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Transgenic *Drosophila* models of Alzheimer's disease and tauopathies

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Abstract

Alzheimer's disease (AD) is the most common form of senile dementia. Aggregation of the amyloid-β42 peptide (Aβ42) and tau proteins are pathological hallmarks in AD brains. Accumulating evidence suggests that Aβ42 plays a central role in the pathogenesis of AD, and tau acts downstream of Aβ42 as a modulator of the disease progression. Tau pathology is also observed in frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) and other related diseases, so called tauopathies. Although most cases are sporadic, genes associated with familial AD and FTDP-17 have been identified, which led to the development of transgenic animal models. *Drosophila* has been a powerful genetic model system used in many fields of biology, and recently emerges as a model for human neurodegenerative diseases. In this review, we will summarize key features of transgenic *Drosophila* models of AD and tauopathies and a number of insights into disease mechanisms as well as therapeutic implications gained from these models.

Keywords

Alzheimer's disease; Tauopathies; Amyloid-β42; Microtubule associated protein tau; *Drosophila*

Introduction

The fruit fly *Drosophila* has been widely used to investigate many aspects of biology. Analysis of the *Drosophila* genome has revealed that approximately 70% of human disease-related genes have homologs in *Drosophila* (Fortini et al. 2000; Reiter et al. 2001). This observation suggests that *Drosophila* may also be used to study the pathomechanisms of human diseases and to identify disease modifier genes.

Recently, *Drosophila* has emerged as a powerful genetic model to study human neurological and neurodegenerative disorders. Many late-onset neurodegenerative diseases including Alzheimer's disease (AD), tauopathies, Parkinson's disease, Huntington's disease and other polyglutamine diseases, amyotrophic lateral sclerosis (ALS), and prion disease are

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characterized by accumulation of misfolded proteins (Soto 2003). Molecular genetic studies revealed the genes associated with familial forms of such diseases, and a transgenic overexpression approach has been used to model those diseases caused by toxic gain-of-function mechanisms. Overexpression of some of those genes in *Drosophila* neurons has been shown to recapitulate key features of human neurodegenerative diseases (Crowther et al. 2005; Deleault et al. 2003; Feany and Bender 2000; Finelli et al. 2004; Greeve et al. 2004; Iijima et al. 2004; Jackson et al. 1998; Jackson et al. 2002; Raeber et al. 1995; Ratnaparkhi et al. 2008; Warrick et al. 1998; Watson et al. 2008; Wittmann et al. 2001). A number of excellent reviews regarding modeling of human neurodegenerative diseases in the fly have been published (Bilen and Bonini 2005; Fortini and Bonini 2000; Khurana 2008; Lu and Vogel 2009; Marsh and Thompson 2004; Sang and Jackson 2005; Shulman et al. 2003). In this review, we will summarize key features of transgenic *Drosophila* models of AD and tauopathies and findings in pathogenic mechanisms and therapeutic implications gained from these models.

Drosophila models of Alzheimer's disease

The role of the A*β* peptide in the pathogenesis of Alzheimer's disease

AD is a fatal disorder and, in its later stages, global cognitive functions are disrupted and associated motor disabilities lead patients to become bedridden (Cummings 2003). Short-term memory impairment is detected in the early stage of the disease along with other psychiatric problems such as sleep disorders and increased agitation, which distinguishes AD from neurodegenerative conditions such as Parkinson's disease, tauopathies, or Huntington's disease (Cummings 2003; Selkoe 2002).

At the level of cellular pathology, extensive neuron loss and two characteristic hallmarks, senile plaques (SPs) and neurofibrillary tangles (NFTs), are observed in the AD brain (Selkoe 2001). SPs are extracellularly deposited protein aggregates that are referred to as amyloid deposits. Biochemical studies have revealed that the major components of SPs are two peptides, the 40 or 42 amino acid amyloid-*β* 40 or 42 (A*β*40 or A*β*42) (Glenner and Wong 1984; Masters et al. 1985). Although a small number of SPs are detected in normal aged brains, this lesion is relatively specific to AD. In contrast, NFTs, which are intracellular protein inclusions composed of the hyper-phosphorylated microtubule-associated protein tau (Lee et al. 1991), are observed in many other neurological diseases.

The majority of AD cases are sporadic, with disease onset after 65 years of age. Less than 10% of all AD cases are inherited in an autosomal dominant manner (Bertram and Tanzi 2005). A*β* peptides are physiological metabolites of the amyloid *β*-precursor protein (APP) and result from sequential cleavage by the *β*-secretase and *γ*-secretase complexes, whose catalytic subunits are Presenilin 1 (PS1) and Presenilin 2 (PS2) (Gandy 2005). Molecular genetic studies of early-onset familial AD (EOFAD) patients have identified causative mutations in the *APP*, *PS1*, and *PS2* genes, and these mutations promote A*β*42 production, aggregation, and stabilize the protein against clearance (Tanzi and Bertram 2005). Therefore, accumulation of A*β*42 in the brain is generally accepted as causing AD (Hardy and Selkoe 2002). However, the mechanism by which the A*β*42 peptide initiates the pathogenesis of AD remains elusive.

Drosophila models to study human amyloid-*β*42 toxicity

Drosophila has a clear homolog of APP, APP-like protein (dAPPL) (Fig. 1) (Luo et al. 1992). Flies deficient for dAPPL exhibit behavioral abnormalities (phototaxis deficiency), which can be rescued by a human APP transgene, indicating a functional conservation between dAPPL and human APP (Luo et al. 1992). dAPPL is involved in synaptic differentiation (Torroja et al. 1999b), synaptic development (Ashley et al. 2005), and neurite arborization (Leyssen et al. 2005). dAPPL overexpression causes axonal transport defects (Torroja et al.

1999a). Similarly, overexpression of human APP induces axonal transport defects and increases cell death in the larval fly brain (Gunawardena and Goldstein 2001).

dAPPL shares about 30% overall sequence identity with human APP, however, the region in dAPPL that corresponds to the Aβ peptide lacks significant homology with the human peptide (Luo et al. 1992).

In the non-amyloidogenic pathway, APP is cleaved within the Aβ domain by α-secretase precluding deposition of intact Aβ peptide. Kuzbanian (Kuz) is the *Drosophila* ortholog of α-secretase ADAM10 (Allinson et al. 2003; Rooke et al. 1996) and cleaves dAPPL (Fig. 1) (Carmine-Simmen et al. 2009). Very recently, Carmine-Simmen et al. identified *Drosophila* β-secretase-like enzyme (dBACE, Fig. 1), which has 25% identity to human BACE1 and 28% identity to human BACE2. dBACE does cleave human APP but not at β-site (Carmine-Simmen et al. 2009; Greeve et al. 2004). Interestingly, dBACE overexpression cleaves dAPPL and produces a fragment containing the region in dAPPL that corresponds to the Aβ peptide, which aggregate into intracellular fibrils, amyloid deposits, and cause age-dependent behavioral deficits and neurodegeneration (Carmine-Simmen et al. 2009). These results suggest that not only the biological functions but also amyloidogenic and non-amyloidogenic proteolytic processing may be conserved across species.

Transgenic flies to study human Aβ peptide-induced amyloid formation and neurodegeneration have been generated by several approaches using the GAL4/UAS system (Fig. 2) (Brand and Perrimon 1993). *Drosophila* has all the components of the γ-secretase complex (Fig. 1) (Periz and Fortini 2004;Takasugi et al. 2003), while *Drosophila* has very low β-secretase activity (Fossgreen et al. 1998;Yagi et al. 2000). To overproduce Aβ peptides from human APP in flies, Greeve et al. (2004) generated triple transgenic flies expressing human APP, human β-secretase, and *Drosophila* presenilin (dPsn) or dPsn with point mutations that correspond to the EOFAD mutants N141I, L235P, and E280A (Ye and Fortini 1999). These flies produced modest levels of Aβ peptides, including Aβ40 and Aβ42, and an additional Aβ peptide (δ-Aβ), as well as intracellular fragments of APP. The flies developed β-amyloid plaques and age-dependent neurodegeneration as well as semilethality. The neurodegeneration phenotype was enhanced in flies expressing dPsn carrying the EOFAD mutations, while the phenotype was suppressed by a genetic reduction in fly endogenous dPsn, suggesting that neurodegeneration was dependent on γ-secretase activity.

In contrast, we and others took a more direct approach to overproduce human Aβ42 peptides in fly neurons. To express human Aβ40 or Aβ42 in the secretory pathway, Aβ peptide sequences were directly fused to a signal peptide at the N-terminus. This artificial construct produced intact Aβ40 or Aβ42 peptides in the fly brain (Finelli et al. 2004; Iijima et al. 2004), which allowed us to evaluate the toxicity of Aβ40 and Aβ42 separately in the brain for the first time in an animal model. Both the Aβ40 and Aβ42 peptides accumulate during aging in the fly brain, but only Aβ42 formed amyloid deposits (Iijima et al. 2004). This result is consistent with previous observations of in vivo amyloid deposits in AD or Down syndrome patient brains, which have shown that Aβ42 first accumulates in amyloid plaques in the brain parenchyma (Iwatsubo et al. 1994).

Sensitive and quantitative assays using different cues, such as courtship and heat stress, as well as olfactory, taste, and visual cues, have been established to study memory in the fly (Heisenberg 2003; Quinn et al. 1974; Tully and Quinn 1985; Waddell and Quinn 2001). In the Pavlovian olfactory classical conditioning assay (Quinn et al. 1974; Tully and Quinn 1985), flies learn to avoid one of two odors because it is associated with an electric shock. During the training phase, flies are exposed to one odor together with an electric shock after which the second odor is delivered without electric shock. Temporally distinct memories such as learning,

short-term memory, and middle-term memory can be tested at various time points after the training by allowing flies to choose between the two odors presented without electric shock.

Using this assay, we detected an age-dependent short-term memory defect in Aβ42 flies. High levels of Aβ40 expression also caused short-term memory defects, which suggest that an excess of Aβ40 was also toxic to synaptic plasticity. Sensory motor activity was not significantly affected in Aβ flies, suggesting that the observed defects were attributable to short-term memory defects.

At later stages, Aβ42, but not Aβ40, caused locomotor defects, premature death, and age-dependent neurodegeneration in the brain (Fig. 3). An electron microscopic analysis of Aβ42 fly brains revealed that most degenerating neurons showed necrotic cell death. Neither amyloid fibril, nor NFTs were detected in this model (Iijima et al. 2004). Finelli et al. (2004) showed that Aβ42 peptides expressed in fly eyes developed amyloid deposits and caused retinal degeneration. Using a similar approach, Crowther et al. (2005) expressed the wild-type and EOFAD-related Arctic mutant (E22G) Aβ42 peptides in *Drosophila* neural tissue, and showed that neuronal dysfunction and degeneration induced by the Arctic Aβ42 were more severe than that induced by wild-type Aβ42.

N-terminally truncated and, in particular, pyroglutamate (pE)-modified Aβ42 peptides have been suggested to be important in the initiation of pathological cascades because of their abundance, resistance to proteolysis, rapid aggregation, and neurotoxicity. The pE-modification of Aβ42 is catalyzed by glutaminyl cyclase. A recent report showed that transgenic *Drosophila* expressing pyroglutamate (pE)-modified Aβ42 (Aβ3(pE)-42), which were fed an inhibitor of glutaminyl cyclase, exhibited a reduced accumulation of Aβ3(pE)-42 (Schilling et al. 2008). In this model, expression constructs containing Aβ42N-terminally fused to the prepro-sequence of murine thyroliberin (TRH) were used, and liberation of Aβ was accomplished by prohormone convertase processing in the secretory pathway (Schilling et al. 2008). Neurotoxicity induced by Aβ3(pE)-42 in these flies has not been reported yet.

Comparison of Aβ42 flies to mouse models of Alzheimer's disease

There are many animal models of AD including nematodes, fly, mouse, rat, rabbit, canine, and non-human primates (Woodruff-Pak 2008). Interestingly, each model seems to recapitulate somewhat different aspects of AD. Most transgenic mice that overproduce Aβ successfully recapitulate several pathological lesions including extracellular amyloid deposits, behavioral deficits and memory defects, but they do not exhibit global neuronal loss (Gotz and Ittner 2008; McGowan et al. 2006). In contrast, progressive neurodegeneration was induced, and extensive intracellular accumulation of Aβ42 within neurons was observed in our transgenic Aβ42 flies (Iijima et al. 2008, 2004). Human AD brains show significant accumulation of intraneuronal Aβ42 (Gouras et al. 2000; Takahashi et al. 2002), and, a few mouse models of AD have shown intraneuronal accumulation of Aβ42 that was associated with memory defects (Oddo et al. 2003), significant neuron loss (Casas et al. 2004; Oakley et al. 2006), and axonopathy (Wirths et al. 2006). These results suggest that intraneuronal Aβ42 may contribute to neuronal dysfunction and degeneration in our fly model.

Another unique feature of our fly model is that the toxicity of Aβ40 and Aβ42 can be dissected in vivo. Since most of the mouse models of AD overexpress APP, which produces a series of Aβ species including Aβ40 and Aβ42 after proteolysis, it is difficult to study the toxicity of either species individually. In contrast, we found that while both Aβ40 and Aβ42 could cause memory defects, only Aβ42 caused neurodegeneration with amyloid deposits. Recently established transgenic mice separately expressing Aβ40 or Aβ42 have shown that extracellular amyloid deposit formation is induced by Aβ42, but not by Aβ40. However, no neuronal losses have been observed in Aβ42 mice (McGowan et al. 2005). The Aβ42 fly model thus provides

a unique tool for studying the mechanism underlying intraneuronal Aβ42 accumulation and neurodegeneration.

Implications for anti-Aβ therapies from fly models

An increase in Aβ42 levels above a given threshold in the brain is generally regarded to be the primary event in AD pathogenesis, and approaches to develop disease-modifying therapies have focused on lowering Aβ42 levels. In contrast to EOFAD, in which Aβ42 production and/or aggregation is enhanced as a result of genetic factors, the mechanisms by which Aβ42 reaches pathological levels in the brains of late-onset AD (LOAD) patients are not well understood. The steady state level of Aβ42 reflects the balance between production and clearance of Aβ42, and an imbalance of these activities could be sufficient to raise Aβ42 levels. Thus, reducing Aβ42 levels can be achieved either by attenuating production, or by facilitating degradation and/or clearance of Aβ42 from the brain (Iwata et al. 2005; Tanzi et al. 2004).

Targeting β- and γ-secretases

In order to reduce the Aβ42 level in the brain, the prevention of amyloidogenic processing of APP by application of inhibitors of β-secretase (BACE) and γ-secretase is a viable option (Citron 2004). Both β- and γ-secretase inhibitor treatments ameliorate Aβ-induced toxicity, including semi-lethality and premature death, in triple transgenic flies expressing human APP, human β-secretase, and fly presenilin (dPsn) (Greeve et al. 2004). Recently, Rajendran et al. (2008) developed a β-secretase inhibitor peptide conjugated to a sterol moiety as a membrane anchor, which markedly increased the potency of the inhibitor. Feeding sterol-linked β-secretase inhibitor to the triple transgenic flies increases the survival rates, indicating a reduction in toxicity in vivo.

Drosophila has been used as a functional in vivo system to search for genetic and pharmacological modifiers of γ-secretase activity. *Drosophila* has all the components of the γ-secretase complex, which is responsible for intramembranous cleavage of several transmembrane proteins (Fig. 1), including APP, DCC, ErbB4, N-Cadherin, E-Cadherin, Notch, and Delta (Brunkan and Goate 2005;Periz and Fortini 2004;Takasugi et al. 2003). Psn is the enzymatic component of the γ-secretase complex, and EOFAD mutations in Psn affect the function of the γ-secretase complex in *Drosophila* (Ye and Fortini 1999). Ubiquilin 1 (UBQLN1) is a Psn interactor that promotes the accumulation of PS1 and regulates its endoproteolysis (Mah et al. 2000). The *Drosophila* ortholog of human UBQLN1, dUbqln, modifies the eye phenotype induced by overexpression of dPsn, indicating that the proteins have a functional interaction in vivo (Ganguly et al. 2008;Li et al. 2007). Using a transgenic reporter of γ-secretase-mediated APP processing (Guo et al. 2003), Gross et al. showed that dUbqln and the *Drosophila* homolog of X11 (Hase et al. 2002), an APP interacting protein that modulates Aβ production (Sano et al. 2006), affects APP processing (Gross et al. 2008).

In *Drosophila*, Notch signaling is required during development and functions in many cell fate specification events. The modifier screens of Psn-dependent Notch-related phenotypes identified many genes, and some of these modifiers genetically interact with APP (Mahoney et al. 2006; van de Hoef et al. 2009). Inhibitors of γ-secretase induce developmental defects in *Drosophila* that are remarkably similar to those caused by genetic reduction of *Notch*, indicating that the three-dimensional structure of the drug-binding site(s) in *Drosophila* γ-secretase is remarkably conserved vis-à-vis the same site(s) in the mammalian enzyme (Micchelli et al. 2003).

In summary, these reports suggest that fly models may be a sensitive and functional in vivo system for the validation of newly developed and the prescreening of drug candidates for β- and γ-secretases.

Enhancing A*β*-degrading enzymes

Several peptidases have been identified as candidate A*β*-degrading enzymes, including neprilysin (NEP) (Iwata et al. 2000), insulin-degrading enzyme (IDE) (Qiu et al. 1998), endothelin-converting enzyme 1 (ECE1) (Eckman et al. 2001), cathepsin D (Hamazaki 1996), and serine protease-*α*2-macroglobulin (Qiu et al. 1996). Interestingly, the expression of NEP is reduced in both LOAD patient brains and normally aging brains (Yasojima et al. 2001a; Yasojima et al. 2001b), suggesting that a reduction in A*β*-degrading activity may contribute to the onset and/or progression of LOAD.

Among the A*β*42 degrading enzymes, NEP has been identified as one of the major rate-limiting A*β*-degrading enzymes in the brain (Iwata et al. 2001, 2000). A deficiency in NEP accelerates formation of extracellular amyloid deposits (Farris et al. 2007), amyloid angiopathy (Farris et al. 2007), synaptic dysfunctions (Huang et al. 2006), and memory defects (Huang et al. 2006) caused by human A*β* in transgenic mice. Transgenic (Leissring et al. 2003; Poirier et al. 2006), viral (Iwata et al. 2004; Marr et al. 2003), or ex vivo (Hemming et al. 2007) delivery of NEP to brains of APP transgenic mice reduces extracellular amyloid deposits, synaptic dysfunction, and premature death. Thus, activation of NEP in the brain could be a potential disease modifying therapy for AD by reducing extracellular A*β*.

In addition to extracellular A*β*, intraneuronal A*β*42 may contribute to AD pathogenesis. However, the protective effects of neuronal NEP expression on intraneuronal A*β*42 accumulation and neurodegeneration was not clear. To investigate the effects of NEP on intraneuronal A*β*42 accumulation and neuron loss induced by A*β*42 in vivo, we have recently established transgenic flies expressing human NEP and examined the effects of NEP on A*β*42-induced toxicity in the A*β*42 fly model (Iijima-Ando et al. 2008).

Expression of NEP significantly reduced the level of A*β*42 in both the detergent soluble and insoluble fractions of A*β*42 brains. Additionally, neuronal expression of NEP prevented formation of intraneuronal Thioflavin S-positive A*β*42 deposits in the cell body. Furthermore, NEP dramatically suppressed neuron loss induced by A*β*42. These protective effects were not observed in transgenic flies expressing an inactive mutant form of human NEP (NEPmut) with the amino acid substitution E585V in the zinc-binding motif of NEP (Hama et al. 2001; Shirotani et al. 2001), suggesting that the observed effects are attributable to the enzymatic activity of NEP (Iijima-Ando et al. 2008). These results are consistent with a recent study using APP transgenic mice (Spencer et al. 2008).

Interestingly, neuronal expression of NEP significantly shortened the lifespan of flies, in part through reduced CREB activity. NEP expression also caused axon degeneration in the aged fly brain. These results suggest that the transgenic fly model may also be used to detect potential side effects of a given manipulation (Iijima-Ando et al. 2008).

Targeting A*β* aggregation

A*β* is a hydrophobic and self-aggregation prone peptide (Lansbury and Lashuel 2006). In its native and soluble states, A*β* is an unfolded polypeptide consisting primarily of a random-coil structure (Kelly 2005; Rochet and Lansbury 2000), and its aggregation in vivo is affected by a combination of genetic (DeMattos et al. 2004), environmental (Cherny et al. 2001), and aging (Cohen et al. 2006a) factors. Aggregation of the A*β*42 peptide in the brain parenchyma is a hallmark of AD pathology, and the prevention of A*β* aggregation has been proposed as a therapeutic intervention in AD.

The azo-dye Congo Red binds to *β*-amyloid and inhibits fibrillization of A*β*42 in vitro. Feeding Congo Red to flies resulted in a significant increase in longevity, reduction in plaque formation, and delay in vacuolation in neurons, as well as preservation of the architecture of the brain and

retinal tissues (Crowther et al. 2005). In a recent study, ligands were designed to specifically target aggregation of Aβ by binding and stabilizing the α-helical conformation of amino acids 13–26 in Aβ. These inhibitors reduced fibril formation of Aβ40 in vitro, and ameliorated Aβ42 toxicity to cells in culture and to hippocampal slice preparations. Additionally, feeding these inhibitors to flies expressing Aβ42 in the central nervous system prolonged lifespan, increased locomotor activity, and reduced neurodegeneration (Nerelius et al. 2009).

In addition to mature amyloid fibrils, Aβ can form a variety of misfolded structures in vitro, including multiple monomer conformers, different types of prefibrillar assemblies including small oligomers (Walsh et al. 2002), higher molecular weight complexes known as Aβ-derived diffusible ligands (ADDLs) (Lambert et al. 1998), oligomers composed of 15–20 monomers (Kayed et al. 2003), dodecameric oligomers (Aβ*56) (Lesne et al. 2006), and protofibrils (Harper et al. 1997). Some of these intermediate Aβ species can also be found in the cerebrospinal fluid and brains of AD patients (Georganopoulou et al. 2005; Kuo et al. 1996). Since aggregation of Aβ42 in the brain parenchyma is a hallmark of AD pathology (Thal et al. 2006), the neurotoxicity of Aβ42 was initially correlated with the tendency of Aβ42 to form insoluble mature amyloid fibrils (Murakami et al. 2003; Yankner et al. 1989). However, recent experimental data indicates that the soluble prefibrillar assemblies cause more severe synaptic dysfunctions, cognitive defects, and neurodegeneration than do the mature fibrils (Haass and Selkoe 2007).

A study using the *Drosophila* model in combination with computational predictions of Aβ42 aggregation has further demonstrated that there is a strong positive correlation between the magnitude of neurotoxicity, as manifested in locomotor deficits and reduced lifespan, and the propensity of Aβ42 to aggregate into protofibrils (Luheshi et al. 2007). These results indicate that soluble prefibrillar assemblies, but not mature amyloid fibrils, of Aβ42 are the primary neurotoxic species, which is consistent with the observation that the level of soluble Aβ is better correlated with the severity of cognitive impairment than the density of insoluble amyloid deposits (Lue et al. 1999; Naslund et al. 2000).

The complexity of Aβ42 aggregation and toxicity

In addition to the “quantitative” changes in toxicity, the structural diversity of Aβ42 species may also “qualitatively” influence the pathogenicity of Aβ42, as has been well established in the pathogenesis of prion disease mediated by various PrP species (Prusiner 2004). Deshpande et al. (2006) reported that both Aβ oligomers and ADDLs induced rapid and massive neuronal death, with ADDLs exhibiting their effects with a slightly slower time course. In contrast, Aβ fibrils induced progressive dystrophy and modest cell death. Recently, Chiang et al. reported that Aβ42 oligomers and larger aggregates have different effects on synaptic transmission and LTD at the neuromuscular junction of *Drosophila* larvae (Chiang et al. 2009).

We investigated the correlation between levels of aggregation of Aβ42 and memory defects and neurodegeneration through genetic manipulation of Aβ42 aggregation in the *Drosophila* model. We have established transgenic fly lines carrying human Aβ42 mutants with differing tendencies to aggregate (Iijima et al. 2008). Human Aβ42 with the Arctic mutation (E22G, Aβ42Arc), which causes EOFAD (Nilsberth et al. 2001), is more aggregation-prone than wild-type Aβ42 (Cheng et al. 2004; Johansson et al. 2006; Lord et al. 2006; Whalen et al. 2005). In contrast, an artificial mutation, L17P, Aβ42art, suppresses amyloid fibril formation (Fay et al. 1998; Morimoto et al. 2004; Murakami et al. 2003). Aβ42Arc accumulated in the insoluble fraction more readily than Aβ42. In contrast, Aβ42art accumulated primarily in the soluble fraction and was greatly reduced in the insoluble fraction.

Remarkably, Aβ42, Aβ42Arc, and Aβ42art each induced a distinct pathology. Aβ42Arc fly brains showed more extensive cell body loss than Aβ42 or Aβ42art brains. In contrast, the level of neuropil degeneration was greatest in Aβ42art flies. Thioflavin S staining, which labels aggregated Aβ42, revealed that the degenerated structures in Aβ42, Aβ42Arc, and Aβ42art flies were closely correlated with intraneuronal accumulation of each Aβ peptide (Fig. 4). Aβ42Arc accumulated primarily as deposits in the cell soma, while Aβ42art was distributed primarily in the neurites. Aβ42 was detected both in the cell body and in the neurites, but to a lesser extent than the mutants (Fig. 4). Thus, the differences in aggregation of Aβ42 correlated with qualitative shifts in pathology in the fly brain (Iijima et al. 2008).

The increase in Aβ42 aggregation proneness associated with the pathogenic Arctic mutation (E22G, Aβ42Arc) correlated with more severe effects on memory, locomotor ability, and lifespan relative to the wild-type Aβ42. These data are consistent with the fact that Aβ42Arc causes EOFAD (Nilsberth et al. 2001) and indicates that aggregation proneness contributes to Aβ42 toxicity in vivo. In contrast, an artificial mutation (L17P) that decreased Aβ42 aggregation proneness suppressed the toxic effects on locomotor function and lifespan, but caused an earlier onset of memory defects, indicating that not all pathogenic effects of Aβ42 are directly correlated with aggregation proneness.

In summary, these results demonstrate that manipulation of the aggregation propensity of Aβ42 modified the pathogenicity of Aβ42 in vivo, and suggest that the partial prevention of Aβ42 amyloidgenesis by aggregation inhibitors may result in qualitative shifts in the pathogenic effects of Aβ42 (Iijima and Iijima-Ando 2008). Furthermore, the tendency of Aβ42, which is normally an unfolded polypeptide consisting primarily of a random-coil structure in the native, soluble state (Kelly 2005; Rochet and Lansbury 2000), to aggregate may be affected by a combination of genetic (DeMattos et al. 2004), environmental (Cherny et al. 2001), and aging factors (Cohen et al. 2006b), and the resultant Aβ42 conformers or species may contribute to the heterogeneous pathogenesis of AD (Cummings 2000).

Insights into pathomechanisms and genetic modifiers of Aβ42-induced toxicity from fly models

The power of genetics in *Drosophila* provides in vivo experimental platforms to examine whether gain or loss of function of a given gene can modify a disease phenotype in a time efficient and cost effective manner (Shulman et al. 2003). Since *Drosophila* generally does not possess as many redundant gene families as mammals, studying the consequence of a single gene disruption is easier in the fly. This enables us to systematically test hypotheses derived from biochemical or histopathological analysis of human disease tissues, genetic association studies performed on particular disease phenotypes, or findings in in vitro model systems. In addition to hypothesis testing, one of the most powerful advantages of using a *Drosophila* model is the ability to conduct large scale, genome-wide, forward genetic screens to identify novel genes and pathways that modify a given disease phenotype. The findings from this unbiased approach have great potential to open novel research areas.

Retinal toxicity has been widely used in genetic screens in *Drosophila* models to identify modifiers of neurode-generative disease. Expression of Aβ42 in the fly eye results in a visible reduction in eye size and a roughened eye surface due to the death of intrinsic photoreceptors and supporting cells (Cao et al. 2008). Using eye degeneration as the read-out phenotype, Cao et al. (2008) have tested the effects of 1,963 EP transposon insertions in the fly genome. The EP transposon has a GAL4 activated promoter and, depending upon the site and orientation of the insertion, the transposon will upregulate or downregulate gene activity (Rorth et al. 1998). Using this approach, 23 modifiers were identified, including genes involved in the secretory pathway (e.g., the human orthologs of carboxypeptidase D and the AP3 subunit δ-1),

cholesterol homeostasis (AMP kinase γ-subunit), copper transport (ATP7), and ubiquitin/proteolysis (ubiquitin-conjugating enzyme E2Q and NEP 2), which have been implicated in AD pathogenesis. This screen also identified genes related to the regulation of chromatin structure as a potential pathway mediating Aβ42 toxicity. Loss-of-function mutations of several components of the Sin3A complex, which regulates transcription and chromatin remodeling through interactions with other transcription factors, including SAP130, and the histone deacetylases HDAC1 and 4, all enhanced the rough eye phenotype in Aβ42 flies (Cao et al. 2008).

Another screen for loss-of-function mutations that enhance or suppress the eye degeneration phenotype induced by Aβ42 identified that the Toll/NFκB signaling pathway as mediating human Aβ42 toxicity, suggesting the involvement of innate immunity/inflammatory pathway components in Aβ42 toxicity (Tan et al. 2008). In addition, recent study showed that Aβ42-induced toxicity was enhanced by the activation of autophagy and partially rescued by inhibition of autophagy, suggesting that autophagic-lysosomal injury is involved in Aβ42 toxicity (Ling et al. 2009).

Rival et al. conducted a genome wide gene-expression analysis and complementary genetic screen. Microarray analysis identified changes in the expression of oxidative stress-related genes induced by Aβ42 expression, and a subsequent genetic screen confirmed the importance of oxidative stress. The iron-binding protein ferritin and the H2O2 scavenger catalase are the most potent suppressors of wild-type and Arctic (E22G) Aβ42 toxicity, and oxidative stress mediated by the Arctic Aβ42 was reversed by ferritin despite an increase in the levels of Aβ42. Likewise, treatment with the iron-binding compound clioquinol increased the lifespan of flies expressing Arctic Aβ42 (Rival et al. 2009).

Deficiencies in the retromer sorting pathway have been linked to late-onset Alzheimer's disease (Small and Gandy 2006). Using transgenic flies expressing human APP and BACE, the role of the retromer sorting pathway in Aβ-induced neurodegeneration was studied. When APP and BACE are overexpressed, flies deficient in retromer complex components developed neuronal loss and Aβ aggregates (Muhammad et al. 2008). These results suggest that retromer deficiency observed in late-onset AD may contribute to the disease pathogenesis.

Recent reports suggest that, in EOAD caused by Psn mutations, more global perturbations in pathways involving other γ-secretase substrates should be considered in addition to Aβ42 toxicity (Baki et al. 2004; Marambaud and Robakis 2005; Marambaud et al. 2003; Saura et al. 2004). Recently, choosing from over 130 EOFAD mutations in *Presenilin-1*, Seidner et al. (2006) introduced 14 corresponding mutations at conserved residues in *Drosophila* Psn and assessed the Notch-cleavage activity of the mutants in transgenic flies. They found that the activity levels of the Psn mutants were tightly linked to their age-of-onset values, providing evidence that disease severity in humans primarily reflects differences in Psn lesions rather than contributions from unlinked genetic or environmental modifiers.

Drosophila models of tauopathies

Tau abnormalities are associated with AD and other neurodegenerative diseases

Neurofibrillary tangles are intracellular protein inclusions composed of abnormally hyperphosphorylated tau protein (Grundke-Iqbal et al. 1986a, b; Kosik et al. 1986; Wood et al. 1986). NFTs are associated with AD and a range of neurodegenerative diseases called tauopathies. These include corticobasal degeneration (CBD), Pick's disease (PiD), progressive supranuclear palsy (PSP), sporadic frontotemporal dementia (FTD), inherited frontotemporal dementia, and Parkinsonism linked to chromosome 17 (FTDP-17) (Lee et al. 2001).

Multiple tau gene mutations are identified in FTDP-17 patients, and tau polymorphisms appear to be genetic risk factors for other tauopathies (Lee et al. 2001). To date, tau mutations are not associated with any known form of familial AD. However, tau haplotypes driving slightly higher tau expression increase the risk of AD (Myers et al., 2005). More recent genetic association studies showed that tau variants associated with higher tau protein levels affect age onset of AD in the presence of Aβ pathology (Kauwe et al. 2008). These reports suggest that tau may play a role in the pathogenesis of AD as a modulator of disease progression.

Tau is a microtubule associated protein that is expressed in neurons and predominantly localizes in axons. Tau is also expressed in astrocytes and oligodendrocytes at lower levels, and, in some disease conditions, tau forms aggregates in these glial cells (Tashiro et al. 1997). In the adult human brain, alternative mRNA splicing produces six tau isoforms, which contain three repeats (3R tau) or four repeats (4R tau) of the microtubule-binding domain at the C-terminus. The major function of tau is to regulate the assembly and stability of microtubules, and 4R tau is approximately 40-fold more efficient at binding microtubules than 3R tau. In addition, 4R tau is more prone to form filaments than 3R tau (Lee et al. 2001).

Tau mutations that have been identified in FTDP-17 are missense, deletion, or silent mutations. These mutations change the relative tau isoform ratio, reduce the level of tau-microtubule binding, and/or increase tau aggregation. The autosomal dominant inheritance pattern of familial FTDP-17 and the prominent tau pathology in the brains of sporadic tauopathy patients suggest a gain-of-function mechanism in tau toxicity (Lee et al. 2001). However, the mechanisms by which tau toxicity mediates neuronal dysfunction and degeneration in the AD and other tauopathies are unknown.

The identification of pathogenic mutations in human Tau in familial FTDP-17 cases has greatly advanced the development of transgenic animal models of tauopathies. A variety of transgenic animal models including *C. elegans*, *Drosophila*, and mouse expressing human wild-type or FTDP-17-linked tau mutants have been generated (Gotz and Ittner 2008; Lee et al. 2005). These animal models recapitulate many important aspects of human diseases, and have been used to explore pathogenic mechanisms as well as to test and develop novel therapeutic strategies. We will briefly summarize *Drosophila* tauopathy models and a number of insights obtained from these models.

Modeling tauopathies in *Drosophila*

Human tau-induced neuronal dysfunction and degeneration have been successfully modeled in *Drosophila*. Tau-induced neurotoxicity in flies was first suggested by an anatomical study showing that transgenic expression of a fusion protein of bovine tau-green fluorescent protein in sensory neurons caused axonal degeneration (Williams et al. 2000). Subsequently, fly models of human tauopathy were created by expressing human wild-type or FTDP-17-linked tau mutants, R406W and V337M (Jackson et al. 2002; Wittmann et al. 2001). Expression of human wild-type or mutant tau in neurons caused an age-dependent progressive neurodegeneration characterized by nuclear fragmentation and vacuole formation in neurons of the cortex and neuropil (Nishimura et al. 2004; Wittmann et al. 2001). In addition, tau expression in fly eyes induced retinal toxicity in adult flies, which appears as a “rough” eye phenotype, characterized by a reduced external eye size and retina thickness, loss of the regular ommatidial organization and neuropil degeneration in the medulla (Jackson et al. 2002; Nishimura et al. 2004). Accumulation of disease-associated phospho- and conformational tau epitopes is observed in brains and eyes. Tau transgenic flies also have a reduced lifespan (Wittmann et al. 2001). Most pathological phenotypes induced by mutant tau are more severe than those induced by wild-type tau (Wittmann et al. 2001), suggesting that this fly model system recapitulates an important pathobiological aspect of tau.

In Drosophila genome, there is a single endogenous tau gene. The fly tau protein accumulates in axonal processes, as is the case with mammalian tau (Heidary and Fortini 2001). Interestingly, overexpression of the fly tau in neurons or eye induced apoptotic neuronal cell death (Chen et al. 2007).

Human tau- or fly tau-induced synaptic dysfunctions have also been studied in *Drosophila* larval motor neurons and neuromuscular junctions. Expression of tau within motor neurons causes morphological abnormalities in the presynaptic terminals and defects in synaptic transmission and microtubule-based axonal transport (Chee et al. 2005; Mudher et al. 2004). In addition, expression of human or fly tau in the mushroom body neurons, a center for olfactory learning and memory in flies, impairs associative olfactory learning and memory prior to the onset of neurodegeneration (Mershin et al. 2004).

Neurofibrillary tangle formation, a hallmark of AD pathology and tauopathies, is not observed in the fly neurons (Wittmann et al., 2001), indicating that tau toxicity is not conferred by large insoluble aggregates of tau in the *Drosophila* models. Studies in mouse models of tauopathy have shown that tau-induced memory defects and neuro-degeneration can be dissociated from NFT formation (Le Corre et al. 2006; Santacruz et al. 2005). These results suggest that some soluble, non-tangled form of phosphorylated tau is intrinsically toxic and that *Drosophila* models of tauopathies may recapitulate early, pretangle events of tau-associated neurodegeneration (Lee et al. 2005).

Insights into pathomechanisms and genetic modifiers of tau-induced toxicity from fly models

The rough eye phenotype is especially useful because the exterior eye size can be easily scored. Moreover, since the eye is not vital for fly survival, the tau eye phenotype provides a highly sensitive and robust readout for assessing phenotypic effects of genetic manipulations (Fig. 5) (Fortini and Bonini 2000). As summarized in this section, many groups have demonstrated that this system can lead to powerful insights into the pathophysiological mechanisms underlying tau toxicity.

Roles of tau phosphorylation at disease-associated sites in tau toxicity

Tau protein is abnormally hyperphosphorylated and aggregated into NFT in the brains of AD and tauopathy patients. There are 79 putative Ser/Thr phosphorylation sites in the longest isoform of tau (441 amino acids), and at least 30 of those sites are phosphorylated in NFTs (Buee et al. 2000). In vitro studies have revealed that tau phosphorylation at some of these sites decreases microtubule binding or promotes tau aggregation (Gong et al. 2005). Since many of the mutations that cause FTDP-17 cluster around the microtubule binding domain and reduce microtubule binding or promote tau aggregation, an imbalance in phosphorylation and/or dephosphorylation of tau may initiate the abnormal metabolism and toxicity of tau in disease pathogenesis (Ballatore et al. 2007; Buee et al. 2000; Lee et al. 2001).

A number of studies have revealed that many kinases can phosphorylate tau in vitro (Gong et al. 2005). Among the 30 disease-associated phosphorylation sites, approximately half are targets for Serine/Proline (SP) or Threonine/Proline (TP) kinases (Gong et al. 2005). In vitro, these sites can be phosphorylated by glycogen synthase kinase-3β (GSK-3β), cyclin-dependent kinase-5, cyclin-dependent kinase-1 (Cdk1), cyclin-dependent kinase-2 (Cdk2), mitogen-activated protein kinase (MAPK), and c-Jun N-terminal kinase (JNK). In contrast, other non-proline directed sites are phosphorylated by protein kinase A (PKA), protein kinase B (Akt), protein kinase C, calcium/calmodulin-dependent protein kinase II, or microtubule affinity-regulating kinase (MARK). These sites can be dephosphorylated by protein phosphatases 1,

2A, 2B, and 2C (Gong et al. 2005). In contrast, little is known about the role of kinases and phosphatases in the phosphorylation/dephosphorylation of specific tau sites, or the effects on aggregation and toxicity in vivo.

A forward genetic screen using *Drosophila* tauopathy models has identified kinases and phosphatases as modifiers of tau toxicity (Shulman and Feany 2003). In addition, candidate testing has revealed Cdk5 and GSK-3*β* as tau kinases (Jackson et al. 2002; Shulman and Feany 2003). Moreover, when fly GSK-3*β* is coexpressed with tau, large NFT-like aggregates form in photoreceptor neurons, and neurodegeneration is enhanced (Jackson et al. 2002), providing in vivo evidence that GSK-3*β* is involved in NFT formation.

To directly establish the role of tau phosphorylation at SP/TP sites in mediating toxicity, forms of tau with phosphorylation-incompetent or phosphorylation-mimicking Ser/Thr kinase sites were expressed (Nishimura et al. 2004; Steinhilb et al. 2007b). A tau construct in which all 14 SP/TP kinase target sites are mutated to alanine, thus rendering the protein phosphorylation incompetent, has greatly reduced neurotoxicity.(Steinhilb et al. 2007b). Conversely, a construct pseudo-phosphorylated at these 14 sites, by substitution of glutamate for the serines and threonines, substantially increases toxicity in both the fly eye and brain (Fulga et al. 2007; Steinhilb et al. 2007b). Moreover, single- or double-phosphorylation mutants, or a mutant with five altered residues (S202/205, T212, and T231/S235), did not exhibit substantially lower toxicity or accumulation of disease-associated conformational epitopes, compared to wild-type tau. These results suggest that the SP/TP sites work in concert to promote toxicity (Steinhilb et al. 2007a).

Among the non-proline directed phosphorylation sites, S262 and S356 have been shown to play critical roles in tau toxicity. Introducing alanine mutations at these sites dramatically reduced tau-induced neurodegeneration in fly eyes and brains (Nishimura et al. 2004). These sites are located in the microtubule binding domains and phosphorylation at these sites, especially S262, promotes dissociation of tau from microtubules in vitro (Biernat et al. 1993). These sites are phosphorylated by MARK kinases, which regulate microtubule dynamics, epithelial cell polarity, and neuronal differentiation under normal conditions and bind to NFT in AD brains (Lu 2009).

In the fly tauopathy model, overexpression of the *Drosophila* homolog of MARK (dMARK, also called partitioning defective-1, PAR-1) increases tau phosphorylation at S262 and S356 and enhances toxicity (Nishimura et al. 2004). Interestingly, dMARK was found to increase tau phosphorylation at multiple SP/TP sites, and mutating S262 and S356 to alanine (S2A tau) reduces phosphorylation at SP/TP sites. These results suggest that phosphorylation at S262 and S356 by dMARK enhances tau toxicity by promoting tau phosphorylation at the SP/TP sites by proline-directed kinases. However, a more recent study shows that S2A tau is resistant to GSK-3*β* induced phenotypic enhancement but not phosphorylation, suggesting that tau toxicity is not determined solely by phosphorylation (Chatterjee et al. 2009).

The upstream signaling mechanisms that regulate tau phosphorylation under normal and disease conditions are not fully understood. Recent studies have shown that the tumor suppressor protein LKB1 activates dMARK and promotes tau phosphorylation and toxicity in *Drosophila*. Because diverse stress stimuli, such as high osmolarity and human APP-induced neurotoxicity, can promote tau phosphorylation and toxicity through LKB1 and PAR-1 activation (Wang et al. 2007), the LKB1/PAR-1/tau phosphorylation cascade may be involved in AD pathogenesis.

Actin cytoskeleton

Intraneuronal actin-rich inclusions called Hirano bodies are found in many neurodegenerative diseases including AD and tauopathies (Hirano 1994), and also in the brain of mouse models of tauopathy (Fulga et al. 2007). Forward genetic screens with *Drosophila* tauopathy models have identified components of the actin cytoskeleton as modifiers of tau-induced retinal degeneration (Blard et al. 2007; Shulman and Feany 2003). Fulga et al. (2007) demonstrated that R406W tau-induced neurodegeneration is associated with accumulation of filamentous actin (F-actin)-containing rods in the *Drosophila* brain and retina. Actin directly interacts with tau in vivo, and the changes in actin structure occur downstream of tau phosphorylation. Furthermore, Aβ synergistically enhances the ability of wild-type tau to promote alterations in the actin cytoskeleton and neurodegeneration (Fig. 6).

Cell-cycle activation

Abnormal accumulations of cell-cycle proteins are observed in post-mitotic neurons in AD and tauopathies (Herrup and Yang 2007). Khurana et al. (2006) demonstrated that abnormal expression of cell-cycle markers is recapitulated in the brain of wild-type and R406W tau flies. An extensive genetic analysis showed that cell-cycle activators enhanced, and cell-cycle inhibitors suppressed, tau-induced neurodegeneration. Furthermore, this study showed that cell-cycle activation was downstream of tau phosphorylation and that activation of TOR (target of rapamycin kinase) by tau overexpression induced neurodegeneration in a cell-cycle-dependent manner (Khurana et al. 2006).

Oxidative stress

Oxidative stress occurs early in the progression of Alzheimer disease (Moreira et al. 2008). Genetic reduction in antioxidant defense enzymes enhanced, whereas activation of these enzymes or administration of the anti-oxidant α-tocopherol (vitamin E) suppressed, tau-induced neurotoxicity in the fly brain (Dias-Santagata et al. 2007). These manipulations did not affect tau phosphorylation but did alter tau-induced activation of the JNK pathway and cell-cycle activation. Since Aβ causes oxidative stress, these findings support the hypotheses that oxidative stress and activation of cell-cycle components may play a role in AD (Klein and Ackerman 2003).

Autophagy and lysosomal function

AD and tauopathies are associated with the accumulation of misfolded proteins, and the factors that regulate the clearance of these aggregation-prone proteins could be therapeutic targets. For example, TOR inhibition promotes autophagy and upregulation of TOR is observed in the AD brain (Williams et al. 2006). Berger et al. (2006) have shown that rapamycin promotes autophagy in a number of fly models of neurodegeneration, including a tauopathy model. Interestingly, the effect of rapamycin was more prominent to the toxicity induced by R406W tau than wild-type tau, suggesting that rapamycin treatment led to preferential degradation of non-microtubule-bound (and aggregation-prone) forms. In addition to autophagy, a study demonstrated that loss of *Drosophila* benchwarmer (bnch), a lysosomal sugar carrier, strongly enhances tau neurotoxicity, suggesting the importance of lysosomal activity in tau degradation (Dermaut et al. 2005).

Aminopeptidase

Each neurodegenerative disease selectively targets certain vulnerable neuronal populations, and the underlying mechanisms are largely unknown. In AD and tauopathies, the cerebellum is relatively resistant to neurodegeneration. A recent cross-species, functional genomic approach has identified a potential role for the puromycin-sensitive aminopeptidase (PSA) in selective neurodegeneration in tauopathy (Karsten et al. 2006). Several genes, including PSA,

are prominently upregulated in the cerebellum, but not in other regions of the brain, in a transgenic tau P301L-expressing mouse. The upregulation of these genes in a relatively resistant brain region may indicate a protective role against tau-induced neurodegeneration (Karsten et al. 2006). Using the *Drosophila* tauopathy model, the effects of manipulation of these genes was tested. Overexpression of PSA suppressed, whereas PSA loss-of-function exacerbated, tau-induced neurodegeneration. Furthermore, human PSA was found to degrade tau. Finally, PSA was shown to be differentially expressed between human cerebellum and frontal cortex in both frontotemporal dementia cases and controls (Karsten et al. 2006). These data highlight the utility of using both mouse and fly models for genetic screening and functional assessment of modifiers of neurodegeneration.

Conclusions

Drosophila have proven to be excellent models for human neurodegenerative diseases as a powerful tool for both hypothesis-testing approaches and non-biased genome-wide screens (Bilen and Bonini 2005; Fortini and Bonini 2000; Khurana 2008; Lu and Vogel 2009; Marsh and Thompson 2004; Sang and Jackson 2005; Shulman et al. 2003). The fly models of neurodegenerative disease can be used to systematically evaluate the effects of known genetic and environmental risk factors, and candidates of susceptibility genes identified by genetic association studies with humans or that revealed by functional genomics with mouse models. Also, the genes discovered in the forward genetic approach in fly models may help reveal disease susceptibility genes in humans. Furthermore, because of the lack of a stringent blood–brain barrier in the fly, which allows compounds to easily gain access to the nervous system, fly models are also excellent in vivo model for the testing and screening of therapeutic compounds (Marsh and Thompson 2006). We anticipate that, in addition to genetic screens, drug screens using the fly models will play a significant role in the development of therapeutic avenues for AD and tauopathies in the future.

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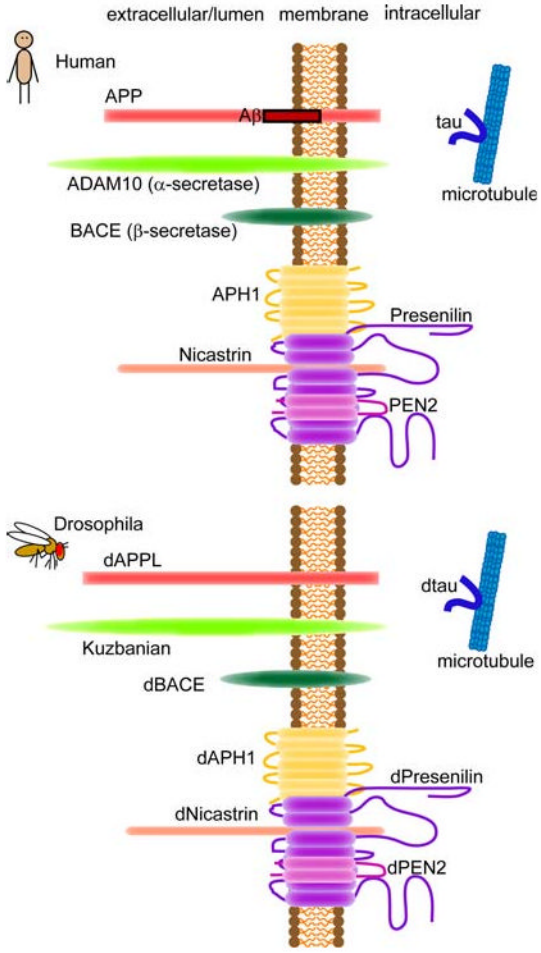

Fig. 1.

Schematic illustration of APP, α- and β-secretase, the components of γ-secretase complex and tau in human and *Drosophila*

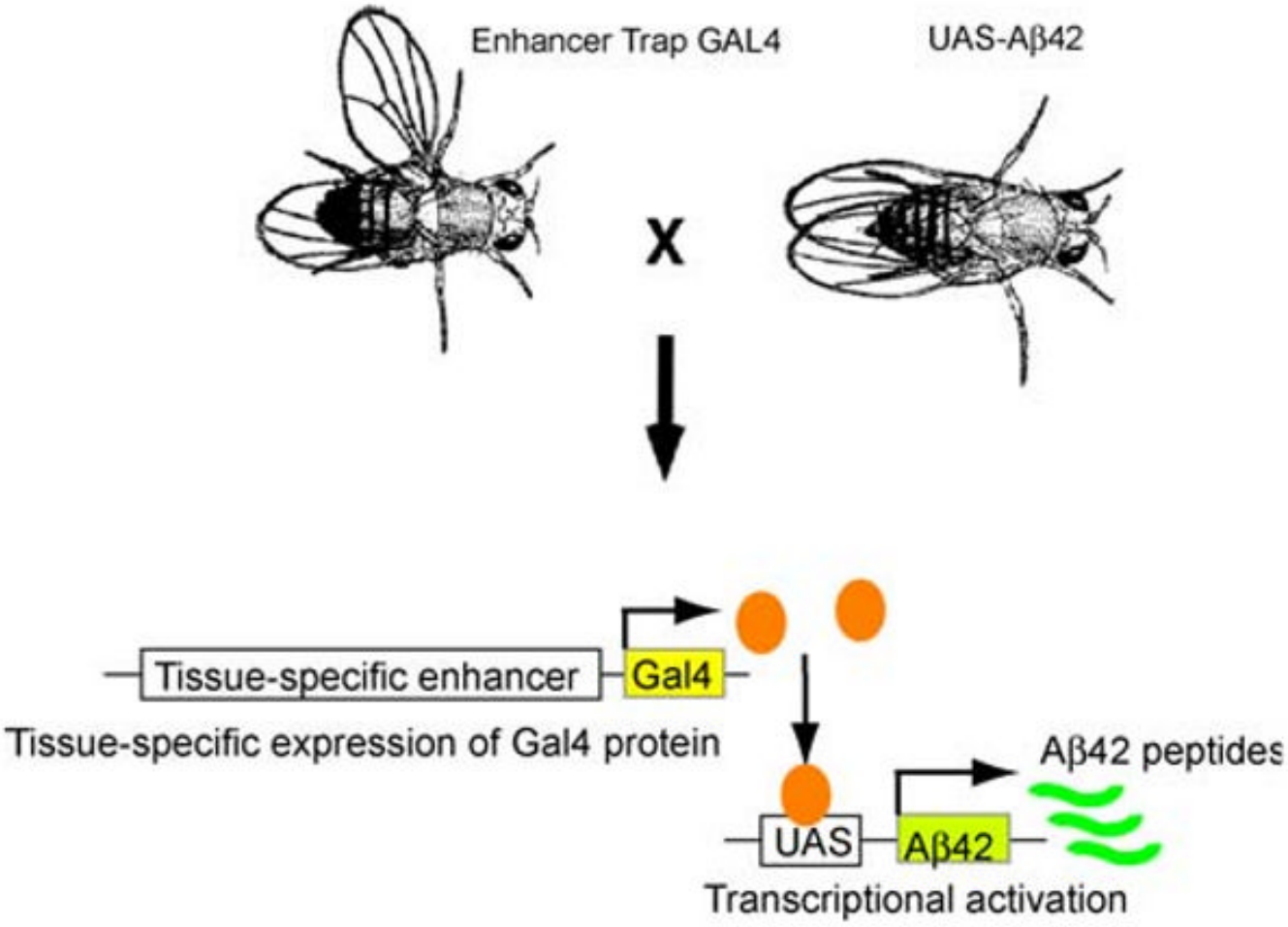

Fig. 2.

Spatial targeting of transgene expression in *Drosophila*. GAL4/UAS system. Driver lines expressing the transcriptional activator GAL4 in a tissue-specific fashion are crossed to UAS-lines with genomic inserts of a target gene fused to five GAL4-binding sites arrayed in tandem (5 × UAS) (shown here as UAS-A*β*42). Adapted from Development, (Brand and Perrimon 1993)

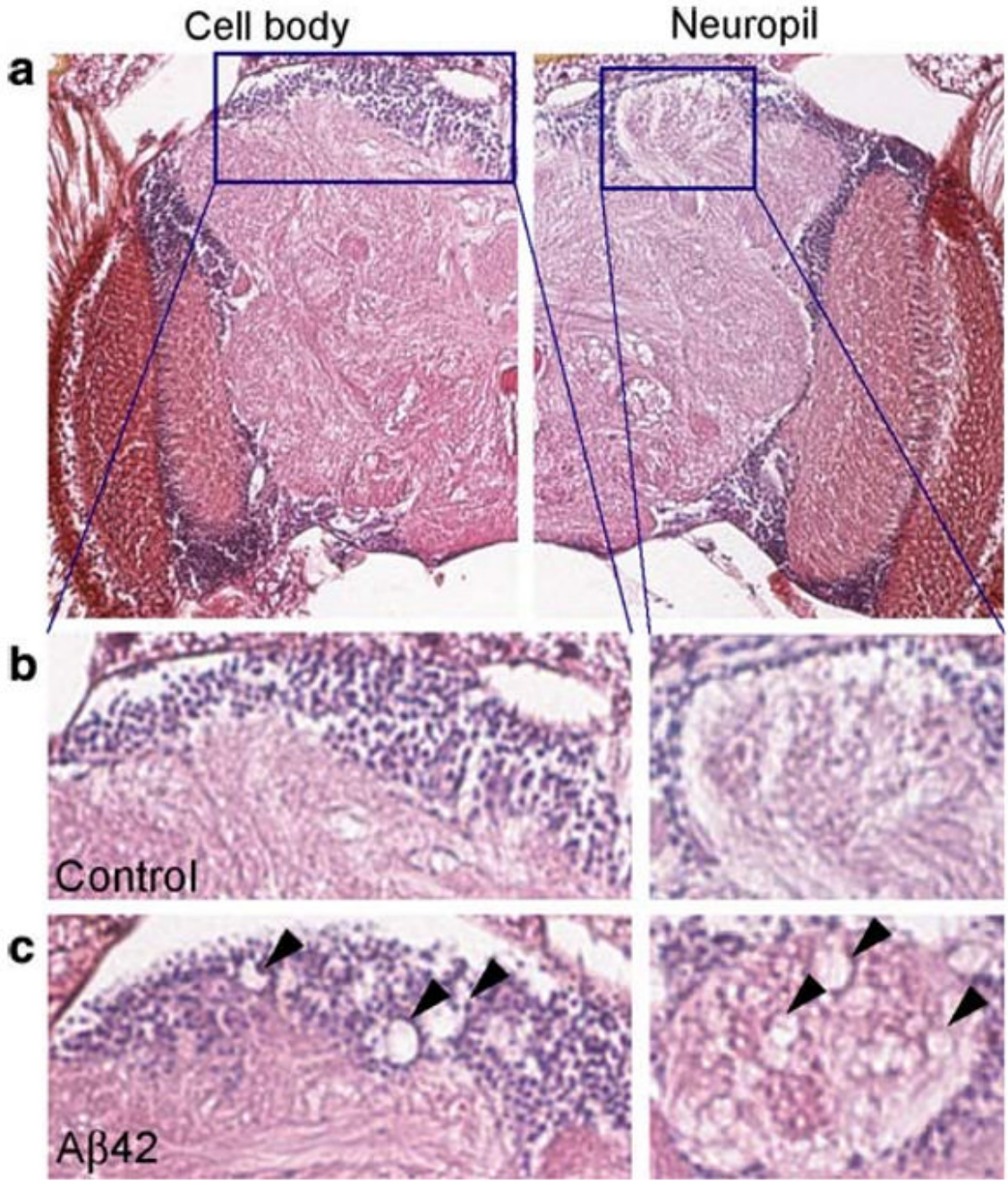

Fig. 3.
Cell body and neuropil degeneration in Aβ42 flies. **a**–**c** Neurodegeneration in Aβ42 flies at 25 days old. The cell body and neuropil region in the mushroom body are enlarged. *Arrowheads* in **c** indicate neurodegeneration. Adapted from PLoS One, (Iijima et al. 2008)

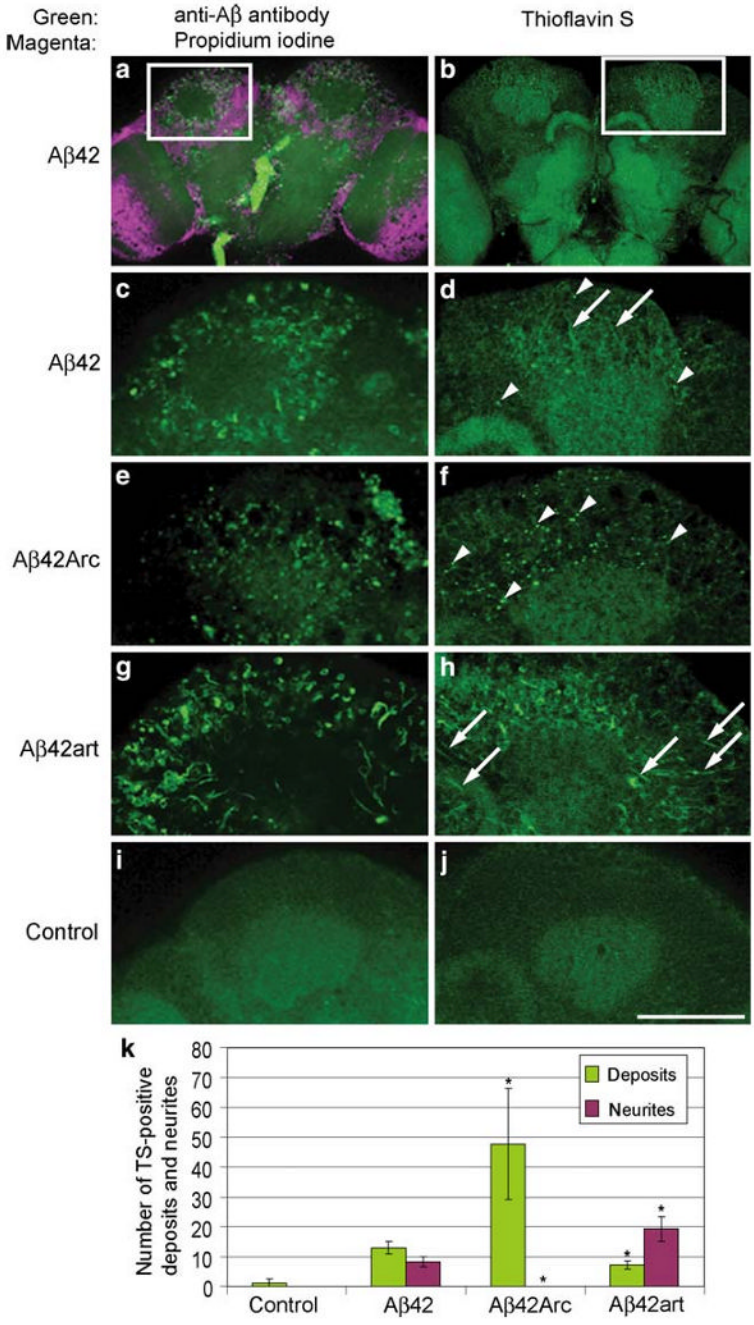

Fig. 4.

Distribution and aggregation of A*β*42, A*β*42Arc, and A*β*42art peptides in fly brains. **a**, **c**, **e**, **g** and **i**, Immunostaining of brains of 25 day-old flies with anti-A*β* antibody (*green*). In **a**, nuclei were stained with propidium iodide (*magenta*). **b**, **d**, **f**, **h** and **j**, Thioflavin S (TS) staining of brains of 25 day-old flies. *Arrowheads* and *arrows* indicate TS-positive deposits and neurites, respectively. No signal was detected in the control **i**, **j**. *Scale bar* in **j**: 50 μm. **c** and **d** are enlarged images of the boxed regions in **a** and **b**, respectively. **k** Numbers of TS-positive deposits and neurites were presented as averages ± SD (n = 6 hemispheres). *Asterisks* indicate significant differences from A*β*42 ($P < 0.05$, Student's *t* test). Adapted from PLoS One, (Iijima et al. 2008)

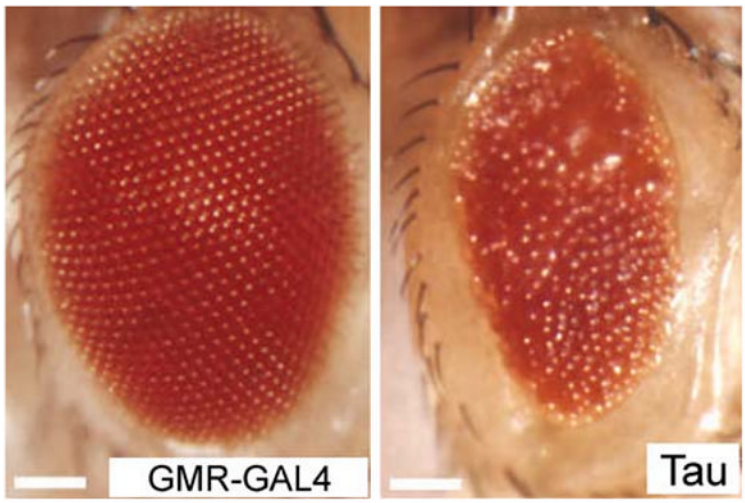

Fig. 5.
Neurotoxicity in the retina of tau transgenic flies. Compare normal eye structure (*left*) with transgenic wild-type tau-induced rough eye (*right*). The *scale bars* represent 10 μm. Adapted by permission from Macmillan Publishers Ltd: Nature Cell Biology, (Fulga et al. 2007), copyright (2007)

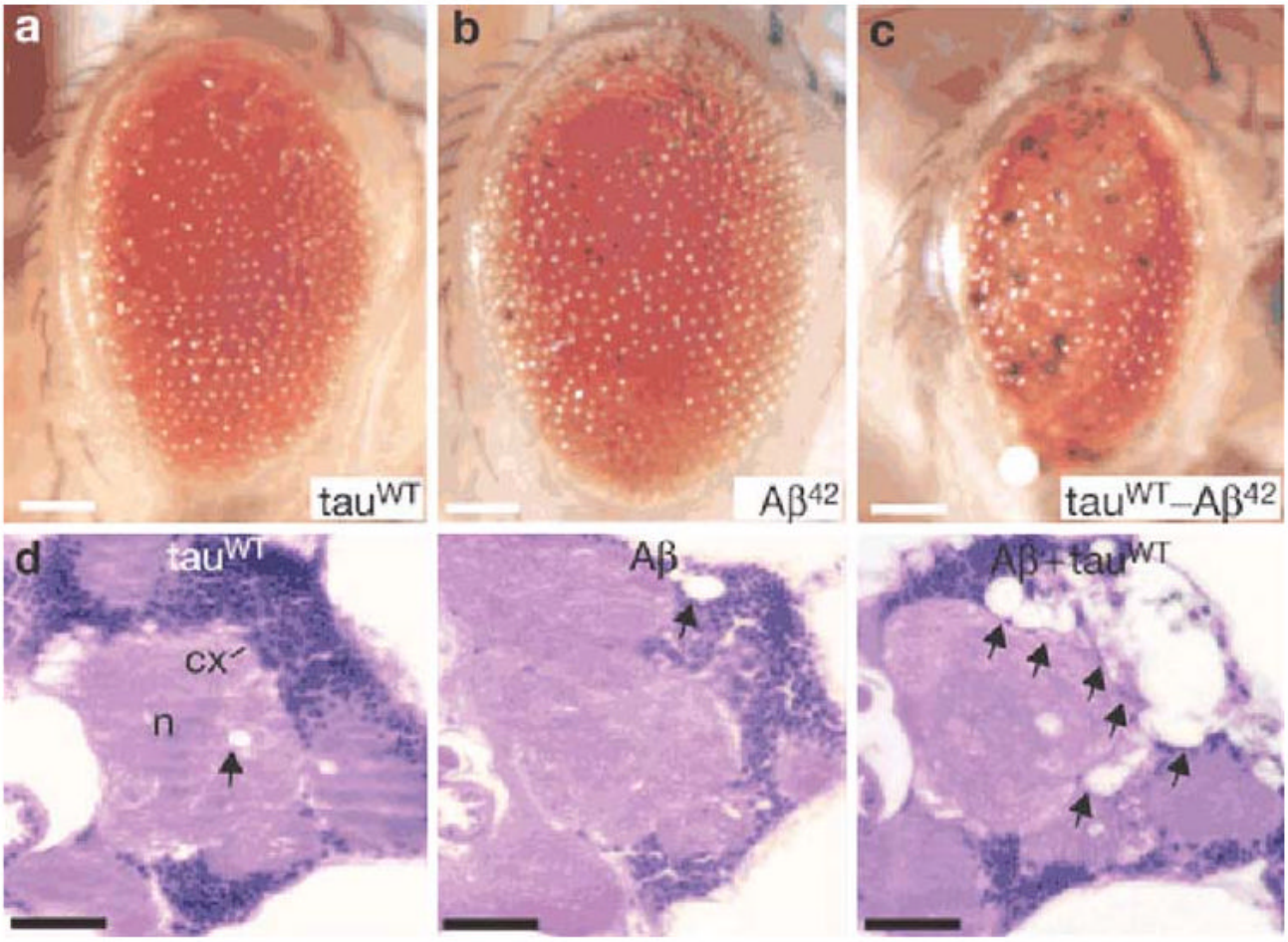

Fig. 6.

A*β* and tau interact in a synergistic manner to promote neuronal degeneration. **a**–**c** Retinal toxicity in flies expressing wild-type tau **a**, A*β* **b** and double-transgenic flies expressing wild-type tau and A*β* **c. d** Hematoxylin and eosin-stained frontal brain sections from flies expressing wild-type tau, A*β* or coexpressing wild-type tau and A*β* indicates that A*β* substantially enhances wild-type tau-induced neurodegeneration. Vacuolization of the neuropil (n) and cortex (cx) is indicated by *arrows*. Adapted by permission from Macmillan Publishers Ltd: Nature Cell Biology, (Fulga et al. 2007), copyright (2007)