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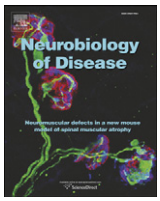

Review

Neurodegenerative disorders: Insights from the nematode *Caenorhabditis elegans*

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ABSTRACT

Neurodegenerative diseases impose a burden on society, yet for the most part, the mechanisms underlying neuronal dysfunction and death in these disorders remain unclear despite the identification of relevant disease genes. Given the molecular conservation in neuronal signaling pathways across vertebrate and invertebrate species, many researchers have turned to the nematode *Caenorhabditis elegans* to identify the mechanisms underlying neurodegenerative disease pathology. *C. elegans* can be engineered to express human proteins associated with neurodegeneration; additionally, the function of *C. elegans* orthologs of human neurodegenerative disease genes can be dissected. Herein, we examine major *C. elegans* neurodegeneration models that recapitulate many aspects of human neurodegenerative disease and we survey the screens that have identified modifier genes. This review highlights how the *C. elegans* community has used this versatile organism to model several aspects of human neurodegeneration and how these studies have contributed to our understanding of human disease.

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Introduction

As average longevity increases, neurodegenerative diseases become more prevalent and problematic, especially as treatments for these diseases are unavailable or ineffective. In many cases, the identity of genes that predispose or cause neurodegenerative disease is clear: huntingtin in Huntington's disease, β-amyloid and tau in Alzheimer's disease, and α-synuclein in Parkinson's disease. While these discoveries have led to improvements in diagnosis, the molecular mechanisms underlying neurodegeneration remain poorly understood. Many neurodegenerative disease genes are broadly expressed throughout the body, yet, in many cases, specific neural tissues are affected which suggests neuron-specific roles for disease proteins. In addition, a hallmark of many age-associated neurodegenerative disorders is aberrant accumulation or aggregation of the

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disease protein and other proteins. Despite a plethora of studies, it is still not clear if the accumulation of mutant huntingtin, β-amyloid, tau, or α-synuclein in their respective diseases is a critical factor leading to neuronal dysfunction and death. Some studies suggest that large protein aggregates are protective and that soluble, misfolded protein monomers or oligomers are the active toxic agent. Due to the complexity of the vertebrate brain and the fact that mammalian model organisms have their own limitations (e.g. cost, laborious transgenesis, long life spans), invertebrate models including *Caenorhabditis elegans* have been used in the past decade to delineate the molecular mechanisms underlying neurodegeneration.

C. elegans is a free-living nematode of ~1 mm in length with a short generation cycle (~3 days) and lifespan (~3 weeks), and a transparent body that allows for the visualization of all cell types at all stages of development (Brenner, 1974). Neuronal cell death and protein inclusions can easily be detected and quantified using optical techniques. Additionally, *C. elegans* have a simple nervous system of 302 neurons in the adult hermaphrodite, in which each neuron has a unique position and identity that is reproducible from animal to animal (White et al., 1986). Furthermore, the synaptic and gap connections between these neurons are also reproducible based on nervous system reconstruction at the electron microscope level of analysis. Major neurotransmitter systems are conserved in *C. elegans* contributing to the appeal of the nematode for neuroscientists. At least 42% of human disease related genes have a *C. elegans* ortholog (Culetto and Sattelle, 2000), suggesting that most biochemical pathways are conserved across evolution. Arguably, the most attractive characteristic of the nematode is its suitability for experimental approaches that are not possible in mammalian models. For example, unbiased forward genetic screens using chemical mutagenesis can reveal genes integral to the biological process of interest. This approach has been used to elucidate the genetic and molecular interactions of various genes/pathways including synaptic vesicle trafficking, apoptosis, and mRNA translational regulation. In addition, reverse genetic analyses or screens can be undertaken by feeding *C. elegans* bacteria expressing double-stranded RNA (RNA interference, RNAi), which leads to almost global knockdown of target gene expression. While neurons are less sensitive to RNAi by bacterial feeding than some other tissues, this powerful strategy nevertheless has been used in numerous screens described below to identify modifier genes. For these reasons, *C. elegans* has emerged as an attractive and powerful *in vivo* model system for studying pathological mechanisms in several major neurodegenerative disorders.

In this article, we review how the *C. elegans* community has used the nematode to model human neurodegenerative disease and how these studies have contributed to our current understanding thereof. Due to space limitations, some *C. elegans* models for human disease are not reviewed herein (Park and Li, 2008; Sym et al., 2000). In addition, *C. elegans* studies addressing the normal function of human neurodegenerative disease orthologs or studies of *C. elegans* neurodegeneration that is not a direct model of human diseases are not reviewed here (Artal-Sanz and Tavernarakis, 2005; Chalfie and Wolinsky, 1990; Driscoll, 1992; Kraemer and Schellenberg, 2007a; Levitan et al., 1996; Springer et al., 2005). In Table 1, the genes pertinent to human neurodegenerative diseases discussed in this article are listed along with their *C. elegans* orthologs.

Table 1
Likely *C. elegans* orthologs of genes associated with neurodegenerative disorders.

Human genes	*C. elegans* genes
huntingtin (Htt)	*n/a*
amyloid precursor protein (APP)	*apl-1*
β-secretase (BACE1)	*n/a*
presenilin-1 and 2 (PS1 and PS2)	*sel-12, hop-1, spe-4*
microtubule-associated protein tau (MAPT)	*ptl-1*
PARK1	*n/a*
PARK2	*pdr-1*
PARK5	*ubh-1*
PARK6	*pink-1*
PARK7	*djr-1.1 & djr-1.2*
PARK8	*lrk-1*
PARK9	*catp-6*
PARK11	*n/a*
PARK13	*n/a*
SOD1	*sod-1*
SMN1/SMN2	*smn-1*

n/a, not applicable.

Polyglutamine repeat diseases

Pathological expansion of CAG trinucleotide repeats in the coding regions of unrelated genes account for at least nine human neurodegenerative disorders, including Huntington's disease (HD) and spinocerebellar ataxias (SCAs) (Bauer and Nukina, 2009). The former is the most frequent autosomal-dominant disorder, characterized by an increased number of glutamines (polyQ) in the N terminus of the huntingtin protein (Htt), a ubiquitously expressed protein of unknown function (MacDonald et al., 1993). The age of onset and disease severity is directly correlated to the number of polyQ repeats, with sequences of 35 or more glutamines invariably resulting in HD neurodegeneration (Bates et al., 2002). The neurons of HD patients contain aggregated N-terminal fragments of mutant Htt in cytoplasmic and intranuclear inclusions in the affected tissues (DiFiglia et al., 1997). Similar aggregates are observed in patient brains in the other polyQ diseases (Bauer and Nukina, 2009). The molecular and cellular mechanisms that lead to neuronal dysfunction in the polyQ disorders are unknown.

The nematode *C. elegans* does not contain an Htt ortholog, but has been widely used to model several aspects of polyglutamine cytotoxicity and to identify novel disease modifiers. Faber and coworkers (1999) used the *osm-10* promoter to drive expression of human huntingtin exon 1 fragments of various polyQ lengths in specific sensory neurons of *C. elegans* (Htn-Q2, Htn-Q23, Htn-Q95 and Htn-Q150). Transgenic animals expressing Htn-Q150 showed evidence of sensory process degeneration based on defective uptake of a lipophilic vital dye *via* the endings of sensory neurons, but no cell death (scored in ASH neurons, based on endogenous OSM-10 immunoreactivity). Co-expression of the same huntingtin polyQ fragments with sub-threshold levels of a second toxic transgene (*OSM-10p::GFP*) enhanced the neurodegeneration phenotype. Htn-Q95 and Htn-Q150 caused ASH cell death and dye-filling defects in addition to defective sensory response to touch, mediated by ASH sensory neurons. Formation of huntingtin-positive cytoplasmic aggregates was also evident and increased with age in the sensory and axonal processes of Htn-Q150 ASH neurons (Faber et al., 1999).

The same *C. elegans* HD model was used in a classical genetic screen to identify mutations that enhanced polyQ neurotoxicity, leading to the discovery of the *polyQ-enhancer (pqe-1)* gene (Faber et al., 2002). In *pqe-1* null animals (completely lacking *pqe-1* function), ASH sensory neurons develop normally, but degeneration and cell death is strongly and specifically exacerbated in the presence of Htn-Q150. Over-expression experiments using the *pqe-1* cDNA demonstrated that the glutamine/proline rich residues (Q/P) and central charged domain in the amino-terminus and central portion of PQE-1, respectively, are sufficient and necessary for protecting ASH neurons from the toxic effects of polyQ tracts (Faber et al., 2002). No clear *pqe-1* ortholog has been identified in humans. However, loss of a similar exonuclease, Rex3p causes synthetic lethality in yeast lacking Rrs1, whose mammalian ortholog has been implicated in Htn polyQ toxicity, suggesting a conserved mechanism may be at work (Carnemolla et al., 2009; Fossale et al., 2002; Nariai et al., 2005). This *C. elegans* polyQ toxicity model has also been used in conjunction with primary cell culture neurons to demonstrate that acetylation of mutant Htt at K444

leads to an increased clearance of mutant Htt and neuroprotection *in vitro* and *in vivo* (Jeong et al., 2009).

An independent HD neuronal model was developed using the *mec-7* β-tubulin gene promoter to drive expression of the first 57 amino acids of human huntingtin with normal and expanded polyQ tracts fused to GFP to the six touch receptor neurons of the body (Parker et al., 2001). The consequent mechanosensory defects (Mec) significantly correlated with increased polyQ expansion (increased from 46% to 72% in *htt57Q88::GFP* and *htt57Q128::GFP*, respectively). Perinuclear aggregates were evident when either normal or expanded polyQ Htt were expressed in the touch receptor neurons at the tail (PLM) and aggregate presence did not correlate with polyQ length or the mechanosensory defects. Analysis of PLM neurons process integrity in Q128 expressing animals revealed various morphological abnormalities, including swelling of axonal processes, but no apoptotic features (Parker et al., 2001). This polyQ toxicity model has subsequently been used to demonstrate a protective role of the *C. elegans* huntingtin-interacting protein 1 ortholog (*hipr-1*) and of other synaptic endocytosis proteins from polyQ toxicity (Parker et al., 2007).

The two HD models (ASH and touch cells) have also been used to examine the role of histone deacetylases (HDACs) in polyQ-induced neurodegeneration. In the touch cells, increased expression of the *C. elegans* ortholog of silent regulator information 2 (*sir-2.1*), a class III HDAC, rescued the Mec phenotype by 38% in the presence of Q128, and this amelioration was dependent on the activity of *daf-16* transcription factor (Forkhead) (Parker et al., 2005). The same authors also saw protection when animals expressing Q128 were treated with resveratrol, a chemical activator of sirtuins. Analysis of HDACs in the ASH polyQ model provided evidence that *sir-2.1* is not unique in rescuing polyglutamine cytotoxicity and that the role of HDACs in polyQ toxicity is complex. Loss-of-function alleles and/or RNAi of the majority *C. elegans* HDAC genes exacerbated Htn-Q150 degeneration (i.e. *hda-1*, *hda-2*, *hda-4*, *hda-5*, *hda-6*, *hda-10*, *hda-11*, *sir-2.1*, *sir-2.2*), while reducing *sir-2.3* activity had no effect (Bates et al., 2006). Interestingly, loss of *hda-3* significantly reduced degeneration when *hda-1* activity was not compromised (Bates et al., 2006).

Genetic manipulations of histone acetylation and deacetylation function in polyQ toxicity have yielded some seemingly discordant results. CBP-acetyltransferase expression or HDAC1 knockdown both increased acetylation of mutant Htt at K444 and facilitated its clearance by autophagosomes (Jeong et al., 2009). And, globally increasing acetylation by treatment with trichostatin A (TSA) which inhibits class I and II HDACs also reduced polyQ toxicity in *C. elegans* neurons (Bates et al., 2006). Yet, decreasing acetylation by over-expression of *sir-2.1* or activation of class III/sirtuins by resveratrol suppressed polyQ toxicity (Parker et al., 2005). These apparently conflicting results can be reconciled by examining the function of each *C. elegans* HDAC individually (Bates et al., 2006). Decreased function of *hda-3*, a class I HDAC, suppressed polyQ toxicity in *C. elegans*; CBP and TSA might act primarily through *hda-3,* which is an apparent ortholog of vertebrate HDAC1. Class III HDACs/sirtuins likely act on different cellular targets than class I and II HDACs and hence, their manipulation with drugs, over-expression or knockdown has strikingly different effects (Parker et al., 2005; Bates et al., 2006). Combined, these studies suggest that different classes of histone deacetylases likely have specific targets which impact polyQ toxicity in different ways.

A pan-neuronal *C. elegans* polyQ model using the *rgef-1* promoter was generated to examine the consequences of expanded polyQ fragments in individual neurons as well as the effect of neurotoxicity on behavioral outputs (Brignull et al., 2006). In addition to neuronal models, muscle-specific expression of polyQ repeats has been used in *C. elegans* to delineate mechanisms underlying polyQ aggregation and toxicity. Satyal and co-workers (2000) used the *unc-54* myosin heavy chain promoter to express short (Q19-GFP) and long (Q82-GFP) fusion proteins in body-wall muscles. The presence of Q82-GFP caused insoluble cytoplasmic aggregates, slowed development, and elevated heat shock response (as observed by elevated β-galactosidase in some cells) (Satyal et al., 2000). These imbalances were partially reversed by expression of the yeast chaperone Hsp104. In the same model, but with polyQ-YFP, the threshold for polyQ expansion protein aggregation was investigated (Morley et al., 2002). Aggregation (observed as focal instead of diffuse fluorescence in muscle cells) was first evident in Q40-YFP animals that exhibited an intermediate motility defect. The threshold for polyQ aggregation was dynamic and age-dependent, with older animals accumulating aggregates at Q33 and Q35. Both aggregation and motility defects were delayed by loss of AGE-1, the *C. elegans* ortholog of phosphoinositide 3-kinase (PI3K) (Morley et al., 2002). This muscle polyQ *in vivo* model has been used in a genetic screen for polyQ modifier genes; 186 genes with diverse functions affected cellular protein homeostasis based on RNAi knockdown studies (Nollen et al., 2004). Indeed, transgene-induced polyQ expression in muscles or neurons of animals carrying temperature-sensitive mutations in genes unrelated to polyQ toxicity exacerbated genetic defects even at under permissive conditions. Additionally, the presence of the temperature-sensitive alleles enhanced the polyQ aggregation phenotype leading to the hypothesis that the cellular pathways critical for protein folding are finite and excessive concentrations of unfolded proteins impact protein homeostasis globally (Gidalevitz et al., 2006).

Additional muscle-specific model systems for polyQ disease related toxicity have been generated in *C. elegans*. One of these used 17 amino acids of the dentatorubural pallidoluysian atrophy (DRPLA) protein to flank polyQ-GFP fusion proteins and reported that CDC-48.1 and CDC-48.2 chaperone proteins normally suppress the polyQ toxicity by direct interaction with Htt pathogenic polyQ repeats (Nishikori et al., 2008; Yamanaka et al., 2004). Furthermore, a separate *in vivo* model used the muscle-specific *unc-54* promoter and found that increased expression of ubiquilin suppressed motility defects induced by N-terminal Htt-polyQ fragment expression (Wang et al., 2006). The latter model has been used to investigate the role of mitochondria dynamics in relation to expanded polyglutamine HD protein toxicity (Wang et al., 2009a). These models confirm that protein folding is critical in the toxicity of expanded polyQ fragments.

Polyglutamine toxicity in *C elegans* is age and polyQ tract length dependent, recapitulating critical aspects of human CAG repeat disorders. However, differences in the polyQ threshold required for neurotoxicity has been observed in *C. elegans* transgenic strains. A polyQ tract of 150 residues is required for ASH neuron degeneration, whereas polyQ repeats of 86 cause aggregates in other neurons (Faber et al., 1999; Brignull et al., 2006). Furthermore, the threshold in muscle cells is even lower with 33 or 35 polyQ repeats leading to cellular dysfunction (Morley et al., 2002). The results obtained could be attributed to the distinct promoters used and/or to differential sensitivity of cell types targeted. In addition, unpublished data suggests that aggregation and toxicity of the huntingtin first exon is increased by addition of GFP in fusion proteins (unpublished data, A. Hart). Nevertheless, all of the *C. elegans* models described herein have unique strengths and have provided insights into polyQ protein neurotoxicity.

Alzheimer's disease (AD)

Alzheimer's disease (AD) is characterized by the presence of senile plaques (SPs) and neurofibrillary tangles (NFTs) in the brains of affected individuals. The major constituents of SPs are toxic species of β-amyloid (Aβ) peptide (mainly $A\beta_{1-42}$), whereas NFTs contain hyperphoshorylated and aggregated forms of the microtubule-associated protein tau (MAPT) (Goedert and Spillantini, 2006). Aβ peptides derive from the sequential cleavage of β-amyloid precursor protein (APP) by β- and γ-secretase, respectively, through the "amyloidogenic pathway" (Jacobsen and Iverfeldt, 2009). The identification of mutations in the *APP* or *presenilin-1* (*PS1)* or *presenilin-2*

(*PS2*) genes (components of the γ-secretase enzymatic complex) in familial forms of AD has provided compelling evidence for the "amyloid cascade" hypothesis in AD pathogenesis (Campion et al., 1995; Selkoe, 1996; Sherrington et al., 1996). It is of interest that mutations in the tau gene do not lead to AD but instead to a different neurodegenerative disorder called FTDP-17 (frontotemporal dementia with parkinsonism chromosome 17 type) (Hutton et al., 1998; Spillantini et al., 1998). The precise mechanism(s) by which tau and Aβ act as toxic agents in AD is currently unclear.

C. elegans β-amyloid peptide models

C. elegans has an APP-related gene, *apl-1*, but the APL-1 protein lacks the Aβ peptide (Daigle and Li, 1993). The *C. elegans* genome encodes three presenilin orthologs (*sel-12*, *hop-1* and *spe-4*) and additionally, *aph-1*, *pen-2*, and *aph-2* (nicastrin) whose genes products combine to form the canonical functional γ-secretase complex. Yet, the *C. elegans* genome does not encode a β-secretase (BACE ortholog). Three engineered *C. elegans* AD models have been described to date, all of which express the longest and most toxic Aβ species ($A\beta_{1-42}$) (Link, 1995, 2006; Link et al., 2003; Wu et al., 2006). Transgenic animals expressing a signal peptide/$A\beta_{1-42}$ fragment under the constitutive *unc-54* body-wall muscle promoter display progressive paralysis and intracellular cytoplasmic Aβ deposits that colocalize with amyloid specific dyes (thioflavin S and Congo Red) (Link, 1995; Link et al., 2001). A fully penetrant paralysis phenotype was obtained in subsequent models by expressing a temperature-inducible Aβ transcript from a carefully designed minigene. The signal peptide/$A\beta_{1-42}$ transcript expression was driven using the *myo-3* muscle-specific promoter and incorporated a long 3' untranslated region (3'UTR) for regulation. In transgenic animals, the abnormal 3' UTR normally leads to an unstable mRNA that is recognized and degraded by the *smg* RNA surveillance pathway. However, expression of the Aβ transgene in animals harboring a temperature-sensitive *smg-1* allele results in stabilization of the $A\beta_{1-42}$ mRNA and substantial translation due to the inactivation of the nonsense-mediated decay pathway at the restrictive temperature (23 °C). Therefore, in contrast to the progressive paralysis phenotype observed in the constitutive Aβ model, the inducible Aβ transgenic animals showed a rapid (~24 h) and dramatic paralysis due to high expression levels and muscle degeneration induced by Aβ toxicity (Link et al., 2003; Link, 2006). The signal peptide/$A\beta_{1-42}$ fragment has also been expressed in the neuronal cells (driven by the ubiquitous *snb-1* promoter). Intraneuronal deposition of Aβ was observed in transgenic animals along with defects in chemotaxis and hypersensitivity to serotonin, but no overt locomotion defects were found (Link, 2006; Wu et al., 2006). Expression of Aβ in muscles and neurons of *C. elegans* AD models has distinct consequences for reasons that remain unclear.

To date, most studies have focused on the constitutive (*unc-54*) and inducible Aβ muscle-specific expression (*myo-3*) models due to methodological advantages (e.g. RNAi is more efficient in muscle cells and less efficient in neurons). McColl and colleagues (2009) have recently demonstrated that in the *unc-54*/signal peptide::$A\beta_{1-42}$ muscle model, animals predominantly express a truncated $A\beta_{3-42}$ protein due to a single cleavage site at position +3 of $A\beta_{1-42}$. All the *C. elegans* AD models utilize the same signal peptide::$A\beta_{1-42}$ fragment and it is therefore, likely that the transgenic animals produce the shorter $A\beta_{3-42}$ form (Teschendorf and Link, 2009).

Cohen et al. (2006) examined the role of the insulin/insulin growth factor (IGF)-1-like signaling (IIS) pathway in *C. elegans* Aβ muscle toxicity (*unc-54*/$A\beta_{1-42}$ strain), and demonstrated that the $A\beta_{1-42}$ toxicity is highly dependent on genes previously implicated in longevity. Knockdown of the insulin/IGF-1 receptor, DAF-2, reduced $A\beta_{1-42}$ toxicity in the *C. elegans* body-wall muscles through the downstream transcription factors, DAF-16 (Forkhead) and heat shock factor-1 (HSF-1) (Cohen et al., 2006). Down-regulation of *daf-16* or *hsf-1* had opposing effects on the $A\beta_{1-42}$ aggregation; the amount of high-molecular weight Aβ species (that are linked to toxicity) was increased upon *hsf-1(RNAi)* knockdown and decreased upon *daf-16* knockdown (Cohen et al., 2006). Toxicity in both polyQ and $A\beta_{1-42}$ *C. elegans* models is impacted by the insulin-like signaling pathway, suggesting that common pathways exist in neurodegenerative disorders.

The temperature-dependent *C. elegans* AD model has been used to study gene expression changes upon Aβ induction (Hassan et al., 2009; Link et al., 2003). DNA microarray analysis identified 67 up-regulated and 240 down-regulated genes upon intracellular Aβ accumulation (Link et al., 2003). Two gene families that were predominantly up-regulated were αB-crystallin (CRYAB), which has been reported to increase Aβ formation (Stege et al., 1999) and the tumor necrosis factor-induced protein 1 (TNFAIP1). These genes were also shown to have increased transcript levels in AD brains (Link et al., 2003). Another transcriptional analysis/microarray study used the same model and demonstrated that over-expression of arsenite-inducible protein (AIP-1) suppressed Aβ toxicity (i.e. decrease in $A\beta_{1-42}$ peptide accumulation and delayed Aβ-induced paralysis) (Hassan et al., 2009). AIP-1 protection was not specific to Aβ and the authors suggested that AIP-1 expression increased protein turnover and clearance of toxic species, consistent with its role in modulating proteasome function (Stanhill et al., 2006; Yun et al., 2008). The same authors examined the human orthologs of AIP-1 and found that AIRAPL, but not AIRAP, suppressed paralysis in *C. elegans* (Hassan et al., 2009).

A number of studies have further investigated the role of chaperones in Aβ toxicity (Fonte et al., 2002, 2008; Wu et al., 2010). Using the *unc-54*/$A\beta_{1-42}$ *C. elegans* model, six Aβ-interacting proteins with a chaperone-like function were identified by mass spectrometry: two HSP70 orthologs (HSP70A and HSP70C), three HSP16 proteins (HSP-16.1, 16.2 and 16.48), and a tetratricopeptide repeat-containing protein, the ortholog of the human SGT protein, suggested to negatively regulate HSP70 (Fonte et al., 2002; Liu et al., 1999). In this study, HSP-16 was found to co-immunoprecipitate and to closely colocalize with intracellular Aβ (Fonte et al., 2002). Fonte and co-workers (2008) further investigated the biological role of HSP-16.2 on Aβ toxicity in the temperature-inducible AD model. HSP-16.2 over-expression partially suppressed the Aβ-induced paralysis phenotype, but did not reduce the total Aβ protein accumulation, suggesting that other chaperones may be involved. Indeed, a subsequent study applied a protective heat shock treatment (2 h, 35 °C) in the same inducible transgenic model and found a remarkable delay in paralysis, increased HSP-16.2 protein expression, and a significant reduction of oligomeric Aβ species but not total Aβ levels (Wu et al., 2010). Combined, all of these studies suggest that protein turnover is a critical determinant in Aβ toxicity reminiscent of results in polyQ models.

C. elegans tauopathy models

Tau aggregates are a common characteristic of Alzheimer's disease, but they are also found in other neurodegenerative disorders that are commonly referred to as tauopathies. *C. elegans* has a single tau ortholog, named *ptl-1* (Goedert et al., 1996; McDermott et al., 1996). *ptl-1* is expressed in many cells including *C. elegans* mechanosensory neurons of the body; loss of *ptl-1* decreases the number of viable progeny and these animals are slightly less sensitive to body touch (Gordon et al., 2008). As *ptl-1* loss-of-function does not recapitulate tau pathology, transgenic *C. elegans* expressing human tau has been used to establish tauopathy models.

Pan-neuronal expression (*aex-3* promoter) of the most abundant human brain tau isoform (4R1N) and mutant tau ($^{V}337^{M}$ and $^{P}301^{L}$) resulted in uncoordinated (Unc) locomotion, followed by progressive

accumulation of insoluble tau aggregates, axonal abnormalities, and loss of GABAergic inhibitory motor neurons (Kraemer et al., 2003). Animals expressing mutant tau were more severely affected in young animals, consistent with the toxicity of FTDP-17 mutations in human disease (Kraemer et al., 2003). In addition, when expressed in *C. elegans*, both normal and mutant human tau were phosphorylated at sites hyperphosphorylated in AD and FTDP-17, although no differences were observed between mutant and normal tau lines (Kraemer et al., 2003). The tau-induced Unc locomotion defect was used as a behavioral read-out in a genome-wide RNAi feeding screen for genetic modifiers that would exacerbate or alleviate the behavioral defects caused by mutant tau expression. A library of RNAi clones corresponding to over 16,000 *C. elegans* genes (Kamath et al., 2003) was screened using the 337^{M}-1 mutant strain. Sixty genes were identified that enhanced only the tau-induced behavioral defect including kinases, phosphatases, chaperones, proteases and genes unique to nematodes (Kraemer et al., 2006). Mammalian orthologs of some of these modifier genes had been previously implicated in human tauopathies (e.g. GSK-3β, CSTE, CHRNA7). Loss-of-function alleles were also used to validate eight candidate modifier genes (Kraemer et al., 2006). A classical chemical mutagenesis, forward genetic screen for modifier genes was also undertaken using the *aex-3*/tau transgenic model. Two novel candidate genes, *sut-1* (Kraemer and Schellenberg, 2007b) and *sut-2* (Guthrie et al., 2009), whose loss-of-function strongly ameliorated the tau-induced neurodegeneration, were identified. SUT-1 binds *in vitro* to the cytoskeletal regulated protein Enabled (ENA), UNC-34, and *sut-1* suppression of the tau-induced Unc phenotype was dependent on *unc-34* activity (Kraemer and Schellenberg, 2007b). SUT-2 interacted directly with ZYG-12, a HOOK protein family member (Guthrie et al., 2009). Interestingly, mammalian SUT-2 (MSUT-2) interacts *in vitro* with a human HOOK ortholog, HOOK2 that plays a role in the formation of centriolar aggresome containing misfolded proteins (Szebenyi et al., 2007).

An additional *C. elegans* model of AD-relevant tau modification utilized the pan-neuronal *rgef-1* promoter to express human tau (fetal form) or pseudohyperphosphorylated (PHP) tau (10 serine/threonine residues were exchanged by glutamate) (Brandt et al., 2009). Human tau was highly phosphorylated in the *C. elegans* neurons reminiscent of the hyperphosphorylation seen in paired helical filaments (PHFs) isolated from AD patients. Uncoordinated locomotion was observed in both the normal and mutant tau transgenic lines, but axonal abnormalities in inhibitory motor neurons were more profound in PHP tau-expressing animals (Brandt et al., 2009). Tau-aggregation was only observed in the PHP tau strains and not in the wild type tau transgenic animals, possibly due to the different normal tau isoform used in the current study (fetal brain) (Brandt et al., 2009).

Another human tauopathy disease *in vivo* model was developed by Miyasaka and co-workers (2005) by expressing human wild type (0N4R and 0N3R) or FTDP-17 mutant tau ($^{P}301^{L}$ and $^{R}406^{W}$) in the six mechanosensory neurons of the body, using the *C. elegans mec-7* promoter. Mutant tau transgenic lines exhibited an age-dependent decline in the touch response, whereas wild type tau lines were only slightly affected. Immunohistochemical and ultrastructural analysis revealed abnormal neuronal morphology in the mutant tau-expressing animals (swellings of cell bodies with thinner processes), accumulation of phosphorylated tau and disorganized microtubules in the PLM mechanosensory neurons (Miyasaka et al., 2005). Furthermore, over-expression of human HSP70 in the mutant tau transgenic animals significantly improved their touch response, whereas over-expression of the mammalian GSK-3β kinase slightly exacerbated tau-induced neurodegeneration (Miyasaka et al., 2005), the latter observation being consistent with *Drosophila* studies (Jackson et al., 2002). Results from these models suggest that classical and RNAi based screening can reveal modifier pathways, that protein turnover is critical, and that microtubule transport may play a role in tauopathies.

Parkinson's disease

C. elegans α-synuclein models

Parkinson's disease (PD) is characterized by the progressive loss of dopaminergic neurons (DA) and the accumulation of protein inclusions in PD patient brains, called Lewy bodies (LB) (Lang and Lozano, 1998; Olanow and Tatton, 1999). The most abundant protein in LBs is α-synuclein (α-syn) which is normally found in presynaptic terminals and nuclei. Multiplications (duplication and triplication) or mutations of the *PARK1* locus that encodes α-syn result in familial PD (Conway et al., 2000; Polymeropoulos et al., 1997; Singleton et al., 2003). Genetic analysis in humans has identified eight additional PD loci: *PARK2*, *PARK5*, *PARK6*, *PARK7*, *PARK8*, *PARK9*, *PARK11* and *PARK13*. Six of these genes have *C. elegans* orthologs, despite the absence of a *C. elegans* α-syn ortholog (Harrington et al., 2010) (Table 1). Since LBs are the pathological hallmark of PD, a plethora of *C. elegans* PD models have been generated to examine α-syn toxicity.

Lakso et al. (Lakso et al., 2003) over-expressed human wild type (WT) and mutant (A53T) α-syn together with GFP using pan-neuronal (*aex-3*), dopaminergic (*dat-1*) and two motor neuron-specific promoters, *unc-30* and *acr-2*. Locomotion deficits (in swimming also known as thrashing) were caused by WT or A53T α-syn expression in all neurons or just motor neurons, but not in dopaminergic neurons. Interestingly, a significant loss of dopaminergic neurons (CEP, ADE and PDE) and their dendritic processes was observed in animals carrying WT or A53T α-syn constructs driven by *dat-1* and *aex-3* promoters, but not by the *acr-2* cholinergic motor neuron promoter (Lakso et al., 2003). This dopaminergic neuron toxicity was subsequently confirmed by Cao and co-workers (2005) using transgenic *C. elegans* over-expressing human α-syn in dopaminergic neurons, which resulted in age-dependent neuronal loss. The *dat-1*/WT α-syn animals were also used to demonstrate the neuroprotective role of the ER-associated protein with chaperone-like activity, TorsinA and Rab1A. The latter is a GTPase involved in ER-to-Golgi transport (Cao et al., 2005; Cooper et al., 2006). TorsinA is found in Lewy bodies in dopaminergic neurons and its perturbation causes early-onset torsion dystonia (Ozelius et al., 1997; Sharma et al., 2001). These studies suggest a link between α-syn toxicity and ER-Golgi vesicular trafficking.

Another *C. elegans* synuclein model found accumulation of α-syn in the cell bodies and neurites of dopaminergic neurons over-expressing wild type or mutant (A53T and A30P) α-syn, but no significant loss of CEP cell bodies (Kuwahara et al., 2006). Interestingly, a subset of WT, A53T and A30P transgenic worms showed positive immunoreactivity to phosphorylated α-syn at Ser-129, a characteristic of α-syn deposited in human Lewy bodies (Fujiwara et al., 2002). This *C. elegans* model was the first to report dysfunction in a previously described dopaminergic behavior, altered response to food, due to A53T and A30P α-syn over-expression in dopaminergic neurons (Kuwahara et al., 2006). A large-scale RNAi feeding screen was conducted in a neuronal RNAi supersensitive background (*eri-1*), by over-expressing α-syn WT or pathogenic mutant (A53T or A30P) constructs under the control of *unc-51* pan-neuronal promoter (Kuwahara et al., 2008). Knockdown of 4 out of 1673 neuronal genes enhanced α-syn-induced neurotoxicity in this model, implicating synaptic endocytosis (*apa-2*, *aps-2*, *eps-8* and *rab-7*). Subsequent studies with loss-of-function endocytosis genes (e.g. *unc-11*, *unc-26*, *unc-57*), pharmacological and immunohistochemical analysis supported the hypothesis that the endocytosis pathway impacts role of α-syn toxicity (Kuwahara et al., 2008).

As described above for polyQ aggregation, some research groups have developed *C. elegans* models that focus on protein misfolding by expressing α-syn::GFP (or YFP) fusion fragments in *C. elegans* body-wall muscles using the *unc-54* promoter (Hamamichi et al., 2008; van Ham et al., 2008). Hamamichi et al. screened 868 genes by RNAi which

were selected based on a bioinformatic analysis of PD-associated genes/pathways; the authors found twenty genes that enhanced α-syn::GFP age-associated aggregation. TOR-2 (*C. elegans* ortholog of TorsinA) was also co-expressed with α-syn in this model in order to maintain α-syn protein at a threshold level of expression. This analysis was subsequently extended to neurons; five genes were identified whose co-expression with α-syn provided significant neuroprotection in dopaminergic neurons (Hamamichi et al., 2008). For one of these genes, a subsequent study demonstrated that expression of the human ortholog, VSP41, which is a lysosomal trafficking protein, protected neuroblastoma cells from the toxic effects of the dopamine-specific neurotoxins 6-OHDA and rotenone (Ruan et al., 2010). Another genome-wide RNAi screen by van Ham and colleagues (2008) identified 80 genes, whose knockdown suppressed age-dependent α-syn::YFP aggregation in inclusions. Some of these genes have functions in vesicular transport, lipid metabolism and aging-pathways, previously suggested to play a role in PD pathogenesis. For three of these genes, *sir2.1*, *lagr-1* and *ymel-1*, their effect was independently confirmed by genetic deletion strains (van Ham et al., 2008). Both aforementioned studies (Hamamichi et al., 2008; van Ham et al., 2008) successfully identified modifiers that were not specific to other forms of aggregation, i.e. polyQ-dependent misfolding but failed to recover common genes, possibly due to technical differences.

C. elegans LRRK2 PD model

Mutations of the *leucine-rich repeat kinase 2* (*LRRK2*/*PARK8*) gene cause autosomal-dominant familial Parkinson's disease (Paisan-Ruiz et al., 2004; Zimprich et al., 2004). LRRK2 is a large protein of unknown function with multiple conserved domains (Zimprich et al., 2004). Pan-neuronal expression of human WT or mutant (G2019S) LRRK2 using the *snb-1* promoter resulted in preferential loss of dopaminergic neurons, consistent with selective sensitivity of dopaminergic neurons in PD (Saha et al., 2009). Furthermore, WT LRRK2 expression increased dopaminergic neuron survival upon rotenone- or paraquat-induced mitochondria stress. Similarly with this result, knockdown of the *C. elegans* LRRK2 ortholog (*lrk-1)* also sensitized animals against rotenone (Saha et al., 2009). The neuronal specificity of this and the aforementioned LRRK2 and synuclein models bodes well for dissection of PD pathological mechanisms in *C. elegans*.

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a progressive neuromuscular disorder and approximately 20% of familial ALS (FALS) cases are associated with mutations in the Cu/Zn superoxide dismutase 1 gene (*SOD1*) (Cudkowicz et al., 1997). Oeda et al. (2001) were the first to generate a *C. elegans* ALS model by introducing human wild type and FALS (A4V, G37R and G93A) *SOD1* constructs under the control of *hsp16-2* heat shock and *myo-3* muscle-specific promoters. No discernable changes in *C. elegans* survival or behavior were observed. However, mutant *SOD1* expression rendered animals more sensitive to paraquat-induced oxidative stress. Furthermore, oxidative stress significantly reduced the degradation rate of mutant SOD1 protein, the latter found to accumulate in the form of discrete aggregates when expressed in muscle cells (Oeda et al., 2001). In a subsequent study, pan-neuronal expression (*C. elegans snb-1* promoter) of a mutant form of SOD1 (G85R or G85R-YFP) did result in defective behavior that correlated with SOD1 aggregation (Wang et al., 2009b). This *snb-1*/G85R-YFP transgenic model was used in a genome-wide RNAi screen in a *eri-1;lin-15B* sensitized background to identify modifier genes. Eighty-one RNAi clones increased the number of SOD1 G85R-YFP fluorescent inclusions; the corresponding genes encode chaperone components, but also other unexpected genes, including TGF-β, SUMO and Topoisomerase I (Wang et al., 2009b). SOD1 toxicity was also examined by Gidalevitz and co-workers (2009) using wild type and mutant SOD1-YFP fusion proteins (G85R, G93A, 127X) expressed in *C. elegans* muscle cells by the *unc-54* promoter. Aggregates were only evident in *C. elegans* expressing the mutant SOD1 proteins (consistent with Wang et al.) and these were associated with limited toxicity in the muscle cells (assessed using phenotypic assays and visualization of actin filaments) (Gidalevitz et al., 2009). Interestingly, animals carrying the three SOD1 mutant transgenes displayed synthetic toxicity with the same unrelated temperature-sensitive (ts) mutants that enhanced polyQ aggregation described above (Gidalevitz et al., 2006). Overall, both studies (Gidalevitz et al., 2006, 2009) suggest that aggregation-prone proteins and mildly destabilized mutations compete for the limited cell folding machinery, which may be of significant import in neurodegenerative disease.

Spinal muscular atrophy (SMA)

Spinal muscular atrophy (SMA) is now the most common genetic cause of infant mortality in North America and is caused by diminished SMN protein function (Cartegni and Krainer, 2002; Crawford and Pardo, 1996; Feldkotter et al., 2002; Kashima and Manley, 2003; Lefebvre et al., 1997; Lorson et al., 1999; Pearn, 1978). In SMA patients, α-motor neurons in the spinal cord become dysfunctional or degenerate with atrophy of the corresponding muscles (Crawford and Pardo, 1996). The *C. elegans* genome harbors a single *smn-1* gene, which encodes a highly conserved SMN ortholog (Miguel-Aliaga et al., 1999). The deletion allele *smn-1(ok355)* results in SMN-1 loss of function and neuromuscular function deficits; few homozygous mutant animals reach adulthood (Briese et al., 2009). Neuronal, but not muscle, expression of *smn-1* rescues the *smn-1(ok355)* phenotype (Briese et al., 2009). We have initiated studies (RNAi genome-wide screen and behavioral assays) by using the loss-of-function *smn-1(ok355)* allele to explore the genetic circuitry affecting SMN neuromuscular function in *C. elegans*. Our preliminary data suggests that the TGFβ/BMP signaling pathway plays a role in SMN loss-of-function neuromuscular pathology, consistent with a previous report in *Drosophila* (Chang et al., 2008).

Conclusions/future perspectives

Identification of many human genes that cause or predispose us to neurodegenerative diseases has been enormously helpful in demystifying these crippling diseases, allowing us to ascribe toxic functions to specific proteins. However, many years of research have not yielded definitive insights into why these genes cause degeneration of specific neuron populations or why disease-associated proteins cause neuronal dysfunction and death. Explaining these conundrums would provide invaluable clues for therapy development. Genetic techniques in invertebrate model organisms have been used to delineate numerous conserved mechanisms including apoptosis, Ras/MAPK and other signaling pathways. It seems likely that a similar approach will expose the conserved pathways critical in neurodegenerative diseases that have eluded us. Forward and reverse genetic approaches in invertebrates have identified and confirmed many novel disease-associated genes/pathways that were not previously implicated in neurodegenerative disorders. These putative modifier genes are interesting candidates for further investigation in vertebrate models. Indeed, when vertebrate orthologs of invertebrate modifiers are tested, they almost invariably are found to impact neurodegeneration across species. This conservation is particularly advantageous as vertebrate models are often expensive and unwieldy for large-scale pathway identification and analysis.

Some of the pathways pertinent to neurodegenerative disease have already been revealed by analyses in *C. elegans* and other invertebrate models. It has become clear that cellular protein homeostasis and

chaperone function is critical in this pathology. Over-expression of specific chaperones can ameliorate neuronal toxicity and cellular death while loss of specific chaperones exacerbates or accelerates degeneration. Additionally, the effects of insulin-like signaling pathway on aging have helped scientists to explore the mechanistic link between aging and the cellular pathways that lead to induced-aggregate toxicity. Interestingly, mammalian miRNA regulation has recently been implicated in neurodegenerative disorders, such as PD, with the dopaminergic neuron-specific miR-133b being down-regulated in the mid-brain tissue from PD patients (Kim et al., 2007) and miR-7 inhibiting α-syn protein levels by directly binding to its 3'-untranslated region (UTR) mRNA (Junn et al., 2009). A recent study (Asikainen et al., 2010) identified twelve miRNAs that were differentially expressed in the dopaminergic *C. elegans* neurons expressing mutant (A53T) α-syn, suggesting that miRNA regulation in PD pathogenesis may be conserved across species and hence, indicating an fruitful field for future research.

Though not described herein, a number of studies have utilized *C. elegans* neurodegenerative disease models to assess the efficacy of pharmacological compounds. Resperine, an FDA approved antihypertensive drug, enhanced longevity, stress tolerance and delayed paralysis in the *unc-54*/Aβ_{1-42} animals (Arya et al., 2009). Furthermore, the *Gingko biloba* leaf extract Egb 761 and its component, ginkgolide A, were tested in all *C. elegans* AD models and were found to alleviate the Aβ-pathological behaviors, including paralysis, reduced chemotaxis, and serotonin hypersensitivity (Wu et al., 2006). Voisine and co-investigators (2007) identified two FDA approved drugs, lithium chloride and mithramycin, that independently and in combination suppressed polyQ-induced neurotoxicity. Despite the technical limitations (i.e. effective compound dose, penetration and bioavailability) that could complicate the use of *C. elegans* models, it is clear that many of the models described above could be used for medium and high-throughput drug screening technologies across multiple genetic backgrounds. Combining invertebrate genetic studies with drug screening may be the most rapid approach possible in identifying pathological pathways and developing therapies for neurodegenerative disease.

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