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C. elegans as a model system to accelerate discovery for Parkinson disease

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The nematode *Caenorhabditis elegans* possesses a wealth of opportunities to explore mechanisms which regulate metazoan complexity, basic cellular biology, and neuronal system attributes. Together, these provide a basis for tenable understanding of neurodegenerative disorders such as Parkinson disease (PD) through functional genomic analysis and pharmacological manipulation for the discovery of previously unknown genetic and environmental risk factors. The application of *C. elegans* has proven prescient in terms of the elucidation of functional effectors of cellular mechanisms underlying PD that translate to mammals. The current state of PD research using *C. elegans* encompasses defining obscure combinatorial interactions between genes or between genes and the environment, and continues to provide opportunities for the discovery of new therapeutic targets and disease-modifying drugs.

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Introduction

Some 200 years after primary characterization by James Parkinson, his namesake disease remains the subject of widespread academic and medical interest. Classically, PD associates with progressive cell death of the selectively vulnerable dopaminergic neurons of the midbrain resulting in permanent impairment of motor control [1]. This neurodegeneration associates with seemingly disparate cellular dysfunctions including accumulation of reactive nucleophiles, alterations of dopamine chemistry, abnormal vesicular trafficking, and disrupted protein homeostasis [2,3]. Despite mechanistic advancements, the full etiology of this disorder remains elusive, due in part to deficiencies in our understanding of molecular and cellular sensitizing risk factors. Further, because PD associates with aging, and its concomitant societal burden, innovative strategies to address all aspects of potential PD pathology are essential. To address this urgent need, PD research in non-human models has attempted to replicate many determinants of PD pathology through which discovery of genetic or pharmacological interactions can be discerned in a tenable manner and accelerate the translational path to humans.

C. elegans as a cell biology and pharmacogenetics disease model

Pioneered as a model system by Sydney Brenner in the 1970s to explore the genetic basis for neuromuscular activity [4], *C. elegans* has proven an invaluable system to reveal, and at times revolutionize, the understanding of genomics, cell biology, cell death, epigenetics and aging [5,6]. Further, this 959-cell nematode, with a nervous system comprised of only 302 total neurons, possesses the most well characterized cell lineage and neuronal connectivity of any animal and currently provides a framework for study of the mammalian brain [7]. Notably, as it has a dopaminergic circuitry of just 8 anatomically-defined neurons, the capacity to exploit *C. elegans* for PD research allows for unparalleled accuracy in quantification of neurodegeneration influenced by both intrinsic and extrinsic stressors. Furthermore, the manner in which stressors integrate innate genetics with environmental exposure is of great interest, and *C. elegans* offers important insights and opportunities at this intersection in the context of PD.

Modeling genetic determinants of PD-like phenotypes in *C. elegans*

In humans, genetic risk factors for PD have been linked to genetic loci termed PARK for which at least 21 putative sites have been identified, with the prospect of additional risk loci also being identified [8]. Of these, only a few have been conclusively linked to familial, monogenic PD. These comprise the inherently disordered protein α-synuclein, the vesicular trafficking protein VPS35, the multidomain kinase LRRK2, and the mitochondrial stress response proteins PINK1, Parkin, and DJ-1. The *C. elegans* genome encodes genetic homologues to most of these risk factors (Table 1), with the notable exception of α-synuclein. However, since mutation or multiplication of the α-synuclein locus is a known cause of PD [9,10], it follows that transgenic overexpression of human α-synuclein has not at all hindered the utility of *C. elegans* to reveal critical mechanistic insights into PD. These

Table 1

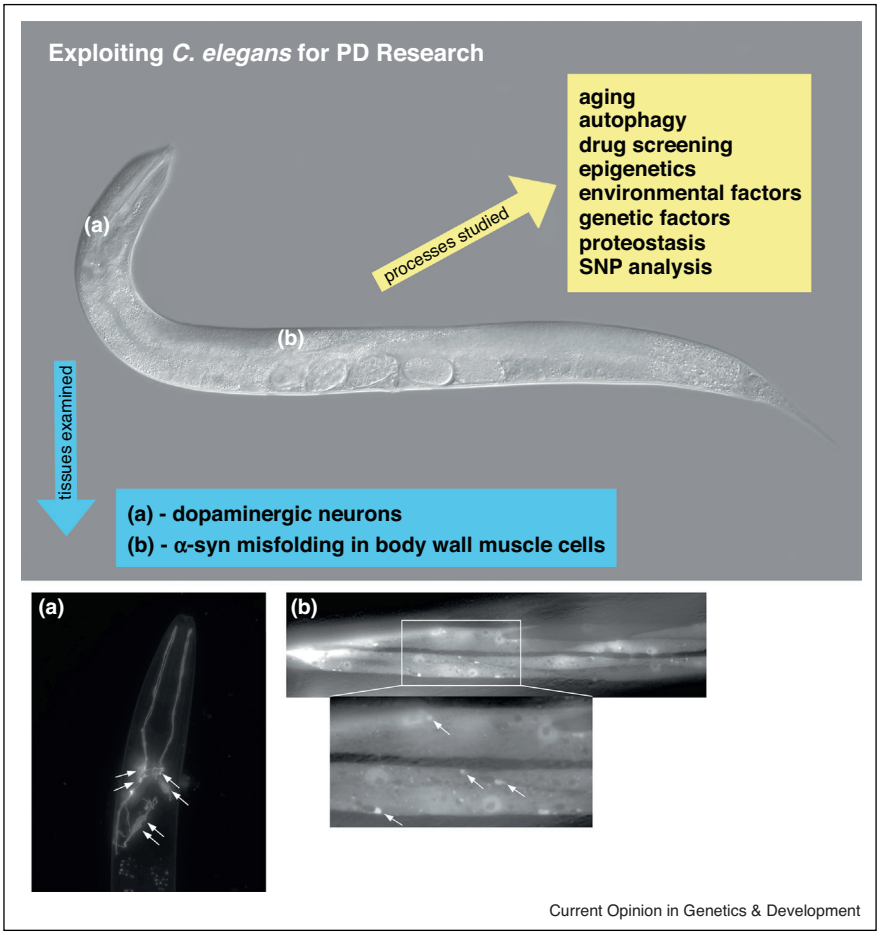
Summary of PARK genes and corresponding *C. elegans* orthologs

Locus	Human gene	Function	*C. elegans* gene	Homology (e value)
PARK1	SNCA (α-synuclein)	Unknown	–	–
PARK2	Parkin	E3 ligase; mitophagy; others	*pdr-1*	1.00E-34
PARK3	Unknown; possibly sepiapterin reductase (SPR)	Biopterin biosynthesis in DA metabolism	*dhs-21*	6.00E-09
PARK4	Regulatory elements of SCNA	Unknown	–	–
PARK5	UCHL1	Hydrolysis of C-terminal ubiquitinyl esters; aging and cellular senescence	*ubh-1*, *ubh-2*, and/or *ubh-3* (together in the CEOP5120 operon)	2.00E-29
PARK6	PINK1	Serine threonine kinase; mitophagy	*pink-1*	1.00E-55
PARK7	DJ-1	Deglycase; chaperone-like function during oxidative stress	*djr-1.1* and *djr-1.2*	2.00E-46
PARK8	LRRK2	Multidomain serine/threonine kinase; intracellular signaling but largely unknown	*lrk-1*	2.00E-45
PARK9	ATP32	Lysosomal-associated cation transporter and ATPase	*catp-6*	1.00E-118
PARK10	Unknown; likely not one gene, but multiple genetic susceptibility factors	–	–	–
PARK11	Unknown; debate centers around GIFYF2	PolyQ protein associated with receptor tyrosine kinases	Uncharacterized protein C18H9.3	3.00E-07
PARK12	Unknown; X-linked	–	–	–
PARK13	Potentially HtrA2	Mitochondrial serine protease	–	–
PARK14	PLA2G6	Calcium independent 2A phospholipase; phospholipid remodeling	Likely a member of the ipla family of proteins (*ipla-2*)	2.00E-67
PARK15	Possibly FBX07	F-box protein; phosphorylation dependent ubiquitination	–	–
PARK16	Unknown	–	–	–
PARK17	VPS35	Vesicular trafficking and retromer sorting	*vps-35*	<1E-120
PARK18	E1F4G1	Translation initiation factor	*ifg-1*	1.00E-23
PARK19	DNAJC6	Heat shock protein; molecular chaperone	*dnj-25*	7.00E-31
PARK20	SYNJ1	Phosphoinositide phosphatase; synaptic transmission and membrane trafficking	*unc-26*	<1E-120
PARK21	DNAJC13	Heat shock protein; molecular chaperone	*rme-8*	<1E-120

include the initial discovery of multiple conserved disease-modifying genes, as well as small molecules for which results uncovered using worms successfully translated to rodent models, as well as human genome-wide association studies and iPSCs derived from PD patients [11–14].

Several different types of α-synuclein overexpression experiments have been developed for both functional and descriptive characterization in various cellular compartments of *C. elegans* (Figure 1). For instance, expression of α-synuclein in the *C. elegans* dopaminergic neurons induces progressive, time-dependent neurodegeneration and motor defects [15]. In addition to neuronal expression, α-synuclein misfolding can be monitored within bodywall muscle cells as translational fusion GFP inclusions. These strains have been studied in large scale RNAi screens for enhancers of α-synuclein misfolding alone [16] as well as in the context of strong misfolding suppressors to contextualize proteostasis decline as surmounting a predefined threshold [11]. In many cases, α-synuclein aggregates and induces toxicity with age, a crucial component of PD in humans. This age-dependent toxicity is thought to arise due to generalized protein homeostasis remodeling and eventual decline during ageing [17]. Furthermore, modulation of the genetic basis of ageing, by framing α-synuclein toxicity within mutations in the worm insulin-like signaling pathway, has been shown to attenuate neurodegeneration and observable α-synuclein misfolding [12•,18•]. These screens have expanded understanding of α-synuclein misfolding and neurodegeneration, in part, as a consequence of impaired vesicular and endosomal trafficking, endoplasmic reticulum stress signaling, autophagy, and altered lipid homeostasis [11,12•,16]. For example, one of these screens uncovered a novel role for the protein VPS-41 in

Figure 1

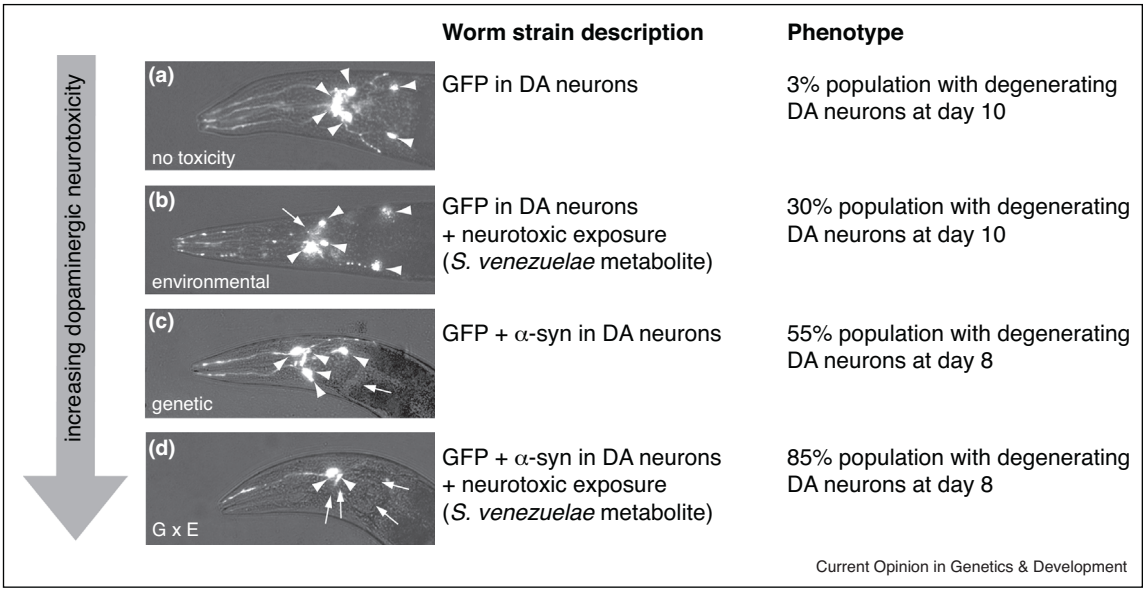


Application of *C. elegans* for research in PD-associated mechanisms. *C. elegans* is a microscopic, transparent nematode with a defined cell lineage and neuroanatomy. Bioassays involving visual inspection of either the six anterior dopaminergic neurons and/or the bodywall muscle (BWM) cells of this animal enables rapid quantifiable analysis of hallmark aspects of PD pathology such as neurodegeneration and protein misfolding. **(a)** Cephalic dopaminergic neurons (6, arrows) can be studied natively in combination with mutations, drugs and/or with transgenic overexpression of human PD-associated proteins such as α-synuclein (α-syn) or pathogenic LRRK2 variants. GFP transcriptional reporters provide a stable system to visualize alterations in neuronal integrity and neuronal cell count, both being indicative of an underlying neurodegenerative process. **(b)** The BWM comprise the largest and most easily visualized cells for the analysis of protein misfolding and aggregation. Expression of α-syn-GFP in the BWM facilitates analysis of proteotoxic foci that increase or decrease in number and size due to genetic or environmental factors. Numerous screens have been conducted in this cell type using RNAi to identify modulators of α-syn protein homeostasis. These two distinct models can both be evaluated for temporal phenotypic changes. For instance, animals with α-syn expression in dopaminergic neurons rarely show extensive degenerative phenotypes in the larval stages but accumulate degenerative phenotypes during adulthood.

regulating α-synuclein toxicity [19]. This protein associates with the HOPS complex, important for lysosomal fusion from the endosomal and trans-Golgi pathway. In some respects, this is related to the known function of the PD-related gene VPS35 (which has been recently identified as interacting with another risk-factor in the scaffolding protein EIF4G1 in yeast and *C. elegans* models [20]) that regulates the retrograde trafficking of vesicles back to the trans-Golgi network and implicates vesicular trafficking as a major regulator for attenuating neuronal toxicity.

C. elegans disease homologues interact with each other in a manner which may converge on a central inclusive pathway, including those homologues of PINK1 (*pink-1*), LRRK2 (*lrk-1*), Parkin (*pdr-1*), and DJ-1(*dnaj-1.1* and *dnaj-1.2*). For instance, loss-of-function to the gene *pink-1* sensitizes animals to paraquat sensitivity and affects neuronal outgrowth whereas loss-of-function to the LRRK2 homologue *lrk-1*, in addition to affecting axonal polarity, sensitizes animals to ER stressors. Combined, these mutants can mask detrimental phenotypes associated with each other and appear to act antagonistically

Figure 2



Gene-by-environment interactions potentiate *C. elegans* dopaminergic neurodegeneration. The cephalic dopaminergic neurons of *C. elegans* provide an investigative platform for rapid quantification of either environmental or genetic causative factors for degenerative phenotypes. The coordination of gene-by-environmental interactions is of interest, as the majority of cases of sporadic PD are idiopathic and could be influenced through undefined factors from both the environment and innate genetics that sensitize individuals to PD. **(a)** Normal *C. elegans* rarely show consistent and statistically significant degenerative phenotypes within dopaminergic neurons, even as animals reach old age (Day 10). Arrowheads indicate intact dopaminergic neurons. **(b)** Addition of environmental susceptibility factors (in this case a bacterial metabolite from the soil bacterium *S. venezuelae*) induces age-dependent accumulation of degenerative phenotypes (arrows). These can be visualized as either the loss of neuron or loss of membrane integrity through neuronal swelling. Similar phenotypes can be observed using other chemicals, such as the ROS-inducing drugs such as 6-OHDA, MPTP, rotenone and paraquat. **(c)** Genetic susceptibility factors (*e.g.*, the overexpression of human α-syn) can induce strong age-dependent neurodegeneration. **(d)** Coordination of both genetic and environmental exposures can be combined to evaluate varied states of neurotoxicity that can be either additive or synergistic depending on dosage or age of animals. In this respect, environmental exposures can be examined to uncover innate genetic susceptibility factors that otherwise would not produce toxic PD-like pathology if not for surpassing a preexisting threshold state.

[21]. Further, LRRK2 overexpression *per se* induces neurodegeneration phenotypes in *C. elegans* through overactive kinase activity [22]. Considering LRRK2 mutations in humans lead to dominant autosomal PD, there is a likely relationship between the over activity of LRRK2 signaling and loss-of-function PINK1 genetic pathways. In addition to this interaction, more attention is also being payed to coordination of PINK1 and Parkin signaling since the identification of both are crucial regulators of mitochondrial autophagy (mitophagy) [23], which is important for preventing accumulation of damaged mitochondria [24]. In *C. elegans* these mutations affect mitochondrial morphology, mitophagic potential, and mitochondrial accumulation [25••,26]. However, Parkin may have functions outside of interactions with PINK1. For instance, Parkin and DJ-1 homologues in worms also show genetic interactions in that loss-of-function states show similar levels of sensitivity to mitochondrial complex I stress [27]. How DJ-1 in *C. elegans* may contribute to the increasingly appreciated role of PINK1 and Parkin towards mitophagy still remains to be seen, although some evidence from cell culture may indicate a parallel role [28]. Collaborative studies conducted in both yeast and worms recently reported that phospholipid content in the mitochondrial membrane plays a role in modulating α-synuclein neurotoxicity and can be attenuated using select chemical modifiers [29•,30]. Moreover, as mutant α-synuclein interacts with the mitochondrial import protein, TOM20, the *C. elegans* system is well-poised to explore the functional consequences of these dynamics and their impact on stress response [31••].

How gene by environment factors influence PD-like pathologies in *C. elegans*

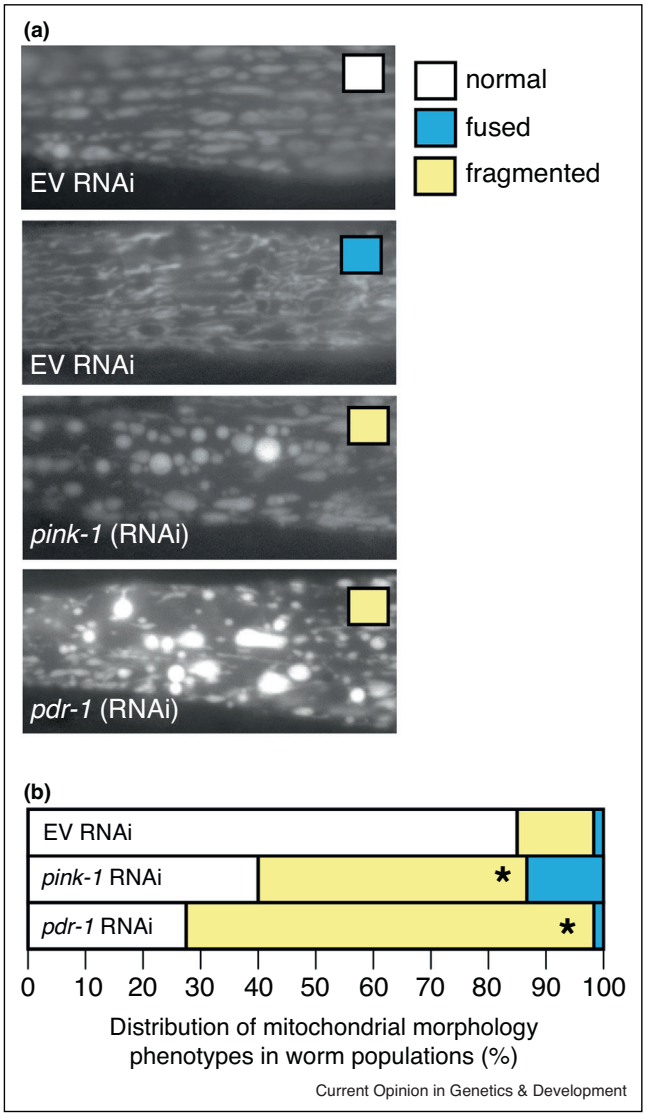
Familial forms of PD are very rare and idiopathic forms, where genetic determinants have not been linked primarily to the progression of PD, are more common but challenging to model. Because it is unlikely that all these idiopathic forms represent spontaneous mutations or an unknown genetic determinant, it stands to reason that they are caused by environmental determinants or combinatorial gene-by-environment (GxE) interactions (Figure 2) [32]. For example, exposure to naturally derived ROS-inducing biocides like rotenone or paraquat

have been correlatively linked to idiopathic PD [33]. Other environmental risk factors include transition metals and other natural sources that impact neurotoxicity [34,35]. *C. elegans* thereby provides a powerful system whereby potential environmental risk factors can be incorporated with genetics to observe and quantify functional effectors of PD-like phenotypes.

One widely used pharmacological model in *C. elegans* is 6-hydroxydopamine (6-OHDA) which is recognized by the dopamine reuptake transporter and induces selective dopaminergic neurodegeneration manifesting as a characteristic blebbing of processes, swelling of neuronal cell bodies and eventual disintegration in a non-apoptotic (or at least not canonically apoptotic) fashion [36]. Dopamine itself is also intrinsically reactive, as overexpression of its rate limiting biosynthetic enzyme tyrosine hydroxylase (TH) induces neurodegeneration but the mechanism of toxicity related to 6-OHDA and TH overexpression may differ [37]. Numerous factors have been identified which regulate 6-OHDA toxicity in *C. elegans*, including dopamine receptor modulation, autophagy inactivation, and ER chaperone function [38,39]. 6-OHDA also induces mitochondria disruption [40], potentially linking 6-OHDA toxicity to other mitochondrial electron transport chain disruptors and generalized ROS producers in the sensitization of *C. elegans* to cell death. Likewise, MPTP, rotenone, and paraquat have been used to model neurodegeneration for high throughput drug screens to identify modulators of cellular death [41,42]. Interestingly, we have discovered that bacterial metabolites can stress mitochondria quality control, induce ROS accumulation and elicit neurodegeneration [43,44].

The coordination of external stressors in GxE interactions is increasingly being appreciated. For instance, toxicity in *C. elegans* models of manganese toxicity is altered in the context of mitochondrial associated PD mutant homologues which sensitize animals in numerous ways, including lifespan reduction and dopaminergic signaling [45–47]. Interestingly, α-synuclein may act in ways that are not strictly toxic as defects and oxidative stress observed during manganese treatment can be attenuated by α-synuclein [46]. GxE interactions may proceed in part through alterations of innate redox homeostasis. For instance, we have reported that a toxic bacterial metabolite can potentiate α-synuclein stress by depressing glutathione homeostasis, resulting in increased *pink*-1-dependent autophagy [48]. Others have found that stressing the glutathione pathway post-translational modification system also interacts with known genetic modifiers such as LRRK-2 overexpression and TH overexpression [49]. In addition to environmentally-derived stress, some newer studies have focused on potential amelioration of neurodegenerative phenotypes (elicited primarily by α-synuclein) by naturally derived substances. For instance, fractionation extracts from a high flavonoid tropical fruit have shown putative neuroprotective molecules and lifespan modulation [50] as well as *n*-butylidenephthalide derived from the East-Asian herb *Angelica sinensis* [51]. Thus, both naturally-derived toxins and protective factors may interact with innate genetic predispositions to alter disease course.

Figure 3



Alterations in *C. elegans* mitochondrial morphology in PD mutant backgrounds. Phenotypic alterations in mitochondrial morphology are an indication of mitochondrial turnover. **(a)** Mitochondrial fragmentation in animals expressing a mitochondrial-targeted GFP localized in body wall muscle cells following empty vector (EV) or *pink-1* or parkin (*pdr-1*) RNAi knockdown by bacterial feeding. Mitochondrial morphology is defined as normal (tubular—white box), fused (elongated—blue box), or fragmented (circular and irregular—yellow). **(b)** Quantitation of mitochondrial morphology phenotypes in *C. elegans* populations. The distribution of fragmented mitochondria is different between all samples. In *pink-1* and *parkin* RNAi populations, increased fragmentation is indicative of damaged mitochondria that cannot be turned over by mitophagy.

Future directions and conclusions

Energy utilization is an important aspect of cellular health and function that changes as organisms age. Insights from both PD genetics and environmental studies have linked ageing to the progression of PD and the decline of protein homeostasis including mitochondrial quality control [17,18•]. Failures of this pathway modulate PD-like pathologies at the genetic, environmental, and GxE levels. One such pathway, mitophagy is being investigated as critical for cellular health and organismal longevity. At the molecular level, mitophagy entails recruitment of the generic autophagy machinery that is adapted for engulfment of a large organelle [23,24,25••]. How mitochondrial-specific targeting for autophagy works is only partially characterized, and largely limited to study of PINK1 and Parkin. Furthermore, the mechanisms required for autophagic destruction of mitochondria are even less characterized. This type of investigative problem is well suited for study in *C. elegans* through both forward and reverse screens in conjunction with reporter strains to measure and observe mitophagic clearance (Figure 3). For instance, colocalization of the *lgg-1* autophagy gene (homolog of human LC3) with mitochondrial GFP signals can roughly approximate autophagy in *C. elegans* [25••]. However, tools for visualizing mitophagy remain limited. Thus, future studies of mitophagy and mitochondrial quality control should expand on the collection of useful reporter strains as well as increase genetic understanding of GxE interactions through a mixture of pharmacology, screening, and targeted genetic investigation.

Because energy utilization is, in many respects, influenced by mitochondrial function it is likely that mitochondrial function, ageing, and energy utilization form a major axis of disease progression. This is especially true of energetically demanding compartments such as neurons where glycolysis is inestimable to neuronal function during stress events [52•] and where alteration of glycolytic potential through changes to insulin signaling affect worm neuronal health [12•,18•] as a function of age. Indeed, insights into these axes in *C. elegans* have found that in addition to genetic, pharmacological extension of ageing through application of anti-ageing drugs slow the progression of toxic phenotypes by restoring and maintaining lysosomal potential indicating a pivotal role for protein clearance in preventing disease progression [53]. However, other forms of organismal life extension may not correlate with increase in quality of life in what is termed healthspan [54••] where, despite increase in lifespan, organisms progressively accumulate old-age phenotypes. In this regard, *C. elegans* is well-positioned to yield additional insights that advance our understanding and hasten the therapeutic trajectory for PD.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Fujita KA, Ostaszewski M, Matsuoka Y, Ghosh S, Glaab E, Trefois C, Crespo I, Perumal TM, Jurkowski W, Antony PMA *et al.*: **Integrating pathways of Parkinson's disease in a molecular interaction map**. *Mol Neurobiol* 2014, **49**:88-102.

2. Cassarino DS, Fall CP, Swerdlow RH, Smith TS, Halvorsen EM, Miller SW, Parks JP, Parker WD, Bennett JP: **Elevated reactive oxygen species and antioxidant enzyme activities in animal and cellular models of Parkinson's disease**. *BBA-Mol Basis Dis* 1997, **1362**:77-86.

3. Lotharius J, Brudin P: **Pathogenesis of Parkinson's disease: dopamine, vesicles and α-synuclein**. *Nat Rev Neurosci* 2002, **12**:931-942.

4. Brenner S: **The genetics of *Caenorhabditis elegans***. *Genetics* 1974, **77**:71-94.

5. Ellis HM, Horvitz HR: **Genetic control of programmed cell death in the nematode *C elegans***. *Cell* 1986, **44**:817-829.

6. Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G: **daf-2, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans***. *Science* 1997, **227**:942-946.

7. Varshney LR, Chen BL, Paniagua E, Hall DH, Chklovskii DB: **Structural properties of the *Caenorhabditis elegans* neuronal network**. *PLoS Comput Biol* 2011, **7**:e1001066 http://dx.doi.org/10.1371/journal.pcbi.1001066.

8. Nalls MA, Pankratz N, Lill CM, Do CB, Hernandez DG, Saad M, DeStefano AL, Kara E, Bras J, Sharma M *et al.*: **Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease**. *Nat Genet* 2014, **46**:989-993.

9. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R *et al.*: **α-Synuclein locus triplication causes Parkinson's disease**. *Science* 2003, **302**:841.

10. Spira PJ, Sharpe DM, Halliday G, Cavanagh J, Nicholson GA: **Clinical and pathological features of a Parkinson syndrome in a family with the Ala53Thr alpha-synuclein mutation**. *Ann Neurol* 2001, **49**:313-319.

11. Hammamichi S, Rivas RN, Knight AL, Cao S, Caldwell KA, Caldwell GA: **Hypothesis-based RNAi screening identifies neuroprotective genes in a Parkinson's disease model**. *Proc Natl Acad Sci U S A* 2008, **105**:728-733.

12. • Knight AL, Yan X, Hamamichi S, Ajjuri RR, Mazzulli JR, Zhang MW, Daigle JG, Zhang S, Borom AR, Roberts LR *et al.*: **The glycolytic enzyme, GPI, is a functionally conserved modifier of dopaminergic neurodegeneration in Parkinson's models**. *Cell Metab* 2014, **20**:145-157.
This paper is one of the first to link energy metabolism and glycolytic potential, as a function of insulin signaling perturbation as a mechanistic determinant of cellular healthspan in models of dopaminergic neurodegeneration. The results of this are further validated by Ref. [52•] which demonstrate the role of glycolytic enzyme recruitment to high energy consuming neuronal compartments during stress.

13. Tardiff DF, Jui NT, Khurana V, Tambe MA, Thomspon ML, Chung CY, Kamadurai HB, Kim HT, Lancaster AK, Caldwell KA *et al.*: **Yeast reveal a druggable Rsp5/Nedd4 network that ameliorates α-synuclein toxicity in neurons**. *Science* 2013, **342**:979-983.

14. Chung CY, Khurana V, Auluck PK, Tardiff DF, Mazzulli JR, Soldner F, Baru V, Lou Y, Freyzon Y, Cho S *et al.*: **Identification and rescue of α-synuclein toxicity in Parkinson patient-derived neurons**. *Science* 2013, **342**:983-987.

15. Lasko M, Vartianen S, Moilanen AM, Sirvio J, Thomas JH, Nass R, Blakely RD, Wong G: **Dopaminergic neuronal loss and motor deficits in *Caenorhabditis elegans* overexpressing human alpha-synuclein**. *J Neurosci* 2003, **86**:165-172.

16. van Ham TJ, Thijssen KL, Breitling R, Hofstra RMW, Plasterk RHA, Nollen EAA: ***C. elegans* model identifies genetic modifiers of α-synuclein inclusion formation during ageing**. *PLoS Genet* 2008, **4**:e1000027 http://dx.doi.org/10.1371/journal.pgen.1000027.

17. Walther DM, Kasturi P, Zheng M, Pinkert S, Vecchi G, Ciryam P, Morimoto RI, Dobson CM, Vendruscolo M, Mann M, Hartl FU: **Widespread proteome remodeling and aggregation in aging *C. elegans***. *Cell* 2015, **161**:919-932.

18. • Visscher M, De Henau S, Wildschut MHE, van Es RM, Dhondt I, Michels H, Kemmeren P, Nollen EA, Braeckman BP, Burgering BMT, Vos HR, Dansen TB: **Proteome-wide changes in protein turnover rates in *C. elegans* models of longevity and age related disease**. *Cell Rep* 2016, **16**:3041-3051.

This article provides information on protein turnover in long-lived daf-2 mutants and α-synuclein expressing animals using mass spectrometry in both young and older animals. Protein turnover is enhanced in daf-2 animals while it is reduced in α-synuclein expressing animals.

19. Harrington AJ, Yacoubian TA, Slone SR, Caldwell KA, Caldwell GA: **Functional analysis of VPS41-mediated neuroprotection in *Caenorhabditis elegans* and mammalian models of Parkinson's disease**. *J Neurosci* 2012, **32**:2142-2153.

20. Dhungel N, Eleuteri S, Li L, Kramer NJ, Chartron JW, Spencer B, Kosberg K, Fields JA, Stafa K, Adame A *et al.*: **Parkinson's disease genes VPS35 and EIF4G1 interact genetically and converge on α-synuclein**. *Neuron* 2015, **85**:76-87.

21. Samann J, Hegermann J, von Gromoff E, Elmer S, Baumeister R, Schmidt E: ***Caenorhabditis elegans* LRK-1 and PINK-1 act antagonistically in stress response and neurite outgrowth**. *J Biol Chem* 2009, **284**:16482-16491.

22. Liu Z, Hamamichi S, Lee BD, Yang D, Ray A, Caldwell GA, Caldwell KA, Dawson TM, Smith WW, Dawson VL: **Inhibitors of LRRK2 kinase attenuate neurodegeneration and Parkinson-like phenotypes in *Caenorhabditis elegans* and Drosophila Parkinson's disease models**. *Hum Mol Genet* 2011, **20**:3933-3942.

23. Vives-Bauza C, Zhou C, Huang Y, Cui M, de Vries RL, Kim J, May J, Tocilescu MA, Liu W, Ko HS *et al.*: **PINK1-dependent recruitment of Parkin to mitochondria in mitophagy**. *Proc Natl Acad Sci U S A* 2010, **107**:378-383.

24. Bess AS, Crocker TL, Ryde IT, Meyer JN: **Mitochondrial dynamics and autophagy aid in removal of persistent mitochondrial DNA damage in *Caenorhabditis elegans***. *Nucleic Acids Res* 2012, **40**:7916-7931 http://dx.doi.org/10.1093/nar/gks532.

25. •• Palikaras K K, Lionaki E, Tavernarakis N: **Coordination of mitophagy and mitochondrial biogenesis during ageing in *C. elegans***. *Nature* 2015, **521**:525-528.

This paper provides a framework for the investigation and understanding of mitophagy in *C. elegans*. By utilizing RNAi of PD-like disease homologues in *pink-1* and *pdr-1* the authors find a novel role for the ROS-induced Nrf2-like protein SKN-1 in the induction of *dct-1* in order to increase mitochondrial bioaccumulation which is uncoupled as animals age.

26. Kamp F, Exner N, Lutz AK, Wender N, Hegermann J, Brunner B, Nuscher B, Bartels T, Giese A, Beyer K *et al.*: **Inhibition of mitochondrial fusion by α-synuclein is rescued by PINK1, Parkin and DJ-1**. *EMBO J* 2010, **29**:3571-3589.

27. Rina Ved, Shamol S, Westlund B, Perier C, Burnam L, Sluder A, Hoener M, Rodrigues CMP, Alfonso A, Steer C *et al.*: **Similar patterns of mitochondrial vulnerability and rescue induced by genetic modification of α-synuclein, parkin, and DJ-1 in *Caenorhabditis elegans***. *J Biol Chem* 2005, **280**:42655-42668.

28. Thomas KJ, McCoy MK, Blackinton J, Beilina A, van der Brug M, Sandebring A, Miller D, Maric D, Cedazo-Minguez A, Cookson MR: **DJ-1 acts in parallel to the PINK1/parkin pathway to control mitochondrial function and autophagy**. *Hum Mol Genet* 2011, **20**:40-50.

29. • Wang S, Zhang S, Liou L, Ren Q, Zhang Z, Caldwell GA, Caldwell KA, Stephan Witt: **Phosphatidylethanolamine deficiency disrupts α-synuclein homeostasis in yeast and worm models of Parkinson disease**. *Proc Natl Acad Sci U S A* 2014, **111**:E3976-E3985.

These authors investigate the role of the inner mitochondrial lipid biosynthetic pathway towards the attenuation of α-synuclein stress in yeast and *C. elegans* disease models. Proper biosynthesis of phosphatidylethanolamine is paramount to proper localization of cytosolic α-synuclein as mutations in the biosynthetic gene phosphatidylserine decarboxylase result in abnormal vesicular trafficking.

30. Wang S, Zhang S, Xu C, Barron A, Galiano F, Patel D, Lee YJ, Caldwell GA, Caldwell KA, Wit SN: **Chemical compensation of mitochondrial phospholipid depletion in yeast and animal models of Parkinson's disease**. *PLoS One* 2016, **11**:e0164465 http://dx.doi.org/10.1371/journal.pone.0164465.

31. •• Di Maio R, Barrett PJ, Hoffman EK, Barrett CW, Zharikov A, Borah A, Hu X, McCoy J, Chu CT, Burton EA *et al.*: **α-Synuclein binds to TOM20 and inhibits mitochondrial protein import in Parkinson's disease**. *Sci Transl Med* 2017. 342:342ra78.

This paper demonstrates how potentiated α-synuclein variants can variably bind to the mitochondrial translocase of the outer membrane (TOM) subunit 20. Interestingly unaltered α-synuclein only weakly interacts with TOM20 but dopamine modulated α-synuclein and phosphomimetic S129E variants bind at a much greater rate providing evidence for compartment and context specific synuclein toxicity.

32. Goldman SM: **Environmental toxins and Parkinson's disease**. *Annu Rev Pharmacol Toxicol* 2014, **54**:141-164.

33. Baltazar MT, Dinis-Oliveira RJ, de Lourdes Bastos M, Tsatsakis AM, Duarte JA, Carvalho F: **Pesticides exposure as etiological factors of Parkinson's disease and other neurodegenerative disease—a mechanistic approach**. *Toxicol Lett* 2014, **230**:85-103.

34. Dixon SJ, Stockwell BR: **The role of iron and reactive oxygen species in cell death**. *Nat Chem Biol* 2014, **10**:9-17.

35. Watkins AL, Ray A, Roberts LR, Caldwell KA, Olson JB: **The prevalence and distribution of neurodegenerative compound-producing soil *Streptomyces* spp**. *Sci Rep* 2016, **6** http://dx.doi.org/10.1038/srep22566.

36. Nass R, Hall DH, Miller DM, Blakely RD: **Neurotoxin-induced degeneration of dopamine neurons in *Caenorhabditis elegans***. *Proc Natl Acad Sci U S A* 2002, **99**:3264-3269.

37. Cao S, Gelwix CC, Caldwell KA, Caldwell GA: **Torsin-mediated protection from cellular stress in the dopaminergic neurons of *Caenorhabditis elegans***. *J Neurosci* 2005, **25**:3801-3812.

38. Marvanova M, Nichols CD: **Identification of neuroprotective compounds of *Caenorhabditis elegans* dopaminergic neurons against 6-OHDA**. *J Mol Neurosci* 2007, **31**:127-137.

39. Toth M, Simon P, Kovacs AL, Vellai T: **Influence of autophagy genes on ion-channel-dependent neuronal degeneration in *Caenorhabditis elegans***. *J Cell Sci* 2007, **120**:1134-1141.

40. Gonzalez-Hunt CP, Leung MCK, Bodhicharla RK, McKeever MG, Arrant AE, Margillo KM, Ryde IT, Cyr DD, Kosmaczewski SG, Hammarlund M, Meyer JN: **Exposure to mitochondrial genotoxins and dopaminergic neurodegeneration in *Caenorhabditis elegans***. *PLoS One* 2014, **9**:e114459 http://dx.doi.org/10.1371/journal.pone.0114459.

41. Braungart E, Gerlach M, Riederer P, Baumeister R, Hoener MC: **Caenorhabditis elegans MPP+ model of Parkinson's disease for high-throughput drug screenings**. *Neurodegener Dis* 2004, **1**:175-183.

42. Chikka MR, Anbalagan C, Dvorak K, Dombeck K, Prahld V: **The mitochondria-regulated immune pathway activated in the *C. elegans* intestine is neuroprotective**. *Cell Rep* 2016, **16**:2399-2414.

43. Ray A, Martinez BA, Berkowitz LA, Caldwell GA, Caldwell KA: **Mitochondrial dysfunction, oxidative stress, and neurodegeneration elicited by a bacterial metabolite in a *C. elegans* Parkinson's model**. *Cell Death Dis* 2014, **5** http://dx.doi.org/10.1038/cddis.2013.513.

44. Caldwell KA, Tucci ML, Armagost J, Hodges TW, Chen J, Memon SB, Blalock JE, DeLeon SM, Findlay RH, Ruan Q *et al.*: **Investigation of bacterial sources of toxicity as an environmental contributor to dopaminergic neurodegeneration**. *PLoS One* 2009, **4**:e7227 http://dx.doi.org/10.1371/journal.pone.0007227.

45. Chen P, DeWitt MR, Bornhorst J, Soares FA, Mukhopadhyay S, Bowman AB, Aschner M: **Age and manganese-dependent modulation of dopaminergic phenotypes in a *C. elegans* DJ-1 genetic model of Parkinson's disease**. *Metallomics* 2015, **7**:289-298.

46. Bornhorst J, Chakraborty S, Meyer S, Lohren H, Brinkhaus SG, Knight AL, Caldwell KA, Caldwell GA, Karst U, Schwerdtle T, Bowman A, Aschner M: **The effects of *pdr1, djr1.1*, and *pink1* loss in manganese-induced toxicity and the role of α-synuclein in *C. elegans***. *Metallomics* 2014, **6**:476-490.

47. Gitler AD, Chesi A, Geddie ML, Strathearn KE, Hamamichi S, Hill KJ, Caldwell KA, Caldwell GA, Cooper AA, Rochet JC, Lindquist S: **α-Synuclein is part of a diverse and highly conserved interation network that includes PARK9 and manganese toxicity**. *Nat Genet* 2009, **41**:308-315.

48. Martinez BA, Kim H, Ray A, Caldwell GA, Caldwell KA: **A bacterial metabolite induces glutathione-tractable proteostatic damage, proteasomal disturbances, and PINK1-dependent autophagy in *C. elegans***. *Cell Death Dis* 2015, **6**:e1908 http://dx.doi.org/10.1038/cddis.2015.270.

49. Johnson WM, Yao C, Siedlak SL, Wang W, Zhu X, Caldwell GA, Wilson-Delfosse AL, Mieyal JJ, Chen SG: **Glutaredoxin deficiency exacerbates neurodegeneration in *C. elegans* models of Parkinson's disease**. *Hum Mol Genet* 2015, **24**:1322-1335.

50. Azevedo JCS, Borges KC, Genovese MI, Correia RTP, Vattem DA: **Neuroprotective effects of dried camu-camu (*Myrciaria dubia* HBK McVaugh) residue in *C. elegans***. *Food Res Int* 2015, **73**:135-141.

[51. Fu R, Harn H, Liu S, Chen C, Chang W, Chen Y, Huang J, Li R, Tsai S, Hung H, Shyu W, Lin S, Wang Y: ***n*-Butylidenephthalide protects against dopaminergic neuron degeneration and α-synuclein accumulation in *Caenorhabditis elegans* models of Parkinson's disease**. *PLoS One* 2014, **9**:e85305 http://dx.doi.org/10.1371/journal.pone.0085305.

52. • Jang S, Nelson JC, Bend EG, Rodriguez-Laureano L, Tueros FG, Cartagenova L, Underwood K, Jorgensen EM, Colon-Ramos DA: **Glycolytic enzymes localize to synapses under energy stress to support synaptic function**. *Neuron* 2016, **90**:278-291.

In this report, the authors find a role for glycolytic genes in the maintenance of proper vesicular clustering during low ATP conditions. More generally, other glycolytic genes are also necessary and cluster to presynaptic termini where they are necessary to maintain proper energetic potential to neurons during synaptic stimulation.

53. Kim DK, Lim HS, Kawasaki I, Shim Y, Vaikath N, El-Agnaf OMA, Lee H, Lee S: **Anti-aging treatments slow propagation of synucleinopathy by restoring lysosomal function**. *Autophagy* 2016, **12**:1849-1863.

54. •• Bansal A, Khu LJ, Yen K, Tissenbaum HA: **Uncoupling lifespan and healthspan in *Caenorhabditis elegans* longevity mutants**. *Proc Natl Acad Sci* 2015, **112**:E277-E286.

The distinction between quality of life and lifespan is most relevant towards the study of degenerative diseases, which often accompany old age. In this study, the authors investigate phenotypic stress sensitivity, movement competency and other signifiers of a healthy organism in four long-lived mutant nematode backgrounds.