Published in final edited form as:

Exp Neurol. 2015 December ; 274(0 0): 58–71. doi:10.1016/j.expneurol.2015.05.013.

Modeling the complex pathology of Alzheimer's disease in *Drosophila*

Pedro Fernandez-Funez^{1,2,3,*}, Lorena de Mena¹, and Diego E. Rincon-Limas^{1,2,3,*}

¹McKnight Brain Institute, Department of Neurology, University of Florida, Gainesville, FL 32611, USA

²Department of Neuroscience and Center for Translational Research in Neurodegenerative Disease, University of Florida, Gainesville, FL 32611, USA

³Genetics Institute, University of Florida, Gainesville, FL 32611, USA

Abstract

Alzheimer's disease (AD) is the leading cause of dementia and the most common neurodegenerative disorder. AD is mostly a sporadic disorder and its main risk factor is age, but mutations in three genes that promote the accumulation of the amyloid- β (A β 42) peptide revealed the critical role of Amyloid precursor protein (APP) processing in AD. Neurofibrillary tangles enriched in tau are the other pathological hallmark of AD, but the lack of causative tau mutations still puzzles researchers. Here, we describe the contribution of a powerful invertebrate model, the fruit fly *Drosophila melanogaster*, to uncovering the function and pathogenesis of human APP, A β 42, and tau. APP and tau participate in many complex cellular processes, although their main function is microtubule stabilization and the to-and-fro transport of axonal vesicles. Additionally, expression of secreted A β 42 induces prominent neuronal death in *Drosophila*, a critical feature of AD, making this model a popular choice for identifying intrinsic and extrinsic factors mediating A β 42 neurotoxicity. Overall, *Drosophila* has made significant contributions to better understand the complex pathology of AD, although additional insight can be expected from combining multiple transgenes, performing genome-wide loss-of-function screens, and testing anti-tau therapies alone or in combination with A β 42.

1. INTRODUCTION

1.1. Etiology and pathology of Alzheimer's disease

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder and the leading cause of dementia among the elderly. The main risk factor for AD is age, with one in nine people above 65 and one-third over 85 suffering AD (Thies et al., 2013). At least 5.2 million

*Correspondence to: PF-F: pedro.fernandez@neurology.ufl.edu, and, DER-L: diego.rincon@neurology.ufl.edu, Department of Neurology 1149 Newell Dr. McKnight Brain Institute University of Florida Gainesville, FL 32611, USA.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Competing Interests: The authors declare that no competing interests exist.

Americans had AD in 2013, most of them over 65 years old. Since the risk for AD is the same across racial groups and geographic location, aging populations in Western Europe and Japan currently face similar economic, social, and medical burdens. Moreover, the AD burden is expected to grow for many emerging economies in Asia and Latin America over the next decades, reaching the level of a global epidemic. At a pathological level, the AD brain is characterized by the shrinkage of several regions, starting with those implicated in memory and cognition - hippocampus and cortex, which explains the early symptoms of memory loss and confusion. In addition to cell loss, the AD brain accumulates two characteristic protein deposits: amyloid plaques enriched in the amyloid- β (A β 42) peptide and neurofibrillary tangles (NFT) enriched in hyperphosphorylated tau (Overk and Masliah, 2014). Both A β 42 and hyperphosphorylated tau are prone to misfold and form soluble assemblies (oligomers and protofibrils), which have shown to be highly toxic in some experimental settings (Benilova et al., 2012; Hardy, 2009). These oligomers continue to aggregate over time into highly organized, insoluble amyloid fibers that have a diagnostic value for AD. Despite the recent emphasis on understanding the role of oligomers in neurotoxicity, both soluble and insoluble A β 42 and tau assemblies may contribute to disease in the complex environment of the human brain; thus, neutralizing the toxicity of different aggregates remains an unmet challenge in the field (Golde et al., 2013).

AD is a complex neurological disorder with sporadic etiology, except for a small fraction of cases with familial inheritance. Familial forms of AD are linked to mutations in three functionally related genes: the *Amyloid precursor protein (APP)* and *Presenilin (PS) 1 and 2*, two key γ -secretase components (Karch et al., 2014). APP encodes a transmembrane protein that is cleaved by combinations of α -, β -, and γ - secretases, which results in the release of several proteolytic fragments, including A β 40 and A β 42 (Fig. 1). Mutations in APP, PS1, and PS2 favor the production of the A β 42 peptide, thus supporting a central role for A β 42 in AD. The presence of A β 42 deposits in the brain of AD patients and the genetic links that favor the production of A β 42 led to the *amyloid cascade hypothesis*, which posits that the accumulation of A β 42 is the triggering event in AD (Hardy and Higgins, 1992). Although this hypothesis was recently revised to recognize the role of both A β 42 and tau oligomers in synaptic toxicity and neuronal loss, its basic premise still drives AD research and the search for elusive therapies (Hardy, 2009). In our current understanding, tau acts downstream of A β 42 and plays a key role in promoting neuronal toxicity and neuronal loss. The experimental evidence for this connection came from the study of mice expressing mutant APP, PS1, and tau, in which clearance of A β 42 reduced tau aggregation, but increasing tau burden had no effect on A β 42 pathology (Oddo et al., 2007). In addition, reduction of tau levels rescued the memory deficits induced by A β 42, supporting the downstream role of tau in AD pathogenesis (Roberson et al., 2007). Recent work has identified the molecular mechanisms linking extracellular A β 42 and intracellular tau (see below), although more work is still needed to define in more detail all the factors implicated in this key pathogenic process. Overall, despite tremendous advances in our understanding of AD pathogenesis, this knowledge has not yet led to the development of disease-modifying therapies. Thus, more research is needed to better understand APP processing and the cellular pathways disrupted by A β 42 and tau.

1.2. The winged supermodel

You may be thinking about the Victoria's Secret angels. But the real winged supermodel is a small fruit fly (*Drosophila melanogaster*) with genetic superpowers. To the untrained eye, fruit flies share little anatomical similarity to humans. But the conservation at the cellular, genetic, and molecular levels is striking based on the common evolutionary origin of all animals. The two Nobel prizes awarded to *Drosophila* researchers in the last 20 years on early development and innate immunity underscore the universality of basic biological principles. Moreover, the tripartite organization of the *Drosophila* brain (proto-, deuto-, and tritocerebrum) is homologous to the forebrain, midbrain, and hindbrain of humans, supporting the ancient origin of the brain (Reichert, 2005). *Drosophila* has driven genetics and developmental studies for over 100 years due to its easy handling in the lab, compact genome distributed in four chromosomes, and short generation time of just ten days from fertilized embryo to adult fly. *Drosophila* was one of the first organisms to be fully sequenced, at last providing the blueprint for mapping thousands of existing mutations (Adams et al., 2000). Comparisons with the human genome revealed that 74% of human genes causative of diseases are conserved in *Drosophila* (Chien et al., 2002), indicating the relevance of studying gene function in flies. The innovative resources for generating mutant strains has helped elucidate many complex biological processes, including the development, differentiation, and function of the nervous system, as well as evolutionarily conserved behaviors such as sleep, learning and memory, foraging, and aggression (Bellen et al., 2010).

In addition, fruit flies are a hotbed for technology development and innovation, which now allows the most sophisticated animal manipulations (Kondo, 2014; Mohr et al., 2014; Venken et al., 2008). The main advantage of *Drosophila* is the ability to manipulate gene expression with extraordinary precision. Despite the slow penetration of gene knock-in technology, recent developments with the fast and flexible CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated protein 9) system adds another powerful weapon to the genetic arsenal of the fruit fly (Bassett et al., 2013; Gratz et al., 2013; Yu et al., 2013). Since its introduction in 1993, massive resources based on the UAS/Gal4 system are now available, including genome-wide RNAi libraries and thousands of Gal4 strains to map single neurons and circuits (Brand and Perrimon, 1993; Jenett et al., 2012). Two new expression systems, Q and LexA, provide added flexibility for creative and sophisticated gene manipulation (Lai and Lee, 2006; Potter et al., 2010). Finally, *Drosophila* has introduced many genetic-based tools to study neuronal circuits and function, including optical tracers, calcium sensors, and engineered channels to regulate neuronal function like the Transient receptor potential A [TrpA1] and Channelrhodopsin. Overall, the wealth of genetic tools for manipulating gene activity and neuronal function make *Drosophila* the most versatile model organism for the discovery of conserved physiological and pathological conditions.

1.3. Modeling neurodegenerative disorders in *Drosophila*

Rodent models of neurodegenerative diseases share many anatomical, pathological, and behavioral features of the respective human disorders. They are also expensive and time consuming, making them optimal for testing candidate genes and therapeutic agents. Here,

Drosophila has proven useful because expression of human genes linked to dominantly inherited disorders perturb endogenous cellular functions, providing a flexible platform for uncovering the cellular mechanisms underlying neuronal loss (Rincon-Limas et al., 2012). These models have been exploited to test candidate pathways (e.g., chaperones, kinases and phosphatases, RNA-binding proteins) and to identify novel genes and pathways in unbiased screens. The first genetic screen revealed the intricate cellular processes implicated in Ataxin1–82Q toxicity, including the ubiquitin-proteasome complex, RNA metabolism, and transcriptional machinery (Fernandez-Funez et al., 2000). Several other large screens have been performed with other disease models, including Ataxin3–78Q, tau, and A β 42 (Ambegaokar and Jackson, 2011; Bilen and Bonini, 2007; Cao et al., 2008; Rival et al., 2009; Shulman and Feany, 2003), providing a comprehensive, unbiased view of the complex cellular pathways underlying common and disease-specific mechanisms.

In addition to mimicking key pathological features of the corresponding human disease at the tissue and cellular levels, the complex repertoire of *Drosophila* innate behaviors, including larval and adult olfactory learning, courtship, grooming, aggression, and circadian rhythms, can also contribute to examine human diseases at the functional / circuit level. These behaviors are highly conserved based on the molecular machinery underlying them. For instance, *Drosophila* has played a key role in deciphering the molecular clock supporting circadian rhythms, including the identification of *period*, which are conserved in humans (Hardin, 2005). The same could be said for the pathways regulating memory formation, including cAMP and Protein kinase A signaling resulting in CREB activation (Davis, 2005). Some of these evolutionarily conserved behaviors have been exploited to characterize disease models and the protective effect of genes and drugs. Unfortunately, these assays are labor intensive despite recent automation efforts, making them impractical for large-scale genetic or pharmacological screens at this time. On the other hand, a simple innate locomotor behavior based on negative geotaxis (climbing) has been extensively used in many neurodegeneration models. This robust behavior is easy to score, allowing the longitudinal analysis of multiple genotypes at once. Since the progressive loss of climbing activity precedes neuronal loss, this simple assay mimics the *progressive neuronal impairment* typical of movement and cognitive disorders alike. Thus, the variety of behavioral assays provides the flexibility to study neurodegeneration at multiple levels, including the activity of conserved neuronal circuits based on shared molecular machinery (e.g., molecular clock, CREB signaling in memory).

Due to its complex etiology, many different *Drosophila* models of AD have been published over the last 15 years. Here, we will review *Drosophila* studies describing the biological function of APP and its *Drosophila* orthologue APP-like (APPL). We will also describe *Drosophila* models overexpressing human APP/BACE1 (β -site APP-cleaving enzyme), A β 42, and tau, three phenotypic models that have contributed to elucidating the molecular mechanisms mediating AD toxicity.

2. APP FUNCTION AND PROCESSING IN DROSOPHILA

2.1. The complex APP processing and its link to AD

APP is the key AD gene that links genetics with pathology. *APP* encodes a type I integral membrane protein, with the 695-residue isoform being the most common in neurons (Nhan et al., 2014; Shariati and De Strooper, 2013). The longer extracellular domain of APP contains the A β 42 fragment, one of many fragments derived from APP and the main component of amyloid plaques (Fig. 1). More than 30 pathogenic mutations on APP cause early onset, familial forms of AD (Karch et al., 2014). These mutations promote A β 42 production through altered γ -secretase activity or increased A β 42 stability, thus providing the genetic link to the amyloid pathology. APP is constitutively cleaved by α -, β -, and γ -secretases during maturation (Coburger et al., 2014). Several α -secretases of the ADAM (a disintegrin and metalloprotease) family can cleave APP close to the membrane in the extracellular domain, producing secreted APP (sAPP α) and a C-terminal fragment (CTF α) (Fig. 1). Since α -secretase activity breaks the A β 42 peptide, this is considered the non-amyloidogenic pathway. Only one β -secretase can cleave APP, BACE1, generating sAPP β and the N-terminus of the A β peptides. Lastly, γ -secretase is a membrane complex containing PS1 or PS2, the other two gene products associated with inherited AD, that cleaves APP in the transmembrane domain to release the amyloid intracellular domain (AICD) and the A β peptides, from A β 36 to A β 43 (Fig. 1). Although the accumulation of A β 42 is proposed to be the main event triggering AD pathogenesis, the biology of APP is highly complex and other APP fragments could have protective or neurotoxic activities. Hence, investigating the function of APP and its close orthologues in *Drosophila* may shed light on the role of APP in AD pathogenesis.

2.2. Evolutionary conservation of APP

The *Drosophila* APP orthologue, APPL, has a 25% identity and a 39% similarity (Luo et al., 1990) and shares the basic structure of human APP (Poeck et al., 2012). *Appl* deficient flies show mild behavioral phenotypes associated with locomotor reactivity that are partially rescued by human *APP*, demonstrating the functional conservation of APP (Luo et al., 1992). *Drosophila* contains all the secretases for APPL processing, including a single PS (Psn) and dBACE (Carmine-Simmen et al., 2009; Greeve et al., 2004). Loss of *dBACE* results in late larval/early pupal lethality, although its substrates are mostly unknown (Bolkan et al., 2012; Soldano et al., 2013). Flies can produce sAPPL α through the activity of the ADAM10 orthologue Kuzbanian, suggesting that the non-amyloid pathway is conserved from flies to humans. A critical difference between APP and APPL is the poor conservation of the A β sequence (Luo et al., 1992; Rosen et al., 1989). However, recent evidence indicates that β - and γ -secretases cleave APPL to generate a 64 residue A β -like fragment that aggregates, is thioflavin-S positive, and is neurotoxic in the eye and the brain (Carmine-Simmen et al., 2009). The evolutionary conservation of the pathway leading to the production of A β fragments suggests that these peptides have a yet unknown but critical function in neuronal physiology.

2.3. APP / APPL in axonal trafficking

Early studies revealed critical functions of APPL in axonal transport, neuromuscular junctions, and brain development in *Drosophila* (Luo et al., 1990; Martin-Morris and White, 1990; Torroja et al., 1996). The axonal trafficking function is one of the most thoroughly investigated since the original description that APPL overexpression caused the axonal retention of vesicles containing synaptic proteins (Fig. 2) (Torroja et al., 1999a). Table 1 contains a list of the constructs expressed in flies for the functional characterization of APP and APPL. The potential role of APPL as a vesicular receptor for kinesin-1 was further confirmed in *Appl* loss-of-function studies. Both *Appl* mutant flies and APP overexpression displayed the same axonal traffic jams as several known axonal transport mutants. Reduced kinesin-1 levels enhanced these phenotypes, whereas reduction of dynein suppressed them, thus supporting the interaction of APP and APPL with the axonal trafficking machinery (Gunawardena and Goldstein, 2001). Surprisingly, the combination of human APP and PS1 harboring familial AD mutations reduced the axonal blockade, indicating that full-length APP, not A β 42, is responsible for these phenotypes (Stokin et al., 2008). Moreover, reduction of Psn levels affected the axonal mobility of APP vesicles, but not of other axonal vesicles, suggesting a potential new link of PS mutations to AD pathology (Gunawardena et al., 2013). A recent study monitored APP-YFP vesicles and found that kinesin-1 and dynein compete for dynein intermediate chain to determine transport directionality (Reis et al., 2012). Another mechanism regulating APP axonal transport involves epigenetic transcriptional regulation by the histone acetyltransferase Tip60. Tip60 forms a transcriptional complex with the intracellular domain of APP and Tip60 loss-of-function leads to axonal transport phenotypes similar to those of APP (Cao and Sudhof, 2001; Slomnicki and Lesniak, 2008). Tip60 overexpression rescued the APP-mediated traffic jams through direct interaction with the C-terminal domain of APP, suggesting that this interaction is critical for preserving neuronal activity (Johnson et al., 2013).

2.4. Other cellular functions of APP / APPL

APPL is widely expressed throughout the brain, but is not homogeneously distributed. APPL accumulates mainly in axonal projections, and appears highly expressed in the mushroom bodies and central complex, which agrees with the locomotor and memory deficits described previously (Luo et al., 1992; Torroja et al., 1996). According to a recent study, APPL acts as a novel, neuronal-specific modulator of the anterograde ***Wnt-PCP signaling*** involved in axon growth and guidance (Fig. 2) (Soldano et al., 2013). In addition, APPL mutant larvae showed fewer synaptic boutons, whereas APPL overexpression increased bouton number and changed synapse structure (Torroja et al., 1999b). This is a highly conserved pathway, suggesting a similar function for APP in mammalian brains.

More details about the function of APPL have emerged in recent years (Fig. 2). APP is known to bind microtubules directly or through tau, but flies lacking sAPPL exhibit severe disorganization of actin filaments and microtubules, suggesting a tau-independent role regulating the ***cytoskeleton*** (Li et al., 2004). sAPPL also regulates neuronal excitability through the modulation of ***K⁺ channel activity*** (Li et al., 2004). In addition, both APP and APPL can induce post-developmental axonal arborizations, which require the conserved C-terminus and interaction with the Abelson (Abl) tyrosine kinase (Leyssen et al., 2005).

APPL is also highly expressed in the R7 photoreceptors, where it is necessary for proper **axonal targeting** into the medulla and UV light discrimination (Mora et al., 2013). Full-length APPL seems to be toxic to glia in the eye lamina, indicating that the activity of BACE is critical to prevent glia cell death (Bolkan et al., 2012). Finally, two recent studies addressed the role of APP/APPL in the **function of brain circuits**. Transient silencing of APPL in the mushroom bodies, the memory center of insects, demonstrated that it is specifically involved in long-term memory, which requires changes in synapse architecture (Goguel et al., 2011). In addition, APP overexpression in the courtship circuit decreased the preference of males to mate with younger females, but the total courtship remained the same, suggesting specific alterations in this circuit (Hu et al., 2014). These results support the hypothesis that APP function is critical to maintain synaptic activity, which could be relevant in AD.

2.5. A novel protective activity for APP / APPL

Although mice lacking APP function are viable, several potential neuroprotective activities have been proposed for APP, particularly from cell culture studies (Nhan et al., 2015). Interestingly, APPL is upregulated after traumatic brain injury, which agrees with the increased post-traumatic mortality in APPL mutant flies (Leyssen et al., 2005). APP/APPL have recently demonstrated an intriguing protective activity against progressive neurodegeneration (Wentzell et al., 2012). The *loechrig* (*loe*) mutant shows spontaneous vacuolization of the *Drosophila* brain, a phenotype that is exacerbated by *Appl* mutants and rescued by APPL overexpression (Tschaape et al., 2002; Wentzell et al., 2012). Actually, sAPP or sAPPL suppresses the degeneration of several well-known neurodegeneration mutants in flies. Interestingly, the protective activity of sAPPL required the direct interaction with full-length APPL (Fig. 2), further suggesting a protective role for APPL, as indicated by the vacuolar changes and reduced longevity in flies lacking APPL function.

2.6. APP + BACE: physiologic models of AD

One of the first *Drosophila* models of AD was based on the expression of wild type human APP-695 (Greeve et al., 2004). APP overexpression showed progressive degeneration in the eye that was dependent on the activity of endogenous Psn. Triple transgenic flies expressing human APP and BACE1 together with Psn carrying pathogenic mutations showed strong amyloid pathology, demonstrating the toxicity of A β 42 in a physiologic fly model of AD (Greeve et al., 2004). Treating these flies with β - or γ -secretase inhibitors reduced toxicity, indicating the potential utility of *Drosophila* to screen for selective β - or γ -secretase inhibitors. More recently, a similar model overexpressing human APP and BACE1 demonstrated neuroanatomical defects in the mushroom bodies, A β 42 accumulation, and deficits in locomotion and memory (Chakraborty et al., 2011). Low levels of APP and BACE1 disrupted the architecture of the adult mushroom bodies and induced progressive memory loss, indicating the physiological relevance of this model (Mhatre et al., 2014). Automated serial electron microscopy followed by 3D reconstruction in 15 day-old flies expressing APP carrying the Swedish mutation (APP^{swe}) revealed autophagy, abnormal axons, and enlarged mitochondria, telltale signs of neurodegeneration suggesting that mutant APP alone displays AD features in flies (Park et al., 2014).

2.7. Clinical relevance of APP models

Detailed work in flies has uncovered a conserved role for APP/APPL in the axonal transport of vesicles through direct and indirect interactions with the kinesin-1 and dynein trafficking machineries. APP loss-of-function can also alter the activity of Tip60, which can result in gene expression changes critical for maintaining healthy axonal trafficking. Additional functions critical for APP include synaptic remodeling and K⁺ channel activity, suggesting that APP mutations can contribute to cognitive decline. Finally, APP has a neuroprotective activity that may be relevant for AD pathology. Overall, these findings suggest that alterations on APP function can have an impact on AD beyond the production of A β 42. If this is the case, then APP function could have a role in sporadic AD as well. Understanding the role of APP endogenous function in AD is critical because it could lead to additional therapeutic approaches based on restoring axonal trafficking, Tip60 transcriptional activity, synaptic architecture, and other neuroprotective activities.

3. DROSOPHILA MODELS OF A β 42 NEUROTOXICITY

3.1. A varied menu of flies secreting A β 42

A more direct approach to modeling AD neurotoxicity is to generate flies expressing a secreted form of the human A β 42 peptide, thus mimicking the extracellular distribution of the main pathogenic trigger in AD. Several independent A β 42 models are based on the fusion of distinct expression strategies and signal peptides (SP), resulting in different toxicity (Table 2). The first model fused the rat pre-proenkephalin SP with A β 40 and A β 42 (Finelli et al., 2004). As expected, A β 40 accumulated in the in SDS-soluble fraction, whereas A β 42 accumulated in the formic acid (insoluble) fraction, confirming its higher amyloidogenicity (Finelli et al., 2004; Iijima et al., 2004). A β 42, but not A β 40, reduced longevity, was thioflavin-S positive, and induced progressive locomotor dysfunction and neuronal degeneration. Although both peptides showed olfactory learning defects starting at 6–7 days of age, A β 42 induced stronger phenotypes than A β 40 (Iijima et al., 2004). Additionally, flies expressing A β 42, but not A β 40, directly under the control of the *Glass Multimer Reporter* enhancers (GMR-A β) exhibited weak eye disorganization, demonstrating their potential utility in large-scale genetic screens (Finelli et al., 2004).

A different model carrying a fusion of human A β 42 with the SP from the *Drosophila* Necrotic protein showed several eye and CNS phenotypes with high accumulation of A β 42 in the secretory pathway (Crowther et al., 2005). Interestingly, another group found that the same Necrotic SP produced A β 42 carrying an additional Gln in the N-terminus, A β Q+42 (Allan et al., 2014). Fusion with the Toll SP produced pure A β 42 that was not toxic, suggesting that the toxicity of A β Q+42 was due to the extra Gln. These results call for caution and validation when generating fusions with small peptides. This group later generated tandem A β 40 and A β 42 peptides and reported that toxicity of the A β 42 dimers correlated with the levels of soluble oligomers (Speretta et al., 2012). We also generated a unique model expressing human A β 42 fused to the *Drosophila* Argos SP, but included two independent copies of the construct in the same vector to induce higher expression level, resulting in stronger eye and CNS phenotypes (Casas-Tinto et al., 2011; Tare et al., 2011). A curious model expressing a fragment of *Drosophila* APPL spanning amino acids 767–831

equivalent to human A β 42 demonstrated that, despite the low sequence conservation with A β 42, the fly A β 64 peptide is also toxic (Carmines-Simmen et al., 2009).

3.2. Intrinsic mechanisms mediating A β 42 toxicity

The small size of A β 42 enables the systematic analysis of how each residue contributes to misfolding, aggregation, and toxicity. One study calculated in silico the aggregation propensity of all possible *amino acid substitutions* in wild type A β 42 and A β 42 carrying the pathogenic arctic mutation E22G (Luheshi et al., 2007). Then, they took advantage of *Drosophila* to test in vivo the in silico predictions in a time- and cost-efficient manner. Expressing 14 mutants in transgenic flies, the authors found a strong correlation between the propensity to form protofibrils and neurotoxicity, and described the protective activity of L17R. Another in silico approach demonstrated that single amino acid substitutions are sufficient to induce A β 40 toxicity (E3R) and to eliminate the toxicity of A β 42-E22G (I31E) (Brorsson et al., 2010). Both mutations alter the relative amount of prefibrillar soluble aggregates, which are thought to be the most toxic species. A more limited study analyzing A β 42-L17P and -E22G reported that pathology correlated with subcellular distribution, with A β 42-E22G inducing neuronal loss and A β 42-L17P neurite degeneration (Iijima et al., 2008). Overall, the study of A β 42 variants in vivo supports the high toxicity of small aggregates, including soluble oligomers and protofibrils.

3.3. Extrinsic factors mediating A β 42 toxicity: metals

The presence of extrinsic factors, including **iron, copper, and zinc**, also affects A β 42 aggregation and toxicity. Overexpression of the iron-binding protein ferritin and the iron chelator YM-F24 rescued A β 42 toxicity, whereas loss-of-function enhanced it, suggesting a direct link between free iron and A β 42 toxicity (Liu et al., 2011; Rival et al., 2009). A similar enhancement of A β 42 toxicity was later reported in flies supplemented with copper and zinc in their diet (Hua et al., 2011; Sanokawa-Akakura et al., 2010). Treatment with specific chelators or overexpression of metal responsive transcription factor 1 (MTF-1) or metallothionein A (MtnA), two detoxifying proteins, prevented the toxicity of copper and zinc. The role of copper in A β 42 toxicity was further confirmed in studies modulating the activity of high-affinity copper importers (Ctr1A, B, and C) and exporters (ATP7, Atox1), and FKBP56, an immunophilin thought to modulate the levels of intracellular copper (Lang et al., 2013; Sanokawa-Akakura et al., 2010). Interestingly, Ctr1C loss-of-function increased the insolubility of A β 42, suggesting that both iron and copper promote A β 42 aggregation into less toxic amyloid fibers. Similarly, overexpression of the zinc transporter Zip1 (Zrt-Irt like protein 1) promoted A β 42 aggregation and rescued its toxicity (Lang et al., 2013). In all, these papers support the ability of iron, zinc, and copper to delay A β 42 aggregation, stabilizing more toxic soluble conformations.

3.4. Disruption of neuronal activity

Several papers have identified A β 42-induced perturbations in *Drosophila* neurons as a means to uncover the pathogenic cascades responsible for neuronal dysfunction and degeneration. For instance, expression of A β 42 in larval motor neurons reduced neurotransmitter release in the neuromuscular junction (NMJ), whereas expression in the

muscle increased neurotransmitter release (Chiang et al., 2009). An anti-A β 42 antibody reversed this effect, indicating that *secreted* A β 42 was responsible for disrupting synaptic activity. In adult flies, expression of A β 42 induced progressive failure of transmission in the giant fiber system together with **depletion of mitochondria** in presynaptic terminals due to slow axonal transport (Fig. 3) (Lin et al., 2014; Zhao et al., 2010). Further studies found reduced number of presynaptic vesicles, probability of vesicle release, calcium channels, and active zones (Huang et al., 2013). These critical changes in the presynaptic terminal preceded and might be responsible for the loss of presynaptic boutons. Similar reduction in synaptic activity was confirmed in cholinergic projection neurons of the olfactory system in pupae, which resulted in altered short-term memory (Fang et al., 2012), a significant finding because AD affects mainly cholinergic systems in the human brain. A study to pinpoint the mechanisms by which A β 42 mediates memory loss found that A β 42 stimulated **PI3K-Akt signaling**, which causes reduced long-term potentiation (LTP) (Fig. 3) (Chiang et al., 2010). Genetic and pharmacologic interventions reducing PI3K signaling rescued the memory defects of flies expressing A β 42, supporting the critical role of this pathway in mediating neuronal dysfunction.

Although not a transgenic model, the treatment of primary cholinergic larval neurons with A β 42 preparations altered the properties of A-type **K⁺ currents** (Kidd et al., 2006; Kidd and Sattelle, 2006). A recent study also described the selective degradation of Kv4 channels in flies expressing A β 42 leading to neuronal hyperactivity (Fig. 3) (Ping et al., 2015). Reducing the levels of Kv4 channels led to locomotor defects whereas Kv4 overexpression rescued A β 42 neurotoxicity, supporting a critical role for K⁺ channel activity in A β 42 pathogenesis.

Other recent reports have documented the **disruption of circuits** responsible for innate behaviors. Although the lack of a molecular clock had no effect on locomotion and survival in flies expressing A β 42, A β 42 disrupted the rhythmic behavior of flies (Chen et al., 2014; Long et al., 2014). Flies expressing A β 42 lose their rhythmicity after several days in the dark despite the integrity of the clock neurons and the molecular oscillators. Interestingly, once flies expressing A β 42 became arrhythmic, they never recovered their rhythm in light-dark cycles (Chen et al., 2014), suggesting that rhythm degradation occurs downstream of the central clock and may involve the synaptic components described above.

3.5. Disruption of endogenous cellular processes

A number of studies have identified endogenous signaling and cellular processes disrupted by A β 42. As described above, A β 42 causes the depletion of **mitochondria** in synaptic terminals and axons, which accumulate in the cell bodies of brain neurons (Iijima-Ando et al., 2009). This mislocalization of mitochondria is likely responsible for memory and locomotor deficits because disrupting the transport of mitochondria alone induced similar behavioral phenotypes. **Autophagy** has been identified as a protective cellular mechanism for recycling damaged organelles and degrading protein aggregates. However, *Drosophila* brain neurons expressing A β 42 accumulate autophagic vesicles fused with lysosomes and their acidic contents seem to leak causing the progressive vacuolation of the cytosol (Fig. 3) (Ling et al., 2009). Although activation of autophagy was protective initially, inhibition of

autophagy partially rescued A β 42 toxicity, supporting a deleterious role for this pathway in A β 42 pathogenesis.

Another critical cellular perturbation induced by A β 42 is **ER stress**, a condition that can result in cell death. In response to ER stress, a coordinated signaling system launches the unfolded protein response (UPR), which inhibits protein translation and promotes refolding and degradative activities in the ER to restore protein homeostasis. We found that A β 42 induces ER stress in the *Drosophila* eye as indicated by the activation of the unconventional splicing of the X-box binding protein 1 (XBP1) (Fig. 3) (Casas-Tinto et al., 2011). A β 42 and other amyloids are known to induce ER stress, but we showed for the first time that overexpression of *Drosophila* XBP1 or murine XBP1s (spliced) suppressed A β 42 toxicity. We found that XBP1s prevented the deleterious effects of releasing calcium from the intracellular stores through ryanodine calcium channels.

A key feature of AD is the progressive **neuronal cell death** in specific brain regions; unfortunately, no APP mouse model shows cell death in the absence of tau transgenes (Hall and Roberson, 2012). In contrast, flies expressing A β 42 show prominent neuronal loss in the developing eyes and in adult brains. Several pathways can promote cell death in response to intrinsic and extrinsic signals, but flies expressing A β 42 activate the c-Jun amino terminal kinase (JNK) pathway (Fig. 3) (Tare et al., 2011). Since genetic and pharmacologic inhibition of JNK signaling rescue A β 42 toxicity in the eye and the brain, JNK signaling plays a key role in A β 42-mediated cell death (Hong et al., 2012; Tare et al., 2011). Moreover, jointly blocking JNK signaling and caspase activation completely restored the normal architecture of the eye, indicating that both pathways contribute to A β 42 toxicity. Overall, these approaches demonstrated the utility of *Drosophila* for identifying pathogenic mechanisms of A β 42 that may be conserved in humans.

3.6. Protective activity of candidate genes

The easy and flexible manipulation of *Drosophila* makes it an ideal system for testing the protective activity of candidate genes or identifying unknown factors in genetic screens. **Neprilysin** is one of the known A β 42-degrading enzymes and its neuroprotective activity has been shown in fly and mouse AD models (Blurton-Jones et al., 2014; Finelli et al., 2004; Iijima-Ando et al., 2008; Marr et al., 2003). Interestingly, Neprilysin overexpression in the brain shortened lifespan and induced neuropathology associated with reduced activity of c-AMP-responsive element-binding protein (CREB), a key stimulus-dependent transcriptional regulator in neurons (Iijima-Ando et al., 2008). The authors also described an age-dependent reduction of Neprilysin expression, which they argue is a critical protective mechanism during aging, but may also contribute to the increased risk for AD. **Neuroserpin** is a serine protease inhibitor that binds A β 42 and is found in amyloid plaques in AD patients (Kinghorn et al., 2006). Whereas this interaction inhibits the activity of neuroserpin, it increases the aggregation of A β 42 into an off-pathway non-toxic species. Co-expression with A β 42 rescued the toxicity of neuroserpin in flies, supporting the biological relevance of this interaction. **Puromycin-sensitive aminopeptidase** (PSA) was initially identified as a suppressor of tau pathogenesis (Karsten et al., 2006) and was later isolated as a suppressor of A β 42 toxicity in a genetic screen, but the mechanisms mediating its protective activity

remain to be elucidated (Kruppa et al., 2013). Despite the robust protective activity of **molecular chaperones** against several intracellular amyloids, particularly polyglutamines, the in vivo role of chaperones against extracellular amyloids such as A β 42 have received less attention. A recent paper demonstrated that secretion of the BRICHOS chaperone domain suppressed A β 42 toxicity and promoted the accumulation of soluble A β 42 aggregates (Hermansson et al., 2014). Similarly, we found that a secreted form of Hsp70 (secHsp70) rescues the glassy eye, locomotor activity, longevity, neuronal architecture, and cell death without obvious changes in A β 42 aggregation (LM, DERL, PFF, unpublished data). Thus, the protective activity of A β 42-binding proteins could be mediated by masking the neurotoxic epitopes of A β 42, hence preventing its interaction with substrates that mediate the deleterious signals.

3.7. Novel mechanisms of A β 42 toxicity

Although several pathways modulating A β 42 toxicity were originally identified in genetic screens (Casas-Tinto et al., 2011; Rival et al., 2009), the Konsolaki lab has published the only genetic screen described in detail (Cao et al., 2008). This gain-of-function screen tested 1,963 randomly inserted enhancer promoter (EP) lines that can overexpress nearby genes (Rorth, 1996). The screen identified several pathways involved in the secretory pathway, cholesterol homeostasis, innate immunity, and chromatin organization.

Some of these modifiers of A β 42, including vesicular transport, chromatin organization, and proteostasis, are common to intracellular amyloids like tau and Huntingtin-93Q, suggesting conserved mechanisms of toxicity for different proteinopathies (Shulman and Feany, 2003). A subsequent screen of loss-of-function alleles identified Toll, a key receptor of the innate immunity pathway, as a suppressor of A β 42 toxicity (Tan et al., 2008). Interestingly, A β 42 activated the Toll signaling pathway, suggesting that Toll, a pattern recognition receptor (PPR), recognizes the structure of A β 42 aggregates as a danger-associated molecular pattern (DAMP), which explains the potent inflammatory response in AD brains (Salminen et al., 2009). More recently, the Crowther lab used a transcriptomic approach to identify the mechanisms mediating A β 42 toxicity (Favrin et al., 2013). The microarrays identified genes involved in normal aging, including oxidative stress, immune response, and protein secretion, due to high levels of A β 42 that altered gene expression in the young flies.

3.8. Pharmacological suppression of A β 42 toxicity

To support the utility of the A β 42 flies, several **proof-of-concept studies** demonstrated the benefits of agents known to directly interact with A β 42 or alter pathways affected by A β 42. For instance, the N-methyl-D-aspartate (NMDA) receptor inhibitor MK-801 and Congo Red, a dye that inhibits A β 42 aggregation, rescue the longevity of A β 42 flies (Crowther et al., 2005). Lithium has been used for treating psychiatric conditions for decades and also inhibits glycogen-synthase kinase 3 β (GSK3 β), which affects both APP processing and tau aggregation. Flies treated with lithium show rescued toxicity through a nonspecific inhibition of translation that reduced A β 42 levels (Sofola et al., 2010; Sofola-Adesakin et al., 2014). Similarly, doxycycline, an antibiotic with demonstrated neuroprotective activity in several models, improved locomotor performance in flies expressing A β 42 and promoted the accumulation of smaller, non-toxic A β 42 aggregates (Costa et al., 2011). The turmeric

root extract curcumin binds A β 42 and has many anti-inflammatory activities. Feeding A β 42 flies with curcumin dramatically rescued lifespan and locomotor activity, and increased A β 42 fibrillation (Caesar et al., 2012). However, curcumin is a known pan-assay interference compound (PAINS) due to its membrane disruption activity, which may explain its multiple reported benefits in laboratory assays and the lack of long-term clinical benefits (Baell and Walters, 2014). Other Chinese traditional medicine extracts such as SuHeXiang Wan, a mix of 15 herbs, and the rhizome of *Gastrodia elata* demonstrated benefits in flies expressing A β 42 (Ng et al., 2013; Park et al., 2013).

Small compounds are the traditional pharmacological therapies because of their superior pharmacodynamics. Two compounds designed to stabilize the α -helix region of A β 42 (residues 13–26) were effective in preventing A β 42 misfolding in vitro (Nerelius et al., 2009). Both the peptide mimetic Pep1a and Dec-DETA rescued longevity and locomotor activity in A β 42 flies, indicating that stabilization of the central A β 42 α -helix can be pursued as a therapeutic strategy in AD.

In addition to classic compounds and extracts, **peptide and protein engineering** are promising approaches for blocking A β 42 toxicity. β -sheet breakers are short peptides designed to bind the amyloidogenic domains of A β 42 and prevent the formation of β -sheet conformations that promote aggregation (Soto et al., 1998). A hexapeptide containing five *N*-methyl sites blocked A β 42 aggregation in vitro and improved longevity and locomotor activity in flies (Pratim Bose et al., 2009). Moreover, small A β 42-binding peptides carrying R residues with inverted sequences (retro-inversal), which increase bioavailability, promoted *Drosophila* hatching and locomotor activity (Acerra et al., 2014). Small A β 42-binding proteins like the Z_{A β 43} affibody can also be selected by phage display. Z_{A β 43} and a dimeric Z_{A β 43} construct suppressed A β 42 toxicity by promoting A β 42 degradation (Luheshi et al., 2010). Another approach consists on expressing antibody fragments that recognize either the A β 42 sequence or specific conformations. The expression of a camelid heavy chain antibody targeting oligomers demonstrated selective interaction with A β 40 instead of A β 42, resulting in increased toxicity in flies (Wacker et al., 2014). This work exemplifies the importance of examining the activity of selective agents in vivo, highlighting the role of the environment in the performance of engineered molecules. We have expressed two known single-chain variable fragment (scFv) anti-A β 42 antibodies to demonstrate that scFvs retain their structure / activity in flies and that *Drosophila* can be used as a platform to screen for effective scFvs (LM, DERL, PFF, unpublished data).

3.9. Clinical relevance of A β 42 models

The robust toxicity of A β 42 in flies has made this model a successful test bed for genetic and pharmaceutical experimentation. These phenotypic models have played a key role in identifying the perturbations of endogenous pathways, including K⁺ channel activity, axonal transport, autophagy, and cell signaling, among others. Restoring the normal activity of these pathways in patients can have symptomatic benefits that improve the quality of life. Additionally, *Drosophila* has provided strong experimental support for the beneficial activity of metal chelators and a few known compounds that may be added to the current standard of care for AD. An important contribution of *Drosophila* is the efficient and cheap

testing of risky therapies that may otherwise not be tested in mice. For instance, three approaches with potential protective activity - autophagy, Neprilysin, and camelid anti-A β 42 antibodies -induced deleterious effects, supporting the use of *Drosophila* as an in vivo filter for new therapies. Overall, different approaches have demonstrated the utility of *Drosophila* as a platform for testing potential AD therapies, which can save time by selecting those that work best in vivo before moving to more expensive mouse trials.

4. DROSOPHILA MODELS OF TAUOPATHIES

4.1. The mystifying role of tau in brain pathologies

Tau is arguably the most puzzling piece of the AD chessboard. Tau pathology in the form of NFT is critical for definitive AD diagnosis, and tau pathology correlates better with cognitive decline than A β 42, but tau mutations do not cause AD (Overk and Masliah, 2014). Tau pathology is also present in diverse neurological disorders collectively known as tauopathies, but tau mutations only cause non-AD dementias, including frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) and Pick's disease (Gendron and Petrucelli, 2009). A single human *microtubule associated protein tau* (*MAPT*) gene on chromosome 17 contains 15 exons and can generate six isoforms in the CNS upon alternative splicing of exons 2, 3, and 10 (Andreadis, 2012). Tau is mainly, but not exclusively, a neuronal microtubule-associated protein (MAP) and its expression is developmentally regulated, e.g., only the shortest isoform is present in human fetal brain. One of the major functions of tau is to stabilize axonal microtubules and promote their polymerization (Beharry et al., 2014). Other functions include interaction with actin cytoskeleton, neurite outgrowth, intracellular vesicle transport, interaction with plasma membrane, and anchoring of enzymes such as protein kinases and phosphatases, to name a few (Morris et al., 2011). Strikingly, tau-deficient mice do not display developmental abnormalities (Harada et al., 1994), suggesting that other MAPs compensate for tau function.

Among the tauopathies, AD is the most prevalent. According to the amyloid hypothesis, A β 42 deposition in the brain triggers a number of cellular perturbations, including tau detachment from microtubules, tau aggregation, and subsequent formation of paired helical filaments that assemble into NFTs (Stancu et al., 2014). Indeed, several landmark articles have confirmed that A β 42 is upstream of tau in AD pathogenesis and induces the conversion of tau into a neurotoxic state (Gotz et al., 2001; Hurtado et al., 2010; Oddo et al., 2007; Roberson et al., 2007). Additionally, recent studies suggest that pathological tau enhances A β 42 toxicity via a feedback loop, suggesting that tau is also critical for A β 42 toxicity (Ittner et al., 2010; Leroy et al., 2012; Roberson et al., 2007). Despite major discoveries on tau structure and assembly, phosphorylation, and interaction with microtubules, the mechanisms triggering tau neuropathology are still poorly understood.

4.2. First tau models and phosphorylation

Over the last 14 years, more than 30 different transgenic fly models expressing human tau have addressed fundamental questions in tau pathobiology. These flies express wild type or mutant tau, contain three or four microtubule binding repeats, and carry mutant

phosphorylation sites for mechanistic studies (Table 3) (Gistelinck et al., 2012). The first *Drosophila* model of human tauopathies targeted tau to the CNS, leading to pathological tau phosphorylation, progressive neurodegeneration, and premature death (Wittmann et al., 2001). However, bona fide NFT were not detected in this model. Interestingly, NFT-like structures appeared when tau was co-expressed with *Drosophila* GSK3 β or upon targeted expression in glial cells (Colodner and Feany, 2010; Jackson et al., 2002).

Given that the longest tau isoform contains more than 80 potential phosphorylation sites (Sergeant et al., 2008), the link between tau phosphorylation and toxicity has received considerable attention. Research in *Drosophila* tau models demonstrated that PAR-1 phosphorylation at S262 and S356 were critical for the subsequent phosphorylation of GSK3 β sites using On4R tau (Fig. 4), suggesting that phosphorylation takes place in a structurally ordered pattern (Kosmidis et al., 2010; Nishimura et al., 2004). However, a different tau isoform - 2N4R - carrying the S262A and S356A substitutions still showed normal phosphorylation at the GSK3 β sites, suggesting that the downstream activity of GSK3 β is isoform-specific (Chatterjee et al., 2009). Another interesting observation is that single and double mutants in S/P or T/P phosphorylation sites had no effect on human tau toxicity, but mutating all 14 sites reduced toxicity, suggesting a cooperative activity in these phosphorylation sites (Steinhilb et al., 2007a; Steinhilb et al., 2007b). Moreover, eliminating 11 of these sites on a different tau isoform had no effect on toxicity, indicating again the critical role of tau isoforms in toxicity (Chatterjee et al., 2009). Overexpression of either human or fly tau led to hyperphosphorylation and microtubule destabilization, phenotypes that could be reversed by GSK3 β inhibitors (Cowan et al., 2010; Mudher et al., 2004). Interestingly, these perturbations of tau and microtubules altered axonal transport without neurodegeneration, thus dissociating these tau activities.

4.3. Candidate approaches

In addition to the phosphorylation studies, tau-expressing flies have been applied towards dissecting the mechanisms associated with tau neuropathology (Fig. 4). For instance, mutant tau interacts with F-actin, and induces its accumulation into actin-rich rods in the fly brain, resembling the Hirano bodies found in AD (Fulga et al., 2007). This group also demonstrated that reduction of F-actin decreased tau toxicity and that **cytoskeleton** abnormalities occurred downstream of tau phosphorylation. Another report showed that silencing antioxidant defense pathways enhanced mutant tau neurotoxicity (Dias-Santagata et al., 2007). The authors found that the antioxidant vitamin E suppressed tau toxicity and the pro-oxidant paraquat enhanced it, confirming that **oxidative stress** is also a critical mediator of tau toxicity. A separate study showed that genetic inhibition of cathepsin D led to an enhancement of tau toxicity, highlighting the role of the **lysosomal pathway** (Khurana et al., 2010). Interestingly, this lysosomal dysfunction was not linked to higher levels or hyperphosphorylation of tau; instead, it was associated with caspase activation and accumulation of a more toxic and insoluble form of truncated tau. Moreover, mutations that disrupt endogenous calpain A or B, or an engineered calpain-resistant tau construct, suppressed tau toxicity (Reinecke et al., 2011).

Another candidate approach study identified the **ER stress** transcription factor XBP1 as potential regulator of tau toxicity. Overexpression of tau activated the XBP1-GFP reporter and loss-of-function of XBP1 exacerbated tau toxicity (Fig. 4), indicating the involvement of the unfolded protein response in tau toxicity (Loewen and Feany, 2010). The same group also demonstrated that tau modulates **mitochondrial dynamics**. Pathological tau inhibited the association of the fission protein DRP1 with mitochondria, resulting in mitochondrial elongation (Fig. 4) (DuBoff et al., 2012). In addition, the NAD synthase and chaperone **NMNAT** was found to interact with tau oligomers and promote their clearance (Fig. 4) (Ali et al., 2012).

More recently, tau neurotoxicity was associated with widespread loss of **heterochromatin** in flies, mice, and human AD brains. This heterochromatin loss occurred downstream of tau phosphorylation, was a consequence of oxidative stress and DNA damage, and induced aberrant gene expression (Frost et al., 2014). *Drosophila* neurons overexpressing tau showed **DNA damage response**, which is characterized by p53 upregulation (Fig. 4) (Khurana et al., 2012). Flies lacking p53 activity displayed enhanced tau neurotoxicity, indicating the protective activity of p53. A search for genes regulated by p53 uncovered targets involved in synaptic transmission and endocytosis, which were also protective in flies expressing tau (Merlo et al., 2014). Additionally, **zinc** triggered tau toxicity by promoting tau phosphorylation and aggregation (Huang et al., 2014). Interestingly, reduction of zinc through diet or the regulation of zinc transporters led to partial rescue of tau toxicity. The authors also found that site-directed mutagenesis of zinc-binding sites in a pathological tau transgene eliminated its toxicity and that restoration of zinc binding reverted toxicity. Overall, tau interacts with many cellular pathways and understanding how tau perturbs cell homeostasis is critical for designing effective therapies.

4.4. Tau genetic screens

So far, three genetic screens for modifiers of tau toxicity have been reported. The first screen was conducted in flies expressing tau-V337M in the eye (Shulman and Feany, 2003). From a collection of 2,276 EP strains, the authors identified kinases and phosphatases as the most prominent group of modifiers, including the homologues of cyclin-dependent kinase 5 (CDK5) and GSK3 β as well as the Cdc25 phosphatases String and Twine. Other modifiers included inhibitors of apoptosis and cytoskeleton factors. A more recent screen was conducted using tau-V337M and a different collection of 1,250 EP strains (Blard et al., 2007). In this case, components of the cytoskeleton were also identified as modifiers of tau toxicity along with transcriptional cofactors, RNA binding proteins, and different types of transporters, to name a few. The last screen expressed the longest wild type tau isoform and screened ~1,000 P-element lethal insertions and a collection of 900 EY overexpression strains (Ambegaokar and Jackson, 2011). This screen confirmed the expected involvement of kinases and cytoskeleton components, but also identified pathways not previously linked to tau toxicity such as RNA metabolism and chromatin regulation.

4.5. Functional interaction of A β 42 and tau

A β 42 and tau have a complex and intricate relationship in AD pathogenesis, which prompted several groups to investigate the functional interactions in flies. Early studies

demonstrated that APPL synergistically enhanced the toxicity of bovine tau in larval motor neurons (Torroja et al., 1999a). Moreover, A β 42 and tau also demonstrated a synergistic interaction that required changes in tau phosphorylation (Fulga et al., 2007). Additionally, co-expression of A β 42 and tau exacerbated the axonal transport and synaptic phenotypes of either construct alone (Folwell et al., 2010). Interestingly, lithium prevented this synergistic effect, suggesting that GSK3 β mediated the functional interaction (Fig. 4). In support of this, flies expressing A β 42-E22G exhibited higher GSK3 β activity and premature death (Sofola et al., 2010). Of note, a null mutant tau background alleviated the toxicity of A β 42-E22G, supporting the hypothesis that tau mediates A β 42 neuropathology through a feedback loop (Bloom, 2014). Flies expressing A β 42 also show tau hyperphosphorylation at Ser262, which is a target for the Checkpoint kinase 2 (Chk2) (Iijima et al., 2010). Importantly, tau-S262A blocked phosphorylation and alleviated A β 42 toxicity. Taken together, these studies provide valuable information regarding the potential mechanisms mediating pathological interactions between A β 42 and tau.

4.6. Clinical relevance of the tau studies

Work on *Drosophila* and other models have identified the main kinases responsible for tau phosphorylation, including their hierarchical activity. Thus, kinase inhibitors should reduce tau phosphorylation and reduce its toxicity. However, these kinases have a multitude of substrates and, thus, blocking their activity may result in deleterious consequences. Developing substrate-specific inhibitors is a more appropriate long-term solution, but there are no guarantees that this strategy will work. Since tau misfolds and aggregates inside the cell, tau shares several pathogenic mechanisms with other intracellular amyloids, including autophagy, ER stress, and molecular chaperones, although these pathways are hard to activate by classic compounds. The most critical therapeutic opportunities for tau may come from the recent discoveries of its implications in heterochromatin maintenance and the DNA integrity. Better understanding of these tau activities can identify therapeutic targets, including p53, with potentially large effects on neuronal survival and function.

Concluding remarks—These studies show that *Drosophila* has significantly advanced our understanding of the biological function of APP and tau as well as the pathways mediating A β 42 and tau neurotoxicity. Additionally, studies on a large number of A β 42 and tau mutants, which are only feasible in invertebrate models due to the short generation time and cost, have provided invaluable in vivo information. Despite these advances, we have only begun to scratch the surface of APP / APPL processing and the biological function of their many proteolytic products. Similarly, tau has many isoforms and complex posttranslational modifications, both physiological and pathological, including glycation, ubiquitination, nitration, deamidation, oxidation, acetylation, and truncation, which are currently not well understood. Furthermore, single and compound *Drosophila* models can explore the role of A β 42 and tau in neuronal loss and the prion-like propagation at a single-cell resolution. These flies can be used for drug screens or to identify novel genes that can block concomitant A β 42/tau pathologies. These are only a few examples of the spectrum of opportunities in which fruit flies may provide unique insights in the years to come.

Acknowledgements

We apologize to those whose work we could not cite in this review. This work was supported in part by the 1R21NS081356 grant to DERL, an Ed and Ethel Moore AD research grant from the Florida Department of Health (4085924232), and funds from the UF department of Neurology to PF-F and DER-L.

References

- Acerra N, Kad NM, Griffith DA, Ott S, Crowther DC, Mason JM. Retro-inversion of intracellular selected beta-amyloid-interacting peptides: implications for a novel Alzheimer's disease treatment. *Biochemistry*. 2014; 53:2101–2111. [PubMed: 24601543]
- Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, Scherer SE, Li PW, Hoskins RA, Galle RF, George RA, Lewis SE, Richards S, Ashburner M, Henderson SN, Sutton GG, Wortman JR, Yandell MD, Zhang Q, Chen LX, Brandon RC, Rogers YH, Blazej RG, Champe M, Pfeiffer BD, Wan KH, Doyle C, Baxter EG, Helt G, Nelson CR, Gabor GL, Abril JF, Agbayani A, An HJ, Andrews-Pfannkoch C, Baldwin D, Ballew RM, Basu A, Baxendale J, Bayraktaroglu L, Beasley EM, Beeson KY, Benos PV, Berman BP, Bhandari D, Bolshakov S, Borkova D, Botchan MR, Bouck J, Brokstein P, Brottier P, Burtis KC, Busam DA, Butler H, Cadieu E, Center A, Chandra I, Cherry JM, Cawley S, Dahlke C, Davenport LB, Davies P, de Pablos B, Delcher A, Deng Z, Mays AD, Dew I, Dietz SM, Dodson K, Doup LE, Downes M, Dugan-Rocha S, Dunkov BC, Dunn P, Durbin KJ, Evangelista CC, Ferraz C, Ferriera S, Fleischmann W, Fosler C, Gabrielian AE, Garg NS, Gelbart WM, Glasser K, Glodek A, Gong F, Gorrell JH, Gu Z, Guan P, Harris M, Harris NL, Harvey D, Heiman TJ, Hernandez JR, Houck J, Hostin D, Houston KA, Howland TJ, Wei MH, Ibegwam C, Jalali M, Kalush F, Karpen GH, Ke Z, Kennison JA, Ketchum KA, Kimmel BE, Kodira CD, Kraft C, Kravitz S, Kulp D, Lai Z, Lasko P, Lei Y, Levitsky AA, Li J, Li Z, Liang Y, Lin X, Liu X, Mattei B, McIntosh TC, McLeod MP, McPherson D, Merkulov G, Milshina NV, Mobarry C, Morris J, Moshrefi A, Mount SM, Moy M, Murphy B, Murphy L, Muzny DM, Nelson DL, Nelson DR, Nelson KA, Nixon K, Nusskern DR, Pacle JM, Palazzolo M, Pittman GS, Pan S, Pollard J, Puri V, Reese MG, Reinert K, Remington K, Saunders RD, Scheeler F, Shen H, Shue BC, Siden-Kiamos I, Simpson M, Skupski MP, Smith T, Spier E, Spradling AC, Stapleton M, Strong R, Sun E, Svirkas R, Tector C, Turner R, Venter E, Wang AH, Wang X, Wang ZY, Wassarman DA, Weinstock GM, Weissenbach J, Williams SM, Woodage T, Worley KC, Wu D, Yang S, Yao QA, Ye J, Yeh RF, Zaveri JS, Zhan M, Zhang G, Zhao Q, Zheng L, Zheng XH, Zhong FN, Zhong W, Zhou X, Zhu S, Zhu X, Smith HO, Gibbs RA, Myers EW, Rubin GM, Venter JC. The genome sequence of *Drosophila melanogaster*. *Science*. 2000; 287:2185–2195. [PubMed: 10731132]
- Ali YO, Ruan K, Zhai RG. NMNAT suppresses tau-induced neurodegeneration by promoting clearance of hyperphosphorylated tau oligomers in a *Drosophila* model of tauopathy. *Hum Mol Genet*. 2012; 21:237–250. [PubMed: 21965302]
- Allan K, Perez KA, Barnham KJ, Camakaris J, Burke R. A commonly used *Drosophila* model of Alzheimer's disease generates an aberrant species of amyloid-beta with an additional N-terminal glutamine residue. *FEBS Lett*. 2014; 588:3739–3743. [PubMed: 25171862]
- Ambegaokar SS, Jackson GR. Functional genomic screen and network analysis reveal novel modifiers of tauopathy dissociated from tau phosphorylation. *Hum Mol Genet*. 2011; 20:4947–4977. [PubMed: 21949350]
- Andreadis A. Tau splicing and the intricacies of dementia. *Journal of cellular physiology*. 2012; 227:1220–1225. [PubMed: 21604267]
- Baell J, Walters MA. Chemistry: Chemical con artists foil drug discovery. *Nature*. 2014; 513:481–483. [PubMed: 25254460]
- Bassett AR, Tibbit C, Ponting CP, Liu JL. Highly efficient targeted mutagenesis of *Drosophila* with the CRISPR/Cas9 system. *Cell reports*. 2013; 4:220–228. [PubMed: 23827738]
- Beharry C, Cohen LS, Di J, Ibrahim K, Briffa-Mirabella S, Alonso Adel C. Tau-induced neurodegeneration: mechanisms and targets. *Neuroscience bulletin*. 2014; 30:346–358. [PubMed: 24733656]

- Bellen HJ, Tong C, Tsuda H. 100 years of *Drosophila* research and its impact on vertebrate neuroscience: a history lesson for the future. *Nat Rev Neurosci*. 2010; 11:514–522. [PubMed: 20383202]
- Benilova I, Karran E, De Strooper B. The toxic A β oligomer and Alzheimer's disease: an emperor in need of clothes. *Nat Neurosci*. 2012; 15:349–357. [PubMed: 22286176]
- Bilen J, Bonini NM. Genome-wide screen for modifiers of ataxin-3 neurodegeneration in *Drosophila*. *PLoS Genet*. 2007; 3:1950–1964. [PubMed: 17953484]
- Blard O, Feuillet S, Bou J, Chaumette B, Frebourg T, Campion D, Lecourtis M. Cytoskeleton proteins are modulators of mutant tau-induced neurodegeneration in *Drosophila*. *Hum Mol Genet*. 2007; 16:555–566. [PubMed: 17309878]
- Bloom GS. Amyloid-beta and tau: the trigger and bullet in Alzheimer disease pathogenesis. *JAMA neurology*. 2014; 71:505–508. [PubMed: 24493463]
- Blurton-Jones M, Spencer B, Michael S, Castello NA, Agazaryan AA, Davis JL, Muller FJ, Loring JF, Masliah E, LaFerla FM. Neural stem cells genetically-modified to express neprilysin reduce pathology in Alzheimer transgenic models. *Stem cell research & therapy*. 2014; 5:46. [PubMed: 25022790]
- Bolkan BJ, Triphan T, Kretschmar D. beta-secretase cleavage of the fly amyloid precursor protein is required for glial survival. *J Neurosci*. 2012; 32:16181–16192. [PubMed: 23152602]
- Brand AH, Perrimon N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development*. 1993; 118:401–415. [PubMed: 8223268]
- Brorsson AC, Bolognesi B, Tartaglia GG, Shammas SL, Favrin G, Watson I, Lomas DA, Chiti F, Vendruscolo M, Dobson CM, Crowther DC, Luheshi LM. Intrinsic determinants of neurotoxic aggregate formation by the amyloid beta peptide. *Biophysical journal*. 2010; 98:1677–1684. [PubMed: 20409489]
- Caesar I, Jonson M, Nilsson KP, Thor S, Hammarstrom P. Curcumin promotes A β -fibrillation and reduces neurotoxicity in transgenic *Drosophila*. *PLoS One*. 2012; 7:e31424. [PubMed: 22348084]
- Cao W, Song HJ, Gangi T, Kelkar A, Antani I, Garza D, Konsolaki M. Identification of novel genes that modify phenotypes induced by Alzheimer's beta-amyloid overexpression in *Drosophila*. *Genetics*. 2008; 178:1457–1471. [PubMed: 18245849]
- Cao X, Sudhof TC. A transcriptionally [correction of transcriptively] active complex of APP with Fe65 and histone acetyltransferase Tip60. *Science*. 2001; 293:115–120. [PubMed: 11441186]
- Carmine-Simmen K, Proctor T, Tschape J, Poock B, Triphan T, Strauss R, Kretschmar D. Neurotoxic effects induced by the *Drosophila* amyloid-beta peptide suggest a conserved toxic function. *Neurobiol Dis*. 2009; 33:274–281. [PubMed: 19049874]
- Casas-Tinto S, Zhang Y, Sanchez-Garcia J, Gomez-Velazquez M, Rincon-Limas DE, Fernandez-Funez P. The ER stress factor XBP1s prevents amyloid-beta neurotoxicity. *Hum Mol Genet*. 2011; 20:2144–2160. [PubMed: 21389082]
- Chakraborty R, Vepuri V, Mhatre SD, Paddock BE, Miller S, Michelson SJ, Delvadia R, Desai A, Vinokur M, Melicharek DJ, Utraja S, Khandelwal P, Ansaloni S, Goldstein LE, Moir RD, Lee JC, Tabb LP, Saunders AJ, Marena DR. Characterization of a *Drosophila* Alzheimer's disease model: pharmacological rescue of cognitive defects. *PLoS ONE*. 2011; 6:e20799. [PubMed: 21673973]
- Chatterjee S, Sang TK, Lawless GM, Jackson GR. Dissociation of tau toxicity and phosphorylation: role of GSK-3 β , MARK and Cdk5 in a *Drosophila* model. *Hum Mol Genet*. 2009; 18:164–177. [PubMed: 18930955]
- Chen KF, Possidente B, Lomas DA, Crowther DC. The central molecular clock is robust in the face of behavioural arrhythmia in a *Drosophila* model of Alzheimer's disease. *Dis Model Mech*. 2014; 7:445–458. [PubMed: 24574361]
- Chiang HC, Iijima K, Hakker I, Zhong Y. Distinctive roles of different beta-amyloid 42 aggregates in modulation of synaptic functions. *FASEB J*. 2009; 23:1969–1977. [PubMed: 19255256]
- Chiang HC, Wang L, Xie Z, Yau A, Zhong Y. PI3 kinase signaling is involved in A β -induced memory loss in *Drosophila*. *Proc Natl Acad Sci U S A*. 2010; 107:7060–7065. [PubMed: 20351282]
- Chien S, Reiter LT, Bier E, Gribskov M. Homophila: human disease gene cognates in *Drosophila*. *Nucleic Acids Res*. 2002; 30:149–151. [PubMed: 11752278]

- Coburger I, Hoefgen S, Than ME. The structural biology of the amyloid precursor protein APP - a complex puzzle reveals its multi-domain architecture. *Biological chemistry*. 2014; 395:485–498. [PubMed: 24516000]
- Colodner KJ, Feany MB. Glial fibrillary tangles and JAK/STAT-mediated glial and neuronal cell death in a *Drosophila* model of glial tauopathy. *J Neurosci*. 2010; 30:16102–16113. [PubMed: 21123557]
- Costa R, Speretta E, Crowther DC, Cardoso I. Testing the therapeutic potential of doxycycline in a *Drosophila melanogaster* model of Alzheimer disease. *J Biol Chem*. 2011; 286:41647–41655. [PubMed: 21998304]
- Cowan CM, Bossing T, Page A, Shepherd D, Mudher A. Soluble hyper-phosphorylated tau causes microtubule breakdown and functionally compromises normal tau in vivo. *Acta Neuropathol*. 2010; 120:593–604. [PubMed: 20617325]
- Crowther DC, Kinghorn KJ, Miranda E, Page R, Curry JA, Duthie FA, Gubb DC, Lomas DA. Intraneuronal A β , non-amyloid aggregates and neurodegeneration in a *Drosophila* model of Alzheimer's disease. *Neuroscience*. 2005; 132:123–135. [PubMed: 15780472]
- Davis RL. Olfactory memory formation in *Drosophila*: from molecular to systems neuroscience. *Annu Rev Neurosci*. 2005; 28:275–302. [PubMed: 16022597]
- Dias-Santagata D, Fulga TA, Duttaroy A, Feany MB. Oxidative stress mediates tau-induced neurodegeneration in *Drosophila*. *J Clin Invest*. 2007; 117:236–245. [PubMed: 17173140]
- DuBoff B, Gotz J, Feany MB. Tau promotes neurodegeneration via DRP1 mislocalization in vivo. *Neuron*. 2012; 75:618–632. [PubMed: 22920254]
- Fang L, Duan J, Ran D, Fan Z, Yan Y, Huang N, Gu H, Zhu Y. Amyloid-beta depresses excitatory cholinergic synaptic transmission in *Drosophila*. *Neuroscience bulletin*. 2012; 28:585–594. [PubMed: 23054636]
- Favrin G, Bean DM, Bilsland E, Boyer H, Fischer BE, Russell S, Crowther DC, Baylis HA, Oliver SG, Giannakou ME. Identification of novel modifiers of A β toxicity by transcriptomic analysis in the fruitfly. *Sci Rep*. 2013; 3:3512. [PubMed: 24336499]
- Fernandez-Funez P, Nino-Rosales ML, de Gouyon B, She WC, Luchak JM, Martinez P, Turiegano E, Benito J, Capovilla M, Skinner PJ, McCall A, Canal I, Orr HT, Zoghbi HY, Botas J. Identification of genes that modify ataxin-1-induced neurodegeneration. *Nature*. 2000; 408:101–106. [PubMed: 11081516]
- Finelli A, Kelkar A, Song HJ, Yang H, Konsolaki M. A model for studying Alzheimer's A β 42-induced toxicity in *Drosophila melanogaster*. *Mol Cell Neurosci*. 2004; 26:365–375. [PubMed: 15234342]
- Folwell J, Cowan CM, Ubhi KK, Shiab H, Newman TA, Shepherd D, Mudher A. A β exacerbates the neuronal dysfunction caused by human tau expression in a *Drosophila* model of Alzheimer's disease. *Exp Neurol*. 2010; 223:401–409. [PubMed: 19782075]
- Frost B, Hemberg M, Lewis J, Feany MB. Tau promotes neurodegeneration through global chromatin relaxation. *Nat Neurosci*. 2014; 17:357–366. [PubMed: 24464041]
- Fulga TA, Elson-Schwab I, Khurana V, Steinhilb ML, Spires TL, Hyman BT, Feany MB. Abnormal bundling and accumulation of F-actin mediates tau-induced neuronal degeneration in vivo. *Nat Cell Biol*. 2007; 9:139–148. [PubMed: 17187063]
- Gendron TF, Petrucelli L. The role of tau in neurodegeneration. *Molecular neurodegeneration*. 2009; 4:13. [PubMed: 19284597]
- Gistelink M, Lambert JC, Callaerts P, Dermaut B, Dourlen P. *Drosophila* models of tauopathies: what have we learned? *Int J Alzheimers Dis*. 2012; 2012:970980. [PubMed: 22701808]
- Goguel V, Belair AL, Ayaz D, Lampin-Saint-Amaux A, Scaplehorn N, Hassan BA, Preat T. *Drosophila* amyloid precursor protein-like is required for long-term memory. *J Neurosci*. 2011; 31:1032–1037. [PubMed: 21248128]
- Golde TE, Borchelt DR, Giasson BI, Lewis J. Thinking laterally about neurodegenerative proteinopathies. *J Clin Invest*. 2013; 123:1847–1855. [PubMed: 23635781]
- Gotz J, Chen F, van Dorpe J, Nitsch RM. Formation of neurofibrillary tangles in P301 τ transgenic mice induced by A β 42 fibrils. *Science*. 2001; 293:1491–1495. [PubMed: 11520988]

- Gratz SJ, Cummings AM, Nguyen JN, Hamm DC, Donohue LK, Harrison MM, Wildonger J, O'Connor-Giles KM. Genome engineering of *Drosophila* with the CRISPR RNA-guided Cas9 nuclease. *Genetics*. 2013; 194:1029–1035. [PubMed: 23709638]
- Greeve I, Kretschmar D, Tschape JA, Beyn A, Brellinger C, Schweizer M, Nitsch RM, Reifegerste R. Age-dependent neurodegeneration and Alzheimer-amyloid plaque formation in transgenic *Drosophila*. *J Neurosci*. 2004; 24:3899–3906. [PubMed: 15102905]
- Gunawardena S, Goldstein LS. Disruption of axonal transport and neuronal viability by amyloid precursor protein mutations in *Drosophila*. *Neuron*. 2001; 32:389–401. [PubMed: 11709151]
- Gunawardena S, Yang G, Goldstein LS. Presenilin controls kinesin-1 and dynein function during APP-vesicle transport in vivo. *Hum Mol Genet*. 2013; 22:3828–3843. [PubMed: 23710041]
- Hall AM, Roberson ED. Mouse models of Alzheimer's disease. *Brain Res Bull*. 2012; 88:3–12. [PubMed: 22142973]
- Harada A, Oguchi K, Okabe S, Kuno J, Terada S, Ohshima T, Sato-Yoshitake R, Takei Y, Noda T, Hirokawa N. Altered microtubule organization in small-calibre axons of mice lacking tau protein. *Nature*. 1994; 369:488–491. [PubMed: 8202139]
- Hardin PE. The circadian timekeeping system of *Drosophila*. *Curr Biol*. 2005; 15:R714–R722. [PubMed: 16139204]
- Hardy J. The amyloid hypothesis for Alzheimer's disease: a critical reappraisal. *J Neurochem*. 2009; 110:1129–1134. [PubMed: 19457065]
- Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. *Science*. 1992; 256:184–185. [PubMed: 1566067]
- Hermansson E, Schultz S, Crowther D, Linse S, Winblad B, Westermarck G, Johansson J, Presto J. The chaperone domain BRICHOS prevents CNS toxicity of amyloid-beta peptide in *Drosophila melanogaster*. *Dis Model Mech*. 2014; 7:659–665. [PubMed: 24682783]
- Hong YK, Lee S, Park SH, Lee JH, Han SY, Kim ST, Kim YK, Jeon S, Koo BS, Cho KS. Inhibition of JNK/dFOXO pathway and caspases rescues neurological impairments in *Drosophila* Alzheimer's disease model. *Biochem Biophys Res Commun*. 2012; 419:49–53. [PubMed: 22326868]
- Hu Y, Han Y, Wang X, Xue L. Aging-related neurodegeneration eliminates male courtship choice in *Drosophila*. *Neurobiol Aging*. 2014; 35:2174–2178. [PubMed: 24684795]
- Hua H, Munter L, Harmeier A, Georgiev O, Multhaup G, Schaffner W. Toxicity of Alzheimer's disease-associated Aβ peptide is ameliorated in a *Drosophila* model by tight control of zinc and copper availability. *Biol Chem*. 2011; 392:919–926. [PubMed: 21801085]
- Huang JK, Ma PL, Ji SY, Zhao XL, Tan JX, Sun XJ, Huang FD. Age-dependent alterations in the presynaptic active zone in a *Drosophila* model of Alzheimer's disease. *Neurobiol Dis*. 2013; 51:161–167. [PubMed: 23149068]
- Huang Y, Wu Z, Cao Y, Lang M, Lu B, Zhou B. Zinc binding directly regulates tau toxicity independent of tau hyperphosphorylation. *Cell reports*. 2014; 8:831–842. [PubMed: 25066125]
- Hurtado DE, Molina-Porcel L, Iba M, Aboagye AK, Paul SM, Trojanowski JQ, Lee VM. Aβ accelerates the spatiotemporal progression of tau pathology and augments tau amyloidosis in an Alzheimer mouse model. *Am J Pathol*. 2010; 177:1977–1988. [PubMed: 20802182]
- Iijima K, Chiang HC, Hearn SA, Hakker I, Gatt A, Shenton C, Granger L, Leung A, Iijima-Ando K, Zhong Y. Aβ42 mutants with different aggregation profiles induce distinct pathologies in *Drosophila*. *PLoS ONE*. 2008; 3:e1703. [PubMed: 18301778]
- Iijima K, Gatt A, Iijima-Ando K. Tau Ser262 phosphorylation is critical for Aβ42-induced tau toxicity in a transgenic *Drosophila* model of Alzheimer's disease. *Hum Mol Genet*. 2010; 19:2947–2957. [PubMed: 20466736]
- Iijima K, Liu HP, Chiang AS, Hearn SA, Konsolaki M, Zhong Y. Dissecting the pathological effects of human Aβ40 and Aβ42 in *Drosophila*: a potential model for Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2004; 101:6623–6628. [PubMed: 15069204]
- Iijima-Ando K, Hearn SA, Granger L, Shenton C, Gatt A, Chiang HC, Hakker I, Zhong Y, Iijima K. Overexpression of neprilysin reduces Alzheimer amyloid-β42 (Aβ42)-induced neuron loss and intraneuronal Aβ42 deposits but causes a reduction in cAMP-responsive element-binding

- protein-mediated transcription, age-dependent axon pathology, and premature death in *Drosophila*. *J Biol Chem*. 2008; 283:19066–19076. [PubMed: 18463098]
- Iijima-Ando K, Hearn SA, Shenton C, Gatt A, Zhao L, Iijima K. Mitochondrial mislocalization underlies Abeta42-induced neuronal dysfunction in a *Drosophila* model of Alzheimer's disease. *PLoS One*. 2009; 4:e8310. [PubMed: 20016833]
- Ittner LM, Ke YD, Delerue F, Bi M, Gladbach A, van Eersel J, Wolfing H, Chieng BC, Christie MJ, Napier IA, Eckert A, Staufenbiel M, Hardeman E, Gotz J. Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. *Cell*. 2010; 142:387–397. [PubMed: 20655099]
- Jackson GR, Wiedau-Pazos M, Sang TK, Wagle N, Brown CA, Massachi S, Geschwind DH. Human wild-type tau interacts with wingless pathway components and produces neurofibrillary pathology in *Drosophila*. *Neuron*. 2002; 34:509–519. [PubMed: 12062036]
- Jenett A, Rubin GM, Ngo TT, Shepherd D, Murphy C, Dionne H, Pfeiffer BD, Cavallaro A, Hall D, Jeter J, Iyer N, Fetter D, Hausenfluck JH, Peng H, Trautman ET, Svirskas RR, Myers EW, Iwinski ZR, Aso Y, Depasquale GM, Enos A, Hulamm P, Lam SC, Li HH, Lavery TR, Long F, Qu L, Murphy SD, Rokicki K, Safford T, Shaw K, Simpson JH, Sowell A, Tae S, Yu Y, Zugates CT. A GAL4-Driver Line Resource for *Drosophila* Neurobiology. *Cell Rep*. 2012; 2:991–1001. [PubMed: 23063364]
- Johnson AA, Sarthi J, Pirooznia SK, Reube W, Elefant F. Increasing Tip60 HAT levels rescues axonal transport defects and associated behavioral phenotypes in a *Drosophila* Alzheimer's disease model. *J Neurosci*. 2013; 33:7535–7547. [PubMed: 23616558]
- Karch CM, Cruchaga C, Goate AM. Alzheimer's disease genetics: from the bench to the clinic. *Neuron*. 2014; 83:11–26. [PubMed: 24991952]
- Karsten SL, Sang TK, Gehman LT, Chatterjee S, Liu J, Lawless GM, Sengupta S, Berry RW, Pomakian J, Oh HS, Schulz C, Hui KS, Wiedau-Pazos M, Vinters HV, Binder LI, Geschwind DH, Jackson GR. A genomic screen for modifiers of tauopathy identifies puromycin-sensitive aminopeptidase as an inhibitor of tau-induced neurodegeneration. *Neuron*. 2006; 51:549–560. [PubMed: 16950154]
- Khurana V, Elson-Schwab I, Fulga TA, Sharp KA, Loewen CA, Mulkearns E, Tyynela J, Scherzer CR, Feany MB. Lysosomal dysfunction promotes cleavage and neurotoxicity of tau in vivo. *PLoS Genet*. 2010; 6:e1001026. [PubMed: 20664788]
- Khurana V, Merlo P, DuBoff B, Fulga TA, Sharp KA, Campbell SD, Gotz J, Feany MB. A neuroprotective role for the DNA damage checkpoint in tauopathy. *Aging Cell*. 2012; 11:360–362. [PubMed: 22181010]
- Kidd JF, Brown LA, Sattelle DB. Effects of amyloid peptides on A-type K⁺ currents of *Drosophila* larval cholinergic neurons. *J Neurobiol*. 2006; 66:476–487. [PubMed: 16470685]
- Kidd JF, Sattelle DB. The effects of amyloid peptides on A-type K(+) currents of *Drosophila* larval cholinergic neurons: modeled actions on firing properties. *Invertebrate neuroscience : IN*. 2006; 6:207–213. [PubMed: 17106756]
- Kinghorn KJ, Crowther DC, Sharp LK, Nerelius C, Davis RL, Chang HT, Green C, Gubb DC, Johansson J, Lomas DA. Neuroserpin binds Abeta and is a neuroprotective component of amyloid plaques in Alzheimer disease. *J Biol Chem*. 2006; 281:29268–29277. [PubMed: 16849336]
- Kondo S. New horizons in genome engineering of *Drosophila melanogaster*. *Genes & genetic systems*. 2014; 89:3–8. [PubMed: 24817756]
- Kosmidis S, Grammenoudi S, Papanikolopoulou K, Skoulakis EM. Differential effects of Tau on the integrity and function of neurons essential for learning in *Drosophila*. *J Neurosci*. 2010; 30:464–477. [PubMed: 20071510]
- Kruppa AJ, Ott S, Chandraratna DS, Irving JA, Page RM, Speretta E, Seto T, Camargo LM, Marciniak SJ, Lomas DA, Crowther DC. Suppression of Abeta toxicity by puromycin-sensitive aminopeptidase is independent of its proteolytic activity. *Biochim Biophys Acta*. 2013; 1832:2115–2126. [PubMed: 23911349]
- Lai SL, Lee T. Genetic mosaic with dual binary transcriptional systems in *Drosophila*. *Nat Neurosci*. 2006; 9:703–709. [PubMed: 16582903]

- Lang M, Fan Q, Wang L, Zheng Y, Xiao G, Wang X, Wang W, Zhong Y, Zhou B. Inhibition of human high-affinity copper importer Ctr1 orthologous in the nervous system of *Drosophila* ameliorates A β 42-induced Alzheimer's disease-like symptoms. *Neurobiol Aging*. 2013; 34:2604–2612. [PubMed: 23827522]
- Leroy K, Ando K, Laporte V, Dedeker R, Suain V, Authélet M, Heraud C, Pierrot N, Yilmaz Z, Octave JN, Brion JP. Lack of tau proteins rescues neuronal cell death and decreases amyloidogenic processing of APP in APP/PS1 mice. *Am J Pathol*. 2012; 181:1928–1940. [PubMed: 23026200]
- Leyssen M, Ayaz D, Hebert SS, Reeve S, De Strooper B, Hassan BA. Amyloid precursor protein promotes post-developmental neurite arborization in the *Drosophila* brain. *EMBO J*. 2005; 24:2944–2955. [PubMed: 16052209]
- Li Y, Liu T, Peng Y, Yuan C, Guo A. Specific functions of *Drosophila* amyloid precursorlike protein in the development of nervous system and nonneural tissues. *J Neurobiol*. 2004; 61:343–358. [PubMed: 15389603]
- Lin JY, Wang WA, Zhang X, Liu HY, Zhao XL, Huang FD. Intraneuronal accumulation of A β 42 induces age-dependent slowing of neuronal transmission in *Drosophila*. *Neuroscience bulletin*. 2014; 30:185–190. [PubMed: 24733651]
- Ling D, Song HJ, Garza D, Neufeld TP, Salvaterra PM. A β 42-induced neurodegeneration via an age-dependent autophagic-lysosomal injury in *Drosophila*. *PLoS ONE*. 2009; 4:e4201. [PubMed: 19145255]
- Liu B, Moloney A, Meehan S, Morris K, Thomas SE, Serpell LC, Hider R, Marciniak SJ, Lomas DA, Crowther DC. Iron promotes the toxicity of amyloid beta peptide by impeding its ordered aggregation. *J Biol Chem*. 2011; 286:4248–4256. [PubMed: 21147772]
- Loewen CA, Feany MB. The unfolded protein response protects from tau neurotoxicity in vivo. *PLoS One*. 2010; 5.
- Long DM, Blake MR, Dutta S, Holbrook SD, Kotwica-Rolinska J, Kretschmar D, Giebltowicz JM. Relationships between the circadian system and Alzheimer's disease-like symptoms in *Drosophila*. *PLoS One*. 2014; 9:e106068. [PubMed: 25171136]
- Luheshi LM, Hoyer W, de Barros TP, van Dijk Hard I, Brorsson AC, Macao B, Persson C, Crowther DC, Lomas DA, Stahl S, Dobson CM, Hard T. Sequestration of the A β peptide prevents toxicity and promotes degradation in vivo. *PLoS Biol*. 2010; 8:e1000334. [PubMed: 20305716]
- Luheshi LM, Tartaglia GG, Brorsson AC, Pawar AP, Watson IE, Chiti F, Vendruscolo M, Lomas DA, Dobson CM, Crowther DC. Systematic in vivo analysis of the intrinsic determinants of amyloid Beta pathogenicity. *PLoS Biol*. 2007; 5:e290. [PubMed: 17973577]
- Luo L, Tully T, White K. Human amyloid precursor protein ameliorates behavioral deficit of flies deleted for *Appl* gene. *Neuron*. 1992; 9:595–605. [PubMed: 1389179]
- Luo LQ, Martin-Morris LE, White K. Identification, secretion, and neural expression of APPL, a *Drosophila* protein similar to human amyloid protein precursor. *J Neurosci*. 1990; 10:3849–3861. [PubMed: 2125311]
- Marr RA, Rockenstein E, Mukherjee A, Kindy MS, Hersh LB, Gage FH, Verma IM, Masliah E. Neprilysin gene transfer reduces human amyloid pathology in transgenic mice. *J Neurosci*. 2003; 23:1992–1996. [PubMed: 12657655]
- Martin-Morris LE, White K. The *Drosophila* transcript encoded by the beta-amyloid protein precursor-like gene is restricted to the nervous system. *Development*. 1990; 110:185–195. [PubMed: 2127912]
- Merlo P, Frost B, Peng S, Yang YJ, Park PJ, Feany M. p53 prevents neurodegeneration by regulating synaptic genes. *Proc Natl Acad Sci U S A*. 2014; 111:18055–18060. [PubMed: 25453105]
- Mhatre SD, Michelson SJ, Gomes J, Tabb LP, Saunders AJ, Marenda DR. Development and characterization of an aged onset model of Alzheimer's disease in *Drosophila melanogaster*. *Exp Neurol*. 2014; 261:772–781. [PubMed: 25173219]
- Mohr SE, Hu Y, Kim K, Housden BE, Perrimon N. Resources for functional genomics studies in *Drosophila melanogaster*. *Genetics*. 2014; 197:1–18. [PubMed: 24653003]

- Mora N, Almudi I, Alsina B, Corominas M, Serras F. beta amyloid protein precursor-like (Appl) is a Ras1/MAPK-regulated gene required for axonal targeting in *Drosophila* photoreceptor neurons. *Journal of cell science*. 2013; 126:53–59. [PubMed: 23178937]
- Morris M, Maeda S, Vossel K, Mucke L. The many faces of tau. *Neuron*. 2011; 70:410–426. [PubMed: 21555069]
- Mudher A, Shepherd D, Newman TA, Mildren P, Jukes JP, Squire A, Mears A, Drummond JA, Berg S, MacKay D, Asuni AA, Bhat R, Lovestone S. GSK-3beta inhibition reverses axonal transport defects and behavioural phenotypes in *Drosophila*. *Mol Psychiatry*. 2004; 9:522–530. [PubMed: 14993907]
- Nerelius C, Sandegren A, Sargsyan H, Raunak R, Leijonmarck H, Chatterjee U, Fisahn A, Imarisio S, Lomas DA, Crowther DC, Stromberg R, Johansson J. Alpha-helix targeting reduces amyloid-beta peptide toxicity. *Proc Natl Acad Sci U S A*. 2009; 106:9191–9196. [PubMed: 19458258]
- Ng CF, Ko CH, Koon CM, Xian JW, Leung PC, Fung KP, Chan HY, Lau CB. The Aqueous Extract of Rhizome of *Gastrodia elata* Protected *Drosophila* and PC12 Cells against Beta- Amyloid-Induced Neurotoxicity. Evidence-based complementary and alternative medicine : eCAM. 2013; 2013:516741. [PubMed: 24174977]
- Nhan HS, Chiang K, Koo EH. The multifaceted nature of amyloid precursor protein and its proteolytic fragments: friends and foes. *Acta Neuropathol*. 2014
- Nhan HS, Chiang K, Koo EH. The multifaceted nature of amyloid precursor protein and its proteolytic fragments: friends and foes. *Acta Neuropathol*. 2015; 129:1–19. [PubMed: 25287911]
- Nishimura I, Yang Y, Lu B. PAR-1 kinase plays an initiator role in a temporally ordered phosphorylation process that confers tau toxicity in *Drosophila*. *Cell*. 2004; 116:671–682. [PubMed: 15006350]
- Oddo S, Caccamo A, Cheng D, Joulé B, Torp R, LaFerla FM. Genetically augmenting tau levels does not modulate the onset or progression of Abeta pathology in transgenic mice. *J Neurochem*. 2007; 102:1053–1063. [PubMed: 17472708]
- Overk CR, Masliah E. Pathogenesis of synaptic degeneration in Alzheimer's disease and Lewy body disease. *Biochemical pharmacology*. 2014; 88:508–516. [PubMed: 24462903]
- Park SH, Lee S, Hong YK, Hwang S, Lee JH, Bang SM, Kim YK, Koo BS, Lee IS, Cho KS. Suppressive effects of SuHeXiang Wan on amyloid-beta42-induced extracellular signal-regulated kinase hyperactivation and glial cell proliferation in a transgenic *Drosophila* model of Alzheimer's disease. *Biological & pharmaceutical bulletin*. 2013; 36:390–398. [PubMed: 23238278]
- Park SJ, Schertel A, Lee KE, Han SS. Ultra-structural analysis of the brain in a *Drosophila* model of Alzheimer's disease using FIB/SEM microscopy. *Microscopy*. 2014; 63:3–13. [PubMed: 24047568]
- Ping Y, Hahm ET, Waro G, Song Q, Vo-Ba DA, Licursi A, Bao H, Ganoe L, Finch K, Tsunoda S. Linking abeta42-induced hyperexcitability to neurodegeneration, learning and motor deficits, and a shorter lifespan in an Alzheimer's model. *PLoS Genet*. 2015; 11:e1005025. [PubMed: 25774758]
- Poeck B, Strauss R, Kretschmar D. Analysis of amyloid precursor protein function in *Drosophila melanogaster*. *Experimental brain research*. 2012; 217:413–421. [PubMed: 21912928]
- Potter CJ, Tasic B, Russler EV, Liang L, Luo L. The Q system: a repressible binary system for transgene expression, lineage tracing, and mosaic analysis. *Cell*. 2010; 141:536–548. [PubMed: 20434990]
- Pratim Bose P, Chatterjee U, Nerelius C, Govender T, Norstrom T, Gogoll A, Sandegren A, Gothelid E, Johansson J, Arvidsson PI. Poly-N-methylated amyloid beta-peptide (Abeta) C-terminal fragments reduce Abeta toxicity in vitro and in *Drosophila melanogaster*. *J Med Chem*. 2009; 52:8002–8009. [PubMed: 19908889]
- Reichert H. A tripartite organization of the urbilaterian brain: developmental genetic evidence from *Drosophila*. *Brain Res Bull*. 2005; 66:491–494. [PubMed: 16144638]
- Reinecke JB, DeVos SL, McGrath JP, Shepard AM, Goncharoff DK, Tait DN, Fleming SR, Vincent MP, Steinhilb ML. Implicating calpain in tau-mediated toxicity in vivo. *PLoS One*. 2011; 6:e23865. [PubMed: 21858230]

- Reis GF, Yang G, Szpankowski L, Weaver C, Shah SB, Robinson JT, Hays TS, Danuser G, Goldstein LS. Molecular motor function in axonal transport in vivo probed by genetic and computational analysis in *Drosophila*. *Mol Biol Cell*. 2012; 23:1700–1714. [PubMed: 22398725]
- Rincon-Limas D, Jensen K, Fernandez Funez A. *Drosophila* models of proteinopathies: the little fly that could. *Curr Pharm Des*. 2012; 18:1108–1122. [PubMed: 22288402]
- Rival T, Page RM, Chandraratna DS, Sendall TJ, Ryder E, Liu B, Lewis H, Rosahl T, Hider R, Camargo LM, Shearman MS, Crowther DC, Lomas DA. Fenton chemistry and oxidative stress mediate the toxicity of the beta-amyloid peptide in a *Drosophila* model of Alzheimer's disease. *Eur J Neurosci*. 2009; 29:1335–1347. [PubMed: 19519625]
- Roberson ED, Scarce-Levie K, Palop JJ, Yan F, Cheng IH, Wu T, Gerstein H, Yu GQ, Mucke L. Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science*. 2007; 316:750–754. [PubMed: 17478722]
- Rorth P. A modular misexpression screen in *Drosophila* detecting tissue-specific phenotypes. *Proc Natl Acad Sci U S A*. 1996; 93:12418–12422. [PubMed: 8901596]
- Rosen DR, Martin-Morris L, Luo LQ, White K. A *Drosophila* gene encoding a protein resembling the human beta-amyloid protein precursor. *Proc Natl Acad Sci U S A*. 1989; 86:2478–2482. [PubMed: 2494667]
- Salminen A, Ojala J, Kauppinen A, Kaarniranta K, Suuronen T. Inflammation in Alzheimer's disease: amyloid-beta oligomers trigger innate immunity defence via pattern recognition receptors. *Progress in neurobiology*. 2009; 87:181–194. [PubMed: 19388207]
- Sanokawa-Akakura R, Cao W, Allan K, Patel K, Ganesh A, Heiman G, Burke R, Kemp FW, Bogden JD, Camakaris J, Birge RB, Konsolaki M. Control of Alzheimer's amyloid beta toxicity by the high molecular weight immunophilin FKBP52 and copper homeostasis in *Drosophila*. *PLoS One*. 2010; 5:e8626. [PubMed: 20084280]
- Sergeant N, Bretteville A, Hamdane M, Caillet-Boudin ML, Grognet P, Bombois S, Blum D, Delacourte A, Pasquier F, Vanmechelen E, Schraen-Maschke S, Buee L. Biochemistry of Tau in Alzheimer's disease and related neurological disorders. Expert review of proteomics. 2008; 5:207–224. [PubMed: 18466052]
- Shariati SA, De Strooper B. Redundancy and divergence in the amyloid precursor protein family. *FEBS letters*. 2013; 587:2036–2045. [PubMed: 23707420]
- Shulman JM, Feany MB. Genetic modifiers of tauopathy in *Drosophila*. *Genetics*. 2003; 165:1233–1242. [PubMed: 14668378]
- Slomnicki LP, Lesniak W. A putative role of the Amyloid Precursor Protein Intracellular Domain (AICD) in transcription. *Acta neurobiologiae experimentalis*. 2008; 68:219–228. [PubMed: 18511958]
- Sofola O, Kerr F, Rogers I, Killick R, Augustin H, Gandy C, Allen MJ, Hardy J, Lovestone S, Partridge L. Inhibition of GSK-3 ameliorates Abeta pathology in an adult-onset *Drosophila* model of Alzheimer's disease. *PLoS Genet*. 2010; 6:e1001087. [PubMed: 20824130]
- Sofola-Adesakin O, Castillo-Quan JI, Rallis C, Tain LS, Bjedov I, Rogers I, Li L, Martinez P, Khericha M, Cabecinha M, Bahler J, Partridge L. Lithium suppresses Abeta pathology by inhibiting translation in an adult *Drosophila* model of Alzheimer's disease. *Frontiers in aging neuroscience*. 2014; 6:190. [PubMed: 25126078]
- Soldano A, Okray Z, Janovska P, Tmejova K, Reynaud E, Claeys A, Yan J, Atak ZK, De Strooper B, Dura JM, Bryja V, Hassan BA. The *Drosophila* homologue of the amyloid precursor protein is a conserved modulator of Wnt PCP signaling. *PLoS Biol*. 2013; 11:e1001562. [PubMed: 23690751]
- Soto C, Sigurdsson EM, Morelli L, Kumar RA, Castano EM, Frangione B. Beta-sheet breaker peptides inhibit fibrillogenesis in a rat brain model of amyloidosis: implications for Alzheimer's therapy. *Nat Med*. 1998; 4:822–826. [PubMed: 9662374]
- Speretta E, Jahn TR, Tartaglia GG, Favrin G, Barros TP, Imarisio S, Lomas DA, Luheshi LM, Crowther DC, Dobson CM. Expression in *drosophila* of tandem amyloid beta peptides provides insights into links between aggregation and neurotoxicity. *J Biol Chem*. 2012; 287:20748–20754. [PubMed: 22461632]

- Stancu IC, Vasconcelos B, Terwel D, Dewachter I. Models of beta-amyloid induced Tau-pathology: the long and "folded" road to understand the mechanism. *Molecular neurodegeneration*. 2014; 9:51. [PubMed: 25407337]
- Steinhilb ML, Dias-Santagata D, Fulga TA, Felch DL, Feany MB. Tau phosphorylation sites work in concert to promote neurotoxicity in vivo. *Mol Biol Cell*. 2007a; 18:5060–5068. [PubMed: 17928404]
- Steinhilb ML, Dias-Santagata D, Mulkearns EE, Shulman JM, Biernat J, Mandelkow EM, Feany MB. S/P and T/P phosphorylation is critical for tau neurotoxicity in *Drosophila*. *J Neurosci Res*. 2007b; 85:1271–1278. [PubMed: 17335084]
- Stokin GB, Almenar-Queralt A, Gunawardena S, Rodrigues EM, Falzone T, Kim J, Lillo C, Mount SL, Roberts EA, McGowan E, Williams DS, Goldstein LS. Amyloid precursor protein-induced axonopathies are independent of amyloid-beta peptides. *Hum Mol Genet*. 2008; 17:3474–3486. [PubMed: 18694898]
- Tan L, Schedl P, Song HJ, Garza D, Konsolaki M. The Toll-->NFkappaB signaling pathway mediates the neuropathological effects of the human Alzheimer's Abeta42 polypeptide in *Drosophila*. *PLoS One*. 2008; 3:e3966. [PubMed: 19088848]
- Tare M, Modi RM, Nainaparampil JJ, Puli OR, Bedi S, Fernandez-Funez P, Kango-Singh M, Singh A. Activation of JNK signaling mediates amyloid-ss-dependent cell death. *PLoS One*. 2011; 6:e24361. [PubMed: 21949710]
- Thies W, Bleiler L, Alzheimer's A. 2013 Alzheimer's disease facts and figures. *Alzheimers Dement*. 2013; 9:208–245. [PubMed: 23507120]
- Torroja L, Chu H, Kotovsky I, White K. Neuronal overexpression of APPL, the *Drosophila* homologue of the amyloid precursor protein (APP), disrupts axonal transport. *Curr Biol*. 1999a; 9:489–492. [PubMed: 10322116]
- Torroja L, Luo L, White K. APPL, the *Drosophila* member of the APP-family, exhibits differential trafficking and processing in CNS neurons. *J Neurosci*. 1996; 16:4638–4650. [PubMed: 8764652]
- Torroja L, Packard M, Gorczyca M, White K, Budnik V. The *Drosophila* beta-amyloid precursor protein homolog promotes synapse differentiation at the neuromuscular junction. *J Neurosci*. 1999b; 19:7793–7803. [PubMed: 10479682]
- Tschape JA, Hammerschmied C, Muhlig-Versen M, Athenstaedt K, Daum G, Kretzschmar D. The neurodegeneration mutant lochrig interferes with cholesterol homeostasis and Appl processing. *Embo J*. 2002; 21:6367–6376. [PubMed: 12456644]
- Venken KJ, Kaspruwicz J, Kuenen S, Yan J, Hassan BA, Verstreken P. Recombineering-mediated tagging of *Drosophila* genomic constructs for in vivo localization and acute protein inactivation. *Nucleic Acids Res*. 2008; 36:e114. [PubMed: 18676454]
- Wacker J, Ronicke R, Westermann M, Wulff M, Reymann KG, Dobson CM, Horn U, Crowther DC, Luheshi LM, Fandrich M. Oligomer-targeting with a conformational antibody fragment promotes toxicity in Abeta-expressing flies. *Acta neuropathologica communications*. 2014; 2:43. [PubMed: 24725347]
- Wentzell JS, Bolkan BJ, Carmine-Simmen K, Swanson TL, Musashe DT, Kretzschmar D. Amyloid precursor proteins are protective in *Drosophila* models of progressive neurodegeneration. *Neurobiol Dis*. 2012; 46:78–87. [PubMed: 22266106]
- Wittmann CW, Wszolek MF, Shulman JM, Salvaterra PM, Lewis J, Hutton M, Feany MB. Tauopathy in *Drosophila*: neurodegeneration without neurofibrillary tangles. *Science*. 2001; 293:711–714. [PubMed: 11408621]
- Yu Z, Ren M, Wang Z, Zhang B, Rong YS, Jiao R, Gao G. Highly efficient genome modifications mediated by CRISPR/Cas9 in *Drosophila*. *Genetics*. 2013; 195:289–291. [PubMed: 23833182]
- Zhao XL, Wang WA, Tan JX, Huang JK, Zhang X, Zhang BZ, Wang YH, YangCheng HY, Zhu HL, Sun XJ, Huang FD. Expression of beta-amyloid induced age-dependent presynaptic and axonal changes in *Drosophila*. *J Neurosci*. 2010; 30:1512–1522. [PubMed: 20107079]

Highlights

- We summarize the molecular pathogenesis of Alzheimer's disease.
- We describe the relevance of *Drosophila* to understand the basis of human diseases.
- Many conserved functions of APP were identified in *Drosophila*.
- *Drosophila* expressing human A β 42 uncovered mechanisms of pathogenesis.
- *Drosophila* identified pathways regulating human tau aggregation and toxicity.

Figure 1. APP processing

APP is a single-pass transmembrane protein (purple) that is cleaved by three types of proteases. Cleavage by α -secretase (green) releases the secreted APP α (sAPP α) fragment. The C-terminal fragment α (CTF α) is then cleaved by the γ -secretase complex (black) to produce the amyloid intracellular domain (AICD). This is considered the non-amyloidogenic pathway because the activity of α -secretase breaks the A β fragment. Cleavage of APP by the β -secretase activity of BACE1 releases a smaller secreted fragment (sAPP β) and CTF β , which is a substrate for γ -secretase. This final cleavage releases AICD and A β fragments of different sizes, from 36 to 43 amino acids, with A β 42 being the pathogenic trigger.

Figure 2. APP/APPL functions uncovered in Drosophila

The main function for APP/APPL is related to the transport of specific vesicles along axons (green spheres). These vesicles transport critical synaptic proteins, including neurotransmitters, and return proteins for recycling or signaling. APP/APPL functionally interacts with the axonal trafficking machinery composed of the kinesin and dynein protein complexes. The direct interaction of APP/APPL with kinesin explains the sensitivity of axonal trafficking to APP/APPL expression levels. Additionally, APP/APPL interacts physically with tau, which links APP to the microtubules and suggests potential new mechanisms for AD pathogenesis. In addition to the role of APP in vesicular transport, APP seems to exert other critical functions at the synapse, including the regulation of signaling across the synaptic space that controls axonal pathfinding and the formation of synaptic boutons. The AICD also interacts with Tip60 to regulate transcriptional activity and axonal transport. As shown in figure 1, APP/APPL can be cleaved and produce several fragments, including the toxic A β 42 and the neuroprotective sAPP, which regulates the actin cytoskeleton and the activity of K⁺ channels.

Figure 3. Mechanisms of Aβ42 toxicity and aggregation described in Drosophila

Aβ42 monomers form soluble oligomers that can continue to aggregate into insoluble fibers. Metals slow down this last step, maintaining higher levels of toxic oligomers. Aβ42 have multiple deleterious activities in synapses, glia, and can penetrate in cells through both active and passive mechanisms. Aβ42 assemblies induce ER stress, activates JNK signaling leading to cell death, blocks clearance of autophagic vesicles, induces tau phosphorylation and blocks axonal transport, and results in the selective degradation of the Kv4 potassium channels. In synapses, Aβ42 oligomers reduce the number of mitochondria and synaptic vesicles, disrupt signaling, and alter neurotransmitter release, resulting in reduced synaptic activity. In glia, Aβ42 assemblies activate Toll receptors and launch anti-inflammatory responses. Some of the remedies against Aβ42 include proteases like Neprilysin and selective Aβ42-binding agents such as single chain antibodies and secreted chaperones.

Figure 4. Tau function and toxicity described in *Drosophila*

Tau binds microtubules and is critical for microtubule stability. Tau phosphorylation causes conformational changes and oligomerization that reduce binding to microtubules, resulting in microtubule destabilization and disruption of axonal trafficking. Tau contains numerous phosphorylation sites that are substrates for many kinases and phosphatases. Work in *Drosophila* suggests a hierarchical activity of kinases, with Par-1 (MARK) playing a priming role at Ser 262 and 356. This activity is critical for the activity of other kinases, in particular GSK3β, although the priming activity of Par-1 seems to be isoform-specific. Tau is also a substrate for CDK5, JNK, Chk2, and MAPK, although the hierarchy and functional interaction of these kinases are not fully known at this time. Chk2 is a negative regulator of the phosphatase String/Cdc25 and MAPK activates GSK3β, two examples of the hidden complexity of tau phosphorylation. Tau interacts with and causes the accumulation of F-Actin. Tau also induces ER stress and prevents the fusion of mitochondria. Finally, cathepsin and caspases cleave tau, causing the accumulation of a highly toxic truncated tau (t-tau). The chaperone NMNAT is one of the few proteostatic mechanisms capable of degrading tau aggregates.

Table 1**Drosophila A β 42 models**

Human APP	Features	Relevance	References
APP	WT	Full-length APP, 695 amino acid long	Merdes 2004 Fossgreen 1998
APP- CT	C-terminal deletion	C-terminal deletion	Fossgreen 1998 Gunawardena 2001
APP- NT	N-Terminal deletion	N-Terminal deletion	Fossgreen 1998
APP Swedish	K670N + M671L	Full-length APP with Swedish mutations	Fossgreen 1998
APP London	V717L	Full-length APP with London mutation	Gunawardena 2001
APP-N684A	N684A	N684A mutation in the NPTY domain	Merdes 2004
APP-Y682A	Y682A	Y682A mutation in the NPTY domain	Merdes 2004
APP- NPTY	NPTY deletion	Deletion of internalization sequence essential for ICD function	Merdes 2004
APP/C99	Lacks N-terminal extracellular domain	Mimics the BACE1 cleaved protein isoform of APP	Finelli 2004 Takasugi 2003
BACE	WT	Full length BACE	Carmine-Simmen 2009 Greeve 2004 Gross 2008
Drosophila APPL			
Appl	WT	Full-length Appl	Torroja 99
Appl-S	TM and CT	Secreted, neuroprotective	Merdes 2004 Carmine-simmen 2009
Appl- CT	CT	Secreted, neuroprotective	Gunawardena 2001
Appl- ECD 1	Deletion 85–231	Membrane-bound Appl deleted for the extracellular domain, including E1	Merdes 2004 Carmine-Simmen 2009
Appl- ECD 2	Deletion 449–740	Deletion of the extracellular domain, including most of E2.	Merdes 2004
Appl- ICD	Deletion ICD	Membrane-bound Appl deleted for ICD	Merdes 2004
Combinations			
dAppl + htau	Both WT	Stronger axonal trafficking phenotypes	Torroja 1999
hAPP/hAPLP2	Chimeric	Chimeric hAPP carrying A β 42 and CT from hAPLP2	Gunawardena 2001 Merdes 2004
hAPP and hBACE1	Both WT	A β 42 production and increased toxicity	Carmine-Simmen 2009 Greeve 2004 Chakravorty 2011

Table 2**Drosophila A β 42 models**

Transgene	Signal peptide	Insertion	Features	Reference
GMR-A β 40, -A β 42	Rat pre-proenkephalin	random	Weak eye phenotype	Iijima 2004
A β 40 [*] , A β 42 [*]	Rat pre-proenkephalin	random	A β 42 $\uparrow$ toxic A β 40 $\downarrow$ toxic	Finelly 2004
A β 42, -E22G, -L17P	Rat pre-proenkephalin	random	Different subcellular phenotypes	Iijima 2008
A β 40 [*] , A β 42 [*] , A β 42-E22G [*]	dNecrotic	random	A β 42-E22G $\uparrow$ toxic	Crowther 2005
A β 42-E22G, I3 1E A β 40-E3R	dNecrotic	random	A β 42-E22G non toxic A β 40-E3R toxic	Bronsson 2010
A β 40, A β 42, A β 42-E22G	dNecrotic	attP-51D	Locomotor dysfunction	Jahn 2011
A β 42 + A2F, R5Y, D7R, E11G, H14W, K16W, L17R, F20E, N27W, M35F	dNecrotic	random	Aggregation/toxicity	Luheshi 2007
A β 42-E22G + F4D, H6W, G9T, I3 1E	dNecrotic	random	Aggregation/toxicity	Luheshi 2007
A β 42-E22G	No SP	attP-51D	Cytoplasmic	Kruppa 2013
Tandem A β 40 or A β 42	dNecrotic	attP-51D	A β 42 with linker are toxic. A β 40 is not	Speretta 2012
A β Q+42	dNecrotic	attP-96E	Toxic	Allan 2014
A β 42	dToll	attP-96E	Not toxic	Allan 2014
dA β	No SP	random	Forms amyloids and toxic	Carmine 2009
A β 42-2x	dArgos	random	Two independent copies Highly toxic	Casas-Tinto 2011
A β 42	dHedgehog, No SP	Random		Hua 2011
A β 42, 3H>R	dHedgehog	attP-86F	Mutant metal binding site	Hua 2011

* Highly used models

Table 3**Drosophila Tau models**

Transgene	Features	Relevance	References
Tau, -R406W, -V337M	WT and FTDP-17 mutations	Tau neurotoxicity	Wittmann 2001
GMR-Tau 4R	WT expressed in eye	Tau neurotoxicity	Jackson 2002
Tau 3R, 4R	3 or 4 microtubule-binding repeats	Tau neurotoxicity	Mudher Williams 2000
Tau-S2A, -S202A	S262, S356, or S202 > A	Tau phosphorylation and neurotoxicity	Nishimura 2004
Tau-T/S>A	14 T/S individually > A	Tau phosphorylation and neurotoxicity	Steinhilb 2007a
Tau-AP, -E14	All 14 T/S > A or E	Tau phosphomimetics and neurotoxicity	Khurana 2006; Steinhilb 2007b
Tau-S2A, -S11A	S262 and S356 > A, 11 S/T > A	Tau phosphorylation and neurotoxicity	Chatterjee 2009
Tau-1-421	Truncated	Tau cleavage and neurotoxicity	Khurana 2010
Tau-K44Q/R-230Q	Calpain-resistant	Calpain-mediated Tau proteolysis	Reinecke 2011
Tau- ZB	Zinc-binding deficient	Zinc-mediated toxicity	Huang 2014
Tau- 1-44, 45-441, 45-230, 231-441, 1-421, 1-394, 395-441	Deletions from 2N4R	Tau proteolysis, myc-tagged	Geng 2015
dTau	Fly Tau	Memory deficits	Mershin 2004
bovTau-GFP	Tau reporter	Axonal tracing	Murray 1998
A β 42 + Tau	Tau 1N4R	A β 42, Tau interaction	Folwell 2010; Fulga 2007
A β 42 + Tau, Tau-S262A	Tau 0N4R WT and S262A	A β 42-dependent Tau phosphorylation	Iijima 2010

All constructs are human tau unless specified. Described in more detail in Gistelink 2012