conserved across species and for the purpose of this review we will highlight only a few of these.

In one study examining the neuroprotective properties of ATP13A2/CATP-6, it was initially shown that the yeast homolog of ATP13A2 could protect against α-synuclein toxicity in a colony growth assay [108]. It was subsequently shown that overexpression of CATP-6 in *C. elegans* decreases α-synuclein-mediated neuronal loss, while *catp-6* RNAi causes increased aggregation of α-synuclein [95]. Finally, this same phenomenon was examined in a mammalian system. In rat primary midbrain neurons, it

was shown that expression of ATP13A2 could protect against α-synuclein(A53T) toxicity [95]. Thus, the ability of ATP13A2/CATP-6 to protect against α-synuclein toxicity is conserved from yeast to worm to rodent.

Similarly, another group examined the interaction between glucocerebrosidase (GBA) and α-synuclein. Mutations in *GBA1* are the most common genetic risk factor for PD: about 10% of people with PD have mutations in *GBA1*. Glucocerebrosidase acts to degrade glycolipids and homozygous mutations in *GBA1* lead to the development of a lysosomal storage disorder called Gaucher disease. Based on the observation of α-synuclein positive Lewy bodies in patients with Gaucher disease [109], Mazzulli et al. explored the relationship between GBA and α-synuclein. They found that knocking down GBA is primary cortical neurons or human iPS neurons resulted in increased levels of α-synuclein [110]. Similarly, RNAi against *gba-2* caused increased aggregation of α-synuclein in *C. elegans*, while a mouse model of Gaucher disease also showed increased α-synuclein accumulation [110].

It is important to note that the corroboration of findings between organisms is not always observed and validating the results from one species in another can be complicated by the absence of an ortholog and deciding which assay to use. In a recent study seeking to prioritize loss of function variants that were found by whole exome sequencing in 1148 PD patients, only 10 of the 27 genes identified had an ortholog in *C. elegans* [111]. The effect of 9 of these genes on developmental lethality and survival was tested and it was found that 3 of the genes exhibited a detrimental effect. Interestingly, two of those genes, *DIS3* and *KALRN*, were known to also reduce viability in *Drosophila* [112, 113] and rodents [114, 115]. The fact that 6 of the 9 genes did not reduce survival does not exclude the possibility that these genes contribute to PD, since most worm models of PD exhibit normal longevity. It is possible that knocking down these genes would impact dopamine neuron survival or dopamine-dependent behavior but these outcomes were not assessed.

ENVIRONMENT FACTORS CONTRIBUTING TO PD AND GENE-ENVIRONMENT INTERACTIONS

While monogenetic forms of PD facilitate studying the molecular mechanisms underlying the disease, most cases of PD likely result from a complex interaction of genetic and environmental risk factors [116]. Work in *C. elegans* has been able to connect exposure to environmental toxins such as pesticides, Mn or methylmercury to the degeneration of dopamine neurons [102–104]. Interestingly, it has also been shown that specific bacterial metabolites can also contribute to neurotoxicity in dopamine neurons [117–119]. The availability of genetic models of PD permits studying gene-environment interactions in *C. elegans*. For example, it was shown that genes implicated in PD (*SNCA, pdr-1, djr-1.1*) cause increased susceptibility to mitochondrial complex I inhibitors, such as rotenone [102]. Similarly, others have examined the effect of PD-causing mutations on Mn toxicity [81, 120] and showed that exposure to pesticides can exacerbate α-synuclein aggregation [121].

DRUG SCREENING AND DISCOVERY

C. elegans models of PD can also be used as a tool for drug discovery. Compounds can be added to the solid agar plates on which the worms are maintained, or worms can be grown in liquid culture and the compounds added directly to the culture medium. While worms have a thick cuticle that can limit the ability of compounds to enter the worm, genetically modified strains are available with increased drug permeability [122]. Previous work has examined the beneficial effect of specific compounds in genetic and toxicant models of PD, including acetaminophen [123] and valproic acid [124]. In some cases, the compounds investigated are specific to certain genotypes, such as testing kinase inhibitors in LRRK2 mutants [77, 78]. In addition, multiple studies have performed targeted drug screens most commonly for compounds that protect against neurotoxin induced deficits [100–102].

RECENT ADVANCES IN PARKINSON'S DISEASE RESEARCH IN *C. ELEGANS*

C. elegans models to dissect the mechanism of cell-to-cell transfer of α-synuclein

Recent work has suggested that the cell-to-cell transfer of α-synuclein may be a key step in the pathogenesis of PD [125]. While the transfer of α-synuclein has been demonstrated in multiple model systems [126, 127] and importantly in neurons grafted into PD patients [128, 129], the mechanisms

involved have yet to be elucidated. To explore the underlying mechanisms of α-synuclein transfer, two groups have developed worm models using a bimolecular fluorescence (BiFC) approach. In this approach, two parts of a fluorescent protein (e.g., GFP) are fused to α-synuclein such that they will only emit fluorescence if two molecules of α-synuclein interact thereby bringing the two parts of the fluorescent protein close enough together to act as one protein [130]. In order to detect neuron-to-neuron transfer of α-synuclein, the two different BiFC-α-synuclein fusion proteins were expressed in separate populations of neurons, which are synaptically connected [131]. The observation of fluorescence in these worms indicated that the BiFC-α-synuclein molecules move from one cell to another. Having shown that neuron-to-neuron transfer occurs in *C. elegans*, this model can now be used to screen for modifiers of α-synuclein transfer. As a proof of principle, it was shown that genes involved in autophagy, endocytosis and exocytosis all modulate α-synuclein transfer [131]. Interestingly, silencing *C. elegans* orthologs of PD-related genes *PRKN, PINK1, DJ-1, ATP13A2, VPS-35*, and *LRRK2* using RNAi increased α-synuclein accumulation in these animals [131]. Another study that used a different BiFC strain to visualize α-synuclein transfer between neuron and muscle demonstrated that transfer of α-synuclein increased with age and could be decreased by genes that have been shown to delay aging [132].

Targeting aging pathways is protective in C. elegans models of Parkinson's disease

While it has long been known that aging is the greatest risk factor for the development of PD [133, 134], the role of aging in PD is still poorly defined. The large number of similarities that exist between the normal aging process and the development of PD indicates a strong association [135]. However, in order to demonstrate a causative role of aging in the pathogenesis of PD, it is necessary to experimentally modulate aging and show an effect on PD. Because *C. elegans* has been used extensively to study the genetics of aging, it provides the ideal organism to study the relationship between aging and PD. To test this idea, two groups examined the effect of modulating molecular pathways that have been shown to extend longevity in *C. elegans* models of PD. Both groups chose to test the effect of decreasing insulin-IGF1 signaling, which has been shown to double the lifespan of a worm [136]. It was found that delaying aging by decreasing insulin-IGF1 signaling decreased aggregation and protected against dopamine neuronal loss in multiple different worm models of PD [60, 137]. Decreasing insulin-IGF1 signaling also reduced the cell-to-cell propagation of α-synuclein [132]. Importantly, a beneficial effect of delaying aging was also observed in a *Drosophila* model indicating conservation across species [137]. Since decreasing insulin-IGF1 signaling has also been shown to increase lifespan in mice [138], and genetic variants in this pathway are associated with longevity in humans [139], targeting this pathway may provide an effective treatment for patients with PD.

CONCLUSIONS

C. elegans provides a number of advantages as an animal model for the study of PD. The most significant advantages include the wide array of genetic tools available and the ability to screen for disease modifiers in a rapid and cost effective manner. On the other hand, the main limitations of using *C. elegans* for the study of PD include the lack of a homolog to α-synuclein, the challenge of performing molecular biology specifically on dopamine neurons (which make up only 8 of 959 cells), and the fact that neuronal connectivity differs from humans. With these limitations in mind, the model system has enabled numerous significant contributions to our understanding of PD. For example, although *C. elegans* lack an ortholog to α-synuclein, this has not precluded using this model to identify numerous modifiers of α-synuclein aggregation and toxicity, which are shared with mammals. As with all model systems, in using *C. elegans* for PD research, it is important to take advantage of its strengths while using other models to complement its limitations.

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CONFLICT OF INTEREST

The authors have no conflict of interests to declare.

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