From fruit fly to bedside: translating lessons from *Drosophila* models of neurodegenerative disease

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Purpose of review

Fly models have been developed for a variety of neurodegenerative disorders, and the field is beginning to harness the power of *Drosophila* genetics to dissect pathways of disease pathogenesis and identify targets for therapeutic intervention. In this review, we emphasize the most recent accomplishments and chart the potential rewards in translating lessons from *Drosophila* models to clinical therapeutics.

Recent findings

The conservation of human disease genes in the *Drosophila* genome forms the basis for several recent investigations of the normal biological functions of genes implicated in neurodegenerative disease. In addition, transgenic approaches continue to expand the list of diseases modeled in *Drosophila* that now includes Parkinson's disease, Alzheimer's disease, Huntington's disease, and several spinocerebellar ataxias. Studies based on these models suggest that protein folding and degradation pathways play an important role in Parkinson's disease and the polyglutamine repeat disorders, and that kinases and apoptotic pathways may modulate neurodegeneration in tauopathies.

Summary

Ongoing genetic studies with *Drosophila* neurodegenerative disease models promise to enhance our understanding of disease pathogenesis and generate target lists for future investigational research and drug development. The next challenge will be distilling a growing number of possible targets into a shortlist for fast-track drug design and clinical trials. With the advent of neurodegenerative disease models, the fruit fly is rapidly assuming a unique niche in bench to bedside research.

Keywords

Drosophila melanogaster, animal models, neurodegenerative diseases, translational research, drug design, Parkinson's disease, Alzheimer's disease, tauopathy, Huntington's disease, spinocerebellar ataxia, polyglutamine repeat

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Abbreviations

APP	amyloid precursor protein
GSK-3β	glycogen synthase kinase-3β
HSP	heat shock protein
SAHA	suberoylanilide hydroxamic acid
SCA	spinocerebellar ataxia

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Introduction

Although clinically heterogeneous, neurodegenerative diseases share intriguing parallels in their natural history and neuropathology. These disorders are characterized by adult-onset, progressive loss of specific neuronal subpopulations, and the ultimate consequence of incapacitation and early death. Pathologically, these diseases are associated with intra or extracellular deposits of aggregated proteins. Remarkably, genetic studies during the last decade have demonstrated that familial forms of many disorders are caused by mutations in the genes encoding the predominant protein constituent of these aggregates. Clinicians who manage the complexities and subtleties of these disorders may be incredulous that these diseases can be modeled in a fruit fly. Discomfort with the concept of a Huntington's or Alzheimer's fly may stem from the cognitive and psychiatric manifestations of these disorders that make them uniquely human afflictions. The *Drosophila* central nervous system, however, is built from neurons and glia that operate on the same fundamental principles as their mammalian counterparts, and many neurotransmitter systems, including dopamine, acetylcholine, glutamate, and GABA, are conserved from flies to humans. While the *Drosophila* brain has fewer neurons and synapses, it is still capable of producing many complex behaviors, including learning and memory, that we might otherwise expect to be the exclusive domain of more highly evolved organisms. Human genetic studies teach us that familial neurodegenerative syndromes can be triggered by the dysfunction of single genes within neurons, and fly models aim to recapitulate this fundamental cellular insult and the subsequent pathway of neuronal death, but within the context of a simpler, more experimentally tractable organism. *Drosophila* offers the power of rapid genetic analysis, with a generation time 10 times faster than in mice, as well as flexible ectopic expression and the ability to perform large-scale mutagenesis screens which are not yet possible in mammalian models. This review will introduce approaches to studying neurodegenerative disease in the fruit fly, *Drosophila melanogaster*,

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emphasizing the accomplishments of the last 12 months, and appraising the direction in which the field is heading. We critically evaluate where *Drosophila* fits into the continuum of 'bench to bedside' research and highlight the challenges that lie ahead. Several excellent and comprehensive reviews have also recently surveyed neurodegenerative disease models in *Drosophila* [1,2].

Dissecting neurodegenerative disease gene function in *Drosophila*

Several neurodegenerative diseases are strictly familial syndromes, dominantly inherited as simple Mendelian traits, and thus triggered by single gene defects. This group is exemplified by the polyglutamine repeat disorders, including Huntington's disease and the spinocerebellar ataxias, in which the nature of the gene mutation is an expanded trinucleotide repeat encoding an elongated glutamine tract in the mutant protein. By contrast, other neurodegenerative disorders, such as Alzheimer's disease and Parkinson's disease, most commonly present in a sporadic form, although rare familial cases have been described. Significantly, the similar clinical and neuropathological features of familial and sporadic forms of neurodegenerative disease suggest a common mechanism of pathogenesis, whether triggered by a single gene mutation or a combination of genetic and environmental influences. Once a disease gene has been identified, clues to its function may be provided by sequence homology and expression pattern; however, in the case of many neurodegenerative disease genes, precise functions often remain elusive without genetic studies of mutant animals. For example, while it has been known for many years that dysfunction of the *amyloid precursor protein* and *huntingtin* genes is associated with Alzheimer's and Huntington's disease, respectively, relatively little is yet known about the normal neuronal functions of these genes. Such knowledge might provide important clues as to how gene function is perturbed in disease states and also suggest potential strategies for therapeutic intervention. Cross-species comparisons indicate that more than half of all known human disease genes have homologs in the *Drosophila* genome [3,4]. Given this high degree of evolutionary conservation, the relative ease of generating mutant animals, and minimal genetic redundancy, *Drosophila* is an ideal system for dissecting the normal functions of genes associated with neurodegenerative disease.

The potential contribution of *Drosophila* genetics to understanding neurodegenerative disease gene function is exemplified by recent studies of fly homologs of genes implicated in Alzheimer's disease. The amyloid precursor protein (APP) is abnormally processed to the 42 amino acid Aβ peptide that aggregates to form the amyloid plaques of Alzheimer's disease. Intensive research has focused on characterizing the proteases responsible for APP processing in the hope that pharmacologic inhibition might prevent the onset or progression of disease. Rare, dominant mutations in *app* or *presenilin*, encoding a component of the γ-secretase proteolytic enzyme, alter APP processing to increase Aβ production and cause familial Alzheimer's disease. Studies of γ-secretase activity in *Drosophila* have emerged from unexpected parallels between the proteolysis of APP and the notch transmembrane receptor, long the subject of intense research by developmental biologists for its role in cell fate determination. Analogous to its action on APP, the γ-secretase complex mediates an essential cleavage of the notch receptor during signal transduction. The *Drosophila* homolog of presenilin and that of another γ-secretase component, nicastrin, were both identified by genetic screens for mutations which produce notch-like phenotypes, and both proteins are similarly required for proteolytic cleavage and release of the notch intracellular fragment [5–7,8•,9•,10]. Studies of notch processing in invertebrate models can be generalized to our understanding of APP processing, providing an investigative approach that is complementary to in-vitro and cell culture-based studies aimed at reconstituting γ-secretase activity. In addition to dissecting the genes involved in its pathologic processing, studies of a *Drosophila* APP homologue, APPL, have provided clues to the normal neuronal function of this protein. *appl* mutant flies develop axonal vesicular accumulations, highly suggestive of defects in axonal transport [11,12]. These results are consistent with the recent demonstration of a direct interaction between mammalian APP and the kinesin motor that mediates anterograde axonal transport [13]. Besides amyloid plaques, Alzheimer's neuropathology is characterized by neurofibrillary tangles consisting of aggregates of the neuronal microtubule-associated protein, tau, and mutations in the human *tau* gene cause familial frontotemporal dementia syndromes. A *tau* homolog was also recently identified in *Drosophila*, and ongoing studies on this system should enhance our understanding of tau function in both normal and disease states [14].

Familial forms of Parkinson's disease have been associated with either dominant mutations in the *α-synuclein* gene or recessive mutations in *parkin*. The function of α-synuclein remains unknown, but this protein aggregates to form the Lewy bodies that characterize Parkinson's disease brains. Studies of mammalian *parkin* have been more informative, identifying this gene as encoding a ubiquitin ligase involved in targeting cellular proteins for proteolysis [15]. Although the *Drosophila* genome apparently lacks an *α-synuclein* homolog, a *Drosophila parkin* gene was recently identified based on primary sequence conservation [16•]. Significantly, *parkin* mutant flies developed apoptotic

muscle degeneration associated with prominent mitochondrial swelling and disruption of the inner membrane. These results bolster models of Parkinson's disease in which mitochondrial dysfunction plays an important role. Nevertheless, dopaminergic neurons were preserved in aged *parkin* mutant flies, and it will thus be important in future work to understand the difference in vulnerabilities to loss of *parkin* between dopaminergic cells in human and *Drosophila* brains.

Genes associated with polyglutamine repeat neurodegenerative diseases have also been the subject of recent loss of function studies in *Drosophila*. Dentatorubral-pallidoluysian atrophy is caused by dominantly inherited mutations in the *atrophin-1* gene, and two recent reports on a *Drosophila* atrophin homolog reveal that this protein functions as a versatile transcriptional repressor in multiple developmental contexts [17,18]. Atrophin thus joins a growing list of polyglutamine repeat disease proteins which appear to function in the cell nucleus as transcriptions factors, including huntingtin and the androgen receptor, implicated in Huntington's disease and spinal and bulbar muscular atrophy, respectively. Loss of function studies have therefore identified an important parallel amongst a subset of polyglutamine repeat diseases, leading to a unifying model of pathogenesis in which expanded polyglutamine tracts may produce a derangement of neuronal transcriptional regulation.

Transgenic models of neurodegenerative disease in flies

Consistent with the findings of gene knockout studies in mice, flies mutant for disease genes cannot in most cases be considered bona fide disease models as they do not demonstrate neurodegeneration. The absence of neurodegeneration in loss-of-function animals, however, might be explained by the hypothesis that most neurodegenerative diseases involve toxic gain-of-function mechanisms, a view that is also suggested by the dominant inheritance pattern of most familial neurodegenerative syndromes. Transgenic approaches have therefore been used to express human disease genes in *Drosophila* in an effort to recapitulate the pathological features of disease. Despite the differences between the *Drosophila* and human brains, the parallels between a transgenic model and its cognate disease can be uncanny. Similar to human Parkinson's disease, transgenic expression of human α-synuclein in the *Drosophila* nervous system causes adult onset, progressive loss of dopaminergic neurons, formation of Lewy body-like inclusions, and locomotor defects [19]. In other cases, distinctions between a disease and its model may also be informative. As in Alzheimer's disease and tauopathy, expression of human tau in the *Drosophila* nervous system causes progressive neurodegeneration and a truncated lifespan [20]. In addition, a number of tau conformational and phosphoepitopes which are known to show relative specificity for disease states increase in association with neurodegeneration. Interestingly, however, unlike human disease, neurodegeneration in *Drosophila* can occur in the absence of tau aggregation into neurofibrillary tangles. These results suggest that tau acquires toxic properties prior to its macromolecular aggregation, and that therapies may therefore need to target this still elusive pre-tangle species in order to be effective.

A number of polyglutamine repeat diseases have also been successfully modeled in transgenic *Drosophila*. Expanded polyglutamine stretches have been expressed alone as well as within the context of *huntingtin*, *SCA1*, *SCA3*, and the androgen receptor [21–24,25•,26,27]. In each case, transgene expression was directed to the *Drosophila* retina and resulted in a reduction in eye size and a roughened surface due to the death of intrinsic photoreceptors and supporting cells. As in human disease, polyglutamine toxicity in the *Drosophila* retina correlates well with the length of the polyglutamine repeat. Spinal and bulbar muscular atrophy, or Kennedy's disease, is an example of a neurodegenerative disease in which pathogenesis involves both loss and gain-of-function elements. Expansion of a polyglutamine repeat disrupts androgen receptor function, leading to androgen insensitivity, but also imbues a novel toxic activity to this protein which triggers neuronal death in an androgen-dependent manner. In a recently developed *Drosophila* model, reporter genes responsive to the androgen receptor were used to demonstrate how a polyglutamine repeat expansion both disrupts transcriptional activation and simultaneously endows neurotoxic properties in a strictly androgen-dependent fashion [25•]. Thus, in addition to demonstrating a toxic gain-of-function, this model elegantly recapitulates the loss of androgen receptor function that leads to androgen insensitivity in the human disease. While polyglutamine stretches have intrinsic toxicity, Kennedy's disease demonstrates how the specific protein context in which an expanded polyglutamine tract appears can exert an important influence on the unique clinical and neuropathological features of a particular polyglutamine repeat disorder. With a variety of polyglutamine repeat disease models now available, it should be possible to begin differentiating those mechanisms of neurotoxicity that are common to the models, and thus likely related to the intrinsic polyglutamine expansion, from determinants that are model-specific, and therefore likely dependent on the protein context.

The reliance of many *Drosophila* models on phenotypic endpoints in the eye raises important questions about how closely a model should mirror a disease in order to be useful for translational research. Although we have

emphasized parallels between *Drosophila* models and their cognate human diseases, a model's success ultimately hinges on whether lessons learned are validated as relevant in humans. Given the amount of research time and effort required for such validation, one approach is to build as much confidence as possible that the pathological features observed in a model closely mirror those characteristic of the human disease prior to initiating further study. This might be accomplished by directing transgene expression to the *Drosophila* brain, recapitulating relative cell type specificity, documenting degenerative cell death, and demonstrating appropriate histopathological markers. Although recapitulating such features of neurodegenerative diseases can bolster confidence in a *Drosophila* model, a rapid screening substrate is also required to fully take advantage of the genetic potential of the system. As described further below, genetic screens for modifiers of retinal toxicity have now been widely used and, encouragingly, some of the initial findings are consistent with results from mouse models of neurodegenerative disease.

Genetic modifiers of neurodegeneration

With numerous fly models of neurodegenerative disease now available, investigators are beginning to harness the power of *Drosophila* genetics for the rapid identification and dissection of pathways that modulate neuronal loss. The usual strategy is to study whether gain or loss of function of a given gene in the context of a particular neurodegenerative disease model can enhance or suppress neurodegeneration. Two complementary approaches are utilized. The candidate approach tests a specific hypothesis that a genetic pathway plays an important role in a given disease process. For example, coexpression of apoptotic inhibitors can suppress both polyglutamine and tau toxicity in flies, implicating programmed cell death pathways as important in both of these models [21,28•]. An alternative approach is to use genetic screens to conduct an unbiased survey of the *Drosophila* genome for genetic modifiers. Such screens have been used to validate an important role for protein misfolding in polyglutamine toxicity [27,29].

Since large protein aggregates are a common neuropathological feature of neurodegenerative disease, it has been postulated that cellular derangements in protein folding and degradation pathways might be an important determinant of neuronal loss. The heat shock proteins and chaperones are key regulators of protein folding and serve as an important defense mechanism against protein denaturation during periods of cellular stress. In addition, misfolded proteins which accumulate in the cytosol are often targeted for destruction by the proteasome, a large, proteolytic machine that recognizes ubiquitinated proteins. Thus, it was postulated that derangements in either of these cellular systems might be important in neurodegenerative diseases. Several genetic modifier studies with *Drosophila* models of polyglutamine repeat diseases have now provided in-vivo support for this hypothesis. In two candidate studies, coexpression of the human heat shock protein, HSP70, attenuated the retinal degeneration observed in *Drosophila* models of spinocerebellar ataxia, type 3 (SCA3) and spinal and bulbar muscular atrophy [24,30]. In addition, endogenous *Drosophila* HSP70 was found to colocalize with nuclear inclusions formed by polyglutamine expanded forms of SCA3 or the androgen receptor. To test the hypothesis that proteasome function might also participate in neurodegeneration, a dominant negative proteasome subunit was coexpressed along with the polyglutamine expanded androgen receptor [24]. Disruption of proteasome function significantly enhanced toxicity and appeared to correlate with a decrease in monomeric androgen receptor, supporting increased aggregation. These results have been complemented by two unbiased genetic screens for modifiers of polyglutamine toxicity, one based on a model expressing an isolated polyglutamine stretch and the other using a mutant form of SCA1 with an expanded polyglutamine stretch [27,29]. Both studies recovered a *Drosophila* homolog of human HSP40, which activates HSP70, as potent suppressors of polyglutamine-induced retinal degeneration. Suppression by *Drosophila* HSP40 was shown to correlate with a reduction in size of SCA1 nuclear inclusions, suggesting a link between the effect of modification and protein aggregation [29]. The SCA1 genetic screen also recovered mutations in the *Drosophila* genes for ubiquitin and ubiquitin ligase as enhancers of retinal degeneration, further supporting a role for the ubiquitin–proteasome degradation pathway as a determinant of polyglutamine toxicity. Consistent with observations in *Drosophila* models, expression of HSP70 or mutation of a ubiquitin ligase has also been found to enhance or suppress polyglutamine toxicity in mice, respectively [31,32].

The preponderance of genetic evidence implicating protein folding and degradation pathways in polyglutamine repeat diseases has naturally raised the question of whether these mechanisms might also be important in other neurodegenerative diseases where protein aggregates are a prominent neuropathological feature, such as the Lewy body seen in Parkinson's disease or the neurofibrillary tangles in Alzheimer's disease and frontotemporal dementia. Indeed, an important role for chaperones was recently generalized to a *Drosophila* model of Parkinson's disease with the demonstration that coexpression of HSP70 can protect dopaminergic neurons against α-synuclein toxicity [33••]. Conversely, expression of a dominant-negative form of HSP70 appeared to accelerate dopaminergic cell loss. One distinct advantage of *Drosophila* disease models is the

capability to rapidly test genetic modifiers among several systems with the potential to reveal unifying features or key distinctions between the pathogenesis of these diseases.

The candidate approach to identifying genetic modifiers is hypothesis-driven. While this strategy is powerful as a rapid method to test an idea, it is limited by bias from preconceptions about mechanisms of disease pathogenesis. Thus, one advantage of unbiased genetic screens is their unique ability to identify completely novel and unexpected gene modifiers. For example, a genetic screen for modifiers of polyglutamine toxicity identified a conserved *Drosophila* homolog of human myeloid leukemia factor-1 as a potent suppressor of retinal degeneration [34•]. Significantly, this protein was colocalized to polyglutamine nuclear inclusions, and was able to extend the life-span of polyglutamine expressing flies. Compared with gene candidates that fit neatly into preexisting hypotheses about disease pathogenesis, it can be much harder to understand how novel genes modulate neurodegeneration. The ability to implicate entirely novel or unexpected genes as in-vivo modulators of neurodegeneration, however, is a unique feature of *Drosophila* models. Whereas candidate testing is possible, though slower, in mouse models, genetic screens are not yet technically practical in mammals. Thus, a unique niche for *Drosophila* in neurodegenerative disease research lies in comprehensive genome-wide screens.

Translational research with *Drosophila* models

Where do *Drosophila* models of neurodegenerative disease fit in the spectrum of bench to bedside research? Rapid and whole-genome genetic approaches available in flies are ideally suited for generating lists of candidate genes for further investigation and possible drug development. Modifier genes may also be excellent candidates for susceptibility loci for human neurodegenerative disease. One paradigm is to exploit *Drosophila* models to generate target lists that can subsequently be tested in mammalian models before entering development for clinical therapeutics or human genetic studies. For example, this potential synergy between *Drosophila* and mouse models is exemplified by recent studies of a link between histone acetyltransferases and neurodegeneration in Huntington's disease. Following the discovery that mutant huntingtin protein directly binds and inhibits histone acetyltransferases, a *Drosophila* Huntington's disease model was used to test *in vivo* whether changes in histone acetyltransferase activity might modulate huntingtin neurotoxicity [23]. Mutations in *sin3A*, encoding a component of a histone deacetylase complex, attenuated the retinal degeneration and early adult lethality caused by the expression of polyglutamine-expanded huntingtin. Feeding the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA, Aton Pharma: Tarrytown, New York, USA) had a similarly beneficial impact on huntingtin transgenic flies. Following these encouraging results in *Drosophila*, SAHA has been further tested as a therapeutic agent in a mouse model of Huntington's disease [35•]. After administering SAHA to mice with drinking water, SAHA crossed the blood–brain barrier, increased histone acetylation in the brain, and significantly ameliorated motor impairments. Since SAHA and similar histone deacetylase inhibitors are currently being tested in clinical trials as anticancer agents, similar trials in patients with Huntington's disease should begin shortly.

If past is prologue, however, success in an animal model is not always a predictor of efficacy in human clinical trials. For example, despite promising results in mouse models, gabapentin and vitamin E both subsequently failed as therapies for amyotrophic lateral sclerosis in humans [36]. Perhaps success in two different species will be a stronger predictor of drugs that will survive the leap to human trials. Nevertheless, once convinced that a particular fly model is as good as existing mammalian models, should insights from *Drosophila* models be the trigger for drug design and clinical trials, such as when a mammalian model does not yet exist or results have been equivocal? For example, a recent study in a *Drosophila* model of tauopathy demonstrated that the well-known tau kinase, glycogen synthase kinase-3β (GSK-3β), could promote tau phosphorylation, aggregation, and retinal toxicity [28•]. Given the ability of GSK-3β to promote pathologic tau phosphoepitopes, it has long been a potential candidate for pharmacological inhibition; however, similar in-vivo tests in murine models have produced conflicting results [37,38]. In another example, a successful 'preclinical trial' in a *Drosophila* model of Huntington's disease was based on the identification of peptides that interfere with huntingtin aggregation in cultured cells [39•]. Coexpression of these peptides attenuated aggregation, retinal toxicity, and lethality caused by expanded polyglutamine repeats. Similar approaches with modified peptides or agents with similar activities might therefore be a viable strategy for intervention in human polyglutamine repeat disease.

Successful preclinical trials of therapeutic agents in flies presage the use of *Drosophila* models for large-scale, comprehensive screens of drug and chemical 'libraries'. Such in-vivo drug screening would be a powerful complement to existing in-vitro or cell culture-based approaches. Whereas existing methods for drug screening usually target a specific enzyme or biochemical pathway implicated in disease, drug screening in *Drosophila* can more broadly target agents which disrupt any step in the pathogenetic cascade since the ultimate

read-out is a phenotypic endpoint of neurodegeneration *in vivo*. Indeed, once confident that a *Drosophila* model recapitulates key pathological features of a human disease, it is not even necessary to understand the mechanisms underlying toxicity in order to begin screening for potential drugs. One potential downside to this approach is that once an effective drug is identified its molecular target may not be immediately obvious. The results of drug screens, however, can be cross-matched with the results of genetic screens conducted in parallel: modifier genes may thus provide clues to the biochemical pathways targeted by candidate drugs.

Conclusion

Although still a relative newcomer to neurodegenerative disease research, *Drosophila* is rapidly making significant contributions. The extensive homology of the human and *Drosophila* genomes forms the basis for ongoing loss-of-function studies, and transgenic approaches have facilitated the development of *Drosophila* models that recapitulate key neuropathological features of Alzheimer's disease, Parkinson's disease, Huntington's disease, and several spinocerebellar ataxias. Genetic modifier studies based on these models have identified protein folding and degradation pathways as in-vivo determinants of neurotoxicity in both polyglutamine repeat diseases and Parkinson's disease. *Drosophila* has thus begun to assume an important niche alongside the mouse for modeling neurodegenerative disease. Despite their great promise, however, the ultimate potential for translating lessons from animal models to the bedside in the form of new therapies has yet to be fully realized. The development of a novel agent for pharmacotherapy, its subsequent testing in animals, and its ultimate validation in human clinical trials is a long and expensive process. Over the next few years, the advent of large-scale genetic screens in *Drosophila* disease models will likely result in an overwhelming list of modifier genes for further research as potential drug targets. A major challenge for the future will be determining which targets should be prioritized for further investigation.

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