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Understanding the molecular basis of Alzheimer's disease using a *Caenorhabditis elegans* model system

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Abstract

Alzheimer's disease (AD) is the major cause of dementia in the United States. At the cellular level, the brains of AD patients are characterized by extracellular dense plaques and intracellular neurofibrillary tangles whose major components are the β-amyloid peptide and tau, respectively. The β-amyloid peptide is a cleavage product of the amyloid precursor protein (APP); mutations in *APP* have been correlated with a small number of cases of familial Alzheimer's disease. APP is the canonical member of the APP family, whose functions remain unclear. The nematode *Caenorhabditis elegans*, one of the premier genetic workhorses, is being used in a variety of ways to address the functions of APP and determine how the β-amyloid peptide and tau can induce toxicity. First, the function of the *C. elegans APP*-related gene, *apl-1*, is being examined. Although different organisms may use APP and related proteins, such as APL-1, in different functional contexts, the pathways in which they function and the molecules with which they interact are usually conserved. Second, components of the γ-secretase complex and their respective functions are being revealed through genetic analyses in *C. elegans*. Third, to address questions of toxicity, onset of degeneration, and protective mechanisms, different human β-amyloid peptide and tau variants are being introduced into *C. elegans* and the resultant transgenic lines examined. Here, we summarize how a simple system such as *C. elegans* can be used as a model to understand APP function and suppression of β-amyloid peptide and tau toxicity in higher organisms.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder affecting over 5 million Americans and over 26 million people worldwide (Association 2008; Brookmeyer et al. 2007). As the US population lives longer, the prevalence of AD will increase and become more of a health concern and financial burden (Association 2008; Hebert et al. 2003). Thus far, AD is incurable and its etiology is unknown.

Inheritance of AD

One of the major risk factors for AD is family history and genetic predisposition (Association 2008). No mutation has been linked to sporadic AD, which is late in onset (>65 years) and accounts for most AD cases. In contrast, several mutations have been linked to familial AD (FAD), which has an early onset (<40 years) (Campion et al. 1999; Chartier-Harlin et al. 1991; Murrell et al. 1991) and can be seen in patients as young as 25 (Miklossy et al. 2003). The brains of AD patients are characterized by the accumulation of dense plaques and neurofibrillary tangles (Kidd 1964; Krigman et al. 1965; Luse and Smith 1964; Terry et al. 1964). The major components of the dense plaques is the β-amyloid peptide (Aβ; (Glenner and Wong 1984; Masters et al. 1985), which is a cleavage product of the amyloid precursor protein (APP; Kang et al. 1987); the major component of the neurofibrillary

tangles is hyperphosphorylated tau, a microtubule-associated protein (MAP; Goedert et al. 1989). Mutations in the genes encoding APP (Chartier-Harlin et al. 1991; Goate et al. 1991; Murrell et al. 1991; Hardy 2009a) and presenilins (PSEN1 and PSEN2; (Levy-Lahad et al. 1995a, b; Rogaev et al. 1995; Sherrington et al. 1995; Hardy 2009b, c), which are proteases that are part of the γ-secretase complex responsible for cleaving APP (Kimberly et al. 2003;Li et al. 2000), have been correlated to FAD. Moreover, a duplication of the APP locus can lead to FAD (Cabrejo et al. 2006; Rovelet-Lecrux et al. 2006; Sleegers et al. 2006). These data suggest that disruption of APP metabolism is one of the causative factors in the disease.

The APP gene is alternatively spliced to give rise to a single-pass transmembrane domain protein (Kang et al. 1987; Ponte et al. 1988; Tanzi et al. 1988; Yoshikai et al. 1990). APP is cleaved through two major proteolytic pathways: the α-or β-secretase pathways (Fig. 1; for reviews, see (Gralle and Ferreira 2007; Nunan and Small 2000; Selkoe 1999). Initial cleavage by the α- or β-secretase releases an extracellular fragment sAPPα or sAPPβ, respectively. Subsequent cleavage in the β-secretase pathway by γ-secretase leads to the production of Aβ and release of a cytoplasmic fragment (AICD). In contrast, because α-secretase cleaves within the Aβ sequence, the subsequent γ-secretase cleavage releases the cytoplasmic AICD fragment, but does not produce Aβ. Hence, the β/γ-secretase pathway is likely favored in the pathogenesis of AD (for reviews, see Nunan and Small 2000; Selkoe 1999). Presenilin 1 (PSEN1) and 2 (PSEN2) are part of the γ-secretase complex that releases Aβ (Kimberly et al. 2003; Li et al. 2000). The specificity of the secretase cleavage sites can vary. Normally, 90% of the derived Aβ consists of 40 amino acids (=Aβ40) and the other 10% consists of 42 and 43 amino acids (=Aβ42 and Aβ43, respectively; Haass and Selkoe 1993). Aβ42 and Aβ43 are highly fibrillogenic and readily aggregate (Haass and Selkoe 1993). Deposition of Aβ, and particularly Aβ42, is presumed to be neurotoxic (Pike et al. 1993; Yankner et al. 1990).

Understanding the role of APP: use of a mouse model system

A powerful approach to elucidate the in vivo function of a protein is to inactivate the gene and observe the defects caused in the organism. Inactivation of APP in Mus musculus revealed several deficits, such as reduced brain and body weight, a malformation of forebrain commissures, a deficit in grip strength, an alteration in circadian locomotor activity, a hypersensitivity to seizures, and impairments in spatial learning and long-term potentiation (Li et al. 1996; Magara et al. 1999; Perez et al. 1997; Steinbach et al. 1998; Tremml et al. 1998; Zheng et al. 1995). Overexpression of APP in mice also resulted in several phenotypes, which varied in severity, presumably because of varying levels of APP expression. These phenotypes include lethality, neophobia, impaired spatial alteration, reactive gliosis, and an increase in the number of synaptophysin and GAP-43 immunoreactive presynaptic terminals (Hsiao et al. 1995; Mucke et al. 1994).

APP is the canonical member of the APP family of proteins that includes APLP1 and APLP2, which share high sequence similarity to APP within the extracellular and cytoplasmic domains, but do not contain Aβ (Slunt et al. 1994; Sprecher et al. 1993; Wasco et al. 1992, 1993a, b). The different members of the APP family probably originated from one ancestral gene, which was duplicated and translocated. Over time, the duplicated genes evolved to have slightly different, but still overlapping functions. Mice in which APLP1 is inactivated show a postnatal growth defect (Heber et al. 2000), whereas mice in which APLP2 is inactivated appear wild type (Von Koch et al. 1997). However, APLP1-APLP2 and APLP2-APP, but not APLP1-APP, double knockouts show early postnatal lethality (Heber et al. 2000). Furthermore, in addition to postnatal lethality, the triple APP-APLP1-APLP2 knockout mice also show a smoothened brain resembling human lissencephaly type

II and neuronal ectopias (Herms et al. 2004). These experiments highlight an essential role for the APP family proteins in development and viability.

Understanding the role of APP: use of the *C. elegans* model system

The nematode *Caenorhabditis elegans* contains only one APP-related gene, *apl-1* (Daigle and Li 1993). Because genetic manipulations are easier and faster in *C. elegans* than in a mammalian system, *C. elegans* presents an attractive alternative model to examine APP function and its mechanisms of action. APL-1 is also a single-pass transmembrane domain protein and shares many conserved domains with the mammalian APP family members; however, like APLP1 and APLP2, APL-1 does not show any sequence similarity in the Aβ peptide region (Fig. 1). Nevertheless, a lack of sequence similarity does not exclude the possibility of APL-1 having an "Aβ peptide equivalent." The *Drosophila* APP-related protein (Appl) also lacks sequence similarity to the mammalian Aβ peptide region (Rosen et al. 1989), but contains an Aβ peptide equivalent that upon cleavage can form neurotoxic plaques (Carmine-Simmen et al. 2009). Animals carrying mutations in *Drosophila appl* are viable (Luo et al. 1992), but unhealthy (D. Kretzschmar, personal communication).

Similar to the APP gene family, *apl-1* has an essential function. Knockout of *apl-1* causes 100% lethality during early larval development (Table 1; Hornsten et al. 2007). The onset of *apl-1* lethality in *C. elegans* is comparable developmentally to the postnatal lethality seen in the APP family mouse knockouts. In wild-type *C. elegans*, each larval stage is punctuated by a molt, when the old cuticle is sloughed off and replaced by a new cuticle. *apl-1* mutants synthesize a new cuticle, but have difficulty shedding their old cuticle; hence, *apl-1* mutants die during the transition from the first to second larval stage (L1–L2). Several other phenotypes were seen, some at low penetrance, including arrest as L1 larvae and/or severe morphogenetic defects, and some at high penetrance, such as the presence of large vacuoles in hypodermal cells. All phenotypes were rescued by germline transformation with a genomic fragment containing the *apl-1* region as well as an APL-1 translational fusion with green fluorescent protein (GFP) at the 3′end (APL-1::GFP; Table 1). The lethality was not rescued with a genomic fragment containing a premature stop codon in the coding region. Hence, APL-1 is an essential protein for postembryonic development. The goal of examining *apl-1* in *C. elegans* is to gain insight into the functional domains and pathways of APL-1 and translate these findings to mammals.

Functional domains within APL-1

In mammals, Fe65 binds to the APP cytoplasmic tail (Sabo et al. 2001). FEH-1, the *C. elegans* ortholog of Fe65, also binds directly to the cytoplasmic tail of APL-1 (Zambrano et al. 2002). Knockdown of *apl-1* or *feh-1* by RNAi causes hyperactive pharyngeal pumping (Zambrano et al. 2002); knockdown of *feh-1* also causes an incompletely penetrant embryonic lethality or L1 arrest (Zambrano et al. 2002). These findings suggest that APL-1 acts as a receptor, which transduces a signal through FEH-1 during postembryonic development. However, germline transformation of the transmembrane and cytoplasmic domains of APL-1 did not rescue the *apl-1* loss-of-function lethality, although this finding does not preclude that APL-1 signals through the cytoplasmic domain for other functions. In contrast, the extracellular domain of APL-1 was able to rescue the loss-of-function *apl-1* lethality (Hornsten et al. 2007). Furthermore, *apl-1(yn5)* is a viable deletion allele that produces only the extracellular domain of APL-1 (APL-1EXT), indicating that only the extracellular part of APL-1 is necessary and sufficient for viability (Hornsten et al. 2007). APL-1EXT corresponds to the entire APL-1 extracellular domain and is not further cleaved by α-secretase (Hornsten et al. 2007). These findings lead to a new model whereby APL-1 is a multifunctional protein, and in one of its functions, APL-1 is cleaved and the extracellular

fragment (sAPL-1) acts as a ligand. Similarly, a knockin of only the extracellular domain of mammalian APP (sAPPα) was able to rescue the phenotypes of an APP knockout mouse (Ring et al. 2007) and the lethality of APP-APLP2 double knockout mice (U. Muller, personal communication). Knockdown of *apl-1* by RNAi in wild-type animals shortened body size (Niwa et al. 2008), which is consistent with the reduced body size seen in APP knockout mice (Tremml et al. 1998). Hence, these results highlight how findings in the *C. elegans* model can be translated back to mammals.

To identify functional domains within sAPL-1, different domains were tested for their ability to rescue the *apl-1* loss-of-function lethality. The extracellular domain of the APP family contains two conserved domains, a cysteine-rich E1 domain and an E2 domain rich in acidic residues (Wang and Ha 2004; Zheng and Koo 2006); the E1 and E2 domains do not appear to share any structural similarities. Surprisingly, the presence of either the E1 or E2 domain was sufficient to rescue the *apl-1* loss-of-function lethality (Hornsten et al. 2007). The apparent functional redundancy between the two domains is unexpected, but suggests that the extracellular APL-1 fragment interacts through multiple domains.

Functional localization of APL-1

Similar to the ubiquitously expressed human APP (Ponte et al. 1988; Tanzi et al. 1988), *apl-1* is expressed during all developmental stages and in many cell types, such as muscles, glial cells, hypodermal cells, and neurons (Fig. 2; Hornsten et al. 2007). To determine in which cell-types *apl-1* expression is sufficient for viability, APL-1 expression was driven by different cell-type specific promoters. Because the *apl-1* loss-of-function lethality is due to a molting defect, APL-1 expression in hypodermis cells, which produce the cuticle during molting, was expected to rescue the lethality; however, no rescue was detected. In contrast, pan-neuronal APL-1 expression was able to rescue the *apl-1* loss-of-function lethality. Furthermore, pan-neuronal expression of only the extracellular domain of APL-1 was also sufficient to rescue lethality, suggesting that after APL-1 is cleaved, sAPL-1 is released from neurons to promote molting (Hornsten et al. 2007). These results raise several questions, including the fate of the extracellular sAPL-1 fragment, the identity of its binding partners, and whether sAPL-1 can be released from a subset of neurons for rescue.

The secretases in *C. elegans*

Although APL-1 has not been directly tested for cleavage by different secretases, *C. elegans* has several orthologues to the mammalian secretases. The secretases will be discussed briefly here, although in the reverse order in which they act. As mentioned above, APP undergoes two cleavages to release Aβ. The second cleavage is mediated by the γ-secretase complex, which consists of at least four proteins: presenilins (PSEN1 and PSEN2), APH-1, Nicastrin/APH-2, and PEN-2 (Kimberly et al. 2003). Mutations in the presenilins were first correlated to FAD in 1995, but their cellular functions were initially unclear (Levy-Lahad et al. 1995a; Rogaev et al. 1995; Sherrington et al. 1995). A few months after these initial reports, the *C. elegans* ortholog SEL-12 PSEN was identified as a suppressor of LIN-12 Notch signaling (Levitan and Greenwald 1995). This work was significant to the AD field in two ways. First, it provided the first insights into the function of presenilins: presenilins were mediators of Notch signaling, which was well characterized for its critical role in cell-fate decisions during development (reviewed in Greenwald 2005). Second, it raised the possibility that presenilins mediated the activity of multiple proteins in addition to APP and Notch; hence, therapeutic strategies to disrupt presenilins would disrupt not only APP and Notch, but other proteins as well.

Some clues as to how SEL-12 PSEN was acting were made from analysis of *sel-12* PSEN suppression of different *lin-12* Notch alleles. Gain-of-function mutations in *lin-12* Notch cause production of ectopic pseudovulvae (Greenwald et al. 1983), whose formation can be suppressed by decreased *sel-12* PSEN activity (Levitan and Greenwald 1995); however, when LIN-12 Notch was constitutively activated by removal of its extracellular domain, decreased sel-12 PSEN activity no longer suppressed formation of pseudovulvae, suggesting that sel-12 PSEN acts either directly on the LIN-12 Notch receptor or upstream in the Notch pathway (Levitan and Greenwald 1998). Loss of *sel-12* PSEN caused defects in vulval morphogenesis, leading to an egg-laying defect (Levitan and Greenwald 1995) and deficits in thermal memory (Wittenburg et al. 2000). Germline transformation of *sel-12* PSEN mutants with human presenilins restored the egg-laying defect to wild type, indicating that the human and *C. elegans* genes are functionally homologous (Levitan et al. 1996). Subsequent screening in *C. elegans* revealed that, like the mammalian systems, *C. elegans* has multiple presenilins: SEL-12, HOP-1 (Li and Greenwald 1997; Westlund et al. 1999), and SPE-4 (L'Hernault and Arduengo 1992). SPE-4 is required only for spermatogenesis (Arduengo et al. 1998) and loss of *sel-12* PSEN cannot be rescued by *spe-4* (Eimer 2003). In contrast, loss of *sel-12* PSEN can be rescued by *hop-1* and knockout of *sel-12* and *hop-1* in a double mutant caused a synthetic lethality, indicating that the two genes are not only functionally homologous, but have functional overlap (Li and Greenwald 1997; Westlund et al. 1999).

By cleverly tagging each transmembrane domain with a lacZ reporter tag, the Greenwald group demonstrated that SEL-12 PSEN undergoes an obligatory endoproteolysis after the sixth transmembrane domain (Li and Greenwald 1996), similar to the obligatory endoproteolysis of mammalian presenilins (Thinakaran et al. 1996). Mutations in *sel-12* PSEN or human presenilins that lead to its own miscleavage increased the levels of Aβ42 in human cell cultures (Okochi et al. 2000). Four years after the initial identification of the presenilins, several groups, including a *C. elegans* and *Drosophila* group, proposed that the presenilins were the proteases within the γ-secretase complex (De Strooper et al. 1999; Struhl and Greenwald 1999; Wolfe et al. 1999; Ye et al. 1999).

Multiple lines of evidence, however, indicated that γ-secretase was a complex of proteins; mammalian PSEN1, for instance, co-fractionated in a high molecular weight complex (Li et al. 2000). Further immunoextraction of proteins tightly associated with PSEN1 yielded nicastrin, a glycosylated transmembrane protein that binds PSEN1/2 and APP, suggesting that nicastrin's role was to target a substrate to the γ-secretase complex (Yu et al. 2000). Loss of a*ph-2*/nicastrin in *C. elegans* resulted in maternal effect embryonic lethality, similar to the phenotype seen in *glp-1* Notch mutants (Goutte et al. 2000; Levitan et al. 2001). Given the high molecular weight complex that co-frac-tionated with PSEN1, other components in addition to nicastrin had to be present. The power of the genetic approaches available in *C. elegans* again contributed to the identification of these other components. Genetic screens for enhancers of sel-12 PSEN phenotypes in *C. elegans* pulled out APH-1 and PEN-2 (Francis et al. 2002; Goutte et al. 2000), whose mammalian orthologues were the major missing components within the γ-secretase complex. Loss of *aph-1* or *pen-2* conferred maternal effect embryonic lethality, similar to the phenotypes seen in *glp-1* Notch and *aph-2*/nicastrin mutants (Francis et al. 2002; Goutte et al. 2000). APH-1, a seven transmembrane domain protein, and PEN-2, a two transmembrane domain protein, bind PSENs and nicastrin to facilitate assembly and maturation of the γ-secretase complex (Francis et al. 2002; Goutte et al. 2000, 2002; Gu et al. 2003; Levitan et al. 2001). An additional gene identified in a genetic screen for suppression of *sel-12* PSEN activity was *sel-10*, which encodes a protein of the CDC4/CUL-1 E2-E3 ubiquitin ligase family (Hubbard et al. 1997). SEL-10 physically interacts with SEL-12 PSEN, presumably to target it for degradation (Wu et al. 1998). Similarly, the human homolog SEL-10 physically

interacts with and facilitates ubiquitination of human PSEN1 and affects Ab42 production in mammalian cells (Li et al. 2002).

Two α-secretases, *sup-17* ADAM10 and *adm-4* ADAM17/TACE, have been identified in *C. elegans* (Jarriault and Greenwald 2005; Tax et al. 1997). These two proteases act redundantly to process LIN-12 Notch (Jarriault and Greenwald 2005) and presumably APL-1. Loss of *sup-17* ADAM10 results in lethality, which can be rescued by germline transformation with either *sup-17* ADAM10 or *adm-4* ADAM17/TACE (Jarriault and Greenwald 2005; Tax et al. 1997; Wen et al. 1997). Thus far, no β-secretase activity that can cleave human APP has been identified in *C. elegans* (Link 2006).

Regulation of APL-1 in *C. elegans*

Regulation of APL-1 expression has only been explored for the time point of the L4 larval-to-adult transition. In wild-type animals, the seams cells, a specialized type of hypodermal cells, serve as stem cells to generate new hypodermal cells during the different molts; however, during the L4 larval-to-adult transition, the seam cells undergo terminal differentiation and join the hypodermal syncytium (Sulston and White 1980). The L4 larval-to-adult transition is regulated by the heterochronic genes *hbl-1*, *lin-41*, and *lin-42* (Abrahante et al. 2003; Fay et al. 1999; Jeon et al. 1999; Slack et al. 2000). Loss of these heterochronic genes caused adult fates to be executed precociously and an incompletely penetrant molting defect from L4 to adult (Abrahante et al. 2003; Jeon et al. 1999; Slack et al. 2000); in contrast, overexpression of these genes caused a reiteration of larval cell fates (Abrahante et al. 2003; Slack et al. 2000). Hence, expression of these heterochronic genes must be down-regulated for entry into the adult stage. This down-regulation occurs in part through negative regulation by *let-7*, a heterochronic microRNA that has been shown to suppress human cancers (Kumar et al. 2008). *hbl-1* negatively regulates *let-7*, such that expression of *let-7* does not occur before L3 (Roush and Slack 2009). *mir-48* and *mir-84*, heterochronic microRNAs of the *let-7* family, are responsible for controlling the L2–L3 larval transition partially by negatively regulating *hbl-1* (Abbott et al. 2005). Loss-of-function *let-7* mutations cause a supernumerary fifth molt during the L4 to adult transition, resulting in the production of extra seam cells, and an adult lethality due to vulval bursting, presumably due to the extra hypodermal cells (Reinhart et al. 2000).

During the L4 larval-to-adult transition, *apl-1* expression appears in seam cells (Niwa et al. 2008). Knockdown of *apl-1* by RNAi reduced *apl-1* levels to 40% of wild type and suppressed the vulval bursting and additional seam cells in *let-7* mutants (Niwa et al. 2008). These data suggest that APL-1 is regulated by *let-7* at the L4 larval-to-adult transition to allow proper molting. If *apl-1* acts downstream of the *let-7* targets *hbl-1*, *lin-41*, and *lin-42*, then loss of *apl-1* should enhance the molting phenotype of *hbl-1*, *lin-41*, and *lin-42* mutants. Knockdown of *apl-1* by RNAi indeed enhanced the L4 to adult molting defects in the *hbl-1*, *lin-41*, and *lin-42* single mutants, suggesting that *apl-1* acts downstream and is a potential target of these heterochronic genes (Niwa et al. 2008). A cold-sensitive mutation of *mir-48* causes a low penetrance supernumerary molt at the young adult stage, presumably because *hbl-1* is not down-regulated (Reinhart et al. 2000). The penetrance of this phenotype is enhanced in a non-temperature dependent manner in a *mir-84* loss-of-function background and the *mir-48; mir-84* double mutant shows two cuticles (Abbott et al. 2005). Knockdown of *apl-1* in *mir-48; mir-84* double mutants is sufficient to suppress the supernumerary molt and formation of a double cuticle (Niwa et al. 2008). These results indicate that APL-1 expression is temporally regulated by heterochronic microRNAs and regulators during the L4 larval-to-adult transition.

Overexpression of APL-1 induces several phenotypes

A duplication of the APP locus is correlated with FAD (Cabrejo et al. 2006; Rovelet-Lecrux et al. 2006; Sleegers et al. 2006), suggesting that increased Aβ levels contributes to the FAD pathology. However, overexpression of the APP extracellular fragment, hyper-signaling through the APP cytoplasmic domain, or toxicity of the APP cytoplasmic fragment may also contribute to the FAD pathology. For instance, the extracellular domain of human APP (sAPP) can bind to the non-canonical DR6 death receptor to induce cell and axonal degeneration in the absence of trophic factors (Nikolaev et al. 2009). In addition, transgenic mice overexpressing an FAD version of human APP with an additional mutation (D664A) in a caspase cleavage site to inhibit the release of the cytoplasmic peptide (APP-C31) show reduced behavioral defects, despite increased Aβ deposits compared to the FAD version alone (Galvan et al., 2008), implicating an important role for the cytoplasmic tail in FAD pathogenesis. Hence, the effects of APL-1 overexpression were examined in *C. elegans* through the use of transgenic animals (Table 1). When generating transgenic animals, microinjected or co-injected DNA appear as extrachromosomal arrays, which are inherited by progeny at a low frequency because the arrays can be lost during cell division. The arrays, which are generally multiple tandem copies of the single or co-injected DNA, can be integrated into the genome, which allow their Mendelian transmission (Mello and Fire 1995). To determine whether APL-1 overexpression causes any phenotypes, several transgenic lines in which different *apl-1* transgenes were present as arrays or integrated into the genome in an otherwise wild-type background were examined. By Western blot analysis, the transgenic lines expressed APL-1 or APL-1::GFP at levels from 15-to 180-fold higher than wild type (Hornsten et al. 2007).

The overexpression lines had defects in brood size, movement, and viability (Table 1); the severity of these defects was strongly correlated with the level of APL-1 overexpression (Hornsten et al. 2007). Wild-type animals generally lay between 250 and 300 eggs (Byerly et al. 1976). Animals overexpressing APL-1 laid significantly fewer eggs than wild type (Hornsten et al. 2007). Because *apl-1(yn5)* APL-1EXT animals also showed a decreased number of progeny, brood size may be decreased by elevated levels of sAPL-1, perhaps because of interference with cell–cell interactions or adhesion defects that disrupt morphogenesis and/or gonadal development (Hornsten et al. 2007). All *apl-1* transgenic overexpression strains also showed significantly reduced swimming and crawling rates compared to wild type (Table 1). Increasing levels of APL-1, therefore, inhibit movement, perhaps by interfering with motor neuron functions.

The overexpression line that had the highest levels of APL-1 (~180-fold higher than wild type), *ynIs79* APL-1::GFP, exhibited the most severe phenotypes, including an incompletely penetrant (70%) larval lethality (Table 1; Hornsten et al. 2007). One other overexpression line, *ynIs86* APL-1, also showed lethality, but at a much lower rate (5.5%). *ynIs79* APL-1::GFP overexpression animals appear morphologically wild type at hatching. At variable times during L1, *ynIs79* APL-1::GFP animals became translucent and large gaps became evident between organs. These phenotypes are consistent with disruptions in cell adhesion whereby elevated levels of APL-1 interfere with normal adhesion contacts between cells. Alternatively, APL-1 overexpression could cause defects in osmoregulation. Mutations in *clr-1*, which encodes a phosphatase involved in regulating osmotic pressure (Kokel et al. 1998), also cause a translucent phenotype (Hedgecock et al. 1990). Decreased *sel-12* PSEN activity partially suppressed the *apl-1* overexpression lethality, suggesting that the interactions between the presenilins and the APP family are conserved between worms and mammals (Hornsten et al. 2007).

Loss-and gain-of-function *apl-1* lethality are not mediated by caspases

Mammalian APP can regulate apoptosis (for review, see Chen 2004) as well as be cleaved by different caspases (Barnes et al. 1998; Gervais et al. 1999). The *apl-1* loss-of-function and overexpression-induced lethality were examined for activation of an apoptotic or necrotic cell death pathway. *ced-3* encodes a caspase that is essential for execution of apoptosis in *C. elegans* (Horvitz 1999) and *crt-1* encodes calreticulin, which is essential for execution of necrotic cell deaths (Xu et al. 2001) in *C. elegans*. Neither loss of *ced-3* caspase nor loss of *crt-1* calreticulin activity rescued the *apl-1* loss-of-function lethality, indicating that this lethality is not due to ectopic activation of either cell death pathway. Similarly, the APL-1 overexpression lethality of *ynIs79* APL-1::GFP is not due to activation of the *ced-3* apoptotic pathway (Hornsten et al. 2007). Whether the *apl-1* lethality is mediated by an autophagic pathway is unknown.

The amyloid cascade hypothesis: *C. elegans* models to induce degeneration

Hardy and Allsop first proposed the amyloid cascade hypothesis in 1991 to explain the pathology seen in AD (Hardy and Allsop 1991). Briefly, they postulated that imbalances in APP metabolism lead to increased levels of Aβ, and in particular $A\beta_{42}$, which can then aggregate into plaques. Formation of these plaques can in turn lead to the other pathologies, such as neurofibrillary tangles and neurodegeneration, seen in AD. *C. elegans* has been used in several ways to test the amyloid cascade hypothesis (Table 2).

Mutations correlated with FAD favor production of $A\beta_{42}$ and $A\beta_{43}$ and this increased $A\beta_{42}$ production presumably leads to a higher accumulation of Aβ into neurotoxic extracellular plaques (Lorenzo and Yankner 1996). In mice, introduction of $A\beta_{42}$ leads to neurodegeneration and a severe decrease in lifespan (Laferla et al. 1995). To determine whether Aβ plaques are toxic in *C. elegans*, human $A\beta_{42}$ was introduced into muscle cells by germline transformation (Table 2; Link 1995). The resultant transgenic animals showed a progressive, irreversible paralysis (Link 1995). Staining these transgenic worms with a human $A\beta_{42}$ specific antibody (mAbG8) revealed an accumulation of $A\beta_{42}$ deposits, which reacted with Congo red, thioflavin S, and X-34, all markers of $A\beta_{42}$ deposits (Fay et al. 1998; Link 1995). Ultrastructural examination of the $A\beta_{42}$ deposits, however, revealed that the deposits were located in the cytoplasm of the muscle cells (Link et al. 2001) rather than as extracellular deposits or plaques as seen in AD brains (Selkoe 2001); the ultrastructural images were insufficient to resolve whether the $A\beta_{42}$ deposits were in the cytoplasm or, more likely, within intracellular inclusions. This intracellular localization was somewhat surprising given that the human Ab42 construct was made with an artificial signal sequence that should have led to the extracellular release of $A\beta_{42}$. The artificial signal sequence is functional, although cleavage occurs after the signal sequence so that the Aβ sequence corresponds to amino acids 3–42 rather than 1–42; the $A\beta_{3\text{-}42}$ species appears more toxic than $A\beta_{1\text{-}42}$ species (McColl et al. 2009). Collectively, these results demonstrated that after production, $A\beta_{42}$ is targeted either by the endoplasmic reticulum (ER) quality control system and retrotranslocated for degradation (Link 2006) and/or sequestered into intracellular inclusions by autophagic vesicles (Florez-McClure et al. 2007). $A\beta_{42}$ co-immunoprecipitated with several chaperone-related proteins, including two HSP70 ER chaperone proteins, in *C. elegans* (Fonte et al. 2002). Intracellular $A\beta_{42}$ accumulation seems to be crucial in AD pathogenesis (Gouras et al. 2000; Laferla et al. 1997), and some $A\beta_{42}$ transgenic mouse models that display AD behavioral phenotypes also show intracellular $A\beta_{42}$ accumulation rather than extracellular plaque formation (Chui et al. 1999; Kuo et al. 2001; Laferla et al. 1995; Li et al. 1999; Oddo et al. 2003b; Wirths et al. 2001). Hence, intracellular $A\beta_{42}$ accumulation could be a first step in AD pathogenesis, and the *C. elegans*

human $A\beta_{42}$ transgenic animals are an excellent model to examine intracellular $A\beta_{42}$ toxicity and metabolism.

Investigating $A\beta_{42}$ toxicity in *C. elegans*

The amyloid cascade hypothesis implicitly implies that $A\beta_{42}$ plaques and not soluble forms of $A\beta_{42}$ are the toxic species in vivo. The human $A\beta_{42}$ transgenic worms offer several advantages to investigating the intracellular toxicity of Aβ species in vivo. To determine which residues are relevant to fibril formation in vivo, transgenic lines containing different human $A\beta_{42}$ variants that do not form fibrils and plaques in vitro were generated in *C. elegans* and examined for their fibril formation and intracellular deposits (Table 2; Fay et al. 1998). Transgenic worms expressing human $A\beta_{42}$ variants containing $Leu^{17}Pro$ and $Met^{35}Cys$ substitutions or an artificial single chain $A\beta_{42}$ dimer formed no thioflavin S-reactive deposits, although some of the strains had $A\beta_{42}$ variant levels comparable to lines expressing wild-type $A\beta_{42}$ (Fay et al. 1998). Hence, $A\beta_{42}$ residues Leu^{17} and Met^{35} are required for fibril formation and $A\beta_{42}$ deposits in vivo as well. To determine whether presence of intracellular $A\beta_{42}$ deposits is correlated with paralysis, an inducible system for human $A\beta_{42}$ expression in muscles was developed (Link et al. 2003). Within several hours of $A\beta_{42}$ induction, animals showed paralysis without any detectable $A\beta_{42}$ deposits (Drake et al. 2003). Hence, $A\beta_{42}$ deposits are not required to induce the $A\beta_{42}$ paralysis phenotype in *C. elegans*, suggesting that either intracellular $A\beta_{42}$ deposits are not the toxic species or a small $A\beta_{42}$ aggregation not visible as a deposit is also toxic. Consistent with these findings, the transgenic AD model mouse (Tg2576) displayed morphological, behavioral and memory deficits months before $A\beta_{42}$ plaque deposition was apparent (Jacobsen et al. 2006). Furthermore, plaque prevalence does not strictly correlate with dementia in AD (Davies et al. 1988; Mann et al. 1992; Schmitt et al. 2000).

How can $A\beta_{42}$ be toxic in *C. elegans* even in the absence of $A\beta_{42}$ deposits? One possibility is that $A\beta_{42}$ induces cells to undergo oxidative stress. Transgenic worms expressing human $A\beta_{42}$, but not the $A\beta_{42}$ $Met^{35}Cys$ variant, showed increased levels of protein oxidation (Yatin et al. 1999). A closer look at the temporal formation of plaques and the oxidative stress response revealed that oxidation of proteins precedes presence of $A\beta_{42}$ deposits (Drake et al. 2003), suggesting that soluble $A\beta_{42}$ or small aggregates of $A\beta_{42}$, rather than deposits, may induce oxidative stress in vivo, thereby causing $A\beta_{42}$ toxicity. Similarly, rat hippocampal cell cultures exposed to exogenous $A\beta_{42}$, but not the $A\beta_{42}$ $Met^{35}Nle$ variant, have increased levels of protein carbonyls and neurotoxicity, presumably due to increased protein oxidation (Yatin et al. 1999). A second possibility for $A\beta_{42}$ toxicity is that intracellular $A\beta_{42}$ inclusions are disrupting the ER-assisted degradation or autophagic degradation pathways (Florez-McClure et al. 2007). Hence, possible mechanisms for $A\beta_{42}$ toxicity in *C. elegans* are oxidative stress and/or disruption of ERAD or autophagy pathways (see also below).

Cellular response to human $A\beta_{42}$ expression in *C. elegans*

Assuming that some form of $A\beta_{42}$ is toxic or disruptive to cells, then a defense mechanism that protects the cells must be initially mounted; such a mechanism is likely conserved among all animals. To identify this $A\beta_{42}$ detoxification pathway in *C. elegans*, two approaches were undertaken. First, as mentioned above, proteins that co-immunoprecipitated with $A\beta_{42}$ were characterized by MALTI-TOF to reveal two HSP70-related proteins, three HSP-16 αB-crystallin-related proteins, and a putative negative regulator of HSP70, 3R05F9.10, all of which are involved in chaperone activity (Fonte et al. 2002). RNAi knockdown of the putative negative regulator of HSP70 (R05F9.10) partially suppressed the paralysis of worms expressing human $A\beta_{42}$ in muscles, suggesting that the

transgenic animals up-regulate heat shock proteins to cope with the $A\beta_{42}$ toxicity (Fonte et al. 2002). Furthermore, HSP-16 co-localizes with $A\beta_{42}$ and binds it directly in vitro (Fonte et al. 2002, 2008). Inducing $A\beta_{42}$ expression leads to a strong transcriptional increase of HSP-16 (Fonte et al. 2002) and targeted over-expression of HSP-16 partially suppresses $A\beta_{42}$ toxicity (Fonte et al. 2008). The second approach to identify components of a degradation pathway was a microarray analysis to find genes that are either strongly up-or down-regulated by induced human $A\beta_{42}$ expression in *C. elegans*. Genes that were up-regulated, as further verified by RT-PCR, included the two HSP-16 chaperone proteins and two proteins with homology to the TNFAIP1 family, which are induced by the tumor necrosis factor α (Link et al. 2003). Interestingly, subsequent RT-PCR of CRYAB, a mammalian ortholog of HSP-16, and TNFAIPI showed their up-regulation in AD brain tissues, suggesting a common $A\beta_{42}$ detoxification pathway in worms and mammals (Link et al. 2003).

Clearance of $A\beta_{42}$ in *C. elegans*

The finding of a potential $A\beta_{42}$ detoxification pathway leads to the question of how $A\beta_{42}$ is cleared from cells in *C. elegans*. One general mechanism could be through autophagy, whereby misfolded, excess, or used proteins or other cellular products are engulfed into a double membrane to form autophagic vesicles, which fuse with lysosomes where the engulfed components are degraded. In AD brains, there is an abnormal accumulation of autophagic vesicles, particularly in the dystrophic neurites of neocortical and hippocampal neurons (Nixon et al. 2005; Yu et al. 2005). In mouse models of AD, autophagic vacuoles contain APP, Aβ, and the APP cytoplasmic terminal fragment CTFβ after β-secretase cleavage (Yu et al. 2004). Similarly, inducible expression of human $A\beta_{42}$ in muscles leads to an accumulation of autophagic vesicles in *C. elegans* (Florez-Mcclure et al. 2007). To determine whether autophagic vesicle formation causes paralysis, autophagic vesicle formation was blocked by RNAi knockdown of *bec-1*, which encodes an APG6/VPS30 protein that is part of the complex that localizes proteins to autophagic vesicles, and *atg-7*, which encodes an E1 ubiquitin-activating-like enzyme orthologous to the autophagic budding yeast protein Apg7p. Such knockdown in $A\beta_{42}$ transgenic animals had no effect on the levels of $A\beta_{42}$ or paralysis rate. In contrast, knockdown of lysosomal component genes, such as aspartyl proteases (*asp-2*, *asp-4*, *asp-5*, and *asp-6*), lysosomal-associated membrane proteins (*lmp-1* and *lmp-2*), and vacuolar proton-translocating ATPase (*vha-15*), by RNAi enhanced the paralysis rate and caused higher $A\beta_{42}$ levels. Hence, $A\beta_{42}$ toxicity in *C. elegans* may result from impaired lysosomal degradation due to defective lysosomal formation and/or vesicular acidification (Florez-Mcclure et al. 2007).

Interestingly, a mutation in *daf-2*, which encodes an insulin/IGF-1 receptor, decreases paralysis and levels of $A\beta_{42}$ peptide, but not $A\beta_{42}$ mRNA levels, in *C. elegans* with inducible expression of human $A\beta_{42}$ in muscles (Florez-Mcclure et al. 2007). These animals also had an increased number of lysosomes and few or no autophagic vesicles. While these data may suggest the presence of an autophagy-independent route for $A\beta_{42}$ degradation in *daf-2* IGF-1/Insulin Receptor mutants, *bec-1* APG6/VPS30 is needed for the suppression of the paralysis and for deceased $A\beta_{42}$ peptide levels (Florez-Mcclure et al. 2007). Hence, $A\beta_{42}$ is targeted to lysosomes in part by autophagic vesicles.

Age-dependent onset of proteotoxicity

Mutations in *daf-2* insulin/IGF-1 receptor extend lifespan, such that animals live twice as long as wild type (Kenyon et al. 1993). DAF-2 insulin/IGF-1 receptor is a negative regulator of the FOXO transcription factor DAF-16 (Lin et al. 1997; Ogg et al. 1997), which activates genes involved in longevity, stress resistance, protein folding, and detoxification (Barsyte et

al. 2001; Honda and Honda 1999; Murphy et al. 2003; Walker et al. 2001). The lifespan extension in *daf-2* insulin/IGF-1 receptor mutants also depends on the presence of the heat shock factor 1 (HSF-1) transcription factor (Hsu et al. 2003). However, although both DAF-16 and HSF-1 are necessary, they are not sufficient for the extended longevity in *daf-2* insulin/IGF-1 receptor mutants (Hsu et al. 2003; Lin et al. 2001) and overexpression of DAF-16 or HSF-1 does not mimic the extreme longevity of *daf-2* insulin/IGF-1 receptor mutants (Henderson and Johnson 2001; Hsu et al. 2003). Interestingly, reducing *daf-2* insulin/IGF-1 receptor activity not only dramatically increases lifespan, but also makes these worms much healthier (Kenyon et al. 1993). Indeed, knockdown of *daf-2* insulin/IGF-1 receptor by RNAi in worms expressing human $A\beta_{42}$ in muscles, not only decreases paralysis, but also dramatically increases their lifespan (Cohen et al. 2006). These effects are dependent on DAF-16 and HSF-1 and do not affect overall mRNA or protein levels of human $A\beta_{42}$ (Cohen et al. 2006). Given that the $A\beta_{42}$ deposits are intracellular, these results suggest that the delay in $A\beta_{42}$ toxicity in *daf-2* knockdown animals is due to $A\beta_{42}$ being removed more efficiently either through clearance or detoxification (Cohen et al. 2006). A possible future approach to AD could be a pharmacological treatment via the insulin signaling pathway. Some AD patients have altered insulin signaling, which manifests in a brain specific diabetes (Steen et al. 2005).

Pharmacological approach to ameliorate $A\beta_{42}$ toxicity in *C. elegans*

Several drugs, particularly ones that decrease reactive oxygen species (ROS) generated by oxidative stress, have been examined for their effects in decreasing toxicity in the transgenic worms expressing human $A\beta_{42}$ in muscles. EGb761, an extract from the Ginkgo biloba that is sold as a dietary supplement, is reported to have several beneficial properties, such as neuroprotection (anti-apoptotic effects in cell culture; Smith et al. 2002), inhibition of Aβ aggregation (Luo et al. 2002), lifespan extension in rats (Winter 1998), cognitive enhancer in mice (Winter 1991), and stabilization of symptoms and improvement of cognitive performance in AD patients (Curtis-Prior et al. 1999; Le Bars et al. 1997;Le Bars et al. 2000; Mix and Crews 2002; Oken et al. 1998). EGb761 was also found to increase lifespan and slow the rate of paralysis in human $A\beta_{42}$ transgenic worms (Wu and Luo 2005; Wu et al. 2006). EGb761 decreases toxic ROS levels induced by $A\beta_{42}$ in *C. elegans* muscles and inhibits $A\beta_{42}$ oligomerization and deposits (Smith and Luo 2003; Wu et al. 2006). However, human $A\beta_{42}$ transgenic worms treated with ascorbic acid, an antioxidant, or ginkgolide GB or GC, two components within EGb761, had low levels of ROS or $A\beta_{42}$ oligomers, respectively, but high rates of $A\beta_{42}$-induced paralysis (Wu et al. 2006). Hence, whether levels of ROS or $A\beta_{42}$ oligomers underlie the increased lifespan and slower rate of paralysis with EGb761 treatment is unclear. Other chemicals that protect against ROS levels are isoflavones (Mahn et al.2005). Glycitein, an isoflavone isolated from soy beans, also suppressed the $A\beta_{42}$-induced toxic hydrogen peroxide levels, attenuated the paralysis rate, and decreased numbers of $A\beta_{42}$ deposits in transgenic $A\beta_{42}$ worms (Gutierrez-Zepeda et al. 2005), indicating that in some instances decreasing ROS levels can decrease $A\beta_{42}$ toxicity.

Reserpine is an FDA approved psychopharmalogical and antihypertensive drug (NDA#009838; Bleuler and Stoll 1955; Vakil 1949) that downregulates biogenic amines through inhibition of the vesicular monoamine transporter (VMAT; Metzger et al. 2002). Surprisingly, in human $A\beta_{42}$ transgenic worms, reserpine increased lifespan and slowed the rate of paralysis without affecting overall Aβ mRNA and protein levels, similar to knockdown of *daf-2* insulin/IGF-1 receptor by RNAi (Arya et al. 2009). However, in contrast to knockdown of *daf-2* insulin/IGF-1 receptor, lifespan extension by reserpine is not dependent on *daf-16* (Srivastava et al. 2008). Whether the protective efficacy of reserpine is mediated through the insulin signaling pathway is not known.

Drugs that inhibit presenilin activity have also been tested in *C. elegans*. The compound BMS AG6B was first discovered in a cell-based screen for compounds that alter Aβ ratios. Applying BMS AG6B on *C. elegans* induced a specific inhibition of *sel-12* PSEN function and thus revealed the mechanism of action for the compound (reviewed in Carroll and Fitzgerald 2003).

Tau in *C. elegans*

Because mutations correlated with FAD were biased toward the amyloid cascade hypothesis, much of AD research has been focused on APP and Aβ. However, a second postmortem criterion in AD is the presence of intracellular neurofibrillary tangles, whose major component is hyperphosphorylated tau, a MAP (Brion et al. 1991; Delacourte and Defossez 1986; Grundke-Iqbal et al. 1986; Kosik et al. 1986). There are several reasons for the neglect of the tau pathology. First, neurofibrillary tangles are not uniquely seen in AD, but also in other neurodegenerative diseases, such as frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17; Hulette et al. 1999; Mirra et al. 1999; Spillantini et al. 1998). Second, there are no FAD cases correlated with mutations in tau (Kwon 2008). Third, $A\beta_{42}$ deposition precedes and can induce hyperphosphorylation of tau (Lewis et al. 2001; Oddo et al. 2003a; Zheng et al. 2002). Nevertheless, the transgenic mouse model that phenocopies the most pathologies seen in AD is a triple transgenic mouse, whose transgenes carry mutations in APP, presenilin (PSEN1), and tau. These triple transgenic mice of mutant APP_{Swe}, $PSEN1_{M146V}$, and tau_{P301L} display plaques and tangles and show behavioral deficits that are characteristic of AD (Oddo et al. 2003b).

Tau-related protein in *C. elegans*

Although no tau homolog is present in *C. elegans*, a ‘protein with tau-like repeats’ (PTL-1) has been identified (Table 3; Goedert et al. 1996; Mcdermott et al. 1996). PTL-1 shares 50% sequence identity within the microtubule tandem repeats with mammalian tau, MAP2, and MAP4 (Goedert et al. 1996; Mcdermott et al. 1996). PTL-1 is expressed in the embryonic epidermis, intestinal muscle, mechanosensory neurons, a number of head neurons, and motor neurons in the ventral nerve cord (Goedert et al. 1996), binds to microtubules, and facilitates microtubule assembly in *ptl-1* transfected COS cells (Goedert et al. 1996). Inactivation of *ptl-1* caused an incompletely penetrant embryonic lethality; escaping animals showed a reduced mechanosensory response and enhanced defects in animals also carrying mutations in *mec-12* and *mec-7*, which encode alpha and beta tubulin subunits, respectively (Gordon et al. 2008). These results indicate that PLT-1 is involved in two main processes: elongation during development and mechanosensation in larval and adult animals; both roles may require PTL-1 to provide structural support for microtubules.

Human tau transgenic models in *C. elegans*

Several transgenic human tau models have been generated in *C. elegans* (Table 2). These include transgenic lines overexpressing wild-type human fetal or adult tau, both of which can be phosphorylated by *C. elegans*, as well as transgenic lines overexpressing human tau containing mutations that cause hyperphosphorylation, correspond to mutations seen in FTDP-17, or correspond to the tau mutation in the triple transgenic mice APP_{swe}, $PSEN1_{M146V}$, and tau_{P301L} (Brandt et al. 2009; Kraemer et al. 2003; Miyasaka et al. 2005). Transgenic lines that overexpress wild-type or mutant tau in neurons show two major phenotypes, which differ in severity and progression depending on whether fetal or adult tau was expressed and on the levels of tau overexpression; in addition, the mutant tau lines showed more severe phenotypes than the wild-type tau lines. First, the transgenic animals were uncoordinated and showed reduced motility (Brandt et al. 2009; Kraemer et al. 2003),

and in the mutant adult tau transgenic lines, this uncoordination became progressively worse with age (Kraemer et al. 2003). Second, the transgenic mutant tau animals showed axonal degeneration whereby gaps and discontinuities appeared in the axonal bundles of the dorsal and/or ventral nerve cords (Brandt et al. 2009; Kraemer et al. 2003); by electron microscopy analysis, axons were dilated, vacuolated, and irregular (Kraemer et al. 2003). In transgenic lines where tau is overexpressed in the mechanosensory neurons, animals lost their sense of touch and this defect became progressively worse with age; the cell bodies of the mechanosensory neurons were swollen, axons were thinner, kinked, and tortuous, and microtubules were broken (Miyasaka et al. 2005). Although all tau over-expressing lines produced insoluble tau, phenotypes were detected even in the absence of insoluble tau. Thus, over-expression of tau either over-stabilizes microtubules or hyperphosphorylated tau is toxic (Brandt et al. 2009). These sets of data show that wild-type human tau is sufficient to induce neurodegeneration in a multicellular organism and its toxicity increases with age.

Mutants that suppress the tau pathology highlight genes that may act in the pathway that mediates tau toxicity. Two suppressors of tau toxicity, *sut-1* and *sut-2*, have been isolated in *C. elegans* using a motility assay. *sut-1* encodes an RNA and snRNP-binding protein (MacMorris et al. 2007), which is localized predominantly in the nucleus (Kraemer and Schellenberg 2007). In *sut-1* mutants, tau-induced toxicity, as assessed by decreased motility, is partially suppressed and levels of tau are decreased (Kraemer and Schellenberg 2007). SUT-1 physically binds to UNC-34/Mena/Enabled, presumably in the cytoplasmic compartment in *C. elegans* (Kraemer and Schellenberg 2007). *unc-34*/Mena/Enabled-*sut-1* double mutants show enhanced tau-induced toxicity and show severely decreased motility (Kraemer and Schellenberg 2007), suggesting that *unc-34* is either needed for the *sut-1* suppression or acts in a parallel pathway to affect motility. In mammals, Mena binds Fe65 (Ermekova et al. 1997) and forms a complex with APP (Sabo et al. 2001). Whether SUT-1, UNC-34/Mena/Enabled, FEH-1/Fe65, and APL-1 form a complex and how loss of *sut-1* mediates detoxification of tau in *C. elegans* is unknown, but could involve shuttling of APP and/or tau to different compartments. *sut-2* encodes a novel subtype of a CCCH zinc finger (Guthrie et al. 2009; Kraemer and Schellenberg 2007), which is similar to the zinc fingers contained within Nab2 in yeast and ZC3H14 in humans (Kelly et al. 2007); these zinc fingers bind polyadenosine RNA with high affinity (Kelly et al. 2007). SUT-2 interacts with ZYG-12, a HOOK cytoskeletal linker protein necessary for the attachment between centrosomes and the nucleus (Guthrie et al. 2009), suggesting that SUT-2 may be involved in tau movement. The role of the SUT proteins in suppressing tau toxicity, however, is still unclear.

Conclusion

The model organism *C. elegans* offers many approaches in understanding the role of genes implicated in AD. First, because the function of APP and related proteins are still unclear, *C. elegans* can be used to probe basic functions and different functional domains of an APP-related protein. The ease of performing genetic screens and generating transgenic strains allows researchers to answer questions that would be much more difficult and time-consuming to perform in a mammalian system. For instance, the in vivo relevance of the extracellular domain of an APP-related protein was determined first in *C. elegans*. By judicious disruption of different domains, the functional domains within the extracellular domain can be determined. In addition, forward genetic screens can be performed to identify genes in the apl-1 pathway; these genes may similarly function in an APP pathway. Second, over 50% of FAD cases have a mutation in the presenilins, which cleave APP. The in vivo function of the presenilins and two components of the γ-secretase complex were first identified in *C. elegans* and genetic screens can continue to identify relevant genes. Third, overexpression of APP and/or processing through the β-/γ-secretase pathway are linked to

AD. Overexpression of *C. elegans* APL-1 is also toxic. Pathways to relieve this toxicity, such as APL-1/APP clearance, may also be conserved. Fourth, artificial introduction of human Aβ in *C. elegans* causes toxicity and this toxicity can be used to probe which residues are critical for toxicity, deposition, and Aβ plaque formation in a multicellular organism. Overexpression of Aβ is linked to oxidative stress, and several genes and drugs that decrease oxidative stress and Aβ toxicity in *C. elegans* may similarly decrease Aβ toxicity in humans. For instance, recent research suggests that autophagy may be a mechanism to clear excess APP and/or Aβ. Lastly, the finding that human tau is hyperphosphorylated by endogenous *C. elegans* enzymes and the resulting neurodegeneration caused by overexpression of human tau opened a new approach to find suppressors of tau pathogenesis. In summary, *C. elegans* is a valuable genetic model system that can provide insights into the function and metabolism of APP and tau and their contributions to AD.

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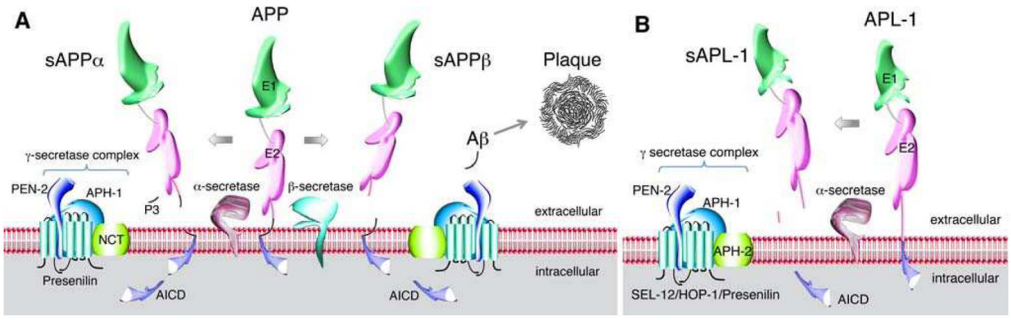

Fig. 1.

Schematic of the processing pathways of human amyloid precursor protein (APP; **a**) and its C. elegans ortholog APL-1 (**b**). **a)** APP undergoes two processing pathways, α/γ-secretase or β/γ-secretase, to produce sAPPα/AICD or sAPPβ/AICD, respectively. Only the β/γ-secretase pathway produces the amyloid peptide (Aβ). α-Secretase corresponds to ADAM17/TACE, β-secretase to BACE. **b)** In *C. elegans*, APL-1 undergoes at least one processing pathway to produce sAPL-1 and presumably AICD. α-Secretase may correspond to SUP-17, ADAM10, or ADM-4 ADAM17/TACE. No β-secretase activity has been described in *C. elegans*. NCT/APH-2 nicastrin; conserved domains: cysteine-rich E1 and acidic residue-rich E2 domain, AICD APP or APL-1 intracellular cytoplasmic domain.

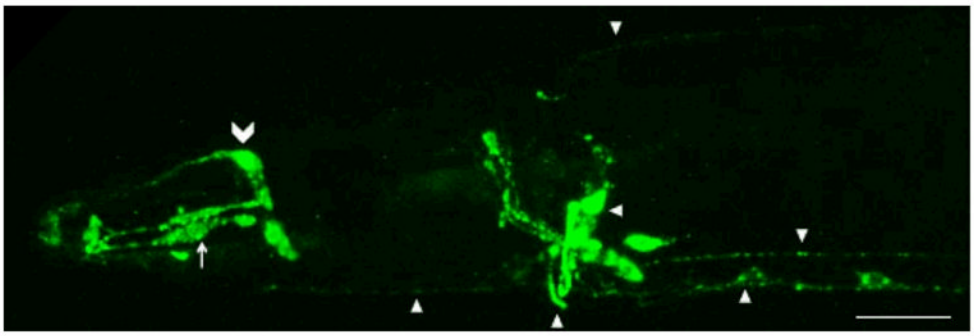

Fig. 2.

Expression of an APL-1::GFP translational fusion in C. elegans. APL-1::GFP is seen in multiple cell types, including neurons and processes (arrowheads), glial sheath cells (arrow), and muscle cells (chevron). Anterior head region shown, ventral side is down. Scale bar = 10 μm

Table 1

Knockouts of *C. elegans* orthologues of human genes implicated in AD

Human		***Caenorhabditis elegans***			
Role	**Gene**	**Gene**	**Knockout (null) alleles**	**Phenotypes of null alleles**	**References**
Amyloid Precursor Protein Family					
APP	*APP/APLP1/APLP2*	*apl-1*	*yn10, yn23, yn28, yn29, yn30, yn31, yn32*	larval lethal; molting defect; vacuoles; morphological defects	(Hornsten *et al*. 2007)
Processing Enzymes of APP					
α-secretase					
	ADAM10	*sup-17*	*n1306, n1315, n1316, n1318, n1319am, n1320*	lethal	(Tax *et al*. 1997)
	ADAM17/TACE	*adm-4*	*ok265*	wild type; functional redundancy between SUP-17 and ADM-4	(Jarriault and GreenwalD 2005)
β-secretase					
	BACE1			no endogenous b-secretase activity that cleaves human APP found in transgenic *C. elegans*	(Link 2006)
γ-secretase complex					
Presenilins	*PSEN1* or 2	*sel-12*	*ar171, ty11*	disrupted vulva morphogenesis; egg laying defective	(Cinar *et al*. 2001; Levitan and Greenwald 1995)
	PSEN1 or *2*	*hop-1*	*ar179*	functionally redundant with *sel-12*	(Wen *et al.* 2000)
APH-1	*APH-1*	*aph-1*	*ep140, ep169, ep170, ep216, ep411, ep413, zu123, or28*	no anterior pharynx; maternal effect embryonic lethal; hypodermis fails to enclose body; egg laying defective; APH-2 localized to cytoplasm rather than cell surface	(Francis *et al.* 2002; Goutte *et al.* 2002)
Nicastrin	*APH-2*	*aph-2*	*zu181*	no anterior pharynx; maternal effect embryonic lethal	(Goutte *et al*. 2000)
PEN-2	*PEN-2*	*pen-2*	*ep219, ep220 ep221, ep336, ep412, ep423*	no anterior pharynx; maternal effect embryonic lethal; hypodermis fails to enclose body; egg laying defective	(Francis *et al.* 2002)
Physical Interactors with APP					
Fe65	*FE65*	*feh-1*	*gb561*	embryonic/larval lethal and larval arrest	(Napolitano *et al.* 2008; Zambrano *et al.* 2002)
Mena	*MENA*	*unc-34*	*e951, gm104, gm114*	uncoordinated; axon guidance defect; reduced brood size	(Withee *et al.* 2004)
Tau and Suppressors of Tau Pathogenesis					
Tau	*TAU*	*ptl-1*	*ok621*	incompletely penetrant embryonic lethal; escapers have mechanosensory defect	(Gordon *et al*. 2008)
		sut-1	*bk79*	suppresses tau pathogenesis	(Kraemer and Schellenberg 2007)
MSUT-2	*MSUT-2*	*sut-2*	*bk741*	suppresses tau pathogenesis	(Guthrie *et al*. 2009)

Not included: *ADAM9* and APOE4, since no orthologues identified in *C. elegans*

Table 2

Overexpression of *C. elegans* proteins implicated in AD

***C. elegans* protein**	***C. elegans* promoter**	**Expression in *C. elegans***	**Fold over-expression**	**Rescuing ability & phenotypes**	**Transgene name/(plasmid)**	**References**
Endogenous expression of APL-1						
APL-1	*apl-1*	head and tail neurons, ventral cord, hypodermis and supporting cells, vulva muscles	125x	rescues *apl-1* null lethality; low level (5.5%) L1 lethality; reduced brood size; sluggish	*ynIs86*	(Hornsten *et al.* 2007)
APL-1::GFP			180x	rescues *apl-1* null lethality; high level (70%) L1 lethality and morphological defects; cell and organ detachment; reduced brood size; sluggish	*ynIs79*	(Hornsten *et al.* 2007)
APL-1 extracellular domain				rescues *apl-1* null lethality; slowed development; reduced brood size; sluggish	*ynIs71, ynEx106, ynIs106A*	(Hornsten *et al.* 2007)
APL-1ΔG$_o$[a]				rescues *apl-1* null lethality		(Hornsten *et al.* 2007)
APL-1ΔE1[b]				rescues *apl-1* null lethality		(Hornsten *et al.* 2007)
APL-1ΔE2[c]				rescues *apl-1* null lethality		(Hornsten *et al.* 2007)
APL-1ΔE1-E2[d]				no rescue of *apl-1* null lethality		(Hornsten *et al.* 2007)
Neuronal expression of APL-1						
APL-1	*snb-1*	constitutively in all neurons; pharynx; arcade cells; distal tip cell; vulval muscle; spermatheca; gonad sheath cells; body wall muscle; hypodermis; seam cells[e]	71x and 17x respectively	rescues *apl-1* null lethality; reduced brood size; sluggishness	*ynIs12, ynIs13*	(Hornsten *et al.* 2007)
APL-1 extracellular domain[f]				rescues *apl-1* null lethality	*ynEx166*	(Hornsten *et al.* 2007)
APL-1::GFP	*rab-3*	constitutively in all neurons		rescues *apl-1* null lethality		(Hornsten *et al.* 2007)
Expression of Proteins in the γ-secretase complex[g]						
SEL-12	*sel-12*	constitutively in most cell types, except intestine		rescues *sel-12* null phenotypes	*byIs100, byIs101,* SEL-12	(Levitan *et al.* 1996; Wittenburg *et al.* 2000)

***C. elegans* protein**	***C. elegans* promoter**	**Expression in *C. elegans***	**Fold over-expression**	**Rescuing ability & phenotypes**	**Transgene name/(plasmid)**	**References**
SEL-12	*ttx-3*	only in AIY neuron		rescues *sel-12* null phenotypes	(pBY478)	(Wittenburg *et al.* 2000)
SEL-12	*egl-13*	Pi cell, neurons, bodywall muscles, intestine		partially rescues egg laying defect and Pi cell fate of *sel-12* null worms	(cHNC2)	(Cinar *et al.* 2001)
HOP-1	*sel-12*	constitutively in most cell types, except intestine		rescues *sel-12* null phenotypes	HOP-1	(Li and Greenwald 1997)
APH-1	*sel-12*	constitutively in most cell types, except intestine		rescues egg laying defect of *aph-1* null worms (Note: no rescue under *aph-1* endogenous promoter)	*Ce3aph-1*	(Francis *et al.* 2002)
PEN-2	*pen-2*	neurons, muscles, intestine, vulva		rescues egg laying defect of *pen-2* null worms	*pen-2 genomic*	(Francis *et al.* 2002)
PEN-2	*sel-12*	constitutively in most cell types, except intestine		rescues egg laying defect of *pen-2* null worms	*Ce pen-2*	(Francis *et al.* 2002)
Endogenous expression of the α-secretase						
ADM-4	*adm-4*	pharynx, intestine, tail		rescues sterility of *sup-17; adm-4* double mutants	*arEx399*, *arEx400*	(Jarriault and Greenwald 2005)

[a] APL-1ΔG$_O$: deletion of G$_O$-binding sequence

[b] APL-1ΔE1: deletion of E1 domain

[c] APL-1ΔE2: deletion of E2 domain

[d] APL-1ΔE1-E2: deletion of E1 through E2 domains

[e] Expression pattern of P*snb-1*::GFP (BC11116; Hunt-Newbury et al. 2007)

[f] APL-1EXT: the entire extracellular domain of APL-1. APL-1EXT is not further cleaved and is slightly larger than sAPL-1

[g] No rescue of maternal effect lethal phenotype for *aph-1*, *aph-2*, *pen-2*, probably due to co-suppression of their endogenous locus in the germline, a general germline effect described in Dernburg et al. (2000)

Table 3

AD toxicity models of human proteins expressed in *C. elegans*

Human protein/peptide	*C. elegans* promoter	Expression in *C. elegans*	Phenotypes	Strain/transgene name/(plasmid)	References
Expression of the human beta amyloid peptide					
$A\beta_{1-42}$ (wild type)	*unc-54*	constitutively in muscles	age-dependent progressive paralysis; forms amyloid deposits; increased oxidative stress	CL2005, CL2006, CL1019, CL1118, CL1119, CL1120, CL1121, CL2120	(Fay *et al*. 1998; Link 1995; Link *et al.* 2001; Yatin *et al.* 1999)
Dimer $A\beta_{1-42}$			no formation of amyloid deposits	CL2109, CL3109	(Fay *et al*. 1998; Link *et al*. 2001)
$Met^{35}Cys$ $A\beta_{1-42}$			no formation of amyloid deposits; no increase in oxidative stress	CL3115	(Fay *et al*. 1998; Yatin *et al.* 1999)
$A\beta_{1-42}$ (with long 3′UTR)	*myo-3*	inducible $A\beta_{1-42}$ in body wall muscles	rapid paralysis; oxidative stress precedes amyloid deposition; autophagosome accumulation	CL4176	(Drake *et al.* 2003; Florez-McClure *et al.* 2007; Link *et al*. 2003)
$A\beta_{1-42}$	*snb-1*	inducible $A\beta_{1-42}$ in all neurons	normal movement; forms amyloid deposits; reduced chemotaxis towards Benzaldehyde; hypersensitive to serotonin	CL2241, CL2355	(Link 2006; Wu *et al.* 2006)
Expression of Components of the γ-Secretase Complex					
PSEN1	*sel-12*	constitutively in most cell types, except intestine	rescues *sel-12* null phenotypes	PS1, (pBY146)	(Levitan *et al*. 1996; Wittenburg *et al.* 2000)
Mutant PSEN1 variants			fails to rescue *sel-12* null phenotypes	PS1ΔE9, PS1M146L, PS1H163R, PS1L266V, PS1A286E, PS1C410Y, A246(pBY147)	(Levitan *et al*. 1996; Wittenburg *et al.* 2000)
PSEN2			rescues *sel-12* null phenotypes	PS2	(Levitan *et al*. 1996)
Nicastrin			rescues egg-laying defect of *aph-2* null	hNCT FL	(Levitan *et al*. 2001)
Mutant Nicastrin variants			partially rescues egg-laying defect of *aph-2* null	DYIGS, AAIGS, Δ340, Δ 69, EC	(Levitan *et al*. 2001)
APH-1			human APH-1 is unable to rescue egg-laying defect of *aph-1* null worms; human APH-1 can partially rescue egg-laying defect of *aph-1* null only in mixture with *Hpen-2, Haph-1a, Haph1b* and *HPSEN1*	*Haph-1a, Haph1b*	(Francis *et al.* 2002)
PEN-2			Partially rescues egg-laying defect of *pen-2* null (with long 3′ UTR)	*Hpen-2*	(Francis *et al.* 2002)

Human protein/peptide	*C. elegans* promoter	Expression in *C. elegans*	Phenotypes	Strain/transgene name/(plasmid)	References
			only in mixture with *Hpen-2, Haph-1a, Haph1b* and *HPSEN1*		
Expression of Human Tau and Variants					
Tau (4R1N isoform, most abundant form in human brain)	*aex-3*	constitutively in all neurons	age-dependent progressive uncoordination and accumulation of insoluble tau; neurodegeneration; reduced lifespan compared to non-transgenic worms	N-1, N-2	(Kraemer *et al.* 2003)
V337M Tau (4R1N) (FTDP-17 mutation)	*aex-3*	constitutively in all neurons	stronger age-dependent progressive uncoordination and accumulation of insoluble tau; neurodegeneration; reduced lifespan compared to non-transgenic worms	337M-1,3337M-2	(Kraemer *et al.* 2003)
P301L tau (4R1N) (FTDP-17 mutation)	*aex-3*	constitutively in all neurons	strong age-dependent progressive uncoordination and accumulation of insoluble tau; neurodegeneration; reduced lifespan compared to non-transgenic worms	301L-1, 301L-2	(Kraemer *et al.* 2003)
Tau WT4R (wild type)	*mec-7*	touch neurons (ALML/R, AVM, PLML/R, PVM); weak in FLP, PVD, BDU	age-dependent progressive impairment in touch response; neurodegeneration; little tau accumulation in PLM neuron	*tmIs82, tmIs83, tmIs84, tmIs85, tmIs171*	(Miyasaka *et al.* 2005)
Tau WT R (wild type)	*mec-7*	touch neurons (ALML/R, AVM, PLML/R, PVM); weak in FLP, PVD, BDU	age-dependent progressive impairment in touch response, neurodegeneration	*tmIs110, tmIs173*	(Miyasaka *et al.* 2005)
P301L tau (FTDP-17 mutation)	*mec-7*	touch neurons (ALML/R, AVM, PLML/R, PVM); weak in FLP, PVD, BDU	strong age-dependent progressive impairment in touch response; neurodegeneration; strong tau accumulation in PLM neuron (as wild type tau WT4R)	*tmIs81, tmIs178, tmIs179*	(Miyasaka *et al.* 2005)
R406W tau (FTDP-17 mutation)	*mec-7*	touch neurons (ALML/R, AVM, PLML/R, PVM); weak in FLP, PVD, BDU	strong age-dependent progressive impairment in touch response; neurodegeneration; strong tau accumulation in PLM neuron (as wild-type tau WT4R)	*tmIs146, tmIs147, tmIs148, tmIs149*	(Miyasaka *et al.* 2005)
Tau_{352} (=fetal, 352aa isoform) wildtype	*rgef-1*	constitutively in all neurons	age-dependent progressive uncoordination; neurodegeneration	VH255, VH1016, VH1018	(Brandt *et al.* 2009)
Tau_{352} PHP (pseudo-hyperphosphorylated)	*rgef-1*	constitutively inall neurons	strong-age dependent progressive uncoordination; neurodegeneration	VH254, VH1014, VH1015,	(Brandt *et al.* 2009)
Tau_{352} Ala (10 Ser/Thr phosphorlyation sites substituted with Ala)	*rgef-1*	constitutively in all neurons	early onset of age-dependent progressive uncoordination and	VH418, VH421	(Brandt *et al.* 2009)

Human protein/peptide	*C. elegans* promoter	Expression in *C. elegans*	Phenotypes	Strain/transgene name/(plasmid)	References
			reduced lifespan compared towild-type tau_{352}		