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MINIREVIEW

Insights from *Caenorhabditis elegans* on the role of metals in neurodegenerative diseases†

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Neurodegeneration is characterized by the cell death or loss of structure and/or function of neurons. Many neurodegenerative diseases including Parkinson's disease (PD) and Alzheimer's disease AD) are the result of neurodegenerative processes. Metals are essential for many life processes, but they are also culpable for several neurodegenerative mechanisms. In this review, we discuss the role of metals in neurodegenerative diseases with emphasis on the utility of *Caenorhabditis elegans* (*C. elegans*) genetic models in deciphering mechanisms associated with the etiology of PD and AD.

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I. Introduction

Neurodegenerative diseases are becoming increasingly more prevalent as the previous generations age and life expectancies increase. Neurodegeneration is a component of Parkinson's disease (PD) and Alzheimer's (AD) disease, as well as diseases of the motor neurons characterized by different pathological hallmarks. PD is a progressive, neurodegenerative disorder afflicting ~2% of the US population.[1] Characteristic features include a gradual loss of motor function due to the degeneration of dopaminergic (DAergic) neurons within the substantia nigra (SN) pars compacta (SNpc) and loss of DAergic terminals in the striatum.[2] The majority of PD

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cases are sporadic in nature with unknown etiology.[3] However, familial early onset PD (with symptoms commencing as early as the 20s) is well described, with both autosomal dominant and autosomal recessive inheritance patterns. The mechanisms underlying the selective degeneration of DAergic neurons are poorly understood; however, genetic factors and environmental and endogenous toxins have been implicated.[4] Epidemiological studies suggest that PD is more common in rural areas, where the increased prevalence is associated with the use of pesticides, herbicides, and heavy metals.[5] Despite the burgeoning knowledge of genetic factors which hasten SN degeneration, there is a dearth of information about the gene-environment interface that sets the stage for the exquisite vulnerability of SN. AD is characterized by senile plaques composed of amyloid-beta peptide, neurofibrillary tangles composed of hyperphosphorylated tau proteins, along with memory deficits leading to profound dementia.[6] AD affects approximately 25 million people worldwide[7] and an estimated 5.3 million Americans.[8] Putative risk factors include genetics, head trauma, lower education, oxidative stress and brain metal accumulation.[9,10] Some studies indicate an autosomal dominant mode of inheritance for familial forms of AD, although the majority of AD cases are more complex and do not follow simple Mendelian genetics.[11] A longitudinal study from Manitoba showed that occupation exposure to environmental toxins such as pesticides, fertilizers, fumigants and defoliants was associated with an increased risk of AD.[12]

While genetics is an important determinant of predisposition to PD and AD, described in a number of reports summarized herein, other factors, such as exposure to environmental contaminants can underlie or exacerbate the pathology and neurodegeneration can be driven by both. The influence of each of these factors can vary, but extensive cell death remains the toxicological hallmark. Improvements in the diagnosis of neurodegenerative disease have enhanced the understanding of the pathology and the relationship between genetics and risk susceptibility. However, the molecular mechanisms underlying neurodegenerative diseases remain elusive. Metals are persistent environmental contaminants that are essential for brain metabolic processes, but the occurrence of metal dysregulation in the brain has been described by a vast literature as being associated with neurodegenerative disease (most recently reviewed in ref. 9 and ref. 13). These reports have ranged from reduced metal supplementation to exposure to toxic doses and have been described in normal ageing, as well as in various disease states.[14] The most studied neurodegenerative diseases in terms of metal-induced etiology are PD and AD.

Some of the most compelling evidence for the contribution of metals to neurodegeneration comes from studies of postmortem tissue implicating metal accumulation in the areas of the brain coincident with cell death in patients with confirmed neurodegenerative disease.[15,16] Although this data is correlational, given that metal accumulation may not reflect a primary mechanism but rather result from the disease itself, these findings are nevertheless intriguing. The mechanism by which metals produce neurodegenerative damage is metal- and dose-dependent; however, they share common mechanisms including free radical production, protein aggregation, bioenergetic dysfunction, calcium dysregulation and metal transport alteration,[17–19] and most likely a combination of these factors ultimately triggers the neurodegenerative process. Based on the association between metals and neurodegenerative disease, the *C. elegans* model emerged as an important tool that allows researchers many advantages in studying neurodegenerative processes.

Some of the advantages afforded by the *Caenorhabditis elegans* (*C. elegans*) model system are small size (~1.5 mm adult), short lifespan (~3 weeks) and rapid lifecycle (~3 days) with worm life cycle starting at hatching (L1 phase), and proceeding after ~12 h to the L2 phase, another 8 h to the L3 stage, and a final round of molting yielding the L4 stage and, after 10 h, young adulthood (~900–940 μm).[20,21] Once they reach adulthood they are capable of laying eggs. Single *C. elegans* hermaphrodites are capable of producing ~300 progeny. The nematode's small genome and anatomical simplicity (less than 1000 cells), including the well-characterized 302 cell hermaphrodite nervous system[22] contribute to the appeal of this model system for genetic manipulation. *C. elegans* are especially powerful as a model system for neurodegeneration as their translucent body allows for *in vivo* visualization of individual neurons labeled with fluorescent probes, as well as any puncta or breaks that may occur in neuronal processes. Many *C. elegans* models have typically described neurodegeneration as strand breaks, formation of puncta and loss of cell bodies (Fig. 1). *C. elegans* are therefore a powerful *in vivo* model system for studying neurodegenerative disease and particularly amenable to studying gene-environment interactions. Sharing homology to mammalian system, *C. elegans* have ~60–80% of human genes,[23] ~40% of human disease-related genes[24] and conserved neurotransmitter systems.

Various heavy metals have been associated with neurodegenerative disease; aluminium (Al), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), mercury (Hg), and zinc (Zn) are highlighted here. In this review we give a brief overview of the ways in which the powerful *C. elegans* model has provided us with insights into the involvement of metals in PD, AD and other types of neurodegeneration.

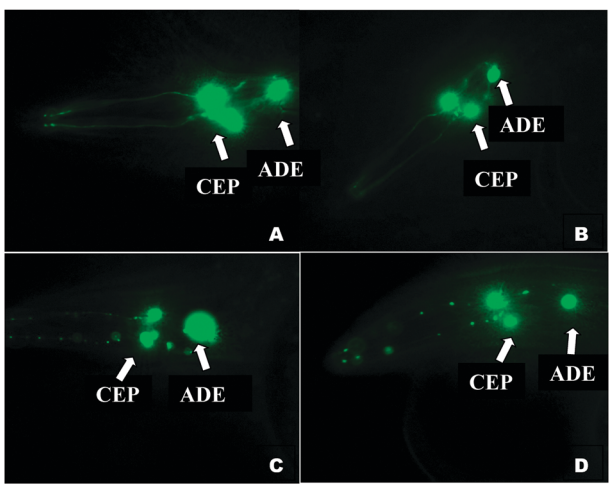

Fig. 1 Degeneration of dopaminergic neurons of transgenic worms expressing Pdat-1::GFP exposed for 30 min to Mn (A) 0 mM; (B) 35 mM; (C) 50 mM; (D) 100 mM. Arrows point to the four cephalic (CEP) neurons, two anterior deirid (ADE). Presence of puncta and strand breaks are visible in CEP neurons at 50 mM and 100 mM doses after 30 min exposure to Mn.

II. Parkinson's disease

Parkinson's disease is the second most common neurodegenerative disease and is characterized by the progressive loss of DAergic neurons within the SN and locus ceruleus, manifesting as muscle rigidity, tremors and bradykinesia. Cellular hallmarks of PD also include the accumulation of proteinaceous intracellular inclusions named Lewy bodies in surviving DAergic neurons. However, some patients lack Lewy bodies, which suggests that the etiology of the disease may involve a complex array of inherited and environmental factors.[25–27]

DA modulates movement, defecation, egg-laying and food sensation in the worm.[28,29] Of the 302 neurons, eight are DAergic in the hermaphrodite: six anterior DAergic neurons (4 cephalic –CEP- and two anterior deirid -ADE neurons) and two posterior neurons (PDE).[30] Additional neurons were noted in the male nematode, specifically in six rays of the male tail, referred to as the R5A, R7A and R9A pairs of neurons. Laser-assisted targeting of the DAergic system disrupted area-restricted searching behaviors employed by nematodes in locating food, thereby demonstrating that this behavior depends on DAergic signaling.[31] The presence of DA in *C. elegans* extracts, including its precursors and metabolites, have been confirmed,[32] and DA levels in synaptic vesicles are similar to those in mammalian neurons.[33] Additionally, all necessary machinery for DA synthesis, storage, release, transport and binding has been shown in *C. elegans*.[29,32,34] The discovery that application of exogenous DA inhibits locomotion and egg laying in this species[28] and the fact that DAergic homeostasis in the worm is very similar to mammals' led to the use of this model to study PD.

IIa *C. elegans* models of PD

C. elegans expresses orthologs for several human genes linked to familial PD, such as Parkin, PARK 9, UCHL-1 (ubiquitin carboxy-terminal hydrolase L1), DJ-1, NURR1 (NUR-Related factor 1), PINK1 (PTEN-INduced Kinase 1) and LRRK2 (leucine-rich repeat kinase 2), but lacks α-synuclein.[35–40] It has been demonstrated that mutations within the *Parkin* gene, an E3 ubiquitin protein ligase that is involved in proteasomal degradation of damaged proteins, has also been associated with an increase in risk for developing PD.[41] Similarly, *pdr-1* knockdown in worms leads to reduced levels of high molecular weight ubiquitin conjugates, indicating that the ubiquitin proteasome system (UPS) is affected in worms.[40] Mitochondria are also a target in PD models in worms since the genetic modulation of *parkin* (*pdr-1*), *djr-1*, *pink-1* and *lrk-1* (the gene that encodes LRRK2) disrupts mitochondrial function in *C. elegans*.[39,40] In addition, pink-1 knockdown causes reduced mitochondrial cristae length in neuronal cells and affects axonal outgrowth of a pair of canal-associated neurons, whereas *djr-1* mutants have decreased oxygen consumption and survival after exposure to rotenone.[39]

Using a microRNA microarray approach to identify potential gene expression changes associated to PD in *C. elegans*, Asikainen and colleagues reported co-regulation of some new genes in different PD models. The models included overexpression of human A53T α-synuclein or mutation in the *cat-1* or *pdr-1* orthologs. The study revealed that microRNAs such as miR-64 and miR-65 family are co-underexpressed in the α-synuclein transgenic and *cat-1* strains, and members of the *let-7* family were co-underexpressed in the α- synuclein and *pdr-1* strains, suggesting that these miRNAs may also have a role in the disease pathogenesis.[42]

C. elegans does not normally express α-synuclein. Expression of wildtype (wt) human α-synuclein in *C. elegans* increases vulnerability to mitochondrial complex-I inhibitors (rotenone, fenperoximate, pyridaben, and stigmatellin), which is reversed by treatment with antioxidants.[40] Live imaging of worms expressing wt α-synuclein in the body wall muscles indicated that there is an increase in mitochondrial fragmentation. Similarly, the investigators found mitochondrial fragmentation in neuronal cell bodies, indicating that α-synuclein leads to mitochondrial damage in a tissue-independent manner.[43]

Wang and colleagues reported that association of α-synuclein expression and MPP+ (a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine MPTP metabolite) caused DA cell death and α-synuclein aggregation and decreased ATP levels.[44] Similarly, studies of 6-hydroxydopamine (6-OHDA) exposure in α-synuclein transgenic worms indicated increased DAergic degeneration, and VPS41, a lysossomal trafficking protein, can rescue the DA cell loss.[45] In humans, there is strong evidence that this protein may be relevant to α-synuclein-related diseases, as lysossomal failure is associated with an increase in α-synuclein toxicity and age of onset of PD is correlated with this toxicity.[46]

Chaperones such as heat shock protein 70 (Hsp70) modulate the pathological conversion of misfolded proteins into cytotoxic species by avoiding misfolding.[47] The Hsp70/α-synuclein complex that is formed in *C. elegans* transgenic worms is structurally compact and necessary for anti-amyloid activity, stimulating the formation of amorphous aggregates and therefore protecting the neurons form neurodegeneration. Studies using worms demonstrated *in vivo* that the ability of Hsp70 to inhibit the aggregation of α-synuclein depends on factors such as nucleotide binding and the presence of the Hip co-chaperone.[48]

Most models for DAergic neurodegeneration in *C. elegans* are based on exposure to the neurotoxins, MPTP and 6-OHDA, which chemically ablate DAergic neurons.[49,50] Metals such as Mn, vanadium(v) and Hg can also affect these neurons (Fig. 1) by disrupting mitochondrial function in DAergic neurons, resulting in energy depletion, increased ROS production and cell death by apoptotic and/or necrotic pathways.[35,51,52] In this review, we will focus on exposures to Mn and MeHg as models for DAergic degeneration and their relation to PD in *C. elegans* (Table 1). The other models were reviewed by several authors.[44,53,54]

IIb Metals and PD

Mn exposure from the environment or in occupational settings such as mining and smelting can cause an extrapyramidal syndrome that resembles PD. The symptoms include rigidity, tremor, gait disturbances and hypokinesia.[55–57] These effects are attributed to a selective interaction of Mn with the basal

ganglia downstream of the nigrostriatal DAergic projection, areas that have the propensity to accumulate Mn.[55,58] Among several toxic mechanisms, such as mitochondrial dysfunction and astrogliosis,[59] Mn can produce the highly toxic reactive metabolite leukoaminochrome o-semiquinone through oxidization of DA.[60,61]

For this reason, Benedetto and colleagues investigated the effects of intra- and extracellular DA in worms exposed to Mn *in vivo*.[62] This study revealed that Mn caused a dose-dependent degeneration in DAergic neurons and that this neurodegeneration required the presence of the reuptake transporter, DAT-1. Mn-induced DAergic GFP-fluorescence loss in *dat-1*;Pdat-1::GFP worms was not seen in *dat-1* knockdown worms. In accordance, worms lacking DAT-1 displayed higher susceptibility to Mn toxicity, whereas this toxicity was prevented by the loss of tyrosine hydroxylase (TH)/CAT-2 function in the double knockout strain, *cat-2*(*e1112*);*dat-1*(*ok157*). Furthermore, it was demonstrated that the absence of vesicular monoamine transporter (VMAT2)/CAT-1 in *cat-1*(*e1111*) mutants, in which DAergic neurons were unable to release DA at the synaptic cleft, resulted in an increased tolerance to Mn exposure. These findings indicate that DA synthesis is required for DAT-1- dependent Mn toxicity and that extracellular DA, and not intracellular DA, is involved in Mn neurotoxicity.[62] Interestingly, the neurotoxic effects were specific to DAergic neurons, and absent from γ-gaminobutyric acid (GABA)ergic, serotononergic or glutamatergic neurons.[62]

The oxidative stress factor is significant in Mn-induced DAergic degeneration in *C. elegans*. Benedetto and colleagues demonstrated that there is extracellular Mn-induced oxidation of synaptic DA, generating ROS thus causing lipid peroxidation.[62] Two other proteins related to antioxidant/oxidant homeostasis were found to be related to Mn-induced neurotoxicity: SKN-1 and BLI-3. SKN-1 is the ortholog of the mammalian NRF-2 (nuclear factor-2 erythroid 2-related factor-2),[60] which loss-of-function caused increased sensitivity to Mn toxicity. On the other hand, wt worms expressing SKN-1::GFP showed translocation of the protein to the nucleus in the ASI neurons, indicating that the protein can induce the expression of antioxidant proteins in response to Mn exposure.[62] BLI-3 is a dual oxidase which is involved in di-tyrosine bond formation in the worm cuticle and pathogen-induced ROS production.[63,64] Loss of function of *bli-3* gene caused hyper-resistance to Mn toxicity and no increase in ROS production from 1 mM to 30 mM Mn exposures.[62] This finding indicates that BLI-3 is required for ROS-associated effects in Mn exposure, potentiating ROS production and oxidative stress.

As in mammals, the sensitivity of DAergic neurons to Mn in *C. elegans* may be attributed to the presence of SMF metal transporters that belong to the NRAMP/divalent metal transport family. The tissue localization of these transporters was determined by the fusion of the three isoforms, SMF-1, SMF-2 and SMF-3 to the green fluorescent protein (GFP), indicating distinct localization and, consequently, distinct roles in relation to Mn uptake.[65] Settivari and colleagues demonstrated that SMF-1 and SMF-2 are expressed *ex vivo* in *C. elegans* DAT-1-expressing neurons, and the deletion of the *smf-1* gene attenuated the DAergic neurodegeneration caused by Mn.[66] Loss-of-function of *smf-1* and *smf-3* caused a significant decrease in Mn accumulation and consequently increased survival after exposure to the metal.[65]

The importance of extracellular DA levels for Mn toxicity in *C. elegans*, if corroborated in human studies, will bear important applications for the treatment for DAergic neurodegeneration. For instance, the use of L-DOPA treatment, although compensating for DA loss, may accelerate or exacerbate DAergic degeneration over a long term. For this reason, modulators of extra- and intracellular DA levels may be a good strategy against Mn-toxicity. If the involvement of dual-oxidases in DA oxidation is confirmed in vertebrates and if L-DOPA is shown to be easily oxidized by dual-oxidases, it would be pertinent to investigate and design alternative DA analogues that have high affinity for DA receptors and DAT, but are poor substrates for dual-oxidases.

MeHg poisoning is characterized by severe neurological deficits due to brain lesions and disruptions of neurotransmitter systems.[67] Major routes of MeHg exposure include seafood consumption, because of the global cycling and accumulation of Hg from industries that reaches the aquatic environment.[68] In *C. elegans*, MeHg causes severe toxic effects such as decreased survival, developmental delay and decreased pharyngeal pumping.[69,70]

MeHg readily causes oxidative stress in *C. elegans* model as indicated by the alterations in glutathione (GSH) levels and by increased expression of heat shock proteins (HSP) and glutathione-S-transferase (GST).[69] In mammals' glial cells, it was recently reported that MeHg increases the mitochondrial translocation of the transcriptional factor Nrf2, thus increasing the expression of antioxidant genes, whereas knockout of Nrf2 greatly reduced the upregulation of these genes, increasing MeHg toxicity.[71] Using this approach, VanDuyn and colleagues demonstrated how important SKN-1/Nrf2 pathway is to MeHg neurotoxicity in *C. elegans*.[72] Reduction in SKN-1 gene expression led to DA neuron degeneration in 30% of the worms exposed chronically to 1 μM of MeHg, whereas no degeneration was observed in wild type animals, indicating that SKN-1 inhibits MeHg DA neuronal degeneration.[72] Notably, SKN-1 is present in DA neurons, as demonstrated *in vivo* and *in vitro* using DA cultured neurons obtained from worms.[72] These studies help to elucidate mechanisms of toxicity and neuroprotection; however further studies are necessary to understand several other pathways that may be involved in these processes.

III. Alzheimer's disease

Alzheimer's disease (AD) is a progressive, neurodegenerative disorder that presents as the most common form of dementia worldwide. The pathological hallmarks of AD include the presence of neurofibrillary tangles and senile plaques, produced from the aggregation of the microtubule-associated tau protein and the β-amyloid peptide (also known as Aß), respectively. More specifically, phosphorylated tau protein normally functions to stabilize microtubules and provides a support and transport system for neuronal survival. In AD cases, however, tau becomes abnormally hyperphosphorylated,

leading to the formation of tangles with extraneous microtubules that cause collapse of the neuronal transport network.[73] Meanwhile, the transmembrane amyloid precursor protein (APP) is cleaved to result in the deposition of dense, extracellular plaques of toxic Aß fibrils around neurons.[74] Nevertheless, the mechanisms behind how such alterations in protein production and folding actually induce neurodegeneration are still poorly understood.

IIIa *C. elegans* models of AD

Various genetic models have been generated in an attempt to enhance our understanding of the disease mechanisms behind AD (Table 2).[75] For example, mutations in the *APP* gene have been linked to a familial, early-onset form of AD. Moreover, *APLP1* and *APLP2* are additional genes related to familial AD that encode for two APP-related proteins (amyloid precursor-like proteins). These APP-related proteins, however, do not contain the Aß peptide, as APP does.[76] *C. elegans* only contains one ortholog of the mammalian *APP* gene, *apl-1*. This gene encodes for a single-pass transmembrane domain protein that contains many conserved domains. Similar to APLP1 and APLP2, APL-1 in worms also does not contain the Aß peptide.[77] Deletion of *apl-1* results in 100% larval lethality,[78] while RNAi knockdown of *apl-1* produces animals with a reduced body size.[79] Other genetic models of AD in *C. elegans* incorporate mutations in worm orthologs of the presenilin proteases responsible for cleaving APP. One such mutation is in the *sel-12* PSEN gene in *C. elegans*, which can cause deficits in thermal memory necessary for proper thermotaxis that is dependent on the activity of AFD sensory neurons and AIZ and AIY interneurons. Interestingly, *sel-12* mutants exhibit axonal abnormalities in AIY neurons that produce acetylcholine, with cholinergic neurons thought to show increased vulnerability in AD.[80] Moreover, additional models of AD involve genetic mutations in the *MAPT* gene that encodes for the tau protein. Although *C. elegans* does not contain any direct orthologs of tau, transgenic worm lines have been produced that overexpress both human wildtype and mutant forms of tau. One transgenic line was created from the introduction of a "pseudohyperphosphorylated" (PHP) human tau mutation into *C. elegans* to simulate the modifications of tau phosphorylation states in AD.[81] These PHP tau transgenic worms exhibit impaired inhibitory motor neuron development that is associated with an age-dependent uncoordinated (Unc) movement phenotype. Moreover, human tau mutations associated with the tauopathy FTDP-17 (frontotemporal dementia with parkinsonism chromosome 17 type) have also been introduced into *C. elegans*, with similar Unc locomotion, impaired cholinergic transmission, and GABAergic axonal degeneration arising from the accumulation of insoluble tau.[82] Moreover, a series of *C. elegans* AD models began with the development of strains expressing human Aβ driven by muscle-specific promoters. The progressive paralysis phenotype in these AD models arises from Aβ aggregation and toxicity in the worms.[83] Nevertheless, the most accurate model of AD would be a triple transgenic presenilin-APP-tau mutated mouse model,[84] which has not yet been generated in *C. elegans*.

IIIb Metals and AD

In addition to the genetic risk factors associated with AD, altered metal homeostasis has also been implicated in its pathophysiology. The link to metal toxicity arises from the evidence of metal enrichment within the senile plaques seen in AD cases, including copper (Cu), zinc (Zn), iron (Fe), and aluminium (Al).[13] Zn was found specifically to be elevated in AD neuropil compared to control subjects, while Cu levels were significantly elevated in the rims of senile plaques.[85] Cu, Zn, and Fe have also been found to either directly or indirectly cause conformational changes in Aß that stabilize it in its most toxic oligomeric form, in addition to enhancing Aß-induced oxidative stress.[86] Controversial findings, however, have clouded the contribution of these metals to the neurodegeneration seen in AD, a topic that has been thoroughly reviewed elsewhere.[13,87] Additional evidence linking the contribution of metal toxicity to AD pathophysiology is derived from studies on the colocalization of the divalent metal transporter DMT1 with Aß plaques in postmortem brain samples from AD patients.[88] DMT1 is a key transporter for various divalent metals, including AD-associated metals like Zn, Fe, and Cu.[89]

The use of *C. elegans* as a model to explore metal toxicity in AD has focused primarily on Cu neurotoxicity and its relationship to APP. This emphasis on Cu has emerged from evidence of a Cu-binding domain (Cu–BD) found within the N-terminal region of APP between residues 135 and 156. This binding site contains two crucial histidines that are conserved in APP-related protein APLP2.[90] The CuBD not only binds Cu, but also strongly reduces it from Cu(II) to Cu(I), potentially allowing for the production of damaging reaction oxygen species (ROS) from the redox reaction's transfer of electrons.[91] Meanwhile, Cu and Zn binding sites are also found in APP's carboxy-terminal that contains Aß. Furthermore, overexpression of this Aß-containing fragment leads to significantly decreased Cu and Fe levels that correspond with increased Mn levels.[92] These results implicate Aß and APP as regulators of metal homeostasis, with aggregations of Aß potentially disturbing proper metal levels in AD. Furthermore, the CuBD of *C. elegans* APL-1 injected into rat dorsal hippocampus was sufficient in conferring protection against Cu^{2+} toxicity *in vivo* by enhancing Cu^{2+} uptake, showing a direct regulation of extracellular Cu^{2+} levels.[93] More specifically, the tyrosine residue at position 147 and the lysine residue at 151 in the *C. elegans* APL-1 CuBD are responsible for this protection against Cu^{2+}-mediated toxicity.[94]

Moreover, *C. elegans* strains overexpressing Aß in their muscle cells are affected by particular point mutations (V18A, E22G) that result in decreased intracellular amyloid aggregation compared to wildtype Aß. Exposure of Cu^{2+}, however, enhances acceleration of wild type Aß aggregation in worms. Remarkably, the Aß transgenic worms show decreased sensitivity to toxic $CuCl_2$ exposures (150–450 μM) compared to control worms, despite evidence of increased amyloid deposits upon Cu^{2+} exposure.[95] These results illuminate a complex and dynamic relationship between copper homeostasis and the role of Aß in *C. elegans*, with

Table 1 *C. elegans* models of Parkinson's disease and contribution of metals

Function/phenotype	Genotypes studied	Major findings/implications	References
Pink-1 knockdown	*pink-1*(*tm1779*)	Reduced mitochondrial cristae length in neuronal cells and affects axonal outgrowth seen in pink-1 mutants rescued by overexpression of lrk-1	39
Lrk-1 overexpression (Gene that encodes LRRK2)	P_{lrk-1}::*lrk-1*::*gfp*		
djr-1 loss of function	Knockout of B0432.2	Decreased oxygen consumption and survival after rotenone exposure	40
A53T α-synuclein overexpression	aex-3::α-synuclein A53T; dat-1::gfp	MicroRNAs miR-64, miR-65 and let-7 underexpressed indicating that they may play a role in disease pathogenesis	42
cat-1 loss of function	*cat-2*(*e1111*)		
pdr-1 loss of function	*pdr-1*(*gk448*)		
Wt α-synuclein	*Transgenic worms expressing Prab-3*::α*S-yfp or Pmyo-3*::α*S-yfp*	Increase in mitochondrial fragmentation	43
Hsp70	zgIs15[P(unc-54)::α-synuclein::YFP fed with ds hsp-70	The ability of Hsp70 to inhibit aggregation of α-synuclein dependent on nucleotide binding and presence of Hip co-chaperone	48
Manganese (Mn)			
Reuptake transporter DAT-1 loss of function mutant	*dat-1* (*ok157*)	Loss of function abolished the Mn-induced DAergic GFP-fluorescence;higher susceptibility to Mn toxicity	62
Tyrosine Hydroxylase (TH)/CAT-2 loss of function double knockout (VMAT2/CAT1)	*cat-2*(*e1112*);*dat-1*(*ok157*)	DA synthesis is required for DAT-1-dependent Mn-induced toxicity; Mn toxicity was prevented	
Absence of vesicular monoamine transporter; DAergic neurons unable to release DA at the synaptic cleft	*cat-1*(*e1111*)	Increased tolerance to Mn exposure; Extracellular DA, not intracellular DA is involved in Mn toxicity	
Loss of function of the *skn-1* gene	*skn-1*(*zu67*) *IV*/*nT1*	Increased sensitivity to Mn; Wildtype worms expressing SKN-1:GFP showed translocation of the protein to the nucleus in ASI neurons, indicating SKN-1 can induce expression of antioxidant proteins in response to Mn exposure.	
Loss of function of *bli-3* gene	*bli-3*(*e767*)	Hyper-resistance to Mn toxicity and no increase in ROS production; BLI-3 is required for ROS-associated effects in Mn exposure; potentiating ROS production and oxidative stress.	
Divalent metal transporter (DMT)	SMF-1:GFP SMF-2:GFP	SMF-1 and SMF-2 found to be expressed *ex vivo* in DAT-1 expressing neurons; Deletion of smf-1 gene attenuated the DAergic neurodegeneration caused by Mn; Loss of function of smf-1 and smf-3 caused significant decrease in Mn accumulation and survival	65
	SMF-3:GFP		
	smf-1 (*eh5*)		
	smf-2 (*gk133*)		
	smf-3(*ok1035*)		
Methylmercury (MeHg)			
Reduction of skn-1	*rrf-3*(*pk1426*) fed with ds *skn-1*	DAergic neurodegeneration in 30% of worms	72

metal-induced changes on the aggregation state of Aß being coupled with protection against Cu^{2+} toxicity from the aggregates themselves. This expression of human Aß in *C. elegans* muscle cells, however, was not found to be in its full-length form of 1–42 amino acids. Rather, a 3–42 truncated Aß product is being expressed, which self-aggregates faster *in vitro* than $Aß_{1-42}$ and is enhanced by the presence of Cu^{2+}.[96]

In regards to Aß-induced toxicity in *C. elegans*, several studies have investigated potential therapeutic drugs that can ameliorate this toxicity. The VMAT inhibitor reserpine,[97] the antidepressant fluoxetine,[98] and tetracyclines[99] have been found to protect against Aß toxicity in worms, with an increase in thermal stress resistance and lifespan[97,98] and decreased oxidative stress.[99] Furthermore, few studies have addressed strategies to counter metal-induced toxicity in *C. elegans* AD models. For example, the Cu chelators histidine and the more potent clioquinol have been shown to diminish the formation of intracellular Aß deposits compared to Cu-treated and untreated control *C. elegans*.[95] These worms, however, were treated with the Cu-chelators continuously from the embryo stage. Thus, it remains to be seen whether treatment with these Cu-chelators later in life could diminish already-established amyloid aggregates in *C. elegans*.

IV. Non-disease specific neurodegeneration

Other neuronal subtypes, not associated with a specific disease, have also been shown to degenerate following metal exposure. Xing and colleagues investigated neuronal loss in

the GABAergic nervous system and synaptic functions after lead (Pb) and mercury (Hg) exposure at different developmental periods in the worm.[100] In these studies neurodegeneration was defined as number of ventral and dorsal cord gaps in addition to neuronal loss of cell bodies. Exposure to Pb and Hg at 2.5, 50 and 100 μM concentrations for 6 h caused a significant increase in neuronal loss compared to controls at the L1 through L3 larval stages. Exposure to Pb and Hg at concentrations of 50 μM and 100 μM at the L4 larval stage and at a concentration of 100 μM at the young-adult stage induced an obvious elevation of GABAergic neuronal loss. Their data suggested that younger nematodes are more sensitive to Pb and Hg-induced neurotoxicity than older nematodes. In addition, they showed deficits in neuronal survival and synaptic function after exposure. The mechanism of which has yet to be revealed.

Table 2 *C. elegans* models of Alzheimer's disease and contribution of metals

Function/phenotype	Genotypes studied	Major findings/implications	References
APL-1 deletion mutant *APL-1* overexpression/ APL-1::GFP	*Apl-1*(*yn10*) *ynIs79*	Larval lethality, defects in molting and morphogenesis Decreased brood size, movement defects (reduced thrashing, head bends), and developmental delay. Partially rescued by loss of *sel-12* (*Presenilin 1* homolog) activity.	78
APL-1 promoter-driven GFP/APL-1 expression *APL-1* knockdown	*apl-1*::*gfp*::*unc-54* *apl-1*(*RNAi*)	APL-1 expression was temporally expressed in seam cells, known to be involved in the molting process. Reduced body size, with some worms exhibiting L4 molting problems.	79
Sel-12 deletion mutants *Sel-12* deletion mutants, expressing neuron-specific GFP transgenes	*sel- 12*(*ar131*) *and sel-12*(*ar171*) Same as above, but crossed with neuron-specific lines: AFD–*gcy-8*::*GFP*, AIZ–*lin-11*::*GFP*, AIY–*ttx-3*::*GFP*	Exhibit thermotaxis defects. No obvious defects in AIZ and AFD neurons, but AIY axonal abnormalities seen in *sel-12* mutants compared to wild-type worms.	80
Human WT and pseudo-hyperphosphorylated (PHP) tau expression	WT & PHP tau strains crossed with *oxIs12*[*unc-47*::*GFP*] line (inhibitory motor neuron control)	Both WT and PHP Tau strains showed movement defects (reduced thrashing) compared to purely wild-type worms. No change in motor neuron viability. However, mutant PHP tau worms exhibit altered motor neuron development (dorsal cord gaps) compared to WT tau or purely wild-type worms.	81
Normal and FTDP-17 mutations-related *MAPT* (encodes tau) mutants	Normal or FTDP-17 mutant *MAPT* lines, also crossed with *unc25*::*GFP* (GABA motor neurons)	Uncoordinated movement, altered presynaptic cholinergic transmission in tau mutants. Strains crossed with *unc25*::*GFP* exhibit GABA motor neuron-specific axonal degeneration (dorsal and ventral cord gaps). FTDP-17 tau mutants show more severe phenotype *vs.* normal tau-expressing worms.	82
Copper (Cu)			
APL-1_{CuBD} (synthetic peptide)	$APP_{135–156}$ *C. elegans*	The CuBD of APL-1 in worms was injected into rats, and used in cell culture. Confers protection against Cu^{2+} neurotoxicity and lipoprotein oxidation by binding and increasing Cu^{2+} uptake.	93,94
Human Aß overexpression	CL2120 (Aß1-42 driven by *unc-54* promoter), CL2122 (minus Aß1-42)	These worms form intracellular amyloid deposits specifically in their muscle cells, driven by the *unc-54* myosin heavy chain promoter. WT Aß worms exhibit heightened resistance to Cu^{2+} toxicity due to increased amyloid aggregates, with a longer lifespan. Cu^{2+} chelators histidine and clinoquol both decrease the number of amyloid deposits from the 96 h timepoint compared to untreated controls.	95
Aß mutants Artic and NIC	E22G, V18A point mutations in strain CL2120	Decreased intracellular amyloid deposition compared to WT Aß. No mention of Cu^{2+} toxicity in Aß point mutants.	
Human WT Aß overexpression	CL2006 (*unc-54*/Aβ 1-42), CL2120	Aß3-42 (*vs.* 1–42) is actually being expressed in these transgenic Aß worms, with Cu^{2+} enhancing its self-aggregation.	96
Human WT Aß overexpression	CL2006	Aß toxicity results in paralysis in worms, which is significantly delayed by reserpine (VMAT inhibitor) administration. Reserpine also increases thermal resistance and lifespan.	97
DAF-16::GFP (Insulin signaling)	TJ356	Fluoxetine promotoes translocation of DAF-16 from the cytosol into the nucleus.	98
Human WT Aß overexpression	CL2006	Fluoxetine delays the paralysis induced by Aß toxicity, along with decreasing Aß olimers.	
Heat-inducible human WT Aß muscle expression	CL4176	Tetracylines protect from Aß-induced paralysis by inhibiting Aß oligomerization and decreasing superoxide production.	99

V. Conclusions

In summary, various genetic strains of *C. elegans* offer a unique perspective on neurodegenerative processes and their etiology. By taking experimental advantage of these models we have gleaned insight into the role of metal transporters and metal homeostasis in the etiology of neurodegenerative disease and tested the efficacy of potential therapeutics in attenuating neurodegenerative changes. The *C. elegans* model has proven invaluable in delineating the importance of genetic perturbations in the etiology of metal-induced neurodegeneration, providing a highly relevant model for studies on the interaction between genes and environment. The *C. elegans* model offers a unique experimental system to further screen metals and other xenobiotics suspected of inducing neurodegeneration. Given its ease of manipulability, the conservation of neurotransmitter biology and the high homology with mammalian systems, *C. elegans* functions well as a platform to study the mechanisms of neurodegeneration and the therapeutic efficacy of novel treatments.

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