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Review

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Genetic and Pharmacological Discovery for Alzheimer's Disease Using *Caenorhabditis elegans*

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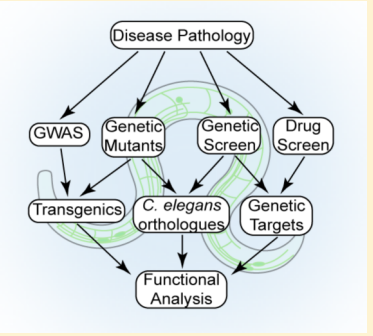
ABSTRACT: The societal burden presented by Alzheimer's disease warrants both innovative and expedient means by which its underlying molecular causes can be both identified and mechanistically exploited to discern novel therapeutic targets and strategies. The conserved characteristics, defined neuroanatomy, and advanced technological application of *Caenorhabditis elegans* render this metazoan an unmatched tool for probing neurotoxic factors. In addition, its short lifespan and importance in the field of aging make it an ideal organism for modeling age-related neurodegenerative disease. As such, this nematode system has demonstrated its value in predicting functional modifiers of human neurodegenerative disorders. Here, we review how *C. elegans* has been utilized to model Alzheimer's disease. Specifically, we present how the causative neurotoxic peptides, amyloid-β and tau, contribute to disease-like neurodegeneration in *C. elegans* and how they translate to human disease. Furthermore, we describe how a variety of transgenic animal strains, each with distinct utility, have been used to identify both genetic and pharmacological modifiers of toxicity in *C. elegans*. As technological advances improve the prospects for intervention, the rapidity, unparalleled accuracy, and scale that *C. elegans* offers researchers for defining functional modifiers of neurodegeneration should speed the discovery of improved therapies for Alzheimer's disease.



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INTRODUCTION

Alzheimer's disease (AD) is characterized by the formation of two distinct types of inclusions, amyloid plaques and neurofibrillary tangles, that are associated with progressive memory loss, cognitive dysfunction, and neurodegeneration. These plaques are insoluble aggregates of amyloid-β (Aβ), the peptide produced by sequential cleavage of the amyloid precursor protein by γ- and β-secretases (Figure 1).[1] Though Aβ plaques were posited to be the source of pathogenesis, more recent work is finding that small soluble aggregates, called oligomers, represent the most toxic Aβ species.[2−5] These oligomers permeabilize the membranes of cellular digestive compartments, causing ions and digestive enzymes to leak into the cytoplasm.[6] Neurofibrillary tangles are intracellular aggregates of tau protein. Normally, tau stabilizes microtubule filaments, but pathogenic phosphorylation and aggregation of tau results in lethal cytoskeletal changes (Figure 1). Unlike Aβ, neurofibrillary tangles are not unique to AD, but are also associated with other tauopathies. Whether tau and/or Aβ are causative agents of AD is still unresolved, yet modeling how these two proteins elicit cellular responses remains a cornerstone in AD research. Here, we review how modeling tau and Aβ in the nematode *Caenorhabditis elegans* has served as a platform for gene and drug discovery directed toward modifiers of AD cellular phenotypes.

ATTRIBUTES OF *C. elegans*

The distinctive features and tractability of *C. elegans* made this metazoan an attractive organism for modeling neuronal connectivity (Figure 2).[7,8] With the comprehensive characterization of cell fate lineage and its complete neuronal connectivity map, *C. elegans* has proven to be an invaluable system for investigating development, apoptosis, and aging.[9−12] Consequently, the genetic tools developed for *C. elegans* have been utilized to construct predictive models of human neurological diseases, such as Parkinson's, Huntington's, amyotrophic lateral sclerosis, dystonia, and ataxia.[13−16] Analysis of multiple genetic databases shows that a number of human genes associated with AD have significant homology to *C. elegans* genes (Table 1).[17] As such, *C. elegans* offers an outstanding platform for investigating the cellular and molecular mechanisms of AD. In this Review, we expound the existing *C. elegans* models and discuss how, collectively, they advance our understanding of cellular aspects of AD.

C. elegans MODELS OF Aβ TOXICITY

Aβ-Induced Paralysis Models. The first *C. elegans* models of AD examined cytotoxicity of Aβ in the body-wall muscle cells of animals.[18] Though toxic human Aβ peptide is produced

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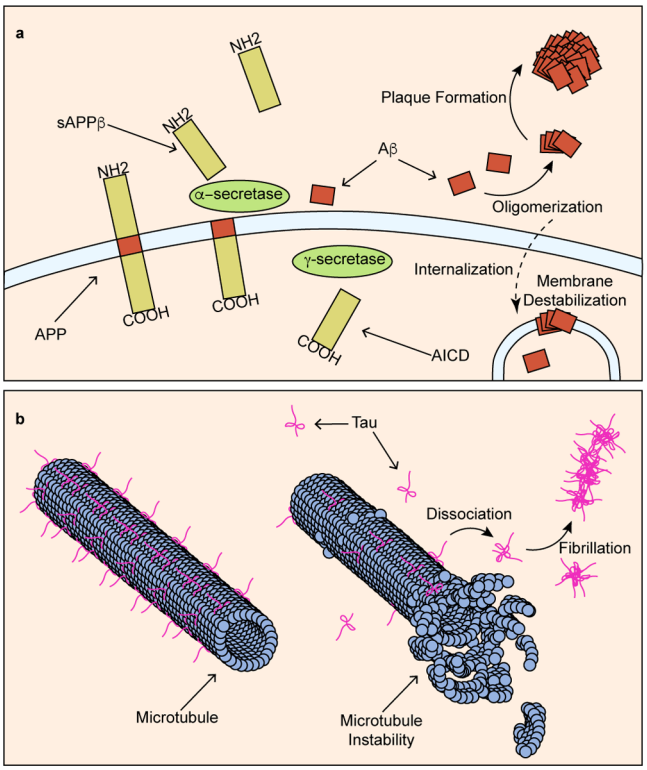

Figure 1. APP processing and tau-mediated microtubule destabilization. (a) At the cell surface, sequential cleavage of APP by α-secretase and γ-secretase releases the Aβ peptide. Extracellular Aβ peptide oligomerizes and is internalized by endocytosis. Alternatively, APP processing occurs within the endosomal compartments subsequent to internalization of cell-surface APP, producing Aβ within the endosomes. At some frequency, Aβ oligomers aggregate into insoluble fractions, which are generated either extracellularly or secreted from intracellular compartments, ultimately forming insoluble plaques. Oligomers of Aβ are considerably more toxic than insoluble plaques, possibly through destabilizing the membranes of digestive compartments. (b) Microtubule assembly is stabilized by wild-type tau protein, but aggregation of tau hinders tau recruitment and mutant tau readily dissociates from microtubules into aggregates, thus destabilizing microtubules and perturbing cytoskeletal structure.

by cleavage of the amyloid precursor protein (APP), transgenic expression of recombinant Aβ circumvented APP processing in *C. elegans*. In doing so, expression of the Aβ cleavage product provides the utility of examining direct modifiers of Aβ toxicity, rather than modifiers of Aβ production as a result of APP processing. Notably, when human APP is expressed in *C. elegans*, products of α- or γ-secretase cleavage were observed, but the products of β-secretase activity could not be detected.[19] Likewise, although the *C. elegans* genome encodes an APP orthologue, *apl-1*, it lacks significantly high sequence identity with Aβ.

Because toxicity of Aβ primarily affects the endosomal system,[5,20] a minigene of Aβ was cloned with the constitutive secretion signal from the *her-1* gene and later corrected to compensate for the cleavage of the secretion signal.[18,21,22] Secreted Aβ is subsequently internalized and, thus, Aβ toxicity is measured as a product of internalized Aβ. This Aβ clone was chromosomally integrated with a *rol-6* phenotypic marker to produce CL2006, which expresses the Aβ minigene constitutively by the muscle-specific *unc-54* promoter. Extracellular Aβ was not visible using immunohistochemistry, but staining

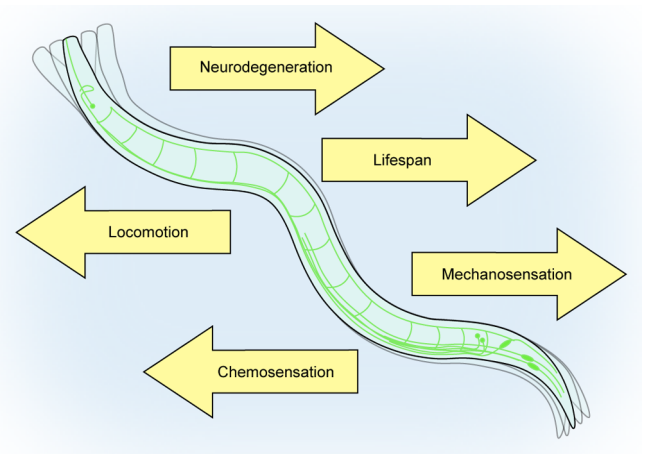

Figure 2. Measurable outputs of neuronal health. As the entire nervous system has been mapped and the cellular lineage of *C. elegans* defined, *C. elegans* behavior is a predictable output of neuronal function. Locomotion, chemosensation, and mechanosensation are elicited by distinct neuronal networks; thus, distinct changes in behavior can be designated to alterations in the function of individual neurons and subtypes. Using tissue-specific expression of fluorescent proteins, changes in neuronal function can be assayed as aberrations in neuronal structure or integrity. As molecular mechanisms of protein stability and neuronal structure are modulated by aging pathways, changes in lifespan primarily reflect macro effects on the animal.

with an amyloid-binding fluorescent dye, X-34, confirmed the presence of extracellular Aβ deposits.[23] Because of the constitutive Aβ in body wall muscles, toxicity of the Aβ peptide induces paralysis of the muscles as the animal ages. With paralysis, animals no longer exhibit a circular, rolling motility phenotype, but become rigid and unresponsive. Thus, paralysis represents a quantifiable behavioral output of Aβ toxicity.

Relationships between Aging and Aβ. Because chronic Aβ paralysis occurs across the lifespan of the animal, age can also be examined as a possible modifier of Aβ toxicity. Since the discovery of insulin signaling as a major regulator of lifespan, longevity of *C. elegans* is typically tested as a variable by modifying expression of the insulin-like receptor and FOXO transcription factor orthologues, DAF-2 and DAF-16, respectively.[10,24,25] The relationship between insulin signaling and aging is reliably conserved between metazoans and mammalian systems, making the short lifespan of *C. elegans* a significant tool in studying the relationship between Aβ and aging. Mutational loss of *daf-2* or depletion by RNAi consistently increase the lifespan of animals. When *daf-2* was depleted in chronic Aβ paralysis animals, not only was lifespan increased, but Aβ-induced paralysis was also attenuated, suggesting that molecular mechanisms of aging impart consequences for Aβ neurotoxicity.[26]

Protein Quality Control. The unfolded protein response (UPR) and ER stress precede neurodegeneration in Alzheimer's brains.[27] Furthermore, their role in AD appears to be conserved from *C. elegans* to mammalian models.[28−30] Using the chronic paralysis model of constitutive Aβ expression, CL2006, heat shock protein 16 (HSP-16) coimmunoprecipitated with Aβ, showing a direct interaction between stress response machinery and Aβ.[31] However, because Aβ toxicity is largely affected by aging, constitutive expression of Aβ is not optimal for investigating modifiers of Aβ independently from aging. Like increased lifespan, reduced Aβ toxicity is dependent on both *hsf-1* and *daf-16*, which

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Table 1. *C. elegans* Strains Modeling Alzheimer's Disease

Aβ models	genotype	strain name (ref)
chronic Aβ paralysis	*dvIs2* [pCL12(*unc-54*/human Aβ peptide 1−42 minigene) + pRF4]	CL2006[18]
Aβ in muscles	*dvIs14* [(pCL12) *unc-54*::Aβ 1−42 + (pCL26) *mtl-2*::GFP]	CL2120[33]
control for Aβ muscles	*dvIs15* [(pPD30.38) *unc-54* (vector) + (pCL26) *mtl-2*::GFP]	CL2122[33]
permissive pan-neuronal Aβ	*dvIs50* [pCL45 (*snb-1*::Aβ 1−42::3′ UTR(long) + *mtl-2*::GFP] I	CL2355[84]
acute Aβ paralysis	*dvIs27* [*myo-3p*::Aβ (1−42)::*let-851* 3′UTR) + *rol-6* (*su1006*)] X	CL4176[32]
Aβ(1−42) in muscles	*dvIs100* [*unc-54p*::Aβ-1−42::unc-54 3′-UTR + *mtl-2p*::GFP]	GMC101[22]
pan-neuronal Aβ(1−40)	*Ex*[pPD49.26 + P*aex-3*::Aβ(1−40); *ttx-3*::rfp p*lin-15*(+)]	MT309[38]
pan-neuronal Aβ(1−42)	*Is*[pTI11.1+P*unc-119*::Aβ(1−42); P*myo-2*::YFP]	(ref 42)
glutamatergic Aβ	[*baInl32*; P*eat-4*::ssAβ 1−42, P*eat-4*::gfp, P*myo-2*::mCherry]	UA166[20]
amphid neuron Aβ	*sesIs25*[P*flp-6*::Aβ1−42; P*gcy-5*::GFP]	(ref 50)
glutamatergic Aβ	*baIn32*[P*eat-4*::ssAβ42,P*eat-4*::GFP,P*myo-2*::mCherry]	UA198[94]

APP model	genotype	strain name (ref)
pan-neuronal *apl-1* overexpression	*ynIs109*[P*snb-1*::*apl-1* cDNA::GFP]	ynIs109[62]
pan-neuronal APP overexpression	*vxSi38* [P*rab-3*::huAPP695::*unc-54*UTR, Cb *unc-119*(+)]	JPS67[65]

tau model	genotype	strain name (ref)
pan-neuronal tau 4R1N	*Is*[P*aex-3*::4R1N; P*myo-2*::mCherry]	(ref 69)
pan-neuronal tau CK10	*bkIs10*[P*aex-3*::h4R1 NTauV337M; P*myo-2*::gfp]	
pan-neuronal tau P301L	*Is*[P*aex-3*::P301L; P*myo-2*::mCherry]	
pan-neuronal tau VH254	*pha-1*(*e2123ts*) III; *hdEx*[F25B3.3::tau352PHP, *pha-1*(+)]	(ref 70)
pan-neuronal tau VH255	*pha-1*(*e2123ts*) III; *hdEx*[F25B3.3::tau352WT, *pha-1*(+)]	
pan-neuronal tau VH421	*pha-1*(*e2123ts*) III; *hdEx181*[F25B3.3::tau352ala, *pha-1*(+)]	
mechanosensory tau 0N4R	*Is*[P*mec-7*::0N4R; P*ges-1*::DsRed]	(ref 71)
mechanosensory tau 0N3R	*Is*[P*mec-7*::0N3R; P*ges-1*::DsRed]	
mechanosensory tau P301L (0N4R)	*Is*[P*mec-7*::P301L (0N4R); P*ges-1*::DsRed]	
mechanosensory tau P406W (0N4R)	*Is*[P*mec-7*::P406W (0N4R); P*ges-1*::DsRed]	
pan-neuronal tau F3ΔK280	*byIs193*[P*rab-3*::F3ΔK280; P*myo-2*::mCherry]	BR5270[72]
pan-neuronal tau F3ΔK280	*byIs162*;[P*rab-3*::F3ΔK280(I277P) (I308P); P*myo-2*::mCherry]	BR5271[72]
low-expression tau	*pirIs3*[P*snb-1*::htau40WT-low;Pmyo-2::gfp]	PIR3[74]
high-expression tau	*pirIs4*[P*snb-1*::htau40WT-high;Pmyo-2::gfp]	PIR4[74]
low-expression tau (A152T)	*pirIs5*[P*snb-1*::htau40A152T-low;Pmyo-2::gfp]	PIR5[74]
high-expression tau (A152T)	*pirIs6*[P*snb-1*::htau40A152T-high; Pmyo-2::gfp]	PIR6[74]
PVD neuron	*kyIs445*:*Is*:[P*des-2*::mCherry::RAB-3; *des-2*::SAD-1::GFP;*odr-1*::DsRED]	(ref 74)

operate downstream of *daf-2* to modulate stress resistance.[26] To address whether cellular stress precedes Aβ toxicity or is a product of it, a *C. elegans* strain was generated in which Aβ expression is restricted by temperature (CL4176). When animals are shifted to the permissive temperature, Aβ levels rapidly increase and paralysis is scored every hour 24 h after temperature upshift.[32] By measuring protein carbonyl levels, oxidative stress was observed before Aβ fibrillation could be discerned at adulthood using the amyloid-specific X-34 fluorescent dye, showing that Aβ toxicity precedes accumulation.[33]

Aβ Structure−Function Studies in Vivo. In work designed to examine the structural implications of the Aβ peptide, *C. elegans* strains expressing residue variants of Aβ were found to differentially affect stress response and deposition.[34−36] Importantly, humans heterozygous for normal Aβ and AβA2V, a mutation of the second alanine within the cleavage product, exhibit a lower incidence of AD than individuals homozygous for the normal Aβ allele.[37] To examine the neuronal effects of this protein, Aβ(1−40) and Aβ(1−40)A2V were subcloned for pan-neuronal expression in *C. elegans*, using the promoter for the *rab-3-like* guanine nucleotide exchange factor, *aex-3*. That Aβ(1−40) was used rather than Aβ(1−42) is an important distinction, as Aβ(1−40) is notably less toxic than Aβ(1−42). In this context, the observer can measure exacerbated toxicity outside a smaller threshold. Expression of Aβ(1−40)A2V in *C. elegans* neurons elicited increased deficits in motility, pharyngeal pumping, and lifespan.[38] Moreover, immunohistochemistry and dot blot analysis of whole-animal lysates showed equivalent expression but increased oligomerization of Aβ(1−40)A2V compared to Aβ(1−40). It appears, then, that in heterozygous individuals, the aggregation of wild-type Aβ may be interrupted by the high propensity of AβA2V to aggregate. When CL2120 or CL4176 animals were fed truncated Aβ(1−6)A2V peptide, oligomerization of Aβ and toxicity-induced deficits were reduced.[39] Notably, as the Aβ(1−6)A2V peptide was constructed with an arginine-rich TAT sequence and D-isomer to allow it to cross blood-brain barriers and be resistant to degradation, it thus had the possibility of being therapeutic in AD patients. The effects of the A2V allele highlight the functional significance of the first few residues of Aβ.

Neurobehavioral Analyses. In contrast to the P*aex-3*::Aβ pan-neuronal model, another transgenic strain, CL2355, utilizes Aβ expression from the pan-neuronal synaptobrevin, *snb-1*, promoter with expression permitted by temperature-sensitive nonsense-mediated RNA degradation. Toxicity of Aβ is assayed by multiple readouts including scoring of chemotaxis, odorant preference, motility, locomotion, lifespan, and egg-laying.[40] Conveniently, a cost-effective multiworm tracking system has been developed to quantify locomotion of CL2355 animals, consistent with other worm tracking methods.[41] Yet, due to the gut fluorescence from the *mtl-1*::GFP coinjection marker, visualization of neurons in CL2355 by GFP is impractical. More recently, a pan-neuronal model was constructed to express Aβ(1−42), taking advantage of a corrected Aβ(1−42) minigene. In contrast to the previous two pan-neuronal models, Aβ(1−42) expression is constitutively driven from the *unc-119*

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promoter. These animals exhibited reduced lifespans, and Western blotting showed Aβ expression in both soluble and insoluble fractions.[42] Like the other neuronal Aβ models, measurable changes in egg-laying and locomotor behaviors were observed. The early onset of middle-aged behaviors in these animals correlated with metabolic decline and electron transport failure that preceded Aβ toxicity. This corroborated a similar finding using CL2006.[43] Though the coinjection marker expressing YFP in the pharynx does not preclude fluorescent visualization of neurons, neuron structure has not yet been visualized in this model. Yet, it provides coherent behavioral readouts for Aβ that are consistent with previous findings.

Evaluation of Aβ-Mediated Neurodegeneration. While deficits in locomotion and behavior are quantifiable outputs of toxicity, they present no direct measurable output for neurodegeneration. To generate a *C. elegans* model of Aβ-mediated neurodegeneration, expression of Aβ and GFP were restricted to the glutamatergic neurons, which comprise 78 out of the 302 neurons.[8,20,44] Of those, 5 are represented distinctly in the tail of the animal and reproducibly degenerate with age in response to constitutive Aβ expression (Figure 3b). Importantly, this model utilized a portion of the CL2006 Aβ minigene for the constitutive secretion of Aβ. Using this model, *C. elegans* orthologues from a genome-wide screen in a yeast model of Aβ toxicity were tested for their effect on Aβ-mediated neurodegeneration.[20] Specifically, overexpression of the Phosphatidylinositol Binding Clathrin Assembly Protein (PICALM/*unc-11*), associated with AD in genome-wide association studies (GWAS), reduced Aβ-mediated neurodegeneration in *C. elegans*. Overexpression of SH3KBP1/*Y44E3A.4*, which interacts with the AD risk factor CD2AP to modulate endocytic cytoskeletal dynamics, also attenuated Aβ-mediated neurodegeneration. Thus, this specific worm model effectively demonstrated its utility in identifying genetic mediators of AD that translate to humans. In another study, the glutamatergic neurodegeneration model was co-opted with Parkinson's and Huntington's models in *C. elegans* to show that a secondary metabolite of a ubiquitous soil-dwelling bacterium, *Streptomyces venezuelae*, exacerbated proteotoxicity across models through induction of mitochondrial dysfunction.[45]

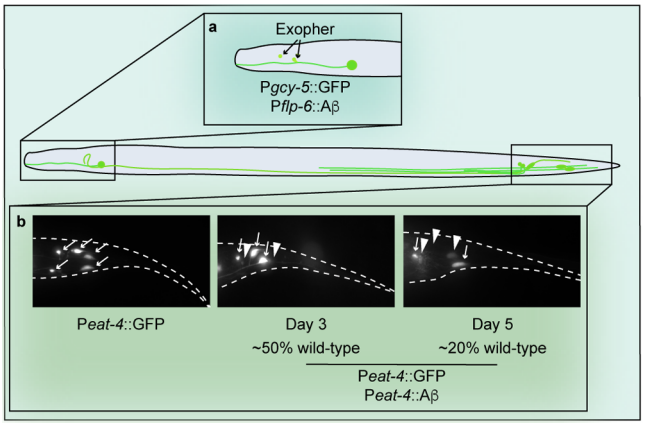

Figure 3. Visualizing effects of Aβ on neurons. Tissue-specific promoters are utilized to express Aβ and GFP from specific subsets of neurons in order to visualize the effect of Aβ on neuronal structure and integrity. (a) The *flp-6* promoter is used to drive expression of Aβ in the anterior amphid neurons and the ASER amphid neuron is visualized by GFP expression from the *gcy-5* promoter. Aggregates of Aβ are sequestered into exopher structures that bleb off the neuron into the coelom. (b) Expression of GFP from the *eat-4* promoter illuminates the 5 posterior glutamatergic neurons (arrows). Coexpression of Aβ induces neurodegeneration of glutamatergic neurons (arrowheads) progressively over time.

Further, this same stably integrated neuronal Aβ transgene model (UA166) has been used to show that age-associated responses to stress modulate neuronal Aβ pathology.[20] Analysis by ChIP-seq found that Repressor Element 1-Silencing Transcription factor (REST), which increases in expression with age, regulated expression of key factors in cell death and autophagy. Not only are these mechanisms perturbed in AD, but AD patients also exhibit decreased REST expression and activity. Normal mice also have increased REST expression with age, but REST-deficient mice have comparatively increased neurodegeneration. These results were recapitulated in *C. elegans* by showing that depletion of the *C. elegans* REST orthologue, *spr-4*, exhibited enhanced vulnerability to oxidative stress and increased mortality.[46] Furthermore, analysis of Aβ-expressing glutamatergic neurons showed that loss of *spr-4* increased neurodegeneration with age. Conversely, overexpression of either SPR-4 or human REST reduced ROS levels in *spr-4* mutants and restored lifespan to wild-type levels in animals treated with paraquat.[46] Thus, as evidenced by the evolutionary conservation of REST functionality, this model provides an observable output for scoring neurodegeneration that reflects the interaction of internalized Aβ with intracellular systems. Taken together, these studies highlight how *C. elegans* can be used for evaluation of both genetic and environmental effectors of Aβ-mediated neurodegeneration.

Aβ Exopher Production. While investigating age-associated neuron restructuring in *C. elegans*, Toth et al. (2012) observed that cell-derived fluorescent markers would appear within extracellular vesicular structures.[47] Because nonprion aggregating proteins have been observed to spread cell-to-cell in a prion-like fashion,[48] these authors examined whether aggregate-prone proteins would be packaged within these extracellular vesicles. Therefore, Aβ expression was restricted to amphid neurons by the *flp-6* promoter and the amphid neuron ASER was visualized by expression of GFP from the *gcy-5* promoter (Figure 3a).[49] When aggregate-prone proteins, including Aβ, are expressed in these neurons, extracellular vesicles, termed exophers, increase in abundance and appear to contain aggregates.[50] By coexpressing the double-stranded RNA transporter, *sid-1*, from the *mec-18* promoter, the amphid neurons were sensitized to RNAi by feeding, allowing identification of modifiers of exopher generation through a systematic RNAi screen. Notably, depletion of polarity genes, *pod-1* or *emb-8*, by RNAi diminished exopher production, showing that genes involved in cell polarity regulated exopher formation. Restricting uptake by phagocytic cells, called coelomocytes, by depletion of an endocytic regulatory gene, *cup-4*, increased the number of exophers observed in the coelom, demonstrating that these aggregates are routinely cleared by coelomocytes, analogous to glia in mammals. Though the toxic hyperexcitation of glia is modified by the calcium-activated protein calcineurin, endocytic clearance of amyloid plaques is also regulated by calcineurin.[51,52] Similarly, the activity of *cup-4* is regulated by calcineurin.[53] Considering recapitulation of calcium dysregulation of AD brains in a *Drosophila* model exacerbated Aβ toxicity, it appears that phagocytic clearance of Aβ is a sensitive and poorly understood process.[54] Notwithstanding, the subject of Aβ exosomes as potential diagnostic biomarkers has been gaining traction.[55,56]

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Thus, how neurons package and expel aggregates to protect the cell, and how migrating phagocytic cells respond to them, can be modeled using *C. elegans* and utilized to further our understanding of how neurons react to amyloidogenic proteins.

■ *C. elegans* MODELS OF APP

As previously described, transgenic expression of human APP in *C. elegans* yielded products of α- or γ-secretase activity but the products of β-secretase activity were not detected.[19] Moreover, the worm APP orthologue, *apl-1*, lacks Aβ homology and is the subject of α-secretase cleavage alone. Still, *apl-1* has been demonstrated to play a critical role in neuronal signaling, as its loss causes lethality in early larval stages that are rescued by restricting expression of *apl-1* to neurons.[57] Further, *apl-1* is recognized to modulate brood size, movement, viability, multiple metabolic pathways and is, itself, regulated by developmental microRNAs.[58−64] To observe how human APP affects neuron health in *C. elegans*, Mos1-mediated Single Copy Insertion (MOSSCI) was used to make a single-copy insertion of human APP driven by the pan-neuronal *rab-3* promoter. With GFP expression restricted to cholinergic neurons, single-copy pan-neuronal expression of APP induced neurodegeneration of the ventral cord cholinergic neurons.[65] Loss of *vem-1*, an orthologue of the Progesterone Receptor Membrane Component 1 (PGRMC1), suppressed neurodegeneration; PGRMC1 itself is thought to form a dimeric complex with sigma 2 receptor (Sig2R).[65]A ligand of Sig2R/PGRMC1, SAS-0132, was selected from a Psychoactive Drug Screening Program as a PGRMC1 modifier that crosses the blood-brain barrier and exhibits low off-target affinity. In mice, SAS-0132 reduced cognitive deficits and synapse loss and in *C. elegans* it suppressed APP-induced cholinergic neuron degeneration.[65] Thus, *C. elegans* is a viable model for investigating the conserved function of APP and pharmacological targets.

■ *C. elegans* MODELS OF TAUOPATHY

In addition to the formation of amyloid plaques, neurofibrillary tangles are another pathological feature of AD. Neurofibrillary tangles are composed of aberrantly phosphorylated protein tau, which is normally responsible for stabilizing microtubule structures (Figure 1). In AD, the destabilization of cytoskeletal elements by the aggregation of hyperphosphorylated tau results in a systematic loss of neuron structure.[66] The *C. elegans* orthologue, *ptl-1*, is also responsible for maintaining cell structural stability, but modulates aging cell-autonomously, depending on the neuronal subtype in which it is expressed.[67] Because *ptl-1* has a similar structure and function to mammalian tau, *C. elegans* represents a practical model for probing tau-induced pathologies.[68]

Functional Analysis of Tau Variants and Hyperphosphorylation. To test the effect of a tau allele associated with Frontotemporal Dementia with Parkinsonism chromosome 17 type (FTDP-17), transgenes encoding either common human tau or one of the two FTDP-17 alleles (V337M or P301L) were driven in *C. elegans* neurons by the pan-neuronal aex-3 promoter and assayed for changes in behavior.[69] Pan-neuronal expression of tau robustly produced multiple phenotypes observed in other neuronal defect mutants, including uncoordinated locomotion, decreased egg-laying, and reduced lifespan. Cholinergic signaling was significantly reduced and GABAergic neurons exhibited degenerative phenotypes. Western blotting, immunostaining, and transmission EM showed accumulation of insoluble tau, the formation of phosphorylated tau inclusions, and progressive neuron loss (Figure 4b). Furthermore, restricted expression of

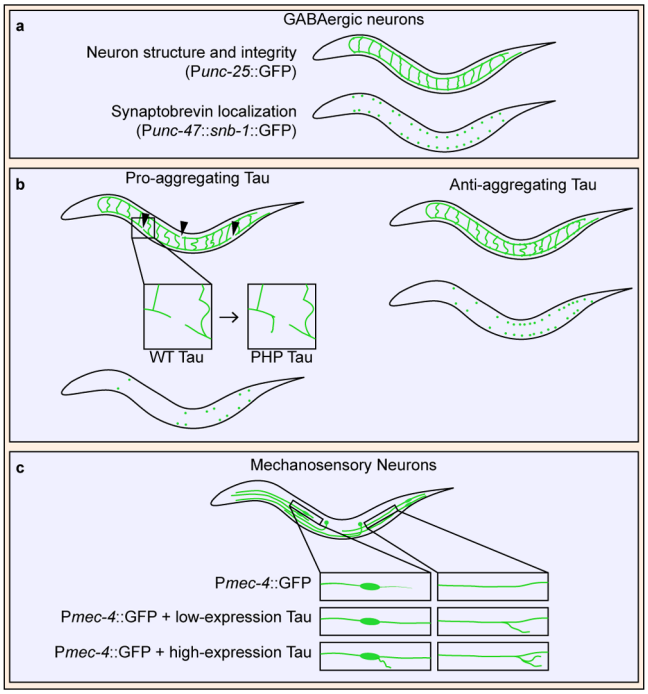

Figure 4. Visualizing effects of tau on neurons. (a) Expression of tau from the *unc-25* promoter illuminates the GABAergic neurons and translational fusion of *snb-1* with GFP expressed from the *unc-47* promoter depicts synaptobrevin localization. (b) Overexpression of tau and tau mutant variants results in neuronal breaks and PHP tau expression induces breaks and abnormal growth. Expression of antiaggregating tau attenuates tau-induced aberrations. (c) Variable expression of tau respectively increases abnormal neurite outgrowth as a result of tau expression in mechanosensory neurons from the *mec-4* promoter.

GFP in GABAergic neurons from the *unc-25* promoter showed discontinuities in axons with tau compared to animals with GFP alone. When a tau isoform with pseudohyperphosphorylation (PHP) mutations to mimic phosphorylated tau was expressed, these phenotypes are exacerbated independently from survival (Figure 4b).[70] Similar models expressing tau from mechanosensory neurons recapitulate comparable behavioral defects and report observed immunoreactive tau and hyperphosphorylated tau deposits that occur independently of apoptosis.[71]

Mechanistic Analysis of Tau Aggregation and Its Impact on Neuronal Integrity. Another study in *C. elegans* sought to investigate inhibitory mechanisms of tau aggregation potentiated by coexpression of the amyloidogenic F3ΔK280 tau fragment with the V337M allele.[72] Coexpression exacerbated locomotor defects compared to animals expressing the transgenes individually. The F3ΔK280-PP variant with I277P and I308P substitutions prevents aggregation and, when coexpressed with V337M tau, locomotor defects are reduced compared to coexpression of F3ΔK280 and V337M. GFP expression in GABAergic and cholinergic neurons showed that coexpression of V337M and F3ΔK280 produced neuronal abnormalities that were largely nonexistent with coexpression of V337M and F3ΔK280-PP (Figure 4b). Expression of

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F3ΔK280, but not ΔK280-PP, resulted in impaired synaptobrevin localization in cholinergic neurons, suggesting perturbations of synaptic structures (Figure 4b). Crossing these animals into mitochondrial reporter strains showed reduced axonal transport and impaired mitochondrial localization that was suppressed by F3ΔK280-PP expression. Furthermore, treatment of animals with small molecule inhibitors of tau aggregation reduced tau-induced phenotypes.[72]

Pan-neuronal expression of tau affords an opportunity to explore the effects of the protein on various aspects of neuronal function by allowing the observer to test different neurons with distinct features. A rare tau variant (A152T) that interferes with microtubule association is a risk factor for frontotemperal dementia, corticobasal degeneration, and AD. This aspect was modeled by expressing it from the *snb-1* promoter and observing its effects on neuronal subtypes and behavior.[73] Animals were uncoordinated, had reduced lifespan, impaired mitochondria localization and trafficking, and defective synaptic signaling.[74] Expression of GFP from either the GABAergic *unc-25* promoter or the mechanosensory neuron promoter, *mec-4*, showed axonal breaks and irregular branching (Figure 4c). These defects increased with increasing tau expression, but tau A152T presented notably more neurodegeneration at even low levels of expression compared to wild-type tau. Using a fluorescent reporter for polarized protein trafficking, axonal components were shown to be mislocalized to dendritic compartments in tau A152T-expressing animals. Though tau A152T exhibited higher cytotoxicity than wild-type tau, it did not form insoluble aggregates and instead formed oligomeric species. Because the A152T mutation lies outside of the β-sheet repeat domain responsible for aggregation, the toxicity of the β-sheet is independent of A152. Consequently, treatment with antiaggregation compounds, bb14 and BSc3094, had no effect. These data support the burgeoning hypothesis that it is not the insoluble aggregates of amyloidogenic proteins that are toxic per se, but rather their soluble oligomeric forms. Furthermore, the finding that expression of the N-terminus of tau alone could incite these effects demonstrates the translational significance of *C. elegans* models of tau pathology.

DISCOVERING GENETIC MODIFIERS OF ALZHEIMER'S DISEASE

Genomic Screening for Functional Modifiers of Aβ Toxicity by RNAi. The ease and expedience of genome-wide screening by RNAi in *C. elegans* cannot be understated. Because the *C. elegans* diet consists of bacteria, animals can be cultivated on small lawns of *Escherichia coli*, while gene expression can be silenced by feeding animals *E. coli* expressing gene-specific double-stranded RNA. Large-scale candidate screens and genome-wide screening in *C. elegans* are therefore attainable.[75] To examine how select genes associated with lifespan regulation affect proteotoxicity across a range of neurodegenerative models, a comparative systematic RNAi analysis was performed in *C. elegans* models of α-synuclein, Aβ, and poly-Q toxicity.[76] Using the acute paralysis model of Aβ, 8 modifiers of Aβ toxicity were identified. Of those, 5 overlapped with α-synuclein toxicity and 1 overlapped with poly-Q toxicity. For example, RNAi targeting the neutral cholesterol ester hydrolase, *nceh-1*, potentiated neurodegeneration and paralysis in both α-syn and Aβ backgrounds, respectively; thus representing an opportune target for pharmacological repression of neurodegeneration.[76,77]

A comparison of human genes and their *C. elegans* orthologues in an RNAi library identified 7970 genes that were subsequently targeted for screening in the acute paralysis model of Aβ toxicity.[78] Though none of these gene candidates were direct orthologues of human genes identified in an AD GWAS, targets within genetic networks a single degree of separation from those candidates were identified as modifiers of Aβ toxicity. Notably, drug targets of the chaperonin complex were more prevalent in the data set and interacted with *C. elegans* orthologues of human genes found to be associated with AD by GWAS.[78]

Screening for Modifiers of Tau Toxicity. To identify new pathways driving tau pathology, Kraemer et al. performed a screen of 16 757 gene targets, representing roughly 85% of the *C. elegans* genome.[79] Of the 60 positive candidates isolated, 38 have human orthologues and 6 were already implicated in tauopathies. The variety of pathways identified demonstrated the complexity of tau pathology and necessitates a clearer understanding of how they work together toward tau toxicity. Using the same model of tau-induced behavioral defects, a forward genetic screen was undertaken to identify mutations that attenuate the *C. elegans* tauopathy. Mutagenesis and screening of the tau strain revealed 72 possible suppressor mutants. One mutant allele, *bk79*, was mapped to an open reading frame, selected for further characterization, and christened the suppressor of tau 1 (*sut-1*). To identify candidates that directly interact with SUT-1, a yeast two-hybrid screen using SUT-1 as bait and a *C. elegans* cDNA library as prey identified *unc-34*, which was further confirmed by pull-down.[80] Epistatic analysis found that *unc-34* and *sut-1* likely have opposing roles in tau toxicity. Though *sut-1* lacks obvious mammalian orthologues, *unc-34* is the *C. elegans* Enabled/VASP orthologue for mediating cell migration and axonal guidance. Multiple interactions with UNC-34/Enabled/VASP have been implicated in tau toxicity and were identified in the RNAi enhancer screen. Similarly, a genetic screen for suppressors of the uncoordinated phenotype in the same tau model identified SUT-2, which shares significant identity with a mammalian orthologue, MSUT-2, a new subtype of zinc-finger protein that binds aggresome components and might represent a novel therapeutic target to attenuate tau toxicity.[81]

IDENTIFYING PHARMACOLOGICAL MODIFIERS OF Aβ AND TAU TOXICITY

In addition to expedient genetic and functional genomic screening, *C. elegans* has been an effective model for drug discovery.[82] An attractive avenue of drug discovery has been the therapeutic advantage presented by compounds found in natural products and foodstuffs.[83−89] Additionally, the translational relevance of stress responses in *C. elegans* has garnered attention in testing herbal medicines through expedient stress response tests in *C. elegans* models of disease. Ethanol extract from a Chinese traditional medicine termed Liuwei Dihuang reduced Aβ toxicity through antioxidant activity, heat shock proteins, and reduced ROS, but did not reduce or inhibit Aβ aggregation.[90] More recently, Dianxianning, another traditional Chinese medicine, was found to reduce toxic Aβ species by a synergistic relationship between its ingredients. This activity was mediated through insulin signaling activated by oxidative stress responses, but independently from chaperone proteins and the Nrf orthologue, *skn-1*.[91] Similarly, phenolic extracts from maple syrup were found to reduce toxicity in human cell culture and *C. elegans*; though the contents of the extract had

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been characterized through HPLC-DAD, the way they coordinate to mediate protection from neurotoxicity of Aβ is unknown.[83]

Drug screening techniques have been employed in multiple *C. elegans* models of neurodegenerative disease.[92−96] Surprisingly, no large-scale screen has been performed for drugs that modulate Aβ toxicity directly in *C. elegans*. Rather, drug candidates from screens in other Aβ models have been tested in *C. elegans*, which has served as a functional bottleneck in the discovery pipeline. In a screen of United States Food and Drug Administration (FDA)-approved drugs that protect against glucose-induced toxicity in primary cortical neuron cultures, 30 candidates that increased viability and reduced cell death were tested in *C. elegans*.[97] Of those, caffeine, tannic acid, and bacitracin attenuated Aβ-induced lifespan reduction. Lifespan extension conferred by caffeine was dependent on *daf-16*, whereas lifespan increase by tannic acid and bacitracin was independent of *daf-16*, indicating distinct and divergent roles in pharmacological mitigation of Aβ toxicity.

A large, high-throughput screen of 140 000 compounds in a yeast Aβ model yielded a large class of clioquinol-related drugs that reduced Aβ toxicity. When treated with clioquinol, glutamatergic neurons expressing Aβ in *C. elegans* exhibited reduced neurodegeneration.[96] However, clioquinol appeared to be toxic to mitochondria at higher concentrations. Another yeast screen identified a dihydropyrimidine-thione (DHPM-Thione) that offers an alternative protective mechanism than clioquinol.[98] Though both reduced neurodegeneration in *C. elegans*, clioquinol alone restored impaired endocytic trafficking in yeast. Furthermore, although both compounds reduced ROS production in yeast, treatment of cells with robust antioxidants had no effect on Aβ toxicity, indicating that reduction of Aβ toxicity was not a consequence of diminished ROS. Together, clioquinol and DHPM-Thione had a synergistic effect in reducing toxicity in yeast and worm neurons.

Computational Screening. An alternative approach to identifying compounds that mitigated Aβ toxicity in *C. elegans* utilized a computational approach before testing compounds in animals. Briefly, multiple chemical databases were screened for candidates based on fragments from compounds reported to interfere with Aβ aggregation. This process yielded 386 potential FDA-approved drugs and two compounds, bexarotene and tramiprosate, were selected for further analysis based on their different chemical scaffolds. Bexarotene, but not tramiprosate, inhibited nucleation of Aβ in a thioflavin T (ThT) fluorescence assay. When exposed to bexarotene at L1 and L4 larval stages, *C. elegans* transgenic strains expressing Aβ in body wall muscles (GMC101) exhibited increased body bend frequency compared to vehicle controls. Furthermore, the amyloid-specific fluorescent dye, NIAD-4, showed fewer Aβ aggregates in animals treated with bexarotene, further suggesting that bexarotene inhibits the initial nucleation of Aβ fibrils.[99] These authors extended their work by screening for chemicals that bind to the ligand-binding domains of proteins that are targets of bexarotene.[100] Using ThT fluorescence and dot blot analysis, 12 candidates were tested for their ability to inhibit Aβ fibril formation. All but one candidate molecule delayed aggregation and kinetic analysis separated the compounds into two groups that distinctly affect nucleation dynamics. Treatment of *C. elegans* found these same compounds also significantly restored motility defects and each drug, with the exception of another one, delayed Aβ-induced paralysis.[100]

Modifying Bioavailability for Drug Test by Increasing Cuticle Permeability. Despite the capacity for high-throughput screening in *C. elegans*, the tough outer cuticle of the nematode is resistant to penetration by many different compounds. To circumvent this, mutations in *bus-8* make the *C. elegans* cuticle more permeable as a result of failure in epidermal organization. Behavior in *bus-8* animals was indistinguishable from wild-type (N2), making this mutant ideal for making *C. elegans* animals more sensitive to drug screens to score behavioral deficits. The *bus-8* allele, *e2698*, was crossed into tau-expressing *C. elegans* to facilitate screening of a library of 1120 compounds to identify FDA-approved compounds that reduce tau-induced defects.[101] From the 1120 compounds, it was determined that the butyrophenone antipsychotics improved locomotion and thrashing of tau-expressing animals reliably in a dose-responsive manner. Additionally, they diminished neurodegeneration and reduced insoluble tau. Importantly, these compounds represent multiple classes of antipsychotic drugs, indicating that attenuation of tau toxicity is being mediated through the common function of dopamine receptor antagonism. These results were further recapitulated in human HEK293 cells, suggesting a conserved role of dopamine antagonism in the mitigation of tau toxicity. The loss of *C. elegans* dopamine receptors *dop-2* and *dop-3* significantly reduced tau-induced deficits and insoluble tau fractions. Systematic crosses between tau-expressing animals and mutants of the dopamine synthesis pathways revealed that *bas-1*, the DOPA decarboxylase (DDC), and dopamine receptors share a common pathway in reducing tau pathology, possibly through reducing the activity of AMP-activated protein kinase.[102]

FUTURE DIRECTIONS AND CONCLUSIONS

The exposition of *C. elegans* in this Review illustrates its capabilities as a transgenic model for probing neurodegeneration by Aβ and tau. Yet, the full potential of *C. elegans* in assessing other major risk factors has not been realized. For example, cholesterol metabolism has been strongly linked with AD. As an auxotroph for choloestrol, *C. elegans* enables strict control of dose−response relationships to be evaluated in vivo. Recent work suggests it may be an ample organism for modeling the relationship between enzymatic regulation of cholesterol metabolism and AD.[76] Moreover, the apolipoprotein E (ApoE), responsible for cholesterol transport, is the strongest risk factor for AD outside of rare APP or presenilin mutations. That *C. elegans* lacks an ApoE ortologue provides the opportunity to examine how ApoE alleles alter neuron biology in response to Aβ, independently from the functional obfuscation of endogenous ApoE variants. Despite genetic correlations identified from GWAS data sets, precise causative relationships with AD remain elusive.

For this reason, *C. elegans* is in a powerful position to be employed in genome-scale screens to identify enhancers or suppressors of Aβ and tau toxicity that could then be swiftly tested for their effects on aging and proteotoxicity. The works highlighted in this review have collectively combed through only a fraction of the *C. elegans* genome, and only a few genes were selected from each study for further analysis. With at least 322 orthologues of AD-associated genes encoded in its genome, *C. elegans* is a wellspring of relatively accessible functional information on the pathogenesis of Aβ and tau.[17] Yet, many of the described pathways highlighted in this Review initially identified candidates from whole-genome screening in

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yeast or high-throughput chemical screening in human cells. The disadvantage of this is that positive candidates could possibly appear negative in yeast or in vitro cell cultures, when they would otherwise incite behavioral changes in response to Aβ or tau, in a metazoan system. With its vast array of behavioral assays and highly conserved genome, *C. elegans* is therefore an exceptional model for unveiling how these relationships emerge into neurological pathology. This ought not be overlooked, as *C. elegans* has proven predictive in determining genetic and pharmacological modulators of neurodegeneration.[13,20] Though other organismal models of AD exist, the characteristics of *C. elegans* and its leadership in the field of aging place this nematode system in a unique position to provide a robust foundation for accelerating our understanding of AD.

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E.F.G. was responsible for generating the primary draft and subsequent updates to the content of the manuscript. E.F.G. also generated the graphics. K.A.C. and G.A.C. contributed to the organization, suggestions on content, and editing of the manuscript.

Notes

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ABBREVIATIONS

AD, Alzheimer's disease; APP, amyloid precursor protein; Aβ, amyloid-beta; RNAi, RNA interference; ThT, Thioflavin T; GWAS, Genome-Wide Association Study; FTDP, Frontotemporal Dementia with Parkinsonism

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