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SPECIAL ISSUE REVIEWS–A PEER REVIEWED FORUM

# *C. elegans* as a Model Organism to Investigate Molecular Pathways Involved with Parkinson's Disease

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**Parkinson's disease (PD) is an age-related movement disorder resulting, in part, from selective loss of dopaminergic neurons. Both invertebrate and mammalian models have been developed to study the cellular mechanisms altered during disease progression; nevertheless there are limitations within each model. Mammalian models remain invaluable in studying PD, but are expensive and time consuming. Here, we review genetic and environmental factors associated with PD, and describe how the nematode roundworm, *Caenorhabditis elegans*, has been used as a model organism for studying various aspects of this neurodegenerative disease. Both genetic and chemical screens have been conducted in *C. elegans* to identify molecular pathways, proteins, and small molecules that can impact PD pathology. Lastly, we highlight future areas of investigation, in the context of emerging fields in biology, where the nematode can be exploited to provide mechanistic insights and potential strategies to accelerate the path toward possible therapeutic intervention for PD. *Developmental Dynamics 239:1282–1295, 2010.* © 2010 Wiley-Liss, Inc.**

**Key words:** *C. elegans*; Parkinson's disease; neurodegeneration; dopamine; alpha-synuclein

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## INTRODUCTION

Since the seminal discovery of PARK1/α-synuclein (α-syn) as a genetic cause of familial Parkinson's disease (PD) in 1997 (Spillantini et al., 1997), human geneticists have meticulously identified, in the span of just over 10 years, eight additional gene products that are linked to this disease. These studies determined a set of mutations, modifying functions as well as expression level and pattern of PARK proteins, which ultimately trigger two most prevalent PD pathological hallmarks: formation of the proteinaceous inclusions called Lewy bodies in PD patient brains and a selective loss of dopamine (DA) neurons in the *substantia nigra pars compacta*. Subsequently, mammalian researchers performed functional analyses of diverse PARK proteins, unveiling a snapshot of potential cellular defects in several common pathways. For example, PARK proteins are implicated in synaptic function (PARK1/α-synuclein), protein degradation (PARK2/parkin, PARK9/ATP13A2, and PARK5/UCHL-1), signal transduction (PARK8/LRRK2 and PARK11/GIGYF2), and protection against mitochondrial/oxidative stress (PARK6/PINK1, PARK7/DJ-1, and PARK13/HTRA2). Collectively, malfunctions in these cellular mechanisms may prompt the onset and progression of PD; limited therapeutic interventions are available, despite being the second most common neurodegenerative disease (Dauer and Przedborski, 2003; Dawson and Dawson, 2003; Fahn, 2003).

While studies on familial PARK proteins have founded the basis for the current understanding of PD etiology, it has been reported that only 5–10% of all PD cases are associated with monogenic forms of PD. Consequently, multiple genetic and environmental susceptibility factors, singly or accumulatively, may contribute to idiopathic form of this disease.

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Nevertheless, human genetic analyses continue to play a pivotal role in terms of illuminating mechanistic insights into PD. Importantly, these findings have led to development of animal models that facilitate evaluation. These findings represent a laborious task of examining potential genetic or physical interactions, screening for susceptibility factors, and identification and validation of putative neuroprotective candidates that may also serve as diagnostic or therapeutic targets. Mammalian PD models remain invaluable tools, especially considering the wide range of clinical features associated with PD, such as muscle rigidity, tremors, depression, and dementia (Lees et al., 2009) that may illustrate underexplored intra- and inter-neuronal misregulation beyond the DA system. Nevertheless, mammalian models may not recapitulate all PD pathological impairments (Fleming and Chesselet, 2006); moreover, these models have limitations and are expensive. In this regard, despite their lack of evolutionary complexity, invertebrate model organisms, such as *Saccharomyces cerevisiae*, *Drosophila melanogaster*, and *Caenorhabditis elegans*, have been used as first pass screens for identifying genes and drugs that might be therapeutically relevant to PD processes (Bilen and Bonini, 2005; Caldwell and Caldwell, 2008; Gitler, 2008). These simple organisms share many conserved molecular pathways and cellular mechanisms with mammals and offer economical, yet strategic experimental paradigms that allow rapid analyses of susceptibility factors that can be predictive of PD pathological mechanisms.

Among the various cellular and transgenic models available for use in studying the pathology of PD, *C. elegans* offers several distinct advantages enabling researchers to dissect avenues leading to this disease. While mammals have billions of neurons in their brain, and even fruit flies have ~10,000 neurons, the adult *C. elegans* hermaphrodite has exactly 302 neurons throughout its body, with fully mapped neuronal circuitry (Bargmann, 1998). Of the 302 neurons, 8 neurons are dopaminergic in the hermaphrodite (Fig. 1A), which include 6 anterior dopaminergic neurons (4 CEP neurons and 2 ADE neurons; Fig. 1B) and 2 posterior DA neurons (PDE neurons; Sulston et al., 1975). With *C. elegans* being a completely transparent animal, neurons are easily visualized by simply expressing a fluorescent protein (e.g., GFP), as previously described by Chalfie and colleagues (1994), who later shared the 2008 Nobel Prize in Chemistry for his groundbreaking research using GFP. This is particularly useful when studying neurodegenerative diseases, such as PD, in that neuronal cell death can be readily observed and quantified within living organisms (Fig. 1C,D). The capacity for immediate assessment for the presence or absence of specific neurons in a living organism is a key attribute in using this system to study neurodegenerative disorders.

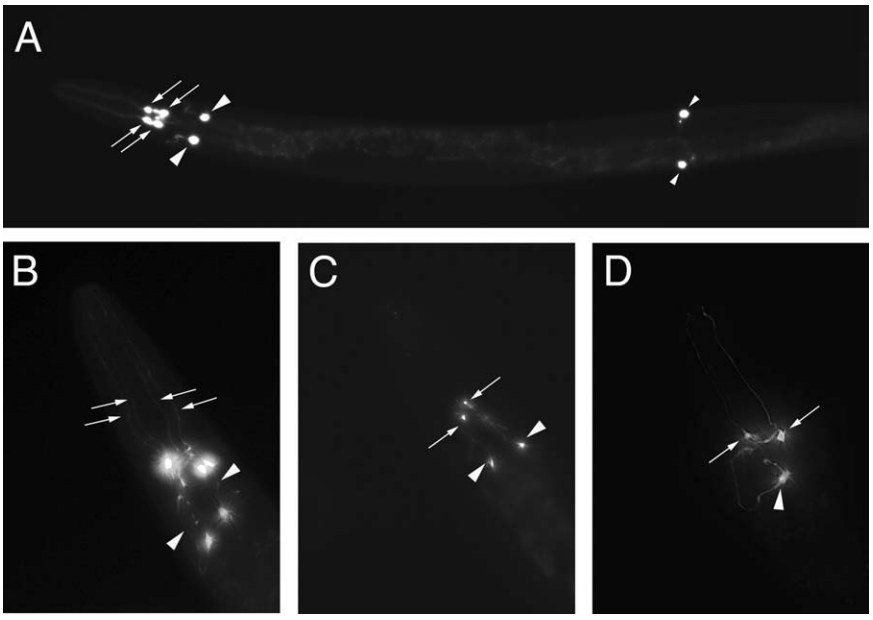


**Fig. 1.** The dopamine (DA) neurons of *C. elegans* hermaphrodites highlighted using green fluorescent protein (GFP) driven from the DA transporter promoter ($P_{dat\text{-}1}$::GFP). **A:** Cell bodies and processes of the six anterior DA neurons include two pairs of cephalic (CEP) neurons (arrows) and one pair of anterior deirid neurons (ADEs; large arrowheads). There are also one pair of posterior deirid neurons in each hermaphrodite (PDEs; small arrowheads). **B:** Magnified view of the anterior region of *C. elegans*, detailing the six anterior-most DA neurons. The dendrites of the four CEP neurons and the axons of the two ADE neurons are labeled with arrows and arrowheads, respectively. **C:** A worm exposed to 6-hydroxydopamine (6-OHDA) exhibiting DA neurodegeneration; two of four CEP cell bodies are present, but degenerating (arrows). The two ADE neurons in this animal are still intact (arrowheads). **D:** A worm co-expressing both GFP and α-syn in DA neurons. Most worms within a population expressing α-syn within the DA neurons are missing anterior DA neurons when they are 7-day-old adults. In this example, only two of the four CEP neurons (arrows) and one of two ADE neurons (arrowhead) remain.

Of interest, the pathways involved in the processing, packaging and transport of DA have been conserved throughout evolution, enabling researchers to use the worm for studying various aspects of DA neurons. By using methods that alter DA signaling in *C. elegans*, several behavioral phenotypes have been identified as being specific for DA signaling. Upon exposure to exogenous DA, worms exhibit a decrease in egg laying, locomotion, and defecation. DA also functions in the basal slowing response, where worms normally decrease locomotion upon entering a bacterial lawn (Sawin et al., 2000). Identification of mutations in *cat-2*, the worm tyrosine hydroxylase that is the rate limiting enzyme for DA synthesis, resulted in no basal slowing response, indicating the role of DA in mechanosensation and food sensing (McDonald et al., 2006). While this mutant phenotype is a viable readout for DA neuronal function, this behavioral effect is difficult to quantify during high-throughput screening, due to the time involved in collecting data.

The most advantageous attribute that *C. elegans* offers is the ability to perform a large-scale genetic analysis. The nematode was the first

multicellular organism to have its complete genome sequenced and made available to the public (*C. elegans* Sequencing Consortium, 1998). In addition, a large number of mutant strains are available within the *C. elegans* community allowing researchers to study the effect of various proteins and pathways. Another tool widely used in *C. elegans* research is reverse genetics. This approach entails the application of the intracellular mechanism of RNA interference (RNAi) to knockdown target genes by simply injecting, soaking, or feeding worms dsRNA which is complementary to the targeted and subsequently silenced gene. Fire et al. (1998) initially described this method to induce selective knockdown of genes in *C. elegans*, for which Andrew Fire and Craig Mello later received the 2006 Nobel Prize in Physiology or Medicine. Along with examining reduced gene expression through RNAi or different loss of function strains, researchers are also able to easily generate transgenic animals to express a protein of interest within specific cells to further analyze the function of the protein. Lastly, a short generation time (3 days from egg to adult) and the availability of various methods to alter expression of protein levels have established *C. elegans* as a valuable organism to dissect different pathways involved in the pathology of disease.

In this review, both genetic and environmental causes of PD and their associated cellular malfunctions are described within the context of *C. elegans* biology (Table 1). As discussed below, these genetic and environmental factors share common pathways that lead to a selective loss of DA neurons. Most notably, α-syn has been studied extensively due to its central role in PD pathogenesis, and multiple disease models have been generated by means of expression of wild-type or mutant α-syn. Among them, *C. elegans* has been exploited to uncover genetic interactions among different worm orthologs of PD genes, discern genetic and environmental susceptibility factors, and discover putative neuroprotective genes against α-syn toxicity. Taken together, the use of this nematode has provided a novel set of therapeutic targets as well as mechanistic insights into PD pathogenesis. Identification of putative genetic susceptibility loci is imperative to determine diagnostic and therapeutic targets to ameliorate clinical symptoms and ultimately halt DA neurodegeneration.

## *C. elegans* MODELS OF α-SYN NEUROTOXICITY

With the completion of the *C. elegans* genomic sequence, many worm orthologs of human genes have been identified including those that are linked to familial PD. Currently, six worm orthologs have been identified (Table 2), with the most notable exception being α-syn. This feature has allowed *C. elegans* researchers to readily overexpress wild-type or mutant α-syn in the α-syn null genetic background without concerns for endogenous α-syn or a dominant negative effect. α-Syn, a main component of Lewy bodies, plays a central role in pathogenesis of both familial and idiopathic forms of PD. Mutations in α-syn, such as A30P and A53T, in addition to multiplication of the α-syn locus have been linked to increased α-syn aggregation, as well as DA neuronal death (Mezey et al., 1998; Conway et al., 2000; Singleton et al., 2003; Ross et al., 2008). In *C. elegans* models, both α-syn and GFP are selectively expressed in a subset of cells. For example, Lakso et al. (2003), Cao et al. (2005), and Karpinar et al. (2009) co-expressed α-syn and GFP in DA neurons and reported that overexpression of either wild-type or mutant α-syn under the control of *dat-1* (DA transporter) promoter resulted in the loss of DA neurons.

Initial hypotheses on PD causality suggested that α-syn aggregation may be toxic, and that formation of Lewy bodies may induce neuronal death by disrupting normal cellular functions. While detection of α-syn aggregation is challenging in worm DA neurons, Kuwahara et al. (2006) observed accumulation of α-syn in the cell bodies and dendrites of DA neurons overexpressing wild-type or mutant α-syn. They also reported that a small fraction of worm DA neurons exhibited a positive immunoreactivity to phosphorylated α-syn, which is suggestive of α-syn deposited in Lewy body-like inclusions. Although the formation of inclusion bodies is widely accepted as a PD pathological hallmark by possibly disrupting normal cellular functions, its role in neurotoxicity remains controversial. However, subsequent studies demonstrated that the inclusion bodies remain undetected in some forms of PD. Furthermore, multiple articles have reported that intermediate protofibrils of α-syn are more toxic than those found in either the monomeric or oligomerized state (Conway et al., 2001; Lashuel et al., 2002), providing an alternative hypothesis that the accumulation of mature protein aggregates may not be solely responsible for neurotoxicity. For example, because α-syn is primarily found in presynaptic nerve termini (Clayton and George, 1998; Recchia et al., 2004) with high propensity for lipid binding (Welch and Yuan, 2003), this natively unfolded protein has been proposed to associate with synaptic vesicle functions. Consistent with this view, Karpinar et al. (2009) elegantly characterized A56P and A76P α-syn variants, both part of β-sheet rich core of α-syn fibrils, which disrupted α-syn aggregate formation in vitro and in vivo. Remarkably, when α-syn variants were overexpressed in worm DA neurons, they observed more robust neurodegeneration than wild-type, A30P, and A53T α-syn, indicating that soluble oligomers, not insoluble aggregates, as toxic species.

To evaluate the effect of α-syn on neuronal activity, changes in worm behavior have been examined as a functional readout. For example, overexpression of both wild-type and A53T α-syn under the control of pan-neuronal promoter (*aex-3*) and motor neuron promoters (*acr-2* and *unc-30*) in *C. elegans* resulted in significant reduction of motor movement (Lasko et al., 2003). Moreover, overexpression of A30P and A53T α-syn in worm DA neurons resulted in modified food-sensing movement, a mechanosensory behavioral response that is specific to the DA neurons, illustrating the alteration of DA neuronal activity (Kuwahara et al., 2006). This behavior was further exacerbated by α-syn with A56P and A76P mutations (Karpinar et al., 2009). It is conceivable that, similar to observations in mammalian

**TABLE 1. Molecular, Genetic, and Chemical Manipulations Used in *C. elegans* Models of PD and Their Corresponding Phenotypes**

| Molecular manipulation | Expression pattern | Phenotype | Reference |
|---|---|---|---|
| $P_{aex-3}$::α-*syn WT* and *A53T* | Pan-neuronal | DA neurodegeneration; Reduced motor movement | Lakso et al., 2003 |
| $P_{aex-3}$::α-*syn A53T* | | Developmental defects in *pdr-1* (*lg103*) | Springer et al., 2005 |
| $P_{snb-1}$::α-*syn WT* | | Mitochondrial stress | Ved et al., 2005 |
| $P_{unc-119}$::α-*syn A53T* | | Mitochondrial stress | Ved et al., 2005 |
| $P_{unc-51}$::α-*syn WT, A30P*, and *A53T* | | Motor/developmental defects; endocytosis defects | Kuwahara et al., 2008 |
| $P_{dat-1}$::α-*syn WT* and *A53T* | DA neurons | DA neurodegeneration | Lakso et al., 2003 |
| $P_{dat-1}$::α-*syn WT* | | DA degeneration enhanced by $Mn^{++}$ | Settivari et al., 2009 |
| $P_{dat-1}$::α-*syn WT* | | DA neurodegeneration | Cao et al., 2005 |
| $P_{dat-1}$::α-*syn WT* | | α-syn accumulation in DA neurons; slightly reduced DA levels | Kuwahara et al., 2006 |
| $P_{dat-1}$::α-*syn A30P* and *A53T* | | α-syn accumulation in DA neurons; reduced DA levels; altered DA neuronal activity | Kuwahara et al., 2006 |
| $P_{dat-1}$::α-*syn A30P, A53T, A56P*, and *A76P* | | DA neurodegeneration; altered DA neuronal activity | Karpinar et al., 2009 |
| $P_{acr-2}$::α-*syn WT* and *A53T* | Motor neurons | Reduced motor movement | Lakso et al., 2003 |
| $P_{mec-7}$::α-*syn WT* and *A53T* | Touch-receptor neurons | Impaired touch response | Kuwahara et al., 2008 |
| $P_{unc-54}$::α-*syn::GFP* | Body wall muscles | α-syn misfolding and accumulation | Hamamichi et al., 2008 |
| $P_{unc-54}$::α-*syn::YFP* | | α-syn misfolding and accumulation | van Ham et al., 2008 |
| $P_{snb-1}$::*LRRK2 WT* | Pan-neuronal | Reduced mitochondrial stress | Saha et al., 2009 |
| $P_{snb-1}$::*LRRK2 R1441C* | | Mitochondrial stress | |
| $P_{snb-1}$::*LRRK2 G2019S* | | Mitochondrial stress; reduced DA levels; DA degeneration | |

| Mutant/RNAi analysis | | Phenotype | Reference |
|---|---|---|---|
| *catp-6* (RNAi) | | α-syn misfolding | Gitler et al., 2009 |
| *djr-1.1* (RNAi) | | Mitochondrial stress | Ved et al., 2005 |
| *lrk-1* (*km17*) and (RNAi) | | Mitochondrial stress | Saha et al., 2009 |
| *lrk-1* (*tm1898*) and (*km41*) | | ER stress sensitive | Sämann et al., 2009 |
| *pdr-1* (*lg103*) | | ER stress sensitive | Springer et al., 2005 |
| *pdr-1* (*XY1046, Parkin KO3*) and (RNAi) | | Mitochondrial stress; decreased lifespan | Ved et al., 2005 |
| *pink-1* (*tm1779*) | | Oxidative stress sensitive; neurite outgrowth defects; mitochondrial cristae defects | Sämann et al., 2009 |

| Chemical treatment | Genetic background | Phenotype | Reference |
|---|---|---|---|
| 6-hydroxydopamine (6-OHDA) | $P_{dat-1}$::*GFP* | DA neurodegeneration | Nass et al., 2002<br>Cao et al., 2005 |
| Manganese ($Mn^{++}$) | N2 | Oxidative stress; mitochondrial stress | Settivari et al., 2009 |
| | $P_{dat-1}$::*GFP* | DA neurodegeneration; reduced DA levels | |
| | $P_{dat-1}$::*GFP*; $P_{dat-1}$:: α-*syn* | Enhanced DA neurodegeneration | |
| MPTP and MPP+ | $P_{cat-2}$::*GFP* | DA neurodegeneration | Braungart et al., 2004 |
| | N2 | Mobility defects; lethality | |
| Paraquat | *pink-1* (*tm1779*) | Oxidative stress | Sämann et al., 2009 |
| Rotenone | *pdr-1* (*XY1046*); $P_{snb-1}$::α-*syn WT*; $P_{unc-119}$::α-*syn A53T* | Mitochondrial stress; reduced viability | Ved et al., 2005 |
| | N2; 262#*lrk-1* (*km17*); $P_{snb-1}$::*LRRK2 R1441C* and *G2019S* | Mitochondrial stress | Saha et al., 2009 |
| *Streptomyces venezuelae* secondary metabolite | $P_{dat-1}$::*GFP* | DA neurodegeneration | Caldwell et al., 2009 |

neuronal culture, intermediate protofibrils may exert toxicity by physically disrupting vesicular membranes (Volles et al., 2001) and causing defective sequestration of DA into synaptic vesicles (Lotharius and Brundin, 2002). In *C. elegans*, Kuwahara et al. (2006) reported A30P and A53T α-syn overexpression resulted in reduced DA levels, but oxidized DA was not examined. Intriguingly, Ved et al. (2005) showed that pan-neuronal (*unc-119*) overexpression of A53T α-syn, after rotenone treatment, increased thioflavine-positive α-syn aggregation and caspase activation through enhanced protein oxidation. Given the fact that DA is readily oxidized, the presence of cytosolic DA may increase oxidative stress, ultimately leading to DA neurodegeneration. Alternatively, as described by Karpinar et al. (2009), A56P and A76P α-syn variants with a reduced ability to form β-sheets exhibited higher neurotoxicity than wild-type, A30P, and A53T α-syn, proposing that the formation of rigid β-structure may not be as critical for PD pathogenesis as previously considered. Additional mechanistic insights are needed to clarify the neurodegenerative capabilities of the α-syn species.

**TABLE 2. Summary of PD Genes and Corresponding *C. elegans* Orthologs[a]**

| PD gene | PD protein | Inheritance | *C. elegans* ortholog | E value |
|---|---|---|---|---|
| *PARK1* | SNCA/α-syn | Autosomal dominant | n/a | n/a |
| *PARK2* | PRKN/parkin | Autosomal recessive | *pdr-1* | 3.4e-38 |
| *PARK5* | UCHL-1 | Autosomal dominant | *ubh-1* | 1.2e-33 |
| *PARK6* | PINK1 | Autosomal recessive | *pink-1* | 7.8e-53 |
| *PARK7* | DJ-1 | Autosomal recessive | *djr-1.1* | 1.6e-45 |
| | | | *djr-1.2* | 8.9e-36 |
| *PARK8* | LRRK2 | Autosomal dominant | *lrk-1* | 5.5e-66 |
| *PARK9* | ATP13A2 | Autosomal recessive | *catp-6* | 2.5e-180 |
| *PARK11* | GIGYF2 | Autosomal dominant | n/a | n/a |
| *PARK13* | HTRA2 | Autosomal dominant | n/a | n/a |

[a]n/a, not applicable.

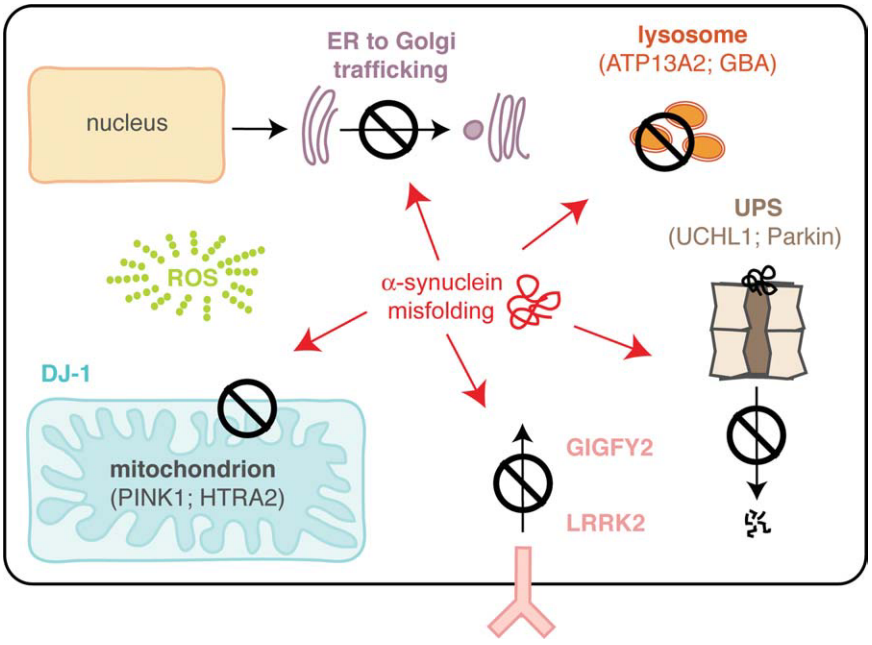


**Fig. 2.** The α-Syn misfolding leads to defective cellular mechanisms. Parkinson's disease (PD) -associated gene products have been shown to influence various aspects of cellular function and some of these gene products are directly affected by α-syn misfolding.

## CELLULAR MECHANISMS ASSSOCIATED WITH PD

### Protein Degradation Machinery

The accumulation and cellular stress induced by misfolded or aggregated proteins may underlie the neurodegenerative mechanisms leading to PD. Thus, intracellular mechanisms that mediate the clearance and degradation of proteins have been widely considered prospective targets for therapeutic intervention.

The primary protein degradation machinery of the cell is the ubiquitin-proteasome system (UPS; Fig. 2). This consists of a variety of proteins including the ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), ubiquitin ligases (E3), multiubiquitylation enzyme (E4), ubiquitin carboxyl-terminal hydrolases (UCHL), and proteasomal subunits. Briefly, E1, E2, E3, and E4 are involved in the processes of activating, transferring, and binding ubiquitin to target proteins that are subsequently degraded by proteasomes. After proteolysis, ubiquitins that are attached to the degraded products are recycled by UCHLs to maintain the cytoplasmic ubiquitin pool. Two PARK proteins, parkin (an E3 ubiquitin ligase) and UCHL-1 are UPS components.

In *C. elegans*, the availability of mutant strains has provided experimental platform for analyzing loss-of-function mutations and potential genetic and environmental interactions. Springer et al. (2005) demonstrated that PDR-1 physically interacted with worm E2 enzymes, UBC-2, UBC-18, and UBC-15, as well as the E4 enzyme, CHN-1, which is consistent with the mammalian findings whereby parkin interacted with Ubch7, Ubch8, and CHIP (Shimura et al., 2000; Zhang et al., 2000; Imai et al., 2002). They isolated one *pdr-1* mutant (*lg103*) that resulted in the expression of truncated PDR-1 and subsequent aggregation, similar to pathogenic *parkin* point mutations in PD patients. Using this mutant strain, they determined that the *pdr-1* mutation enhanced sensitivity to ER stress, and that PDR-1 is regulated by the unfolded protein response (UPR). Of interest, one of the substrates of parkin is a G

protein-coupled Pael receptor wherein accumulation of insoluble receptor leads to UPR-induced cell death in mammalian DA neurons (Imai et al., 2001; Wang et al., 2008). While a worm ortholog of the Pael receptor has not been identified, it is interesting to consider the same conserved pathway might be responsible for mutant parkin-induced toxicity. In a separate study, Ved et al. (2005) studied *pdr-1* knockout strain and observed a reduced level of basal ubiquitination, further establishing PDR-1 as an E3 ligase. They reported that *pdr-1* knockout enhanced vulnerability to mitochondrial complex I inhibitors including rotenone, fenperoximate, pyridaben, and stigmatellin, altering cellular respiration to generate more oxidative stress. Accordingly, in mammals, parkin has been shown to rescue DA neurons against DA or 6-hydroxydopamine (6-OHDA)-induced apoptosis (Jiang et al., 2004; Hasegawa et al., 2008).

Lysosomes are organelles that contain digestive enzymes including lipases, carbohydrases, nucleases, and proteases to break down organelles, macromolecules, and microorganisms. Most notably, recent findings indicate that the bulk of misfolded and aggregated proteins, including α-syn, are degraded by lysosomes by means of macroautophagy and chaperone-mediated autophagy (Webb et al., 2003; Cuervo et al., 2004; Fig. 2). Further supporting a potential role of lysosomal function in PD pathogenesis is a strong association between PD and type I Gaucher disease (Bembi et al., 2003; Sidransky et al., 2009). Type I Gaucher disease is an autosomal recessive lysosomal storage disorder that is caused by reduced activity of glucocerebrosidase (GBA), a lysosomal enzyme that catalyzes the breakdown of glucosylceramide. One PARK gene product, ATP13A2 is a lysosomal P-type ATPase. Ramirez et al. (2006) determined that while wild-type ATP13A2 protein is localized in the lysosomes, PD-associated mutant forms are misfolded and retained in the ER to be degraded by proteasomes. Surprisingly, they observed approximately a 10-fold increase in *ATP13A2* mRNA levels in the surviving DA neurons from the *substantia nigra* of human idiopathic PD postmortem midbrains, suggesting the potential neuroprotective function of this protein.

The importance of lysosomal trafficking and function in suppressing PD pathology has been shown in several studies involving *C. elegans*. Overexpression of α-syn::GFP in body wall muscle cells (under the control of the *unc-54* promoter) led to misfolding and accumulation of α-syn. Co-overexpression of TOR-2 (a worm ortholog of torsinA that is linked to another movement disorder called early-onset torsion dystonia), a protein with chaperone-like activity, ameliorated α-syn misfolding, and maintained the cellular threshold of α-syn at the diffused and soluble state. This genetic background (i.e., α-syn::GFP + TOR-2) has allowed rapid analysis of α-syn misfolding suppressors by scoring the return of α-syn aggregates following the RNAi treatment (Hamamichi et al., 2008). An enhancement in α-syn misfolding was observed when *catp-6*/*ATP13A2* was knocked down, while overexpression of *catp-6* under the control of *dat-1* promoter protected DA neurons against α-syn–induced neurodegeneration (Gitler et al., 2009). Importantly, this genetic interaction was also detected in mouse primary DA neuron culture, indicating the nematode data was predictive of relationships present within the mammalian system. Following the same procedure, another study showed while RNAi knockdown of *asp-4* (a worm ortholog of cathepsin D) enhanced α-syn misfolding, overexpresson of human cathepsin D rescued worm DA neurons from α-syn–induced toxicity (Qiao et al., 2008). Additionally, endogenous α-syn formed aggregates in the mouse cathepsin D knockout, further implicating the importance of protein clearance in preventing α-syn aggregation and neurodegeneration. Taken together, continued investigation of the role degradative processes play in PD is likely to reveal important insights into disease mechanism.

## Signal Transduction

LRRK2 is a signaling component, but precisely which pathways it alters is unclear (Fig. 2). LRRK2 encodes a large, 2527 amino-acid protein with leucine-rich repeat, Roc GTPase, COR, MAPKKK, and WD40 domains, illustrating its putative function in the MAPK signaling pathway. Given the high frequency of G2019S mutation (in the MAPKKK domain) in autosomal dominant (Di Fonzo et al., 2005) and idiopathic (Gilks et al., 2005) PD patients, West et al. (2005) characterized LRRK2 G2019S expressed in HEK293 and SH-SY5Y cells, and determined that the mutant form exhibited significantly higher kinase activity compared with wild-type LRRK2. West et al. (2007) also analyzed 10 PD-linked *LRRK2* mutations, and determined that LRRK2 GTPase activity regulates its kinase activity, and enhanced kinase activity leads to neurodegeneration. This kinase activity was observed with dimeric LRRK2, and disruption of the GTPase or kinase activity prevented dimer formation (Sen et al., 2009). PD-associated mutants which promote the kinase activity showed an increase in dimeric LRRK2, further implicating the importance of dimer formation and kinase activity in LRRK2 toxicity.

In *C. elegans*, overexpression of either wild-type or G2019S LRRK2 under the control of pan-neuronal promoter (*snb-1* promoter) enhanced DA neurodegeneration accompanied by reduction of DA (Saha et al., 2009). Additionally, LRRK2 G2019S, compared with wild-type LRRK2, resulted in enhanced vulnerability to rotenone. Focusing on mitochondrial malfunction, one common PD pathological mechanism, Saha et al. (2009) also reported that reduction of *lrk-1* by RNAi and mutant analysis increased toxicity in the nematodes to rotenone treatment. Conversely, overexpression of wild-type LRRK2 significantly increased resistance against rotenone and paraquat. These findings illustrate an initial step in elucidating how LRRK2 alters cellular vulnerability to various forms of stress inducers.

## Mitochondrial/Oxidative Stress

While cellular stress induced by misfolded or aggregated proteins may shed light on the neurodegenerative mechanisms leading to PD, defects in protein degradation machinery alone may not explain the selective loss of DA neurons. Another cellular defect associated with PD pathogenesis is insufficient response to

mitochondrial/oxidative stress (Fig. 2). Two PARK proteins, DJ-1 and PINK1, appear to combat cellular oxidative stress. For example, overexpression of wild-type DJ-1 significantly rescued DA neurons from hydrogen peroxide, DA, and MPP+ insults, demonstrating the anti-oxidant properties of DJ-1 (Junn et al., 2005). Compatible with the mammalian results, Ved et al. (2005) knocked down worm *djr-1.1* by RNAi, and observed increased vulnerability to rotenone similar to *pdr-1* knockout. Furthermore, d-β-hydroxybutyrate and tauroursodeoxycholic acid treatment reversed the susceptibility of the RNAi-treated worms to altered complex I inhibition.

Valente et al. (2004) demonstrated that, after proteasome inhibitor treatment, wild-type PINK1 enhanced mammalian DA neuron survival without modifying mitochondrial membrane potential whereas a PD-associated mutant displayed no protection with decreased potential. Moreover, Pridgeon et al. (2007) reported that PINK1 protects the mammalian cells from oxidative stress-induced cell death by suppressing cytochrome c release from mitochondria. Using available *C. elegans* mutants, Sämann et al. (2009) reported modified mitochondrial morphology, enhanced sensitivity to paraquat, and defective axon guidance in *pink-1* knockout strain, phenotypes which were rescued by expression of wild-type PINK-1. Of interest, similar to Saha et al. (2009) demonstrating a functional link between LRRK2 and mitochondria, the same rescue was also observed by *lrk-1* knockout mutation, suggesting an antagonistic role of these two kinases. While the genetic interaction between PINK1 and LRRK2 needs to be confirmed in the mammalian models, this study clearly demonstrates the advantages of model organisms for uncovering genetic interactions among different orthologs of PD genes.

## EVALUATING ENVIRONMENTAL CONTRIBUTORS TO PD

With only ~10% of the population of PD patients comprising known genetic mutations, it has been proposed that environmental factors largely contribute to the prevalence of this disease. The use of environmental toxins has been widely applied in a variety of model organisms to induce parkinsonian symptoms. MPTP, which is metabolized to a neurotoxin, 1-methyl-4-phenylpyridinium (MPP+), has been shown to specifically induce DA cell death in both mammalian and invertebrate models of PD (Langston et al., 1983; Braungart et al., 2004). MPTP is a highly lipophilic molecule which easily passes through the blood–brain barrier and cell membranes. DA neurons uptake this molecule through the vesicular DA transporter, where MPP+ inactivates the mitochondrial complex I of the respiratory chain and induces cell death. The DA analogue, 6-OHDA, is another commonly used toxin to specifically induce monoaminergic neuron cell death, because this molecule requires the DA or noradrenergic transporter for uptake.

With many cases of PD being of sporadic nature, *C. elegans* is an excellent organism to examine environmental toxins that may induce the pathological features of PD, including DA neuron toxicity. Nass et al. (2002) showed that exposing *C. elegans* to the neurotoxin 6-OHDA induces specific degeneration of the DA neurons, which is both time- and dose-dependent. 6-OHDA is specifically taken up by monoaminergic neurons and causes free radical formation and oxidative stress, which in turn leads to cell death. Using this 6-ODHA model, several groups have identified genes and drugs that alter the sensitivity of DA neurons to 6-OHDA-induced toxicity (Cao et al., 2005; Nass et al., 2005). One group used forward genetics to identify the DA transporter (*dat-1*) as being a required component for this toxicity (Nass et al., 2005), indicating that 6-OHDA is taken up through the *C. elegans* DA transporter to induce toxicity in vivo. Cao et al. (2005) used this model to identify a human torsinA-related protein, TOR-2, that showed protection against several cellular insults shown to induce degeneration of the DA neurons, including exposure to 6-OHDA, excess DA synthesis, and transgenic overexpression of human α-syn in DA neurons. Maranova and Nichols (2007) extended application of this model to identify specific DA, GABA, and NMDA receptor agonists as neuroprotective in a dose-dependent manner in *C. elegans*. Other toxins that have been widely exploited to induce Parkinson-like symptoms in mammalian models have also been reported to induce DA degeneration in worms, including rotenone and MPTP. Exposure of worms to the neurotoxin MPTP was shown to increase lethality and reduce mobility (Braungart et al., 2004), and treatment of these worms with pharmacological active substrates used in PD therapy reduced this toxicity, further proving this model system to be beneficial in studying PD.

Among the few known risk factors for PD is an association with a rural lifestyle (Priyadarshi et al., 2001). Although the underlying cause for this relationship is unknown, the prevalence of PD seen within the rural population has led to the discovery that several environmental toxins commonly used in farming communities can induce PD symptoms. The organic pesticide rotenone and the herbicide paraquat are two such chemicals that have been studied in both mammalian and invertebrate models and found to cause DA neurotoxicity by inhibiting the mitochondrial complex, similar to MPP+ and 6-OHDA. The aforementioned report from Ved et al. (2005) showed that exposing nematodes to rotenone enhanced the sensitivity of mitochondrial dysfunction in several models of PD including pan-neuronal α-syn expression and mutants in *parkin* and *djr-1.1*. Furthermore, this study identified several compounds, including an antioxidant (probucol), a mitochondrial complex II activator (d-β-hydroxybutyrate), and an anti-apoptotic bile acid (tauroursodeoxycholic acid), that protected against mitochondrial dysfunction in this model. The use of all of these environmental toxins has proven to be an effective method to induce DA degeneration in *C. elegans*, and these models enable screening for chemical and genetic modifiers of cellular toxicity.

Recently, several other environmental factors have been identified which promote dopaminergic cell death in *C. elegans*. Caldwell et al. (2009) identified a highly stable and

lipophilic secondary metabolite produced by a species of common soil bacteria, *Streptomyces venezuelae*. This neurotoxicity, when compared with other neuronal subtypes, was enhanced within the DA neurons. Additionally, the presence of DA exacerbated DA neuron toxicity because neurodegeneration was attenuated in *cat-2* mutants, which were depleted for tyrosine hydroxylase, the rate-limiting enzyme in DA production. Upon further analysis, the secondary metabolite was shown to inhibit the degradation of proteins through the UPS, similar to that observed with the proteasome inhibitor MG-132. The neurotoxic bacterial excretion was further confirmed as being detrimental to mammalian DA neurons as well, suggesting an evolutionarily conserved mechanism involving protein degradation is disrupted by this metabolite. As another possible environmental contributor to PD, Settivari et al. (2009) developed a model in *C. elegans* to study manganese ($Mn^{++}$) toxicity with respect to DA neurodegeneration. Worms briefly exposed to $Mn^{++}$ showed increases in reactive oxygen species, altered mitochondrial membrane potential, and DA neurodegeneration; moreover, the $Mn^{++}$ toxicity was dependent on the divalent metal transporters, *smf*-1/2. Furthermore, $Mn^{++}$ exposure enhanced degeneration of DA neurons expressing human α-syn, consistent with mammalian observations, and indicating the importance of environmental exposure to heavy metals in exacerbating the pathology seen with α-syn in the progression of PD. Taken together, *C. elegans* offers an elegant system to study environmental triggers that may play a pivotal role in the progression of PD and serves to accelerate the identification of molecular pathways involved in the toxicity of environmental factors.

## LARGE SCALE ANALYSIS FOR MODIFIERS OF α-SYN TOXICITY

Because formation of Lewy bodies is a central pathological feature of both familial and idiopathic forms of PD, much current research on PD focuses on α-syn toxicity (protein aggregation, neuronal defects, and DA neurodegeneration) and cellular mechanisms involved in ameliorating it. Presently, the modification of at least four cellular mechanisms has been linked to the enhanced α-syn toxicity including ER to Golgi trafficking, proteasomal and lysosomal protein degradation, signaling pathways, and mitochondrial function (Fig. 2). For example, overexpression of α-syn blocks ER to Golgi trafficking (Cooper et al., 2006; Gitler et al., 2008), and overexpression of mutant α-syn induces ER stress (Smith et al., 2005). α-Syn is degraded by the UPS (Stefanis et al., 2001; Zhang et al., 2008; Nonaka and Hasegawa, 2009) and lysosomes by means of macroautophagy or chaperone-mediated autophagy (Webb et al., 2003; Cuervo et al., 2004), and conversely, treatment with proteasome or lysosome inhibitors enhance its aggregation (Sawada et al., 2004; Lee et al., 2004). α-Syn has also been shown to regulate MAPK signaling and accelerate cell death by reducing the amount of active MAPK (Iwata et al., 2001). Additionally, α-syn may be targeted to mitochondria and impair complex I function by means of cryptic mitochondrial targeting signal (Devi et al., 2008). Functional analyses of the remaining eight PARK proteins highlight involvement of these same cellular mechanisms. As discussed below, large-scale screening for PD susceptibility factors using *C. elegans* has both confirmed and revealed additional pathways altered in PD pathogenesis.

Several groups have developed and used models for α-syn misfolding to screen for genetic modifiers of this process in *C. elegans*. Hamamichi et al. (2008) fused the GFP to wild type human α-syn and expressed the transgene in the body wall muscles of *C. elegans*, where significant accumulation of cytosolic α-syn::GFP puncta was observed over the course of development and aging. Upon co-expression of TOR-2, which exhibits chaperone-like activity, α-syn::GFP was found diffused and soluble throughout the cytoplasm. Through a reverse genetic strategy applying RNAi to knockdown gene expression, Hamamichi et al. (2008) undertook a hypothesis-based approach to identify putative genetic modifiers of α-syn misfolding. This hypothesis-based approach entailed bioinformatic prioritization and subsequent screening of ~900 target genes involved in pathways related to PD, such as protein folding, degradation, and trafficking, or co-expressed with known *C. elegans* PARK gene orthologs. Through an initial screen, 20 genes were identified as having the greatest effect on increasing α-syn misfolding when knocked down. Among these candidate genes were included several PD related genes, such as worm homologues of *DJ-1* and *PINK1*, along with another gene, *ULK2*, that was previously reported as one of only six genes identified in a genome-wide polymorphism screen for candidate SNPs in PD patients (Fung et al., 2006). To further analyze the effect of these candidate genes in an assay with more direct implications for PD, several candidates from the initial RNAi screen for effectors of α-syn misfolding were co-expressed in DA neurons along with human α-syn, and evaluated for their impact on DA neurodegeneration. Strikingly, overexpression of five of seven genes tested protected worm DA neurons against α-syn–induced neurodegeneration. Two of the top neuroprotective candidates, *atg-7* and *vps-41*, have been further validated in mammalian models, in that knockout of *Atg7* (E1 ubiquitin activating enzyme) in mice results in neuronal degeneration (Komatsu et al., 2006), and overexpression of human VPS41 (lysosomal trafficking protein) in mammalian cell culture protects cells against several PD related neurotoxins, including 6-OHDA and rotenone (Ruan et al., 2009). Both of these proteins have been shown to be involved in the autophagy pathway for protein clearance in which cargo is delivered to the lysosome for degradation. Thus, the outcomes of this study exemplify the value of using *C. elegans* to study pathologies characteristic of PD, both α-syn misfolding and DA neurodegeneration, and include the identification of several genes that may represent novel therapeutic targets to combat the progressive degeneration associated with PD.

Another group preformed an extensive RNAi screen to identify processes involved in age-dependent α-syn

inclusion formation. For this screen, van Ham et al. (2008) expressed human wild-type α-syn fused to the yellow fluorescent protein (YFP) in the body wall muscles of *C. elegans*. Upon expression of the fusion protein, but not YFP alone, cytosolic inclusions were observed in young animals, and these inclusions increased in an age-dependent manner. Because one of the pathological hallmarks of PD is accumulation of electron-dense protein material with inclusion formation, fluorescence recovery after photobleaching (FRAP) was used to monitor the mobility of the inclusion contents, and led to the identification of two types of inclusions, one with mostly mobile material and one with immobilized material. The inclusions with immobilized material resemble inclusions seen with aggregated proteins, and these inclusions were not observed till late adulthood, similar to what is observed in human PD cases. Using this system, van Ham and coworkers conducted a genome-wide RNAi screen to identify genetic factors that, when knocked down, increased the number of cytoplasmic α-syn inclusions. They identified 80 suppressors of inclusion formation, with many having human orthologues, and further verified some of these hits by examining *C. elegans* mutant strains corresponding to three of their positive candidates, in which they observed a similar increase of inclusions. Of the 80 suppressors isolated, several were predicted to be involved in similar pathways that have been implicated in having roles in PD, including vesicular transport, lipid metabolism, and aging. Of interest, one of the suppressors identified, *sir-2.1*, has also been shown by others to play a significant role in aging by regulating ER stress genes (Tissenbaum and Guarente, 2001; Viswanathan et al., 2005). Furthermore, the effect of knocking down another known modifier of α-syn toxicity, the G-protein coupled receptor kinase, Grpk2, confirmed data from *Drosophila* studies, wherein an increase in neuronal toxicity was seen when Grpk2 was overexpressed, possibly through Grpk2 phosphorylating α-syn. Accordingly, RNAi of the *C. elegans* orthologues of Grpk2, *grk-1* or *grk-2*, reduced the accumulation of cytoplasmic α-syn inclusions, a result that was further validated by using worm strains mutant in these two genes.

Kuwahara et al. (2008) conducted a similar RNAi screen with the readout for α-syn-neurotoxicity being behavioral defects. This screen involved overexpressing either WT or mutant human α-syn under a pan-neuronal promoter, and a systematic analysis of ~1,700 genes involved in the nervous system or synaptic function was performed for evidence of altered behavior dependent on α-syn expression. Because neurons in *C. elegans* are not highly responsive to RNAi treatment, this screen was conducted in an *eri-1* mutant background, a neuronal RNAi supersensitive mutant, to enhance the genetic knockdown of target genes within neurons. Through this screen, 11 genes were categorized as altering the distinct behavioral phenotypes, including uncoordinated movement (Unc) and growth retardation (Gro), in pan-neuronal expressed α-syn nematodes. Of these 11 candidates, four are reported as being components of the endocytic machinery. Of interest, one of these genes, *apa-2*, was also identified as a positive candidate that enhanced α-syn misfolding when knocked down by RNAi in the Hamamichi et al. (2008) study. Kuwahara et al. further validated their findings of the involvement of the endocytic pathway with α-syn toxicity by testing mutants within the AP-2 complex, involved in endocytosis, and found enhanced behavioral defects only in mutants expressing α-syn, thereby indicating the endocytic pathway is affected by α-syn. Using pharmacological assays that have been well established in *C. elegans* to study acetylcholine (Ach) neurotransmitter release at the neuromuscular junction, it was further shown that α-syn decreased presynaptic neurotransmitter release of Ach, similar to endocytosis-defective mutants. Furthermore, immunohistochemical analysis revealed an increase in phosphorylated α-syn within the cell bodies of neurons upon RNAi knock-down of *apa-2*, resembling the accumulation of phosphorylated α-syn indicative of synucleinopathies. Collectively, these large-scale functional genomic analyses epitomize the substantial value of using *C. elegans* to identify novel proteins and pathways involved in disease mechanisms, specifically in the context of the dysfunction caused by α-syn accumulation and its impact on the pathogenesis of PD.

## PERSPECTIVES

In this review, we have provided an overview of the use of the model organism *C. elegans* to identify putative pathways involved in the cellular pathology seen within PD patients. Indeed, *C. elegans* provides immense opportunity for conducting chemical and genetic screens to identify factors that may be influencing certain pathways putatively implicated in a variety of diseases (Kamath and Ahringer, 2003). The aforementioned examples of large-scale RNAi screens, genetic mutant, and transgenic nematode analyses to identify and characterize genetic factors that influence several main pathological features of PD (Hamamichi et al., 2008; Kuwahara et al., 2008; van Ham et al., 2008) represent prime examples of the utility of the nematode system for advancing PD research. Through these screens, several proteins involved in lysosomal trafficking, autophagy, mitochondrial function, and aging have been identified to protect against α-syn accumulation and toxicity. While α-syn regulation and degradation may play a pivotal role in the pathogenesis of PD, alterations of the mitochondria have also been shown to play a significant role in the progression of this disease. Therefore, the application of *C. elegans* model toward identifying potential therapeutics that restore a homeostatic function to the mitochondria could be used to prevent the pathology seen with either environmental or genetic contributors to PD. In this regard, Ved et al. (2005) used both genetic factors and PD-related mitochondrial toxins to identify several chemical compounds that protected *C. elegans* against mitochondrial toxicity induced by these factors. Likewise, Marvanova and Nichols (2007) used the *C. elegans* system to look at the effect of neurotransmitter agonists and antagonists against 6-OHDA-induced DA neurotoxicity, while Nass et al. (2005) screened for genetic

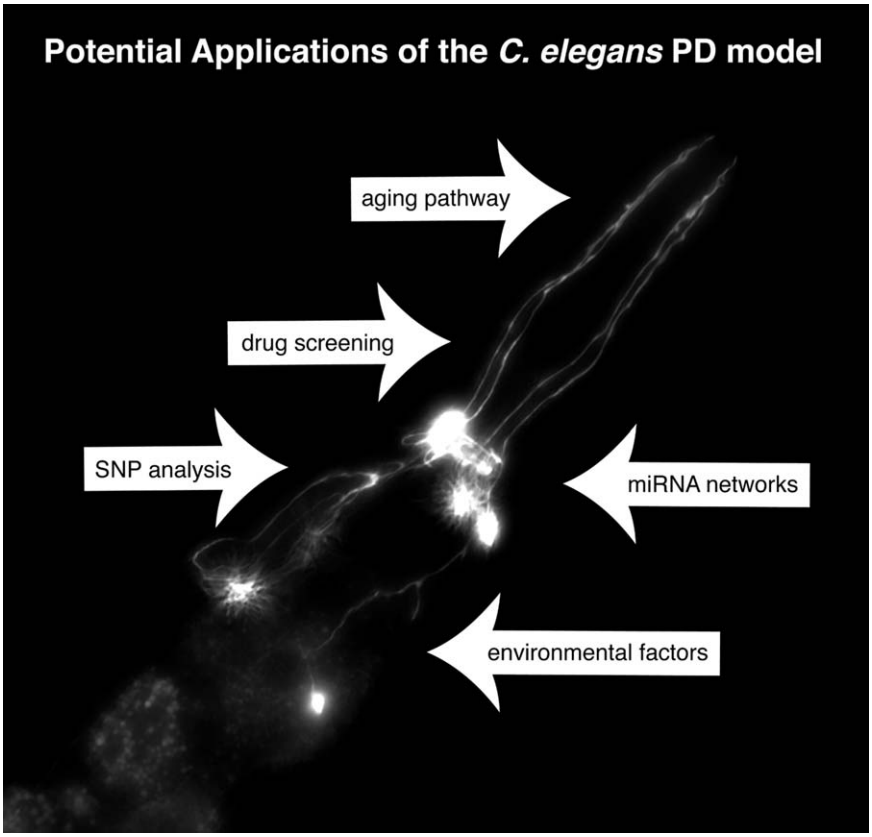


**Fig. 3.** There are numerous applications of *C. elegans* for Parkinson's disease (PD) research, including these potential directions using the dopaminergic system.

factors involved in 6-OHDA-induced neuronal toxicity and death.

As PD research advances, a new and emerging field of biology is the identification of micro RNAs (miRNA), small 21- to 23-bp RNA fragments that function within cells to modify gene expression (Fig. 3). These miRNAs are originally transcribed as ~1,000-bp fragments which are processed by the RISC complex to form mature miRNAs (Moss and Poethig, 2002); these mature miRNAs have been shown in mammalian systems to regulate neuronal differentiation, neurite outgrowth, survival, and synaptic function. Of interest, Kim et al. (2007) reported a decrease in *mir-133b* expression within the midbrain of patients with PD, which regulates the maturation and function of DA neurons through altering the transcription factor Pitx-3. Furthermore, *mir-7* was recently identified to target the 3′ UTR of α-syn and suppress α-syn protein expression (Junn et al., 2009). These findings only scratch the surface regarding the prospective importance of miRNA regulation of gene expression in PD, and are indicative of an exciting field for future exploration. Significantly, the nematode offers valuable tools in which the regulation of genes by miRNAs can be examined, especially in respect to disease. With many genes identified through genetic screens in *C. elegans* to modify disease etiology and the availability of numerous bioinformatics databases that identify putative miRNA gene targets (miRBase, TargetScan, etc.), an interesting avenue to be pursued is the identification of miRNAs that may co-regulate several potential protective genes or molecular pathways in a coordinated manner. Another distinct advantage in studying the effect of miRNAs on the pathogenesis of disease in *C. elegans* is the rapidity with which creating transgenic animals overexpressing miRNAs or crosses with mutants of specific miRNAs may alter the toxicity seen with α-syn expression can be performed.

While both genetic and environmental factors have been identified as being causative agents in the onset and progression of PD, the most common and undisputed risk factor for PD is aging (Fig. 3). Currently, many advances in understanding the mechanisms of aging have come through work in *C. elegans* where several genes have been identified that significantly alter the lifespan of the worm. Of interest, van Ham et al. (2008) identified the age-associated gene, *sir-2.1*, in their genome-wide screen for effectors of α-syn accumulation. This gene acts as a histone deacetylase to regulate the expression of various genes, and it has been shown to function in the well-studied aging pathway involving the insulin-like receptor (*daf-2*) along with the downstream effecter of the insulin-signaling pathway, the FOXO transcription factor, *daf-16*. Mutations within the *daf-2* gene promote the activation of DAF-16, which is transported to the nucleus where it, along with the SIR-2.1 protein, initiates transcription of genes that regulate a variety of cellular pathways including cellular stress-response and metabolic genes (Murphy et al., 2003). However, the relationship between genes involved with the aging pathway and how they may be relevant to the progression of neurodegenerative disease has not been well defined and remains an open an avenue to be further exploited in *C. elegans*.

With the sequencing of the human genome being complete, there has been identification of many single nucleotide polymorphisms (SNPs) throughout the general population, which accounts for 0.1% of the diversity within humans. These SNPs are single base pair changes within the genome which may significantly alter the function of the protein, by altering transcription, splicing, or protein coding or may have an effect on gene expression or transcript stability by being located outside the coding region of a gene. While many SNPs do not necessarily have an effect on an encoded amino acid sequence for a protein, several SNPs have been identified to be either causative agents or susceptibility factors for disease. With many nonsynonymous SNPs identified in the population, the need to study the functional effect of these mutations on the normal activity of a given protein is an avenue to further

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be explored. Previous genome-wide screening with *C. elegans* to identify effector genes of α-syn toxicity, either enhancers or suppressors, have revealed many candidates that may play a role in the pathogenesis of PD. With many candidate genes identified, an interesting direction of research would be to determine if these genes have verified SNPs within human populations, and if these SNPs alter the function of the respective protein, by either enhancing or reducing the functionality in *C. elegans* PD models (Fig. 3). Identification of detrimental SNPs within genes, as well as screening PD patients for these polymorphisms, may represent genetic susceptibility factors for the occurrence of this disease.

While continued identification of genetic factors that may contribute to the progression of PD is critical, environmental factors have shown to play an important role in the etiology of this disease. In this regard, in addition to recapitulation of pre-existing mammalian toxin models that enhance the progressive loss of DA neurons, including MPP+ and 6-OHDA, *C. elegans* represents an indispensable tool for potential identification of toxins which induce neuronal degeneration. The recent report by Caldwell et al. (2009) describing a novel secondary metabolite produced by a species of common soil bacteria that can induce neurodegeneration within *C. elegans*, an effect that is enhanced in the presence of dopamine, represents one such application of the model. Therefore, continued use of *C. elegans* to identify environmental toxins and study mechanisms by which these toxins contribute to neuronal cell death may provide greater understanding of the etiology of this disease (Fig. 3).

Finally, as an intact microscopic animal, one of the greatest advantages of *C. elegans* is the ability to conduct large scale screening of chemical compounds which may alter disease state within a living organism (Fig. 3). Previous studies with nematodes have identified small molecules that prevent or slow the onset and progression of disease through enhancing or inhibiting the activity of proteins or molecular pathways. Braungart et al. (2004) developed a *C. elegans* MPP+ model of PD to be used for high-throughput drug screening and verified their model by treating these worms with pharmacological compounds that are currently being used in therapy for treatment of PD and have protection against MPP+ toxicity. Marvanova and Nichols (2007) conducted a small molecule screen to identify modifiers of 6-OHDA induced DA neurotoxicity and identified two D2 receptor agonists that protected against cell death. Furthermore, Cao et al. (2009) recently used a series of assays in *C. elegans* to identify an established drug which functions to enhance the molecular chaperone-like activity of human torsinA, a gene product that has previously been shown to protect against α-syn-induced DA neurodegeneration in vivo (Cao et al., 2005). Of course, *C. elegans* has limitations with respect to its capacity for accurately predicting aspects of drug discovery, such as effective concentrations, penetration and bioavailability; these constraints are largely due to the thick cuticle of the animal and limited routes of molecular entry. Nevertheless, the means by which *C. elegans* can be readily, and inexpensively, exploited for high-throughput screening, rapid evaluation of cognate targets, and simultaneous testing of chemical modifiers across multiple genetic and/or transgenic backgrounds all point to the value of using the nematode to identify small molecules, which may provide promising therapeutics for the treatment of neurodegenerative diseases.

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