

Drosophila Models of Proteinopathies: the Little Fly that Could

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Abstract: Alzheimer's, Parkinson's, and Huntington's disease are complex neurodegenerative conditions with high prevalence characterized by protein misfolding and deposition in the brain. Considerable progress has been made in the last two decades in identifying the genes and proteins responsible for several human 'proteinopathies'. A wide variety of wild type and mutant proteins associated with neurodegenerative conditions are structurally unstable, misfolded, and acquire conformations rich in β -sheets (β -state). These conformers form highly toxic self-assemblies that kill the neurons in stereotypical patterns. Unfortunately, the detailed understanding of the molecular and cellular perturbations caused by these proteins has not produced a single disease-modifying therapy. More than a decade ago, several groups demonstrated that human proteinopathies reproduce critical features of the disease in transgenic flies, including protein misfolding, aggregation, and neurotoxicity. These initial reports led to an explosion of research that has contributed to a better understanding of the molecular mechanisms regulating conformational dynamics and neurotoxic cascades. To remain relevant in this competitive environment, *Drosophila* models will need to expand their flexible, innovative, and multidisciplinary approaches to find new discoveries and translational applications.

Keywords: Drosophila models, neurodegeneration, protein misfolding, amyloids, Alzheimer, Parkinson, Huntington, Prion.

1. INTRODUCTION

A number of human neurodegenerative conditions are associated with abnormal protein deposition in brain neurons. This group of *protein misfolding* disorders or *proteinopathies* includes some of the most common diseases among the elderly (e.g., Alzheimer's [AD] and Parkinson's [PD] disease), several dominantly inherited diseases (e.g., Huntington's disease [HD], Spinocerebellar ataxias [SCA] 1 and 3), the aggressive amyotrophic lateral sclerosis (ALS) or Lou Gehrig's disease, and the rare and unique Prion diseases (PrD). These protein misfolding disorders belong to a larger class of neurodegenerative diseases characterized by late onset, progressive loss of brain neurons that includes dominantly inherited RNA diseases (e.g., myotonic dystrophy, SCA8 and 10) and recessive loss-of-function disorders (e.g., Fragile X syndrome, Friedreich's ataxia), among others. The proteinopathies constitute a heterogeneous group of brain disorders (and other systemic conditions that will not be reviewed here) that includes dominantly inherited disorders (AD, PD, HD, SCAs, ALS, PrD), sporadic (idiopathic) conditions (AD, PD, ALS, PrD) and infectious diseases (PrD). Some of the disorders have multiple etiologies, which contributed to the identification of the genes and molecular mechanisms that cause them. In the familial forms of the diseases, the mutant genes encode the proteins that aggregate in the sporadic forms of each disease, thus identifying the culprits in all forms of the disease. These proteins become structurally unstable upon mutation (Ataxin1 [Atx1], Cu/Zn superoxide dismutase 1), after abnormal proteolytic cleavage (Amyloid- β 42 [A β 42], Huntingtin [Htt]) or are naturally unstable in their normal state (Prion protein [PrP], α -Synuclein [α -Syn]). Regardless of the origin of the structural perturbations in these proteins, the conformational changes (misfolding) expose hydrophobic residues and increase the β -sheet content (β -state). The β -state is unstable as a monomer because of the need to hide the exposed hydrophobic residues, leading to self-assembly into small, soluble aggregates (oligomers). These soluble assemblies are highly toxic in cell culture models and are proposed to be the neurotoxic species [1, 2]. Over time, oligomers aggregate by a "seeding-nucleation" model [3] into larger, insoluble, fibrillar structures that are detected

by traditional histo-pathological techniques: amyloid plaques and neurofibrillary tangles in AD, Lewy bodies in PD, and nuclear inclusions in HD and SCAs. A testament to the complexity of these disorders is that two decades of tremendous advances in understanding the structural dynamics and biological properties of these proteins have provided no disease-modifying therapies.

Protein misfolding is mostly an intrinsic property of these pathogenic proteins: their amino acid sequences (either wild type or mutant) contain the information that makes them structurally unstable and capable of populating the β -state [3]. Given the intrinsic structural instability and neurotoxicity of these proteins, it is not surprising how easy it was to replicate those properties in transgenic flies and how popular these disease models have become in the last decade. Two breakthrough reports in 1998 paved the way by modeling two dominant conditions in flies, HD and SCA3 [4, 5]. Several more papers came shortly after from laboratories that worked independently on the same idea [6-9], and an explosion of papers followed in the next decade (Fig. 1). Here we review some of the most important contributions of *Drosophila* to unraveling the molecular mechanisms of neurodegeneration and discuss its future applications in gene and drug discovery efforts.

2. THE MIGHTY LITTLE FLY

Drosophila melanogaster is a small fruit fly with worldwide distribution that poses no threat to human health and agriculture. What made *Drosophila* attractive to T.H. Morgan in the early 1900s is that fruit flies have a short life cycle (10 days at 25°C) and produce a large progeny (upwards of 100 eggs per female). The pioneering work of Morgan and Sturtevant with these elegant, golden-colored flies produced the first mutation in 1906, *white*, which eliminated the pigment from the large eyes, and many more mutations followed in the ensuing decades. Many additional reasons make *Drosophila* the model of choice to tackle relevant biological problems: access to large collections of mutant strains, sophisticated genetic techniques, multiple approaches for manipulating gene expression, easy transgenesis, and compatibility with low- to mid-throughput screening [10]. Additionally, *Drosophila* has been a leader in genome sequencing and annotation [11].

Drosophila has been a favored tool for genetic studies for over 100 years and is an excellent model system to study a variety of biological processes, including development, signal transduction, cell biology, and immunity. Moreover, *Drosophila* has provided

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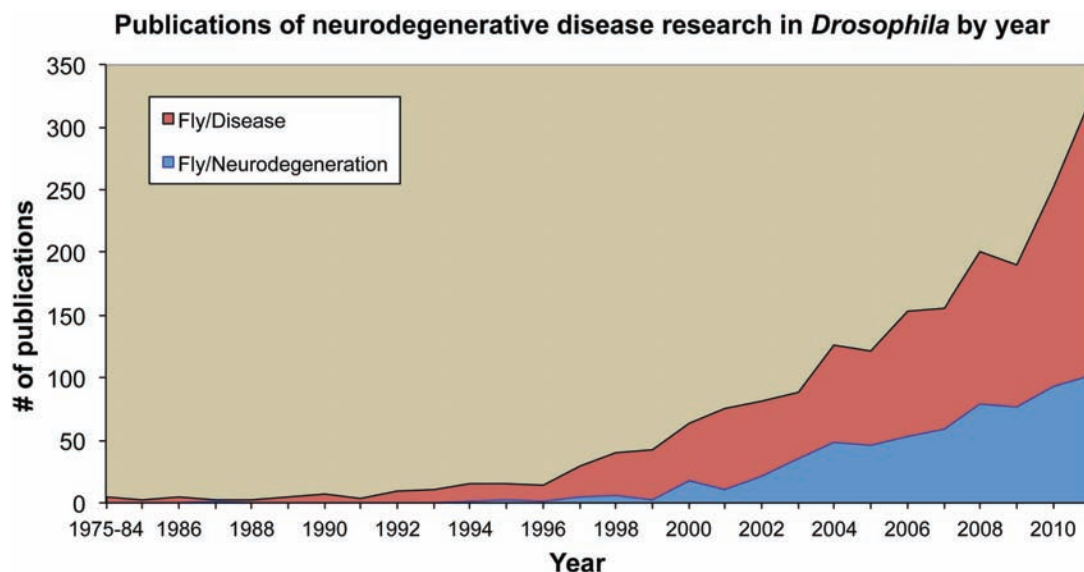


Fig. (1). The upward trend of disease model publications using flies. Publications displayed in PubMed with general *disease* application (red) and those relevant to *neurodegenerative diseases* (blue). The numbers for 2011 are projections based on year-to-date publications. Search parameters: Blue: (neurodegenerative OR neurodegeneration) AND drosophila; Red: (disease OR neurodegenerative OR neurodegeneration) AND drosophila. Search terms were limited to the title and abstract to eliminate false positives. This can lead to underestimation of the number of papers (false negatives), but a brief visual analysis confirmed the sensitivity of the search.

substantial contributions to fundamental questions in neurobiology: nervous system organization and function, information integration and processing, wiring and physiology of neural circuits, neurodegeneration, and the genetic control of behavior, sleep, memory, aggression, mating, and addiction [12]. A transformative moment in *Drosophila* research came with the realization of the extraordinary conservation of gene sequence/function with humans. In fact, some of the most important genes with key regulatory functions were first described in flies [see [12] for historical context], including Homeotic/HOX, decapentaplegic/TGF- β , Wnt/wingless, Hedgehog/Sonic hedgehog. Indeed, over 75% of the human genes implicated in disease display highly significant sequence conservation at the protein level with *Drosophila* genes [13]. This high degree of functional conservation suggests that diseases resulting from disruption of conserved cellular pathways should be easily recapitulated at genetic and molecular levels in fruit flies.

3. THE EVER-EXPANDING TOOLBOX

Drosophila still maintains a healthy edge over other animal models in the ability to manipulate gene expression and function. The genetic toolbox in *Drosophila* is unrivaled due to constant innovation and the selfless sharing of ideas and resources [10, 14]. This is a brief summary of the most relied upon genetic tools in flies.

Gene expression. Following the recombinant DNA revolution of the 80s, which introduced the technology for incorporating transgenes in flies (always first!) and other animals [15], the early 90s brought another game changer: the UAS/Gal4 expression system. The heterologous UAS/Gal4 system imported from yeast is a binary expression system: one strain carries a transgene under the control of the UAS (upstream activating sequence) promoter sequence, and a second strain expresses the transcription factor Gal4 [16]. Transgenes are cloned into a freely available vector (pUAST) downstream of the UAS, followed by microinjection of embryos (now outsourced to commercial services). To induce transgene expression, the UAS-transgene strains are combined with strains expressing Gal4 under the control of highly diverse promoters (from ubiquitous to single cell), offering unparalleled experimental flexibility (Table 1). The UAS/Gal4 system is a terrific tool for directing the spatial expression of transgenes; unfortunately, it pro-

vides poor temporal control. The introduction of the tripartite TARGET (temporal and regional gene expression targeting) system finally provided good temporal control to the UAS/Gal4 system, thus taking full advantage of the tens of thousand of existing UAS-regulated transgenes [17]. TARGET introduces a temperature-sensitive allele of the Gal4 inhibitor Gal80, which regulates gene expression by shifting from permissive temperature (19°C, Gal80^{TS} active, Gal4 inactive) to restrictive temperatures (30°C, Gal80^{TS} inactive, Gal4 active). TARGET has become a wonderful tool for manipulating critical processes in adult flies, particularly those associated with brain function, without affecting central nervous system (CNS) development.

Mutant strains. In addition to the classic mutations generated by chemicals and ionizing radiations, transposable elements (P-elements) have played a key role in the relentless push to mutate every gene in the fly genome (Table 1). P-element-mediated gene discovery has introduced transposable elements near or inside 65% of *Drosophila* genes, making them available for further modification, including excision, *in vivo* tagging, and enhancer capture for Gal4 expression [10, 14]. Also, modified P-elements carrying the UAS enhancer with a minimal promoter allows misexpression of nearby genes, which have proved very useful in genetic screens [18]. Several versions of this transposon have been randomly inserted throughout the *Drosophila* genome, resulting in tens of thousands of invaluable strains stored in various stock centers (Table 1). Moreover, the *Drosophila* field has fully incorporated the powerful RNA interference (RNAi) technology from *C. elegans* [19]. Researchers now have access to genome-wide collections of RNAi molecules (double stranded RNA) for cell culture assays plus three independent genome-wide collections of transgenic flies carrying RNAi constructs under the control of UAS (Table 1). P-element technology has finally allowed the creation of a genome-wide collection of molecularly defined deletions, an invaluable resource for gene mapping.

Latest technologies. The fly genome allows the most sophisticated manipulations of any animal model, from random transposon tagging to site-specific transgenesis. Latest efforts have focused on making gene targeting more efficient in *Drosophila* through the use of zinc-finger nucleases [20]. Also, recombination-mediated genetic

Table 1. *Drosophila* Resources

| Name | Website | Features | Content |
|---|--------------------------------------|--|--|
| Flybase | flybase.org | Genes, alleles, phenotypes, sequence, stocks, images, movies | 55,000+ gene records from 500+ <i>Drosophilids</i> |
| Bloomington <i>Drosophila</i> Stock Center | flystocks.bio.indiana.edu | Collect, maintain, and distribute <i>Drosophila</i> strains for research | 30,810 stocks, 196,930 lines distributed in 2010 |
| DrosDel | drosdel.org.uk | An isogenic deficiency kit for <i>Drosophila</i> | 15,166 total possible deletions |
| <i>Drosophila</i> Genetic Resource Center | dgrc.kit.ac.jp/en | Mutants, aberrations, balancers, insertions | 17,140 stocks |
| <i>Drosophila</i> Species Stock Center, San Diego | stockcenter.ucsd.edu | Diverse array of species | 250 species in 1499 stocks |
| <i>Drosophila</i> Genomics Resource Center | dgrc.cgb.indiana.edu | Cell lines, clones, and vectors | 135 cell lines, 1,000,000+ clones |
| <i>Drosophila</i> RNAi Screening Center | flymai.org | dsRNA for cell culture assays, coding and non-coding RNAs | 13,900 genes, dsRNA in 62 assay plates |
| Vienna <i>Drosophila</i> RNAi Center | stockcenter.vdrc.at/control/main | UAS-dsRNA in flies | 31,896 strains, 13,142 genes (93% coverage) |
| Berkeley <i>Drosophila</i> Genome Project | fruitfly.org | Sequence data, clones, stocks, libraries | 118.4 Mb genome assembly |
| MODEL organism ENCyclopedia Of DNA Elements (modENCODE) | modencode.org | Functional elements in <i>C. elegans</i> and <i>Drosophila</i> genomes | Transcriptome, regulatory elements |
| Textpresso for Fly | textpresso.org/fly | Information extracting and processing for fly literature | Literature on Fly: Title: 43186; Abstract: 36029; Total: 99179 |
| The Interactive Fly | sdbonline.org/fly/aimain/1aahome.htm | Guide to <i>Drosophila</i> development and metazoan evolution | Atlases, gene listings |
| <i>Drosophila</i> Interactions Database | droidb.org | Protein-protein, TF-gene, miRNA-gene, and genetic interactions | 400,031 interactions, 15,201 genes |
| <i>Drosophila</i> Protein Interaction Map | interfly.med.harvard.edu/index.php | Unbiased interaction map of the proteome based on MS analysis | 3,546 interactions |

engineering or recombineering, coupled with the bacteriophage ϕ C31 integrase, has provided a new platform for easy and speedy manipulation of DNA fragments larger than 130,000 bases into the fly genome [14]. Finally, recent methods for the automated mass-injection of fly embryos will result in an expanded ability to test and use sophisticated P-element vectors and RNAi [21, 22]. This area of *Drosophila* research is very hot and continues to provide revolutionary technology every year.

4. FLY MODELS OF NEURODEGENERATIVE DISORDERS

The innovative models of human neurodegenerative diseases that appeared between 1998 and 2000 inspired a new field of research and created a whole generation of researchers dedicated to understand human neurological diseases using *Drosophila*. This community has produced hundreds of research papers in the last few years and is projected to publish more than 100 papers in 2011 (Fig. 1). Current examples of *Drosophila* models of neurodegenerative disease include AD, PD, tauopathies, several polyglutamine disorders (HD, SCA1, SCA3 [a.k.a. Machado-Joseph disease], Spinobulbar muscular atrophy), ALS, PrD, dystonia, non-coding expansions (SCA8, myotonic dystrophy), and several recessive disorders, including Fragile-X syndrome and Friedreich's ataxia, among

others. These models have been reviewed recently in excellent papers and the reader should refer to them for more details [23, 24]. We next present a brief overview of the proteinopathies and how *Drosophila* has contributed to their understanding.

4.1. Alzheimer's disease and Frontotemporal dementias

AD is the most prevalent neurodegenerative disorder affecting 1% of those 65 years old or older and up to 35% of those older than 85. With the rising life expectancy in advanced economies, AD is acquiring the dimensions of an epidemic [25]. AD first manifests by short-term memory loss, progressing to loss of executive functions and full dementia over several years. Upon autopsy, the AD brain is characterized by degeneration of the cortex and hippocampus, and by two types of protein deposits: extracellular amyloid plaques rich in the A β 42 peptide and intracellular neurofibrillary tangles (NFT) containing the microtubule associated protein Tau. According to the "amyloid hypothesis", accumulation of the A β 42 peptide initiates the pathogenic cascade in AD, including Tau hyperphosphorylation, aberrant cellular signaling and, ultimately, cell death [26]. The A β 42 peptide is the result of β - and γ -secretase cleavage of the transmembrane Amyloid Precursor Protein (APP). Mutations associated with familial AD affect APP and two proteins with γ -

secretase activity, Presenilin 1 and 2, supporting the hypothesis that overproduction of A β 42 initiates AD pathogenesis. Only temporary symptomatic treatment for early AD is currently available, but the most promising upcoming therapies may be based on immunodepletion of specific conformers of A β oligomers [27].

The first AD-related studies in flies focused on APP and showed that overexpression of *Drosophila* APPL (APP-Like) and human APP (Table 2) cause axonal transport defects [28, 29]. This axonal dysfunction phenotype was also associated with other disease-related proteins (mutant Huntingtin), hinting at a common mechanism of neurodegeneration in different diseases [30]. Specific APP cleavage is key for accumulation of A β 42 and AD pathogenesis; interestingly, *Drosophila* possesses all the components necessary for both amyloid and non-amyloid processing of APP [31, 32]. The γ -secretase complex is essentially the same in flies and can cleave either APP or APPL at the γ -site in the transmembrane domain [31]. In addition, a *Drosophila* β -secretase-like enzyme (dBACE) cleaves APPL (but not APP) in the β -site, leading to the production of A β 42, formation of amyloid fibers and neurotoxicity [32]. The conservation of the amyloidogenic and non-amyloidogenic pathways in *Drosophila* created ideal conditions for further uncovering new genetic and pharmacologic regulators of APP processing. Although flies do not produce A β 42 naturally, overexpression of human APP and BACE combined with *Drosophila* Presenilin (Psn) results in A β 42 production and neurotoxicity [33]. Using these flies, a β -secretase inhibitory peptide demonstrated increased potency when anchored to the membrane by a sterol moiety [34]. Moreover, known inhibitors of human Presenilins rescue the toxicity of triple transgenic flies and induce Notch-related developmental defects, indicating that these drugs interact with and inhibit *Drosophila* Psn [33, 35]. These observations suggest that fruit flies can be used for *in vivo* validation and toxicity evaluation of novel γ -secretase inhibitors of high importance for the pharmaceutical industry. In addition to these pharmacological applications, *Drosophila* models of AD have been applied to the discovery of novel genetic regulators of APP processing. Ubiquilin was originally identified as a Presenilin-interacting protein with a potential contribution to early-onset AD [36, 37], but its functional interaction with other AD genes was ultimately demonstrated in transgenic flies. These studies demonstrated that Ubiquilin regulates Psn activity and uncovered its direct interaction with and regulation of APP [38–40]. Finally, recent genetic screens have identified new modifiers of Psn activity that also interact genetically with APP [41, 42]. These observations suggest an unexpected level of complexity in the regulation of APP processing, expanding the number of potential targets for AD therapeutics.

An alternative approach to the production of A β 42 through the amyloidogenic pathway is to directly express A β 42 fused to a signal peptide for secretion in flies [43–46]. A β 42 induces strong phenotypes in several assays, including memory and learning deficits, making this model very attractive for uncovering the genetic mechanisms of A β 42 neurotoxicity, and for testing drugs with protective activity. Given the relative ease to generate transgenic animals, different forms of the Amyloid- β peptide, including A β 40, A β 42, A β 42 with the familial AD Arctic mutation, and A β 42 with artificial mutations, have been compared *in vivo*. Overall, these studies confirmed experimentally that A β 42 aggregation propensity correlates with neurotoxicity; thus, strategies that prevent A β 42 aggregation should reduce toxicity [43, 47, 48]. For instance, feeding flies with Congo Red, a dye that binds to amyloids and prevent A β 42 fibrillization *in vitro*, prevents neurotoxicity [45]. In addition, compounds that stabilize the α -helical conformation of A β 13–26 also rescue A β 42 toxicity in flies [49]. These strategies make *Drosophila* a key tool for *in vivo* testing of promising AD compounds. For more details, comprehensive reviews on AD models in flies are available in the recent literature [50, 51].

Frontotemporal dementias (FTD) refer to a complex group of disorders that present with radical personality changes with involvement of either the frontal or the temporal lobes. FTP are mostly sporadic, but one of its forms, frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), is associated with mutations in Tau. This connection explains the role of Tau accumulation in other forms of FTD, which collectively receive the name of tauopathies. The first fly model of tauopathy was based on expression of wild type Tau and mutations linked to FTDP-17 (Table 2) [52]. Human wild type and mutant Tau disrupt the eye, induce locomotor dysfunction and shorten lifespan. Similarly, expression of bovine Tau fused to green fluorescent protein (GFP) caused axonal degeneration, while fly Tau disrupted the eye [53, 54], supporting the key role of Tau in neuronal homeostasis. Additionally, flies contributed to the identification of Actin cytoskeleton as modifiers of Tau-induced eye phenotype [55, 56]. The neurotoxicity of Tau-R406W is associated with accumulation of Actin filaments, which is dependent on Tau phosphorylation [57]. Another pathway that received attention in Tau neurotoxicity was cell cycle regulation. Tau-R406W aberrantly induces activation of cell cycle proteins through activation of TOR (target of rapamycin), which, in turn, mediates Tau neurotoxicity [58]. Finally, several studies on Tau have identified the kinases that phosphorylate Ser and Thr residues, and the consequence of phosphorylation on Tau conformation and aggregations. This is discussed in more detail in section 8.

4.2. Parkinson's disease

Parkinson's disease is the most prevalent movement disorder, affecting four to six million patients worldwide. PD patients suffer from tremors, rigidity, bradykinesia (slow movements), and difficulty in balance, symptoms associated with the loss of dopaminergic neurons in the substantia nigra and locus ceruleus of the brain [59]. The affected neurons are characterized by cytoplasmic protein aggregates known as Lewy bodies and Lewy neurites, which are enriched with α -Syn. However, other proteins such as ubiquitin and heat shock chaperones are also present, suggesting that these neurons may suffer from abnormal protein quality control mechanisms [59, 60]. Although most PD cases are sporadic, genetic defects in rare familial cases have provided valuable insights into the pathogenesis of PD. Notably, three point mutations in α -Syn (A30P, E46K and A53T) are associated with autosomal dominant forms of PD [60]. Allele multiplication of *SNCA*, the gene encoding α -Syn, also cause familial PD, suggesting that excess of wild type α -Syn can lead to disease [60]. Inherited forms of PD have been associated with at least 11 other loci, including Parkin, DJ-1, PINK1, and LRRK2/Dardarin (reviewed in [61]). Due to space limitations, we will focus on α -Syn proteinopathy in flies. For specialized reviews on fly models involving other familial PD genes see [62, 63].

α -Syn is a soluble synaptic protein that has been recently proposed to exist natively as a helical tetramer (Bartels *et al.*, Nature, 2011). α -Syn aggregates easily *in vitro* and its oligomers are very toxic to cultured neurons. However, α -Syn misexpression does not induce neurotoxicity in transgenic mice, leaving a big question about the role of α -Syn in the neurodegenerative cascade of PD. When Feany and Bender showed that normal and mutant forms of α -Syn (Table 3) induce selective degeneration of dopaminergic neurons, the paper was warmly welcomed [9]. These flies also showed locomotor dysfunction as well as α -Syn-rich cytoplasmic inclusions reminiscent of Lewy bodies, recapitulating essential features of PD. However, other models developed independently showed reduced α -Syn neurotoxicity: Bonini and colleagues reported a 50% reduction in dopaminergic cells [64], while other authors reported even lower penetrance [65]. Differences in the experimental approaches seem to explain these discrepancies [62, 63]. New methodologies, including semi-automated quantification of dopaminergic neurons and increased α -Syn expression through optimized translational efficiency, recently confirmed α -Syn toxic-

Table 2. AD/Dementia Models in Flies

| Model | Transgene | Features | Relevance | References |
|----------------|---|--|--|------------|
| APP | dAPPL, hAPP | Full-length APP | Neural differentiation and axon function | [28, 29] |
| | APP-swe APP Δ CT, APP Δ NT | APP KM670/671NL, deletion constructs | PNS development | [135, 136] |
| | Nicastrin, Aph-11, dPsn, hPS2, APP-C99 | In vitro, S2 cells | Description of γ -secretase complex | [31] |
| | dPsn-WT, N141I, L235P, E280A | Fly γ -secretase activity | APP processing, A β 42 toxicity | [33] |
| | dPsn+fAD | dPsn with 14 mutations found in familial AD | γ -secretase activity | [137] |
| | dUbqIn, hUBQLN1, hUBQ-8i | Fly and two isoforms of human UbqIn | Regulation of γ -secretase activity | [38-40] |
| | APP-Gal4, APP-C99-Gal4 | γ -secretase activity sensor | Genetic screen of APP-processing factors | [40, 138] |
| | APP, ADAM10/kuz, dBACE | Fly α -secretase, β -secretase activities | APP processing | [32] |
| | APP-C99 | C-terminal 99 a.a. | APP processing, A β 42 neurotox. | [44] |
| A β | A β 40, A β 42 | Toxicity of A β 40 vs. A β 42 | A β 42 neurotox. | [44] |
| | A β 42, E22G (arctic), L17P (synthetic) | Mutant A β 42 | A β 42 neurotox. | [45, 48] |
| | Pyroglutamate-A β 42 | Posttranslational modification | A β 42 aggregation | [139] |
| | A β 42x2 | Highly expressed | A β 42 neurotox. | [46] |
| Tau | bovTau-GFP | Tau reporter | Axonal tracing | [140] |
| | hTau, -R406W, -V337M | WT Tau and FTDP-17 mutations | Tau neurotox. | [52] |
| | hTau 4R | WT Tau expressed in eye | Tau neurotox. | [93] |
| | dTau | Fly Tau | Memory deficits | [141] |
| | hTau 3R, 4R | Tau with 3/4 microtubule-binding repeats | Tau neurotox. | [53, 142] |
| | hTau-R406W+S2A, S202A | Mutant Tau + S262, 356 or S202 > A | Tau phosphorylation and neurotox. | [104] |
| | hTau-T/S>A | 14 T/S individually > A | Tau phosphorylation and neurotox. | [105] |
| | hTau-AP, -E14 | All 14 T/S > A or E | Tau phosphorylation and neurotox. | [58, 106] |
| | hTau-S2A, -S11A | S at 262 and 356 > A, 11 S/T > A | Tau phosphorylation and neurotox. | [107] |
| Tau, A β | A β , Tau | Tau N4R, A β 42 | A β 42, Tau interaction | [57, 143] |

ity in transgenic flies [66, 67]. Additionally, structure-function studies identified α -Syn regions that play a role in aggregation and neurotoxicity in flies [68], while α -Syn variants generated by rational design demonstrated that mutants with impaired β -state structure (surprisingly) exhibit higher neurotoxicity in flies [69]. Based on these observations, *Drosophila* seems to be the best *in vivo* model of α -Syn neurotoxicity available, making it a powerful tool for gene and target discovery.

4.3. Huntington's Disease and other polyQ Disorders

Polyglutamine (polyQ) diseases are devastating and incurable neurodegenerative conditions with autosomal dominant inheritance

and worldwide distribution. At least nine polyQ disorders have been described, including HD, spinobulbar muscular atrophy (SBMA), dentatorubral-pallidolulsian atrophy (DRPLA) and six Spinocerebellar ataxias (SCA1, 2, 3, 6, 7 and 17) [70]. PolyQ disorders are linked to the expansion of a glutamine-coding CAG repeat within the open reading frame of nine unrelated genes. The polyQ expansion perturbs protein stability leading to misfolding, aggregation, inclusion formation and extensive neurodegeneration. Although these nine polyQ proteins are broadly expressed in the CNS, neurodegeneration occurs in selective regions of the brain, resulting in clinically distinct neuropathologies. In all these disorders, the mechanisms of neuronal cell death are largely unknown

Table 3. PD and other Proteinopathy Models in Flies

| Model | Transgene | Features | Relevance | References |
|-------|--|--------------------------------------|------------------------------|------------|
| PD | α -Syn-WT, A30P, A53T | fPD | α -Syn neurotox. | [9, 64] |
| | α -Syn-WT | Comparative studies of α -Syn | Unravels technical issues | [65] |
| | α -Syn-S129A, S129D, YF | S/Y Phosphorylation mutations | Aggregation and neurotox. | [110, 111] |
| | α -Syn Δ 71-82, 1-120, 1-87 | Deletion constructs | Aggregation studies | [68] |
| | α -Syn-WT | Highly expressed | α -Syn neurotox. | [67] |
| | α -Syn-WT, A53T, A56P | ϕ C31 integrase constructs | Aggregation studies | [69] |
| PrD | MoPrP-WT, P101L | GSS | PrP misfolding and neurotox. | [86, 87] |
| | HaPrP-WT | Sporadic PrD | PrP misfolding and toxicity | [89] |
| | RaPrP-WT | Resistant PrP | PrP misfolding and neurotox. | [90] |
| ALS | SOD1-WT, G41S | fALS | Longevity studies | [79, 80] |
| | SOD1-WT, AV4, G37R, G41D, G93C, I113T | fALS <i>dSod</i> promoter | Longevity studies | [81] |
| | SOD1-WT, A4V and G85R | fALS UAS promoter | Neuronal dysfunction | [82] |

and probably involve a variety of gain-of-function activities. To help understand these issues, several *Drosophila* models of polyQ disorders have been generated (Table 4). For a more comprehensive review of these models, specialized reviews are available [63, 71].

Huntington's disease. HD is the most common polyQ disorder affecting one in 10,000 individuals, and is characterized by involuntary movements (chorea), psychiatric disturbances, dementia, and premature death [72]. HD is caused by a CAG expansion (>36) within exon 1 of the *Huntingtin* (*Htt*) gene. The first *Drosophila* model that recapitulated relevant features of HD pathogenesis was reported in 1998 [5]. These flies expressed an N-terminal fragment of human *Htt* with variable lengths of polyQ tracts under the control of an eye-specific promoter, which led to nuclear inclusions and age- and polyQ length-dependent degeneration of photoreceptor neurons. Since manipulation of these *Htt* transgenes was restricted to the fly eye, a subsequent model expressing a mutant N-terminal fragment (*Htt-ex1*) capitalized on the flexibility of the UAS/Gal4 system [73]. Over the last decade, additional models that express different N-terminal fragments or full-length *Huntingtin* under control of the UAS sequence have appeared (Table 4). Overall, these models have made important contributions to understanding the molecular pathology of the disease: *Htt* aggregates sequester other expanded polyQ proteins in the cytoplasm, leading to disruption of axonal transport [30, 74], while SUMOylation and ubiquitination of the expanded *Httex1p* regulate its neurotoxicity [75]. A new disease mechanism was revealed with the full-length *Htt* fly model, consisting in Ca^{2+} -dependent increase in neurotransmitter release efficiency, which occurs even before expanded *Htt* is imported into the nucleus [76]. Several other contributions of HD models in gene and drug discovery are discussed below.

Spinocerebellar ataxias (SCAs). The six known polyQ-related SCAs (1, 2, 3, 6, 7 and 17) are the most common cause of dominantly inherited ataxia, accounting for over 50% of ataxia patients worldwide [70]. The clinical features of ataxias reflect damage to the cerebellum; however, many SCAs are characterized by their extracerebellar brain involvement [70]. SCA1 and SCA3 fly models

were among the first reported [4, 6]. What made them particularly attractive despite being rare disorders is that (a) SCAs exhibit a purely genetic inheritance pattern and (b) the full-length protein (rather than a proteolytic fragment) is involved in pathogenesis, which facilitated construct design. SCA1 and SCA3 have played a key role in the discovery of disease relevant mechanisms that are described below.

PolyQ-only models. Fly models that express pure polyQ repeats revealed that expanded polyQ tracts alone are intrinsically cytotoxic, form aggregates, and lead to neurodegeneration and premature death [7, 8]. Interestingly, polyQ toxicity was neutralized by protein context [7]. This model was also used to identify genetic modifiers of polyQ neurotoxicity [8]; however, given the influence of the protein context, most laboratories prefer to use disease-related polyQ-containing proteins.

4.4. Amyotrophic Lateral Sclerosis

ALS is an incurable, devastating neurodegenerative disorder with a rapid disease course. ALS affects 5 out of every 100,000 people worldwide and is characterized by the selective death of upper and lower motor neurons, causing progressive muscle atrophy, paralysis, and death within one year of diagnosis. Approximately 5-10% of ALS cases are familial (fALS), and 20% of these patients carry mutations in the gene encoding Cu/Zn superoxide dismutase 1 (SOD1), a ubiquitous cytosolic enzyme that protects against toxic superoxide radicals. Out the 153 amino acids of SOD1, more than 125 distinct mutations have been linked to fALS [77]. The mechanisms involved in motor neuron death are largely unknown; current hypotheses for ALS pathogenesis include oxidative damage, accumulation of intracellular SOD1-positive aggregates, mitochondrial dysfunction, axonal transport defects, and astroglial cell pathology, to name a few [78].

Modeling SOD1-dependent pathology in flies has mirrored some of the difficulties experienced by mouse laboratories. For instance, overexpression of wild type hSOD1 in motor neurons

Table 4. Polyglutamine Disease Models in Flies

| Model | Transgene | Features | Relevance | References |
|-------|--------------------------------------|-------------------------------------|------------------------------------|------------|
| HD | hHtt-ex1-2Q, -75Q, -120Q | Expressed in the eye | Htt neurotox. | [5] |
| | hHtt-ex1-20Q, -93Q | First 90 a.a. | Htt neurotox. | [73] |
| | hHtt-1-171-18Q, -138Q | First 171 a.a. | Htt neurotox. | [144] |
| | hHtt-1-336-16Q, -128Q | First 336 a.a. | Htt neurotox. | [113] |
| | hHtt-1-548-0Q, -128Q | First 548 a.a. | Htt neurotox. | [74] |
| | hHtt-ex1-18Q, -48Q, -152Q | EGFP fusions | Htt aggregation and neurotox. | [120] |
| | hHtt-ex1-25Q, -46Q, -72Q, -103Q | EGFP fusions | Htt aggregation and neurotox. | [121] |
| | hHtt ^{FL} -16Q, -128Q | Full-length | Htt neurotox.and neurobiology | [76] |
| SCA1 | hSCA1-2Q, -30Q, -82Q | Full-length | Atx1 neurotox.and neurobiology | [6] |
| SCA2 | hSCA2 and dSCA2 | Full-length and deletion constructs | Atx2 function | [145] |
| SCA3 | hSCA3-27Q, -78Q | C-terminal fragment | Atx3 neurotox. | [4] |
| | hSCA3-65Q(NLS), hSCA-77Q(NES) | C-terminal fragment | Atx3 neurotox.and axonal transport | [30] |
| | hSCA3-79Q | C-terminal fragment | Atx3 neurotox. | [146] |
| | hSCA3 ^{FL} -27Q, -78Q, -84Q | Full-length | Atx3 neurotox. | [147] |
| SCA7 | hSCA7-10Q, -102Q | First 232 a.a. | Atx7 neurotox. | [148] |
| DRPL | hAtrophin1-26Q, -65Q | First 917 a.a. | At1 neurotox. | [149] |
| SBMA | hAndrogen Receptor-112Q | N-terminal fragment | AR neurotox. | [150] |
| | hAndrogen Receptor-52Q | Full-length | AR neurotox. | [151] |
| PolyQ | 20Q, 127Q | HA tagged | polyQ neurotox. | [8] |
| | 22Q, 108Q | Myc/Flag tagged | polyQ neurotox. | [7] |

dramatically extended lifespan, whereas expression of the fALS mutant hSOD1-G41S was not detrimental (Table 3) [79, 80]. Moreover, expression of human wild type and mutant SOD1 under the control of endogenous *dSod* regulatory sequences failed to induce neuronal dysfunction in *dSod* null flies [81]. These results are consistent with the lack of motor neuron degeneration upon neuron-specific expression of mutant SOD1 in transgenic mice. Since SOD1 is expressed ubiquitously in the CNS, other cell types such as astrocytes may play a role in neuronal degeneration (non-cell-autonomous toxicity), explaining the difficulties in modeling SOD1 neurotoxicity in animal models [77]. More recently, another fly model of ALS showed that wild type and mutant hSOD1, but not dSOD1, induced locomotor dysfunction with abnormal synaptic transmission and aggregation of hSOD1, but no apparent neuronal loss [82]. Other fly models for non-SOD1 fALS, including those expressing TAR DNA-binding protein 43 (TDP43) and vesicle-associated membrane protein B (VAMPB), are extensively reviewed elsewhere [63].

4.5. Prion Diseases

Prion diseases (PrD) are unique among the protein misfolding disorders because they can present with sporadic, genetic, and infectious etiologies. These neurodegenerative disorders affect humans and other mammals and lead to dementia, motor dysfunction, and, eventually, death [83]. The Prion protein (PrP) is a membrane-anchored glycoprotein highly enriched in the brain that has an es-

sential role in the pathogenesis of PrD [84]. PrP undergoes irreversible conformational changes from the cellular, α -helix-rich isoform (PrP^C) to pathological, β -sheet-rich isoforms (PrP^{Sc}) that are partially resistant to protease degradation. It is well accepted that the PrP^C to PrP^{Sc} conversion is a key event leading to aggressive spongiform neurodegeneration and death. Unfortunately, major gaps exist in our understanding of how the conformational conversion of PrP occurs and how it ultimately kills neurons.

The development of *Drosophila* models of PrD has been particularly challenging (Table 3). The first two attempts to induce PrP-dependent neuropathology resulted in no obvious degenerative effects (reviewed in [85]). These models seemed to accumulate low levels of PrP and led the authors to conclude that flies were inadequate for studying PrP biology. Later on, flies expressing a different PrP mutant associated with a genetic PrD were found to induce brain degeneration associated with PrP aggregation [86], and altered synaptic architectures in larval neuromuscular junctions [87]. However, aged flies did not accumulate detergent-insoluble or protease-resistant PrP conformers, thus missing two hallmark features of pathogenic PrP.

In sporadic Creutzfeldt-Jakob disease (CJD), which accounts for 85% of all CJD cases in humans (by far the most common PrD), spontaneous misfolding of wild type PrP is responsible for disease [88]. To gain insights into the mechanisms involved in the spontaneous misfolding of PrP *in vivo*, we created flies expressing wild type PrP from hamster (HaPrP). The hamster is an excellent model

for PrD because it undergoes aggressive disease progression with high amounts of pathogenic PrP conformers, suggesting that HaPrP is highly prone to populate the β -state. In young flies, HaPrP exhibits properties of native PrP^C; in aged flies, HaPrP acquires PrP^{Sc}-like properties, such as resistance to denaturing agents and immunoreaction with PrP^{Sc}-specific conformational antibodies, although it is not resistant to proteases. The accumulation of these PrP^{Sc}-like conformers correlate with severe spongiform degeneration in brain neurons, indicating that the prototypical, infectious PrP^{Sc} conformer is not required to induce spongiform degeneration [89]. To further understand how the primary amino acid sequence of PrP determines its structural dynamics, we created transgenic flies expressing wild type PrP from rabbit (RaPrP), an atypical mammal that is resistant to prions [90]. RaPrP does not accumulate in PrP^{Sc}-like conformations and is not neurotoxic, suggesting that protective amino acid substitutions prevent the population of the β -state [90]. A recent paper identified a RaPrP-specific hydrophobic staple that links a loop to helix 3 and increases the conformational stability of the globular domain [91, 92]. However, *in vivo* evidence for the protective activity of this and other residues is still missing, creating an ideal niche for *Drosophila* research. This type of function/structure analyses will help to better understand the rules governing PrP misfolding and pathogenesis.

5. MODELING PROTEINOPATHIES IN FLIES: MAKING EYE CONTACT

The pathogenesis of the proteinopathies involves gain-of-function mechanisms by either wild type or mutant forms of the human protein. Therefore, misexpression of the disease-related proteins in flies should be highly toxic to brain neurons, but also (potentially) to other tissues and cell types. Following this rationale, several laboratories expressed these proteins in different tissues of the peripheral nervous system, including the eye and the sensory bristles. The compound insect eye has a highly organized lattice that is very sensitive to genetic disruption of various biological processes, can be easily analyzed under a standard stereoscope, and is non-essential for viability. Expression of some disease-related proteins in the eye leads to disorganization of the eye lattice or rough eye phenotypes, while other proteins do not affect the eye (Fig. 2). These phenotypes have been very useful as the basis for large scale, unbiased genetic screens and for testing candidate genes (see section 8). Critics may argue that the rough eye does not adequately reproduce the neurodegeneration of brain neurons in the human disorders; thus, the mechanisms associated with protein misfolding toxicity in the eye would be irrelevant to understanding the disease. We can deflect these critiques with several arguments. (1) While some disease-related proteins induce a rough eye (Atx1-82Q, Atx3-78Q, A β 42, wild type Tau), others do not (Htt-93Q, α -Syn, PrP, SOD1) (Fig. 2). Neither gene overexpression nor cellular overload explains photoreceptor toxicity; therefore, *specific pathways* must be perturbed to disrupt the eye. (2) Another important observation is that when these disease-related proteins affect the eye, they induce very different phenotypes: disorganized lattice, glassy surface, fused ommatidia, lost pigment, or reduced size (Fig. 2, B,C,D, G and H). Thus, each protein interferes with different pathways/networks in the eye that explain the specific eye perturbations. (3) Interestingly, A β and Tau show similar rough eye phenotypes, with reduced size and fusion of ommatidia (Fig. 2, G and H), suggesting that these two AD genes affect similar pathways/networks during eye development. (4) Although mutant Htt does not induce rough eye like mutant Atx1 and Atx3, mutant Htt induces progressive degeneration of the underlying retina. This observation supports the relevance of the protein context in polyQ diseases. Finally, (5) the rough eye induced by A β 42 is the same whether A β 42 is expressed in photoreceptors (neuron only) or in all eye cell types, suggesting that the rough eye is a consequence of A β 42 toxicity on photoreceptor neurons, not on the neighboring support cells [46]. Regardless of the advantages of using the rough

eye for rapid screening purposes, it is advisable to develop assays that support neurotoxicity to central neurons for secondary screens or validation purposes.

Another common approach in fly models of proteinopathies is to use locomotor activity to assess neuronal dysfunction. In fact, all the models described here show progressive locomotor dysfunction, including the wild type alleles of α -Syn, hSOD1, and hamster and mouse PrP. The typical locomotor test measures the ability of flies to climb upward following their innate negative geotaxis, a simple assay easy to set up in the lab, although complex software packages can also analyze more comprehensive behavioral tasks. These locomotor tests are highly relevant to the pathology of movement disorders such as PD, ALS and HD, but they have also been used in models of dementias (AD, PrD). How is this locomotor assay relevant to understand the molecular mechanisms of A β 42 neurotoxicity, a peptide that targets primarily the hippocampus and the cortex? Although A β 42 induces progressive memory deficits in flies [43], these assays are complex and time consuming. Thus, we use locomotor dysfunction as surrogate for *neuronal dysfunction*. Locomotor dysfunction informs us about the ability of any protein to interfere with basic neuronal processes, including cell survival/ apoptosis, oxidative stress and mitochondrial metabolism, cytoskeleton and axonal transport, synaptic architecture and transmission, etc. These cellular activities are essentially the same in all neurons; thus, a locomotor phenotype is an easy indicator of a serious neuronal pathology. In addition to being easy, locomotor assays provide complex information about the underlying cellular pathology. Curiously, A β 42 and Htt induce distinct locomotor phenotypes: whereas flies expressing A β 42 are unremarkably slow, the HD flies exhibit highly uncoordinated movements and shaking, a phenotype reminiscent of the chorea of HD patients. Coincidence? Unlikely. In fact, this locomotor dysfunction indicates that flies undergo specific neurodegenerative changes related to HD pathology, making them an excellent system to understand the molecular mechanisms underlying HD neurodegeneration. The experimental flexibility provided by the locomotor assays allows for fast and efficient characterization of new disease models and of potential genetic and pharmacologic modifiers of the pathology. Many other assays are available for characterizing protein neurotoxicity, including memory and learning, longevity/survival, and analyses of specific neuronal loss such as dopaminergic neurons in PD models. These assays have different applications based on their technical complexity: gene discovery efforts require easy-to-score phenotypes (rough eye), while testing protective genes or drugs demand higher specificity (memory tests, survival of dopaminergic neurons). In any case, the availability of multiple assays is a testament to the flexibility of *Drosophila*.

6. PROTEIN AGGREGATION AND THE NEUROTOXIC PARTICLE

Another key feature of these proteins is their ability to form disease-specific assemblies with characteristic cellular distribution. So far, *Drosophila* models have been very good at reproducing disease-specific protein dynamics: mutant Atx1, Atx3, and Htt accumulate in nuclear inclusions (NI) [4-6], whereas both wild type and mutant α -Syn accumulate in Lewy body-like cytoplasmic aggregates [9]. In addition, wild type PrP accumulates in detergent-soluble aggregates in lipid rafts, although some of it is retained in the secretory pathway as a consequence of early misfolding [89]. Overall, all of these proteins accumulate and aggregate in the right subcellular compartment and lead to neurotoxicity, making these models powerful tools for understanding *in vivo* protein dynamics and neurotoxicity. However, interesting observations in flies have provided relevant cues about the role of protein aggregates in neurotoxicity. Although mutant Atx1, Atx3 and Htt-ex1 form NI, these large aggregates may have a protective role as suggested by the increase in aggregate size by co-expression of chaperones and Atx1 [6]. Moreover, the HD-FL model exhibits signs of neurotoxicity

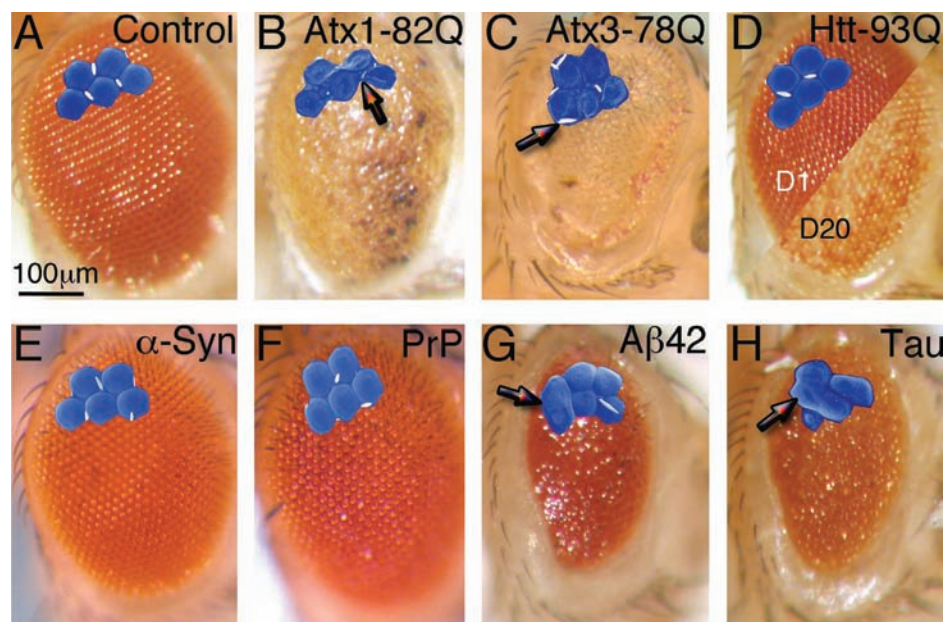


Fig. (2). Disruption of the fly eye by expression of amyloidogenic proteins. (A–H) Microphotographs of the *Drosophila* compound eye. Higher magnifications show a detail from scanning electron microphotographs (artificially colored). (A) Control eye showing normal pigmentation and organization of the ommatidia. (B) Flies expressing Atx1-82Q show highly disorganized (glassy), depigmented eyes, with necrotic spots. High magnification shows poorly differentiated ommatidia with small bristles (arrow). (C) Flies expressing Atx3-78Q show disorganized and depigmented eyes with normal bristles. (D) Flies expressing Htt-93Q have normal eye structure at day 1 (left), but the underlying retina degenerates by day 20, resulting in patchy depigmentation (right). (E and F) Flies expressing 3 copies of wild type α -Syn (E) or wild type hamster PrP (F) have normal eye structure and pigmentation. (G and H) Flies expressing A β 42 (G) or wild type Tau (H) have small, disorganized, bumpy eyes resulting from fusion of ommatidia (arrows).

without obvious NI, supporting the role of other conformers in neurotoxicity [76]. Also, wild type and mutant Tau accumulate in neurites [52, 93], but flies do not accumulate NFT, the typical AD and FTD aggregates containing hyperphosphorylated Tau [52]. This observation suggests that Tau-dependent neurodegeneration does not require NFT and supports the role of soluble assemblies in toxicity. Similarly, flies expressing HaPrP display extensive vacuolar degeneration without accumulation of the protease-resistant PrP^{Sc} isoform, suggesting that different PrP isoforms are responsible for infection and neurotoxicity [89, 94]. In flies, A β 42 aggregates in both extra- and intracellular amyloid aggregates [43], which have also been observed in AD patients [95], despite having strong signal peptides, suggesting that A β 42 is internalized after secretion. Moreover, the intrinsic aggregation propensities of 17 artificial mutations in A β 42 (chosen from 798 variants tested *in silico*) exhibited a strong correlation with their neurotoxicity in flies [47]. The exception was a double mutant with the highest predicted aggregation propensity (E22G, I31E). It turns out that this double mutant rapidly formed fibrillar aggregates, a state less toxic than soluble oligomers and protofibrils. This study demonstrated that A β 42 neurotoxicity is tightly regulated by its fibrillation, and that both too little and too much aggregation could reduce A β 42 neurotoxicity. Overall, *Drosophila* models of proteinopathies have provided important clues for uncovering the connection between protein aggregation and neurodegeneration.

7. LIMITATIONS: THE ELEPHANT-SIZE FLY IN THE ROOM

Disclaimer: Fly models of human diseases are just that, models. The anatomy and physiology of invertebrates are significantly different from those of humans, which impose constraints on translatable research. In the case of neurodegenerative diseases, an obvious limitation of fly models is the reproduction of physiological disease conditions: fly models cannot replicate the decades-long progression of the disease nor the regional- and neuronal-specific brain pathology. Moreover, fly models are based on gene misexpression,

in which transgenes are not regulated by endogenous promoters. These critiques are legitimate, but the same could be said about many popular mouse models based on transgene overexpression (e.g., SCA1-B05, HD-R6/2, AD-Tg2576). Although several knock-in mouse models with late onset and slow progression have been generated in the last decade, they have not eliminated the use of misexpression models because the latter have more robust phenotypes and are easier to use for discovery purposes. Likewise, *Drosophila* models are still relevant towards uncovering disease mechanisms, target discovery, and other innovative applications (see below).

But, is the fly still a relevant model of neurodegenerative disorders? Has it become unfashionable? The evidence says no: the number of publications continues on an upward trend (Fig. 1) and many of those papers continue to appear in high-quality journals. However, both public and private funding agencies seem reluctant to fund studies in *Drosophila*, moving away from basic or exploratory research projects. This may be a shortsighted approach since some of the most spectacular discoveries with human health applications have come from basic research (RNAi in worms, circadian rhythm in flies). Ultimately, it is our responsibility to focus our research towards relevant, innovative, and/or translational areas, and to explain better the relevance of our research to the general public.

8. ROBUST PHENOTYPES LEAD TO GENETIC SCREENS

Fly models of proteinopathies exhibit neuronal dysfunction, neuronal loss, and protein aggregation. These phenotypes have been described in many disease models, including mice and cultured cells. What, then, makes *Drosophila* relevant? Without a doubt, *Drosophila* stands out because of the ability to perform genetic screens for modifiers of neurotoxicity in a cost- and time-efficient manner. Fruit flies are an excellent model system for *in vivo* testing of small sets of interesting genes (candidate approach) by taking advantage of existing loss-of-function or overexpression alleles, or

by creating new transgenic flies in less than 10 weeks. Moreover, flies have also been used for unbiased screens of large sets of mutants that allow the identification of new genes and molecular pathways involved in disease pathogenesis. Here, the eye phenotypes have played a fundamental role because the easy analysis of the rough eye under the dissecting microscope allows testing of hundreds of genes per week and over a thousand genes per month. Other phenotypes such as reduced survival or locomotor dysfunction are also compatible with large genetic screens, although they are more time-consuming and may require the use of advanced engineering and software. The more time-consuming the task, the fewer conditions (genes) can be tested. That explains why the largest modifier screens have been performed in the eye [6, 55, 96, 97]. These are a few examples of the contributions of fly genetics to uncovering disease mechanisms.

Coping with protein aggregation: Hsp70. Several amyloidogenic proteins aggregate inside the cell, suggesting a role for protein quality control mechanisms in pathogenesis. Cell culture studies demonstrated the role of molecular chaperones, particularly the Heat shock protein 70 (Hsp70), in A τ 1 aggregation and neurotoxicity [98]. However, *in vivo* evidence for this protective activity was missing. N. Bonini showed that overexpression of an inducible isoform of human Hsp70 suppressed the rough eye phenotype and A τ 3 aggregation in flies [99]. In an unbiased genetic screen, we found that the Hsp70 co-chaperone Hsp40 suppressed the rough eye of A τ 1 [6]. This ability of molecular chaperones to suppress pathogenic protein aggregation and neurotoxicity was later expanded to include other disease models. For instance, co-expression of Hsp70 and α -Syn alleviated dopaminergic neuronal loss, while disruption of endogenous chaperone function enhanced neuropathology [64]. Not surprisingly, Hsp70 later showed the ability to block A τ 1 and α -Syn aggregation in transgenic mice [100, 101]. Trying to extend this protective activity to extracellular amyloids, we next wondered if Hsp70 could also reduce misfolding of HaPrP, a membrane anchored protein. Interestingly, in flies expressing HaPrP, Hsp70 accumulated in the lipid rafts (a highly specialized membrane domain) and reduced misfolding and neurotoxicity of HaPrP, uncovering a new ability for Hsp70 in preventing misfolding of an extracellular protein [89]. These observations illustrate the value of the chaperone system and its implications in the design of rational therapeutic approaches for several proteinopathies (see below).

Protein misfolding in the ER: XBP1. Protein misfolding in the ER and the secretory pathway induces the unfolded protein response (UPR), which differs significantly from the response to misfolded proteins in the cytoplasm and nucleus. The UPR consists in the activation of three independent pathways (PERK, IRE1 and ATF6) that function to reduce protein synthesis and elimination of misfolded proteins in the ER. One of the UPR branches consists on the ER stress sensor IRE1 and its downstream effector X-box binding protein 1 (XBP1), a transcriptional regulator that induces the expression of ER chaperones and other factors that reduce protein misfolding in the ER [102]. AD brains show signs of ER stress, as indicated by the activation of two sensors and several downstream effectors; however, the protective role of XBP1 and other ER stress-response pathways in AD had not been demonstrated *in vivo*. We recently described that flies expressing a bi-cistronic A β 42 construct induce ER stress and activation of XBP1 [46]. Moreover, XBP1 overexpression rescues and XBP1 loss-of-function enhances A β 42 rough eye phenotype. This protective activity was mediated by the reduction in ryanodine calcium channels in the ER, thus preventing the release of pro-apoptotic levels of calcium in the cytoplasm. Interestingly, many proteinopathies activate ER stress regardless of the localization of the misfolded conformers, suggesting that ER homeostasis may be affected during neuronal degeneration [103].

Posttranslational modifications: Phosphorylation. A recurrent theme in proteinopathies is the role of post-translational modification, in particular phosphorylation, in protein aggregation and neurotoxicity. The AD brain contains NFT in which Tau is hyperphosphorylated. Tau is a long protein with 79 putative Ser/Thr phosphorylation sites, and Tau phosphorylation increases aggregation and recognition by disease-specific, conformational antibodies. *Drosophila* has played a key role in the *in vivo* determination of many phosphorylation sites. Genetic analysis demonstrated that Cdk5 and GSK-3 were Tau kinases that regulate Tau aggregation and neurotoxicity [55, 93]. Also, modification of the phosphorylation sites showed their differential contribution to Tau aggregation and neurotoxicity. Whereas the Ser-Pro/Thr-Pro sites seemed to work in concert (mutating up to five sites had no significant effect, but mutating all 14 sites dramatically increased Tau aggregation), the non Ser-Pro sites S262 and S356 played critical roles in Tau aggregation by promoting the phosphorylation of Ser-Pro/Thr-Pro sites [57, 104-107]. Along the same line, A τ 1 and α -Syn phosphorylation also proved relevant in neuropathology. *In vitro* evidence indicated that A τ 1 phosphorylation by Akt1/PKB at S776 promoted binding of 14-3-3, aggregation, and neurotoxicity [108]. Reduced Akt1 function suppressed the rough eye of A τ 1 flies, while a S776A substitution in mice reduced A τ 1 toxicity, demonstrating the functional relevance of A τ 1 phosphorylation [108, 109]. Finally, α -Syn phosphorylation at S129 and T125 exhibited opposing effects, the first being neurotoxic and the second neuroprotective, suggesting that α -Syn toxicity depended on a balance between these two modifications [110, 111]. Thus, these studies played a critical role in identifying how phosphorylation contributes to the generation of the neurotoxic isoform, a key piece of information for the rational design of neuroprotective compounds.

Unbiased screens. If candidate pathways have found in *Drosophila* the ideal *in vivo* system for fast functional tests, unbiased genetic screens have expanded our understanding of the molecular mechanisms underlying protein misfolding and neurodegeneration. Genetic screens provide the means for unbiased gene discovery without prior knowledge of the involved pathways. Seminal contributions include the confirmation that protein quality control mechanisms, including molecular chaperones and protein degradation, play important roles in polyQ neurotoxicity [6]. More importantly, genetic screens served a unique role towards identifying novel pathways implicated in A τ 1 and A τ 3 neurotoxicity, including RNA metabolism, cellular detoxification, and transcriptional regulation [6, 96]. Not surprisingly, several kinases and phosphatases were identified as modifiers of Tau toxicity in the fly eye [55]. On the other hand, genetic modifiers of A β 42 implicated the secretory pathway, cholesterol homeostasis and copper transport in its neurotoxicity [97]. Thus, four genetic screens based on gene misexpression using the same collection of random insertions identified very different modifiers, revealing the unique mechanisms of neurotoxicity activated in each model. In addition, a loss-of-function screen identified the role of innate immunity and inflammation pathways in A β 42 neurotoxicity [112]. By testing collections of genetic modifiers of one model against other models, common and distinct mechanisms of pathogenesis can be identified [55, 97, 113]. Other key findings show that polyQ expansions in A τ 1 contribute to gain- and loss-of-function mechanisms by specific promotion/disruption of endogenous protein complexes [114]. Interestingly, interaction of A τ 1 and A τ 3 with other proteins bearing polyQ revealed that the activity of A τ 2 (SCA2) is critical for SCA3 and SCA1 pathogenesis [115, 116]. A comprehensive list of genetic pathways and modifiers of polyQ diseases can be found elsewhere [117].

Complex screens. Important contributions to the field of target discovery have come from combining high throughput screening (HTS) in a variety of platforms with *in vivo* validation in flies. For instance, integration of a yeast two-hybrid screen and affinity pull-

down assays with genetic manipulation of an HD fly model led to the identification of several Htt-interacting proteins that were known modifiers of neurodegeneration [118]. Another screen combining biochemical approaches with fly genetics identified three matrix metalloproteases as modifiers of Htt proteolysis and toxicity [119]. More recently, genome-wide RNAi screens for modifiers of Htt aggregation *in vitro* combined with validation in fly models, identified new regulators of Htt aggregation and toxicity, including genes related to nuclear transport, nucleotide processing, and signaling [120, 121]. A different approach consists in identifying aberrant pathways through gene expression analyses (microarrays). Expression of A β 42 in flies led to significant changes in the expression of oxidative stress genes and genetic tests confirmed the role of the iron-binding protein Ferritin and Catalase as strong suppressors of A β 42 neurotoxicity [122]. Given the lack of robust phenotypes in α -Syn flies, genetic screens have not been possible with this model. However, combination of a primary genetic screen in a yeast model of α -Syn aggregation with subsequent validation in a fly model of PD led to the identification of Rab guanosine triphosphatase 1 (Ypt1p/Rab1) as a strong suppressor of dopaminergic cell death [123]. This suppression was further confirmed in primary cultures of mammalian dopaminergic neurons, highlighting the value of simple model systems in the gene discovery process.

9. THE FLY PHARMACY

During the last 20 years, drug research has experienced dramatic changes resulting from newer technologies that include genomics and proteomics, structural biology, informatics, automated imaging, microfluidics, and robotics. Biotechnology companies have combined these emerging technologies to design robust HTS methods for drug discovery. Despite these technological advances, effective new drugs come out at a slow rate due to three major limitations: (1) current HTS methods rely mostly on the availability of a short list of validated targets; (2) inability to reproduce human pathogenic mechanisms *in vitro*; and (3) inefficacy of hits when tested in rodent models. To bypass these limitations, efforts have been directed to screen chemical libraries in simple model organisms, where complex biological processes can be studied in the context of intact living systems. In this regard, *Drosophila* holds tremendous potential in pharmacological research [124, 125]. A considerable amount of FDA-approved drugs act on receptors or downstream effectors of major signal transduction pathways conserved in flies and humans, including those for Wnt/wingless, Hedgehog, TGF- β , Notch, Ras/MAPK and insulin. The availability of several fly models of human diseases may facilitate testing of large compound libraries for the few hits that can revert, prevent, or delay neurotoxicity. Here, we provide a perspective on how *Drosophila* can complement ongoing efforts in drug development.

How to drug a fly. Compounds can be easily provided to larvae or adult flies by mixing them with the food or in a sugary solution placed in a small Whatman paper from which starved flies will drink. Using these strategies, administration of 4-phenylbutyrate throughout adulthood extended lifespan, while compounds that alter dopamine levels modified aggressive behaviors [126]. In other cases, vaporized chemicals such as cocaine or ethanol allowed the simultaneous treatment of large groups of flies, making this method suitable to automation and HTS [126]. However, determination of the actual inhaled dose and the vaporization of active compound can be limiting factors. An alternative approach, which offers better control over administration of pharmacological compounds, is direct intra-abdominal or intra-thoracic injection [127]. Although this procedure requires advanced skills and is more laborious, it allows a precise delivery of compounds under the microscope. Interestingly, microfluidic devices coupled with computer-controlled injection systems have been recently developed to inject fly embryos [21, 22]. These efforts emphasize the interest of the *Drosophila* community for achieving *bona fide* HTS conditions.

From genes to targets: the hit list. *Drosophila* models of proteinopathies have contributed to the development of novel therapeutic approaches [71]. For instance, a seminal work reported that the polyQ domain of Htt directly binds the acetyltransferase domains of Creb-Binding Protein and p300/CBP-associated factor, resulting in reduced histone acetylation and changes in gene expression. To compensate for this, flies were treated with histone deacetylase (HDAC) inhibitors (trichostatin A and suberoylanilide hydroxamic acid), which reversed Htt neurotoxicity [73]. Other important contributions to the suppression of Htt neurotoxicity include the mTOR inhibitor rapamycin (autophagy) [128], the inhibition of transglutaminase 2, a transcriptional repressor overexpressed by mutant Htt, with ZDON [129], ERK activation by polyphenols [130], and Meclizine, an FDA-approved drug with anti-histaminergic activity, that also suppresses mitochondria respiration and reduces oxidative stress [131]. Inhibitors of Sirtuin 2, a member of the histone deacetylase family of proteins, rescued α -Syn-dependent loss of dopaminergic neurons [132] and also showed neuroprotection in Htt flies [133]. Another example of the gene-to-drug transition is the rescue of Atx3 and α -Syn toxicity by Hsp70. The pharmacologic strategy to stimulate Hsp70 expression consists on inhibiting Hsp90, which acts as a negatively regulator of the heat-shock response. This approach worked by feeding flies the Hsp90 inhibitor geldanamycin, which suppressed α -Syn neurotoxicity [134]. Since geldanamycin is very toxic, a new generation of Hsp90 inhibitors with improved activity and lower toxicity has been developed, including 17-AAG, with potential uses in cancer and neurodegenerative diseases. Taken together, these studies illustrate the potential of flies in the discovery and validation of neuroprotective compounds.

10. DROSOPHILA GOES BIOTECH

Several pharmaceutical and biotech companies have embraced fruit flies in their research programs. For instance, Exelixis Inc. created an impressive collection of gene disruption mutants for use in target identification, while Curagen Corporation generated a comprehensive protein interaction database of fly proteins. Similarly, Aktogen Limited aims to accelerate the discovery of drug targets for the treatment of neurodegenerative and neuropsychiatric disorders using flies. Helicon Therapeutics is exploiting an array of proprietary technologies from Cold Spring Harbor Laboratory to identify fly genes that control memory, and Medros grows fruit flies in a standard 96-well format combined with robotics to screen for drugs against human chronic diseases. EnVivo Pharmaceuticals was founded in 2001 to conduct pharmacological screens in fly models of human neurodegenerative disorders using high-speed cameras and a proprietary software known as PhenoScreen. This effort was later transformed into optimized, drug screen services with fly models of neurodegeneration now offered by Vitruvean LLC to pharmaceutical companies and academic labs. Thus, the opportunity to request drug screens with the library of choice may contribute significantly to the drug discovery process in the years to come.

11. CONCLUSIONS

Drosophila models of proteinopathies have provided some of our earliest insights into the mechanisms leading to neuronal cell death. The ability to manipulate the fly genome in a flexible and time-efficient manner, along with an extensive range of genomic resources and genetic tools, allows the study of neurodegenerative processes at a level of resolution not possible in more complex organisms. Thus, fly geneticists are in a unique position to address relevant questions in neurodegenerative diseases, such as: Which are the molecular mechanisms triggering the accumulation of disease-related conformations? What is the identity of the neurotoxic conformer(s) in each disease? Which are the pathogenic cascades leading to neurodegeneration? How do environmental factors and susceptibility genes contribute to neurodegenerative disorders? The extensive genetic arsenal of *Drosophila*, combined with a century of genetic knowledge, will be instrumental to answer these funda-

mental questions and to pose new hypotheses in the years to come. It will be interesting to see how new technologies, including next-generation sequencing, proteomics, metabolomics, *in vivo* imaging, and single cell analysis, will be utilized in flies to develop a more integrated understanding of the cellular perturbations induced during neurodegeneration.

On the other hand, *Drosophila* is gaining momentum as a platform for therapeutics. This is because, after spending hundreds of millions on R&D, pharmaceutical firms discover that promising compounds are ineffective in clinical trials or are toxic in humans. Therefore, inclusion of model organisms in the initial stages of drug discovery is crucial to validate hits and exclude compounds with unfavorable properties. Unfortunately, *in vivo* studies are usually performed after lead optimization due to the complexity of rodent manipulation, and the relative high number of preliminary hits. The pharmacological susceptibility of fly models and the technological advances described above illustrate their potential for rapid and inexpensive drug evaluation under HTS conditions. Moreover, the ability to conduct large-scale genetic screens represents a unique opportunity to identify relevant targets. While flies cannot replace the need for testing in rodent models, the studies described here highlight their potential to speed up the drug discovery process. Given the successful multidisciplinary efforts of *Drosophila* researchers to identify neurodegenerative targets and pathways (that would be missed by more conventional approaches), we augur more significant contributions in the next decade. Therefore, the future looks bright for *Drosophila* as an instrument for genetic and pharmacological discovery in neurodegenerative proteinopathies.

ABBREVIATIONS

| | | |
|--------------|---|-------------------------------------|
| A β 42 | = | Amyloid beta peptide |
| AD | = | Alzheimer's disease |
| ALS | = | Amyotrophic lateral sclerosis |
| APP | = | Amyloid precursor protein |
| Atx | = | Ataxin |
| Psn | = | <i>Drosophila</i> Presenilin |
| DRPL | = | Dentatorubral-pallidoluyian atrophy |
| GFP | = | Green fluorescent protein |
| HD | = | Huntington's disease |
| HTS | = | High-throughput screen |
| Htt | = | Huntingtin |
| PD | = | Parkinson's disease |
| PrD | = | Prion disease |
| PrP | = | Prion protein |
| SBMA | = | Spinobulbar muscular atrophy |
| SCA | = | Spinocerebellar ataxia |
| SOD1 | = | Superoxide dismutase 1 |
| Syn | = | Synuclein |
| UAS | = | Upstream activator sequences |

CONFLICT OF INTEREST

The authors declare that they have no competing financial interests.

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REFERENCES

- Glabe CG, Kaye R. Common structure and toxic function of amyloid oligomers implies a common mechanism of pathogenesis. *Neurology* 2006; 24; 66(2 Suppl 1): S74-8.
- Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol* 2007; 8(2): 101-12.
- Morales R, Green KM, Soto C. Cross currents in protein misfolding disorders: interactions and therapy. *CNS Neurol Disord Drug Targets* 2009; 8(5): 363-71.
- Warrick JM, Paulson HL, Gray-Board GL, *et al.* Expanded polyglutamine protein forms nuclear inclusions and causes neural degeneration in *Drosophila*. *Cell* 1998; 93(6): 939-49.
- Jackson GR, Salecker I, Dong X, *et al.* Polyglutamine-expanded human huntingtin transgenes induce degeneration of *Drosophila* photoreceptor neurons. *Neuron* 1998; 21(3): 633-42.
- Fernandez-Funez P, Nino-Rosales ML, de Gouyon B, *et al.* Identification of genes that modify ataxin-1-induced neurodegeneration. *Nature* 2000; 408(6808): 101-6.
- Marsh JL, Walker JL, Theisen H, *et al.* Expanded polyglutamine peptides alone are intrinsically cytotoxic and cause neurodegeneration in *Drosophila*. *Hum Mol Genet* 2000; 9(1): 13-25.
- Kazemi-Esfarjani P, Benzer S. Genetic suppression of polyglutamine toxicity in *Drosophila*. *Science* 2000; 287(5459): 1837-40.
- Feany MB, Bender WW. A *Drosophila* model of Parkinson's disease. *Nature* 2000; 404(6776): 394-8.
- Matthews KA, Kaufman TC, Gelbart WM. Research resources for *Drosophila*: the expanding universe. *Nat Rev Genet* 2005; 6(3): 179-93.
- Adams MD, Celniker SE, Holt RA, *et al.* The genome sequence of *Drosophila melanogaster*. *Science* 2000; 287(5461): 2185-95.
- 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(7): 514-22.
- Bier E. *Drosophila*, the golden bug, emerges as a tool for human genetics. *Nat Rev Genet* 2005; 6(1): 9-23.
- Venken KJ, Bellen HJ. Transgenesis upgrades for *Drosophila melanogaster*. *Development* 2007; 134(20): 3571-84.
- Rubin GM, Spradling AC. Genetic transformation of *Drosophila* with transposable element vectors. *Science* 1982; 218(4570): 348-53.
- Brand AH, Perrimon N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 1993; 118(2): 401-15.
- McGuire SE, Roman G, Davis RL. Gene expression systems in *Drosophila*: a synthesis of time and space. *Trends Genet* 2004; 20: 384-91.
- Rorth P. A modular misexpression screen in *Drosophila* detecting tissue-specific phenotypes. *Proc Natl Acad Sci U S A* 1996; 93(22): 12418-22.
- Mohr S, Bakal C, Perrimon N. Genomic screening with RNAi: results and challenges. *Annu Rev Biochem* 2010; 79: 37-64.
- Carroll D, Beumer KJ, Trautman JK. High-efficiency gene targeting in *Drosophila* with zinc finger nucleases. *Methods Mol Biol* 2010; 649: 271-80.
- Cornell E, Fisher WW, Nordmeyer R, *et al.* Automating fruit fly *Drosophila* embryo injection for high throughput transgenic studies. *Rev Sci Instrum* 2008; 79(1): 013705.
- Zappe S, Fish M, Scott MP, Solgaard O. Automated MEMS-based *Drosophila* embryo injection system for high-throughput RNAi screens. *Lab Chip* 2006; 6(8): 1012-9.
- Hirth F. *Drosophila melanogaster* in the study of human neurodegeneration. *CNS Neurol Disord Drug Targets* 2010; 9(4): 504-23.
- Lu B, Vogel H. *Drosophila* models of neurodegenerative diseases. *Annu Rev Pathol* 2009; 4: 315-42.
- Alzheimer's Association 2010 Alzheimer's disease facts and figures. *Alzheimer's and Dementia* 2010; 6: 158-94.
- Crews L, Masliah E. Molecular mechanisms of neurodegeneration in Alzheimer's disease. *Hum Mol Genet* 2010; 19(R1): R12-20.
- Wisniewski T, Konietzko U. Amyloid-beta immunisation for Alzheimer's disease. *Lancet Neurol* 2008 Sep; 7(9): 805-11.

- [28] 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* 1999; 9(9): 489-92.
- [29] Gunawardena S, Goldstein LS. Disruption of axonal transport and neuronal viability by amyloid precursor protein mutations in Drosophila. *Neuron* 2001; 32(3): 389-401.
- [30] Gunawardena S, Her LS, Brusch RG, *et al.* Disruption of axonal transport by loss of huntingtin or expression of pathogenic polyQ proteins in Drosophila. *Neuron* 2003; 40(1): 25-40.
- [31] Takasugi N, Tomita T, Hayashi I, *et al.* The role of presenilin cofactors in the gamma-secretase complex. *Nature* 2003; 422(6930): 438-41.
- [32] Carmine-Simmen K, Proctor T, Tschape J, *et al.* Neurotoxic effects induced by the Drosophila amyloid-beta peptide suggest a conserved toxic function. *Neurobiol Dis* 2009; 33(2): 274-81.
- [33] Greeve I, Kretschmar D, Tschape JA, *et al.* Age-dependent neurodegeneration and Alzheimer-amyloid plaque formation in transgenic Drosophila. *J Neurosci* 2004; 24(16): 3899-906.
- [34] Rajendran L, Schneider A, Schlechtingen G, *et al.* Efficient inhibition of the Alzheimer's disease beta-secretase by membrane targeting. *Science* 2008; 320(5875): 520-3.
- [35] Micchelli CA, Esler WP, Kimberly WT, *et al.* Gamma-secretase/presenilin inhibitors for Alzheimer's disease phenocopy Notch mutations in Drosophila. *Faseb J* 2003; 17(1): 79-81.
- [36] Mah AL, Perry G, Smith MA, Monteiro MJ. Identification of ubiquilin, a novel presenilin interactor that increases presenilin protein accumulation. *J Cell Biol* 2000; 151(4): 847-62.
- [37] Bertram L, Hiltunen M, Parkinson M, *et al.* Family-based association between Alzheimer's disease and variants in UBQLN1. *N Engl J Med* 2005; 352(9): 884-94.
- [38] Li A, Xie Z, Dong Y, McKay KM, McKee ML, Tanzi RE. Isolation and characterization of the Drosophila ubiquilin ortholog dUbqln: *in vivo* interaction with early-onset Alzheimer disease genes. *Hum Mol Genet* 2007; 16(21): 2626-39.
- [39] Ganguly A, Feldman RM, Guo M. ubiquilin antagonizes presenilin and promotes neurodegeneration in Drosophila. *Hum Mol Genet* 2008; 17(2): 293-302.
- [40] Gross GG, Feldman RM, Ganguly A, Wang J, Yu H, Guo M. Role of X11 and ubiquilin as *in vivo* regulators of the amyloid precursor protein in Drosophila. *PLoS ONE* 2008; 3(6): e2495.
- [41] van de Hoef DL, Hughes J, Livne-Bar I, Garza D, Konsolaki M, Boulianne GL. Identifying genes that interact with Drosophila presenilin and amyloid precursor protein. *Genesis* 2009 Apr; 47(4): 246-60.
- [42] Mahoney MB, Parks AL, Ruddy DA, *et al.* Presenilin-based genetic screens in Drosophila melanogaster identify novel notch pathway modifiers. *Genetics* 2006; 172(4): 2309-24.
- [43] Iijima K, Liu HP, Chiang AS, Hearn SA, Konsolaki M, Zhong Y. Dissecting the pathological effects of human Abeta40 and Abeta42 in Drosophila: a potential model for Alzheimer's disease. *Proc Natl Acad Sci U S A* 2004; 101(17): 6623-8.
- [44] Finelli A, Kelkar A, Song HJ, Yang H, Konsolaki M. A model for studying Alzheimer's Abeta42-induced toxicity in Drosophila melanogaster. *Mol Cell Neurosci* 2004; 26(3): 365-75.
- [45] Crowther DC, Kinghorn KJ, Miranda E, *et al.* Intraneuronal Abeta, non-amyloid aggregates and neurodegeneration in a Drosophila model of Alzheimer's disease. *Neuroscience* 2005; 132(1): 123-35.
- [46] Casas-Tinto S, Zhang Y, Sanchez-Garcia J, Gomez-Velazquez M, Rincon-Limas DE, Fernandez-Funez P. The ER stress factor XBP1s prevents Amyloid-ss neurotoxicity. *Hum Mol Genet* 2011; 20(11): 2144-60.
- [47] Luheshi LM, Tartaglia GG, Brorsson AC, *et al.* Systematic *in vivo* analysis of the intrinsic determinants of amyloid Beta pathogenicity. *PLoS Biol* 2007; 5(11): e290.
- [48] Iijima K, Chiang HC, Hearn SA, *et al.* Abeta42 mutants with different aggregation profiles induce distinct pathologies in Drosophila. *PLoS ONE* 2008; 3(2): e1703.
- [49] Nereelius C, Sandegren A, Sargsyan H, *et al.* Alpha-helix targeting reduces amyloid-beta peptide toxicity. *Proc Natl Acad Sci U S A* 2009; 106(23): 9191-6.
- [50] Iijima-Ando K, Iijima K. Transgenic Drosophila models of Alzheimer's disease and tauopathies. *Brain Struct Funct* 2010; 214(2-3): 245-62.
- [51] Khurana V. Modeling Tauopathy in the fruit fly Drosophila melanogaster. *J Alzheimers Dis* 2008; 15(4): 541-53.
- [52] Wittmann CW, Wszolek MF, Shulman JM, *et al.* Tauopathy in Drosophila: neurodegeneration without neurofibrillary tangles. *Science* 2001; 293(5530): 711-4.
- [53] Williams DW, Tyrer M, Shepherd D. Tau and tau reporters disrupt central projections of sensory neurons in Drosophila. *J Comp Neurol* 2000; 428(4): 630-40.
- [54] Chen X, Li Y, Huang J, *et al.* Study of tauopathies by comparing Drosophila and human tau in Drosophila. *Cell Tissue Res* 2007; 329(1): 169-78.
- [55] Shulman JM, Feany MB. Genetic modifiers of tauopathy in Drosophila. *Genetics* 2003; 165(3): 1233-42.
- [56] Blard O, Feuillet S, Bou J, *et al.* Cytoskeleton proteins are modulators of mutant tau-induced neurodegeneration in Drosophila. *Hum Mol Genet* 2007; 16(5): 555-66.
- [57] Fulga TA, Elson-Schwab I, Khurana V, *et al.* Abnormal bundling and accumulation of F-actin mediates tau-induced neuronal degeneration *in vivo*. *Nat Cell Biol* 2007; 9(2): 139-48.
- [58] Khurana V, Lu Y, Steinhilb ML, Oldham S, Shulman JM, Feany MB. TOR-mediated cell-cycle activation causes neurodegeneration in a Drosophila tauopathy model. *Curr Biol* 2006; 16(3): 230-41.
- [59] Lees AJ, Hardy J, Revesz T. Parkinson's disease. *Lancet* 2009; 373(9680): 2055-66.
- [60] Cookson MR. alpha-Synuclein and neuronal cell death. *Mol Neurodegener* 2009; 4: 9.
- [61] Hardy J, Lewis P, Revesz T, Lees A, Paisan-Ruiz C. The genetics of Parkinson's syndromes: a critical review. *Curr Opin Genet Dev* 2009; 19(3): 254-65.
- [62] Botella JA, Bayersdorfer F, Gmeiner F, Schneuwly S. Modelling Parkinson's disease in Drosophila. *Neuromolecular Med* 2009; 11(4): 268-80.
- [63] Ambegaokar SS, Roy B, Jackson GR. Neurodegenerative models in Drosophila: polyglutamine disorders, Parkinson disease, and amyotrophic lateral sclerosis. *Neurobiol Dis* 2010; 40(1): 29-39.
- [64] Auluck PK, Chan HY, Trojanowski JQ, Lee VM, Bonini NM. Chaperone suppression of alpha-synuclein toxicity in a Drosophila model for Parkinson's disease. *Science* 2002; 295(5556): 865-8.
- [65] Pesah Y, Burgess H, Middlebrooks B, *et al.* Whole-mount analysis reveals normal numbers of dopaminergic neurons following misexpression of alpha-Synuclein in Drosophila. *Genesis* 2005; 41(4): 154-9.
- [66] Botella JA, Bayersdorfer F, Schneuwly S. Superoxide dismutase overexpression protects dopaminergic neurons in a Drosophila model of Parkinson's disease. *Neurobiol Dis* 2008; 30(1): 65-73.
- [67] Trinh K, Moore K, Wes PD, *et al.* Induction of the phase II detoxification pathway suppresses neuron loss in Drosophila models of Parkinson's disease. *J Neurosci* 2008; 28(2): 465-72.
- [68] Periquet M, Fulga T, Myllykangas L, Schlossmacher MG, Feany MB. Aggregated alpha-synuclein mediates dopaminergic neurotoxicity *in vivo*. *J Neurosci* 2007; 27(12): 3338-46.
- [69] Karpinar DP, Balija MB, Kugler S, *et al.* Pre-fibrillar alpha-synuclein variants with impaired beta-structure increase neurotoxicity in Parkinson's disease models. *Embo J* 2009; 28(20): 3256-68.
- [70] Williams AJ, Paulson HL. Polyglutamine neurodegeneration: protein misfolding revisited. *Trends Neurosci* 2008; 31(10): 521-8.
- [71] Marsh JL, Thompson LM. Drosophila in the study of neurodegenerative disease. *Neuron* 2006; 52(1): 169-78.
- [72] Ross CA, Tabrizi SJ. Huntington's disease: from molecular pathogenesis to clinical treatment. *Lancet Neurol* 2011; 10(1): 83-98.
- [73] Steffan JS, Bodai L, Pallos J, *et al.* Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila. *Nature* 2001; 413(6857): 739-43.
- [74] Lee WC, Yoshihara M, Littleton JT. Cytoplasmic aggregates trap polyglutamine-containing proteins and block axonal transport in a Drosophila model of Huntington's disease. *Proc Natl Acad Sci U S A* 2004; 101(9): 3224-9.
- [75] Steffan JS, Agrawal N, Pallos J, *et al.* SUMO modification of Huntingtin and Huntington's disease pathology. *Science* 2004; 304(5667): 100-4.
- [76] Romero E, Cha GH, Verstreken P, *et al.* Suppression of neurodegeneration and increased neurotransmission caused by expanded full-length huntingtin accumulating in the cytoplasm. *Neuron* 2008; 57(1): 27-40.

- [77] Dion PA, Daoud H, Rouleau GA. Genetics of motor neuron disorders: new insights into pathogenic mechanisms. *Nat Rev Genet* 2009; 10(11): 769-82.
- [78] Rothstein JD. Current hypotheses for the underlying biology of amyotrophic lateral sclerosis. *Ann Neurol* 2009; 65 Suppl 1: S3-9.
- [79] Elia AJ, Parkes TL, Kirby K, *et al.* Expression of human FALS SOD in motoneurons of *Drosophila*. *Free Radic Biol Med* 1999 May; 26(9-10): 1332-8.
- [80] Parkes TL, Elia AJ, Dickinson D, Hilliker AJ, Phillips JP, Boulianne GL. Extension of *Drosophila* lifespan by overexpression of human SOD1 in motoneurons. *Nat Genet* 1998 Jun; 19(2): 171-4.
- [81] Mockett RJ, Radyuk SN, Benes JJ, Orr WC, Sohal RS. Phenotypic effects of familial amyotrophic lateral sclerosis mutant Sod alleles in transgenic *Drosophila*. *Proc Natl Acad Sci U S A* 2003; 100(1): 301-6.
- [82] Watson MR, Lagow RD, Xu K, Zhang B, Bonini NM. A *drosophila* model for amyotrophic lateral sclerosis reveals motor neuron damage by human SOD1. *J Biol Chem* 2008; 283(36): 24972-81.
- [83] Aguzzi A, Baumann F, Bremer J. The Prion's Elusive Reason for Being. *Annu Rev Neurosci* 2008; 31: 439-77.
- [84] Prusiner SB. Prions. *Proc Natl Acad Sci U S A* 1998; 95(23): 13363-83.
- [85] Rincon-Limas DE, Casas-Tinto S, Fernandez-Funez P. Exploring prion protein biology in flies: genetics and beyond. *Prion* 2010; 4(1): 1-8.
- [86] Gavin BA, Dolph MJ, Deleault NR, *et al.* Accelerated accumulation of misfolded prion protein and spongiform degeneration in a *Drosophila* model of Gerstmann-Straussler-Scheinker syndrome. *J Neurosci* 2006; 26(48): 12408-14.
- [87] Choi JK, Jeon YC, Lee DW, *et al.* A *Drosophila* model of GSS syndrome suggests defects in active zones are responsible for pathogenesis of GSS syndrome. *Hum Mol Genet* 2010; 19(22): 4474-89.
- [88] Wadsworth JD, Collinge J. Update on human prion disease. *Biochim Biophys Acta* 2007; 1772(6): 598-609.
- [89] Fernandez-Funez P, Casas-Tinto S, Zhang Y, *et al.* *In vivo* generation of neurotoxic prion protein: role for hsp70 in accumulation of misfolded isoforms. *PLoS Genet* 2009; 5(6): e1000507.
- [90] Fernandez-Funez P, Zhang Y, Casas-Tinto S, Xiao X, Zou WQ, Rincon-Limas DE. Sequence-dependent prion protein misfolding and neurotoxicity. *J Biol Chem* 2010; 285(47): 36897-908.
- [91] Khan MQ, Sweeting B, Mulligan VK, *et al.* Prion disease susceptibility is affected by beta-structure folding propensity and local side-chain interactions in PrP. *Proc Natl Acad Sci U S A* 2010; 107(46): 19808-13.
- [92] Fernandez-Funez P, Zhang Y, Sanchez-Garcia J, Jensen K, Zou W, Rincon-Limas DE. Pulling rabbits to reveal the secrets of the prion protein. *Commun Integr Biol* 2011; 4(3).
- [93] Jackson GR, Wiedau-Pazos M, Sang TK, *et al.* Human wild-type tau interacts with wingless pathway components and produces neurofibrillary pathology in *Drosophila*. *Neuron* 2002; 34(4): 509-19.
- [94] Sandberg MK, Al-Doujaily H, Sharps B, Clarke AR, Collinge J. Prion propagation and toxicity *in vivo* occur in two distinct mechanistic phases. *Nature* 2011 Feb 24; 470(7335): 540-2.
- [95] Gouras GK, Tsai J, Naslund J, *et al.* Intraneuronal Abeta42 accumulation in human brain. *Am J Pathol* 2000; 156(1): 15-20.
- [96] Bilen J, Bonini NM. Genome-wide screen for modifiers of ataxin-3 neurodegeneration in *Drosophila*. *PLoS Genet* 2007; 3(10): 1950-64.
- [97] Cao W, Song HJ, Gangi T, *et al.* Identification of novel genes that modify phenotypes induced by Alzheimer's beta-amyloid overexpression in *Drosophila*. *Genetics* 2008; 178(3): 1457-71.
- [98] Cummings CJ, Mancini MA, Antalffy B, DeFranco DB, Orr HT, Zoghbi HY. Chaperone suppression of aggregation and altered subcellular proteasome localization imply protein misfolding in SCA1. *Nat Genet* 1998; 19(2): 148-54.
- [99] Warrick JM, Chan HY, Gray-Board GL, Chai Y, Paulson HL, Bonini NM. Suppression of polyglutamine-mediated neurodegeneration in *Drosophila* by the molecular chaperone HSP70. *Nat Genet* 1999; 23(4): 425-8.
- [100] Cummings CJ, Sun Y, Opal P, *et al.* Over-expression of inducible HSP70 chaperone suppresses neuropathology and improves motor function in SCA1 mice. *Hum Mol Genet* 2001; 10(14): 1511-8.
- [101] Klucken J, Shin Y, Maslah E, Hyman BT, McLean PJ. Hsp70 Reduces alpha-Synuclein Aggregation and Toxicity. *J Biol Chem* 2004; 279(24): 25497-502.
- [102] Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol* 2007; 8(7): 519-29.
- [103] Yoshida H. ER stress and diseases. *Febs J* 2007; 274(3): 630-58.
- [104] 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(5): 671-82.
- [105] 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* 2007; 18(12): 5060-8.
- [106] Steinhilb ML, Dias-Santagata D, Mulkearns EE, *et al.* S/P and T/P phosphorylation is critical for tau neurotoxicity in *Drosophila*. *J Neurosci Res* 2007; 85(6): 1271-8.
- [107] Chatterjee S, Sang TK, Lawless GM, Jackson GR. Dissociation of tau toxicity and phosphorylation: role of GSK-3beta, MARK and Cdk5 in a *Drosophila* model. *Hum Mol Genet* 2009; 18(1): 164-77.
- [108] Chen HK, Fernandez-Funez P, Acevedo SF, *et al.* Interaction of Akt-phosphorylated ataxin-1 with 14-3-3 mediates neurodegeneration in spinocerebellar ataxia type 1. *Cell* 2003; 113(4): 457-68.
- [109] Emamian ES, Kaytor MD, Duvick LA, *et al.* Serine 776 of ataxin-1 is critical for polyglutamine-induced disease in SCA1 transgenic mice. *Neuron* 2003; 38(3): 375-87.
- [110] Chen L, Feany MB. Alpha-synuclein phosphorylation controls neurotoxicity and inclusion formation in a *Drosophila* model of Parkinson disease. *Nat Neurosci* 2005; 8(5): 657-63.
- [111] Chen L, Periquet M, Wang X, *et al.* Tyrosine and serine phosphorylation of alpha-synuclein have opposing effects on neurotoxicity and soluble oligomer formation. *J Clin Invest* 2009; 119(11): 3257-65.
- [112] 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(12): e3966.
- [113] Branco J, Al-Ramahi I, Ukani L, *et al.* Comparative analysis of genetic modifiers in *Drosophila* points to common and distinct mechanisms of pathogenesis among polyglutamine diseases. *Hum Mol Genet* 2008; 17(3): 376-90.
- [114] Lim J, Crespo-Barreto J, Jafar-Nejad P, *et al.* Opposing effects of polyglutamine expansion on native protein complexes contribute to SCA1. *Nature* 2008; 452(7188): 713-8.
- [115] Lessing D, Bonini NM. Polyglutamine genes interact to modulate the severity and progression of neurodegeneration in *Drosophila*. *PLoS Biol* 2008; 6(2): e29.
- [116] Al-Ramahi I, Perez AM, Lim J, *et al.* dAtaxin-2 mediates expanded Ataxin-1-induced neurodegeneration in a *Drosophila* model of SCA1. *PLoS Genet* 2007; 3(12): e234.
- [117] Mallik M, Lakhota SC. Modifiers and mechanisms of multi-system polyglutamine neurodegenerative disorders: lessons from fly models. *J Genet* 2010; 89(4): 497-526.
- [118] Kaltenbach LS, Romero E, Becklin RR, *et al.* Huntingtin interacting proteins are genetic modifiers of neurodegeneration. *PLoS Genet* 2007; 3(5): e82.
- [119] Miller JP, Holcomb J, Al-Ramahi I, *et al.* Matrix metalloproteinases are modifiers of huntingtin proteolysis and toxicity in Huntington's disease. *Neuron* 2010; 67(2): 199-212.
- [120] Doumanis J, Wada K, Kino Y, Moore AW, Nukina N. RNAi screening in *Drosophila* cells identifies new modifiers of mutant huntingtin aggregation. *PLoS ONE* 2009; 4(9): e7275.
- [121] Zhang S, Binari R, Zhou R, Perrimon N. A genomewide RNA interference screen for modifiers of aggregates formation by mutant Huntingtin in *Drosophila*. *Genetics* 2010; 184(4): 1165-79.
- [122] Rival T, Page RM, Chandraratna DS, *et al.* 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 Apr; 29(7): 1335-47.
- [123] Cooper AA, Gittler AD, Cashikar A, *et al.* Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. *Science* 2006; 313(5785): 324-8.
- [124] Bell AJ, McBride SM, Dockendorff TC. Flies as the ointment: *Drosophila* modeling to enhance drug discovery. *Fly (Austin)* 2009; 3(1): 39-49.

- [125] Rand MD. Drosophotoxicology: the growing potential for Drosophila in neurotoxicology. *Neurotoxicol Teratol* 2010; 32(1): 74-83.
- [126] Mayr LM, Bojanic D. Novel trends in high-throughput screening. *Curr Opin Pharmacol* 2009; 9(5): 580-8.
- [127] Sarantseva S, Timoshenko S, Bolshakova O, *et al.* Apolipoprotein E-mimetics inhibit neurodegeneration and restore cognitive functions in a transgenic Drosophila model of Alzheimer's disease. *PLoS One* 2009; 4(12): e8191.
- [128] Ravikumar B, Vacher C, Berger Z, *et al.* Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. *Nat Genet* 2004; 36(6): 585-95.
- [129] McConoughey SJ, Basso M, Niatsetskeya ZV, *et al.* Inhibition of transglutaminase 2 mitigates transcriptional dysregulation in models of Huntington disease. *EMBO Mol Med* 2010; 2(9): 349-70.
- [130] Maher P, Dargusch R, Bodai L, Gerard PE, Purcell JM, Marsh JL. ERK activation by the polyphenols fisetin and resveratrol provides neuroprotection in multiple models of Huntington's disease. *Hum Mol Genet* 2011; 20(2): 261-70.
- [131] Gohil VM, Offner N, Walker JA, *et al.* Meclizine is neuroprotective in models of Huntington's disease. *Hum Mol Genet* 2011; 20(2): 294-300.
- [132] Outeiro TF, Kontopoulos E, Altmann SM, *et al.* Sirtuin 2 inhibitors rescue alpha-synuclein-mediated toxicity in models of Parkinson's disease. *Science* 2007; 317(5837): 516-9.
- [133] Luthi-Carter R, Taylor DM, Pallos J, *et al.* SIRT2 inhibition achieves neuroprotection by decreasing sterol biosynthesis. *Proc Natl Acad Sci U S A* 2010; 107(17): 7927-32.
- [134] Auluck PK, Bonini NM. Pharmacological prevention of Parkinson disease in Drosophila. *Nat Med* 2002; 8(11): 1185-6.
- [135] Merdes G, Soba P, Loewer A, Bilic MV, Beyreuther K, Paro R. Interference of human and Drosophila APP and APP-like proteins with PNS development in Drosophila. *Embo J* 2004; 23(20): 4082-95.
- [136] Fossgreen A, Bruckner B, Czech C, Masters CL, Beyreuther K, Paro R. Transgenic Drosophila expressing human amyloid precursor protein show gamma-secretase activity and a blistered-wing phenotype. *Proc Natl Acad Sci U S A* 1998; 95(23): 13703-8.
- [137] Seidner GA, Ye Y, Faraday MM, Alvord WG, Fortini ME. Modeling clinically heterogeneous presenilin mutations with transgenic Drosophila. *Curr Biol* 2006; 16(10): 1026-33.
- [138] Guo M, Hong EJ, Fernandes J, Zipursky SL, Hay BA. A reporter for amyloid precursor protein gamma-secretase activity in Drosophila. *Hum Mol Genet* 2003; 12(20): 2669-78.
- [139] Schilling S, Zeitschel U, Hoffmann T, *et al.* Glutaminyl cyclase inhibition attenuates pyroglutamate Abeta and Alzheimer's disease-like pathology. *Nat Med* 2008; 14(10): 1106-11.
- [140] Murray MJ, Merritt DJ, Brand AH, Whittington PM. *In vivo* dynamics of axon pathfinding in the Drosophila CNS: a time-lapse study of an identified motoneuron. *J Neurobiol* 1998; 37(4): 607-21.
- [141] Mershin A, Pavlopoulos E, Fitch O, Braden BC, Nanopoulos DV, Skoulakis EM. Learning and memory deficits upon TAU accumulation in Drosophila mushroom body neurons. *Learn Mem* 2004; 11(3): 277-87.
- [142] Mudher A, Shepherd D, Newman TA, *et al.* GSK-3beta inhibition reverses axonal transport defects and behavioural phenotypes in Drosophila. *Mol Psychiatry* 2004; 9(5): 522-30.
- [143] Folwell J, Cowan CM, Ubhi KK, *et al.* Abeta exacerbates the neuronal dysfunction caused by human tau expression in a Drosophila model of Alzheimer's disease. *Exp Neurol* 2010; 223(2): 401-9.
- [144] Mugat B, Parmentier ML, Bonneaud N, Chan HY, Maschat F. Protective role of Engrailed in a Drosophila model of Huntington's disease. *Hum Mol Genet* 2008; 17(22): 3601-16.
- [145] Satterfield TF, Jackson SM, Pallanck LJ. A Drosophila homolog of the polyglutamine disease gene SCA2 is a dosage-sensitive regulator of actin filament formation. *Genetics* 2002; 162(4): 1687-702.
- [146] Matsumoto M, Yada M, Hatakeyama S, *et al.* Molecular clearance of ataxin-3 is regulated by a mammalian E4. *Embo J* 2004; 23(3): 659-69.
- [147] Warrick JM, Morabito LM, Bilen J, *et al.* Ataxin-3 suppresses polyglutamine neurodegeneration in Drosophila by a ubiquitin-associated mechanism. *Mol Cell* 2005; 18(1): 37-48.
- [148] Latouche M, Lasbleiz C, Martin E, *et al.* A conditional panneuronal Drosophila model of spinocerebellar ataxia 7 with a reversible adult phenotype suitable for identifying modifier genes. *J Neurosci* 2007; 27(10): 2483-92.
- [149] Charroux B, Freeman M, Kerridge S, Baonza A. Atrophin contributes to the negative regulation of epidermal growth factor receptor signaling in Drosophila. *Dev Biol* 2006; 291(2): 278-90.
- [150] Chan HY, Warrick JM, Andriola I, Merry D, Bonini NM. Genetic modulation of polyglutamine toxicity by protein conjugation pathways in Drosophila. *Hum Mol Genet* 2002; 11(23): 2895-904.
- [151] Takeyama K, Ito S, Yamamoto A, *et al.* Androgen-dependent neurodegeneration by polyglutamine-expanded human androgen receptor in Drosophila. *Neuron* 2002; 35(5): 855-64.