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Drosophila in the Study of Neurodegenerative Disease

Review

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As populations benefit from increasing lifespans, neurodegenerative diseases have emerged as a critical health concern. How can the fruit fly, *Drosophila melanogaster*, contribute to curing human diseases of the nervous system? A growing number of neurodegenerative diseases, as well as other human diseases, are being modeled in *Drosophila* and used as a platform to identify and validate cellular pathways that contribute to neurodegeneration and to identify promising therapeutic targets by using a variety of approaches from screens to target validation. The unique properties and tools available in the *Drosophila* system, coupled with the fact that testing in vivo has proven highly productive, have accelerated the progress of testing therapeutic strategies in mice and, ultimately, humans. This review highlights selected recent applications to illustrate the use of *Drosophila* in studying neurodegenerative diseases.

Diseases Can Be Modeled in Flies

Many of the classic studies of brain functional genetics used conventional loss-of-function approaches (Hotta and Benzer, 1970; Lin et al., 1998; Lush et al., 1998; Min and Benzer, 1999), whereas more recent studies have focused on investigating fly homologs of human disease genes. In 1998, the first transgenic *Drosophila* model of a human neurodegenerative disease for spinocerebellar ataxia 3 (SCA3) was described (Warrick et al., 1998), closely followed by a transgenic model for Huntington's disease (Jackson et al., 1998). Over the past several years, the use of fly models of human diseases has emerged at a rapid pace and has significantly contributed to our growing understanding of the molecular basis of these diseases (for reviews, see Bilen and Bonini [2005] and Sang and Jackson [2005]). Dominant gain-of-function (GOF) diseases including the polyglutamine-repeat diseases, Parkinson's disease (PD) (overexpression of mutant and wild-type α-synuclein), and tauopathies have been effectively investigated with transgenic approaches. In addition, *Drosophila* models for diseases associated with genetic mutations that cause a reduction of the normal levels or activity of endogenous proteins such as parkin, presenilin, DJ-1, and others have emerged. Modeling the dominant diseases is accomplished by "humanizing" a fly to produce transgenic animals expressing the pathogenic form of a human disease gene. A binary system is used for this purpose (Figure 1), and transgenic flies containing a tissue-specific promoter fused to the yeast GAL4 transcription factor are crossed to flies containing the gene of interest fused to the yeast upstream activator sequence (UAS), such that offspring will express the human disease gene only in selected tissues in a controlled manner (Brand and Perrimon, 1993; Brand and Dormand, 1995). Typically, expression is driven in neurons (e.g., *elav* driver) or in all cells of the eye (e.g., *gmr* driver), and neurotoxicity is monitored by measuring the loss of visible photoreceptor neurons in the eye, lethality of the organism, or behavioral phenotypes (for reviews, see Bilen and Bonini [2005]; Marsh and Thompson [2004], and Sang and Jackson [2005]), although many other measures of degeneration can be used. Transgenes expressing short hairpin RNAs to interfere with expression of specific genes also make use of this binary system (Figure 1). This system allows one to maintain toxic proteins separate from the elements that drive their expression, thus avoiding the selective pressures that might lead to the accumulation of modifiers or the selection of mutated transgenes. This system also allows one to modulate the levels of transgene expressed by capitalizing on the temperature dependence of the GAL4 system (Duffy, 2002).

How Fly Models Can Complement Other Systems

In studying human neurodegenerative diseases, one typically employs multiple systems, including cell-based models in which one can generate stably expressing lines and phenocopy cellular aspects of disease. However, in many cases, the response of the intact organism is not fully recapitulated in cell lines. In vitro, intersecting physiological pathways and responses (e.g., neurotransmitter circuitry and interactions with support cells, etc.) are eliminated, nonautonomous cellular influences are removed, and new parameters such as those used to immortalize cells, are often introduced, thus reducing the ability of cultured cells to mirror in vivo pathology. It can also be very difficult to obtain a functional measure of the impact of pathogenic proteins in in vitro systems. The possibility that cells in culture may represent a subset of cells that do not reflect the diversity of neurons of the adult brain is illustrated in *Drosophila*. Challenge of the *Drosophila* brain mushroom-body neurons in vivo with expanded repeat Huntington (Htt) leads to approximately 25% loss of cells (volume) (Agrawal et al., 2005). However, dissociation and culturing of those brains from expressing animals yields cells that have a reduced plating efficiency in comparison to nonchallenged cells, although the cells that do become established in culture show no progressive death when they are compared to nonchallenged cells plated in parallel (O'Dowd and J.L.M., unpublished data). It is not clear whether

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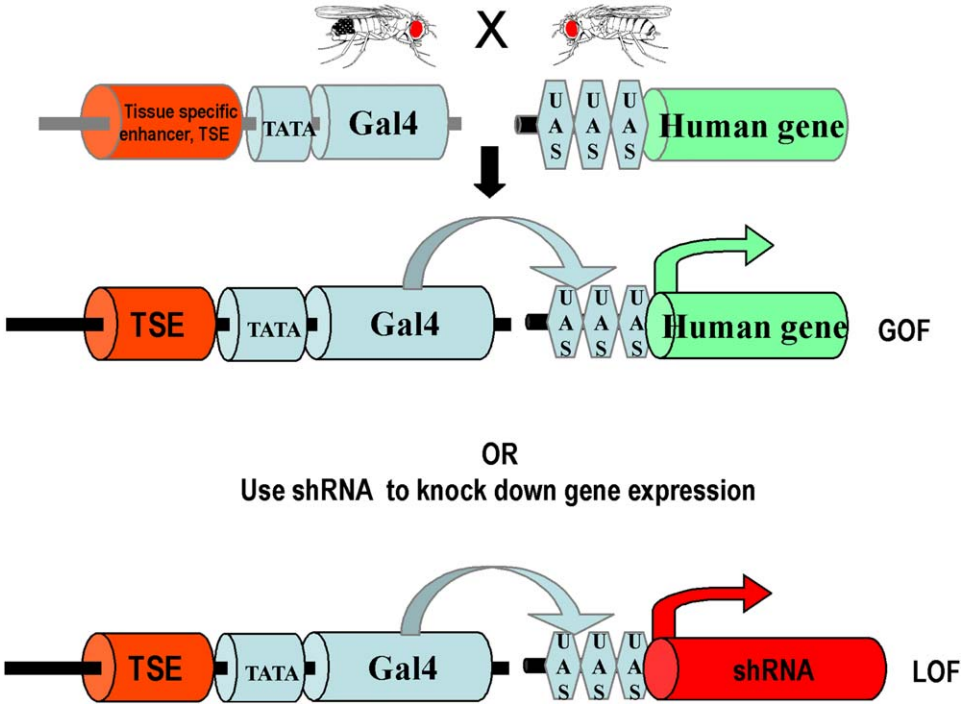

Figure 1. Humanized Flies Express Human Genes in *Drosophila* with the Binary GAL4/UAS System
Genes can be either mutant disease genes or modifiers of human disease genes.

conditions in culture are different from those in vivo or whether culturing selects a subset of neurons with different susceptibilities to pathogenic challenge. In contrast, although mice and other mammalian model systems offer in vivo opportunities and extensive similarity to the human brain, the length of time and cost required to perform experiments comparable to those possible in flies can be prohibitive.

Flies, on the other hand, allow excellent genetic manipulation and in vivo readouts of pathology, and the pathways are considered generally highly conserved with vertebrates, with approximately 75% of human genes known to be associated with disease having a *Drosophila* ortholog (Reiter et al., 2001). *Drosophila* is emerging as a model animal with broad applications for rapidly addressing mechanistic questions and testing therapeutic options because flies can be engineered to exhibit many of the symptoms of human disease. As would be expected from mammalian studies, for instance, decreased glutamate buffering in *Drosophila* is neurotoxic and provides a genetic system for analysis of glutamate-mediated neurodegeneration (Rival et al., 2004). For instance, flies recapitulate characteristic aspects of neurodegenerative diseases such as the polyglutamine-repeat diseases. Fundamental characteristics reflected in the fly include polyglutamine length-dependent pathology that is of later onset (late in larval or pupal stages), is progressive, causes motor abnormalities, and causes early death (Marsh and Thompson, 2004).

Insights from Flies into Human Neurodegenerative Diseases

Drosophila has been used to model neurodegenerative diseases ranging from transgenic models of dominant polyglutamine-repeat diseases, tauopathies, Alzheimer's disease (AD), and Parkinson's disease (PD) to expanded triplet-repeat diseases in noncoding DNA (e.g., fmr1, SCA8). In addition, fly models have been generated to study autosomal-recessive loss-of-function mutations that cause familial forms of PD (e.g., DJ-1, parkin, and PINK1) and AD (e.g., presenilin, APPL) (for reviews, see Bilen and Bonini [2005]; Sang and Jackson [2005]; Seidner et al. [2006], and Whitworth et al. [2006]). Selected examples that illustrate how these models can provide insight into neurodegenerative diseases are described here.

1. Tauopathy

A key feature of AD and other "tauopathies" is the neurofibrillary tangle, characterized by the accumulation of hyperphosphorylated tau, but the range of cellular processes that can impact this process is unclear. Tau pathology has been effectively modeled in flies (e.g., Mudher et al. [2004]; Wittmann et al. [2001]; Karsten et al. [2006], and Shulman and Feany [2003]), producing morphologic effects such as axonal degeneration and swelling. These flies have effectively been used to illustrate the involvement of specific pathologic processes, such as tau phosphorylation, in eliciting these responses. For instance, tau overexpression in combination with phosphorylation by the *Drosophila* GSK-3 ortholog (Shaggy) significantly enhances tau-induced neurodegeneration and leads to the formation of neurofibrillary pathology (Jackson et al., 2002). Furthermore, PAR-1 kinase is involved in the tau phosphorylation process in *Drosophila* (Nishimura et al., 2004).

2. Aβ Toxicity

A second hallmark of AD is the formation of amyloid plaques. Because *Drosophila* do not have endogenous

BACE for complete processing of human APP to the Aβ peptide (flies do encode γ-secretase components), several groups have used misexpression of Aβ in flies to recapitulate amyloid-plaque formation in a genetically tractable model and thus provide models for studying molecular mechanisms underlying Aβ toxicity and identify potential genetic and pharmacologic modifiers. Targeted expression of Aβ42 caused neurodegenerative phentoypes, amyoid deposits, and learning deficits, whereas Aβ40 expression only caused learning deficits (Finelli et al., 2004; Iijima et al., 2004), confirming a requirement for the Aβ42 peptide in pathology. Using an alternate approach, Greeve et al. (Greeve et al., 2004) used targeted expression of APP, the APP-cleaving enzyme BACE, and presenilins, and this approach also led to β-amyloid-plaque formation and age-dependent neurodegeneration. A genetic screen showed that *Drosophila* neprilysin 2 suppressed the Aβ phenotype (Finelli et al., 2004), and secretase inhibitors also suppressed neurodegenerative phenotypes (Greeve et al., 2004), further supporting both the validity of the models and the modifiers themselves as attractive targets. Wild-type and arctic mutant peptides of Aβ42 were also expressed in *Drosophila;* these experiments resulted in intracellular Aβ accumulation, nonamyloid aggregates, progressive locomotor deficits, vacuolation of the brain, and premature death of flies (Crowther et al., 2005). Of note, the extent of the neuronal phentoype was dependent upon the propensity of the expressed Aβ peptide to form oligomers, highlighting the utility of *Drosophila* in modeling key aspects of human disease.

3. Parkinson's Disease

Drosophila models of PD underscore the powerful use of genetics to define pathways that are involved in human disease as well as the effectiveness of using both transgenic and loss-of-function approaches. A number of human genes have been linked to PD and include dominant mutations in α-synuclein, UCH-L1, and LRRK2 as well as autosomal-recessive mutations in parkin, DJ-1, and most recently, PINK1 (for review of fly models of PD and the corresponding human mutations, see (Bilen and Bonini, 2005; Sang and Jackson, 2005; Whitworth et al., 2006). Both gain-of-function and loss-of-function genetic causes of PD can be modeled in flies. Overexpression of either wild-type α-synuclein or mutant forms associated with human disease leads both to motor abnormalities and to loss of dopaminergic neurons in the CNS (Auluck and Bonini, 2002; Feany and Bender, 2000). Loss-of-function mutants of *Drosophila* parkin (Greene et al., 2003; Pesah et al., 2004) have been reported and used to implicate pathogenic mechanisms, including disturbed mitochondrial function. The recent identification of autosomal-recessive mutations in Pink1, which encodes a Ser/Thr kinase with a mitochondrial-targeting signal associated with PD (Valente et al., 2004), offered the opportunity to use *Drosophila* as the first in vivo model of PINK1 to investigate whether reduction of PINK1 function could cause PD-like phenotypes and to determine how this model compares to other loss-of-function models of PD (Clark et al., 2006; Park et al., 2006; Wang et al., 2006; Yang et al., 2006). When dPINK1 function is removed, apoptotic muscle degeneration, defects in mitochondrial morphology, increased sensitivity to oxidative stress, and male sterility develop, and evidence of mitochondrial dysmorphology is observed (Clark et al., 2006). Similarly, a mitochondrial basis for dopaminergic neuronal degeneration accompanied by locomotor defects and indirect flight-muscle degeneration was found (Park et al., 2006). Similar phenotypes were observed in two further studies (Wang et al., 2006; Yang et al., 2006). As additional support for the interchangeability of flies and human systems, human Pink1 could rescue the fly mutants. Expression of human SOD1 and antioxidant treatment suppressed neurodegeneration in these flies (Wang et al., 2006), again supporting a role for oxidative stress and mitochondrial function in the disease. One of the striking aspects of these studies was the unexpected finding that PINK1 and parkin appear to act in a common pathway that influences mitochondrial integrity; flies lacking parkin are nearly identical to flies lacking PINK1, and upregulation of parkin rescued flies lacking PINK1, suggesting that at least some of the genes involved in heritable forms of PD act through common pathways and identifying a pathway that warrants further investigation as a therapeutic target. This is a nice example of how genetic manipulations in *Drosophila* provided insight into a pathogenic pathway.

4. SCA1

A critical question for most neurodegenerative diseases relates to the selectivity observed in regions of the brain most susceptible to neuronal loss. It is thought that specific protein interactions may be responsible for the regional specificity observed. A recent study using *Drosophila* (Tsuda et al., 2005) suggests that this may be true for Purkinje cell degeneration through an investigation of protein interactions with *Drosophila* ataxin-1 (dAtx-1). dAtx-1, which does not have the polyglutamine-repeat domain but does have a conserved AXH domain, interacts through this AXH domain with *Drosophila* and mammalian Senseless/GFi-1. When ataxin-1 is overexpressed in flies, sensory-organ development is inhibited as a result of decreased senseless protein, and hAtx-1 overexpression similarly reduces Gfi1 in Purkinje cells. Significant to the SCA1 purkinje cell phenotype, deletion of the AXH domain abolishes reduction of Gfi-1, and targeted reduction of Gfi-1 mimics the SCA1 phenotype. Similarly, another ataxin-1 interactor, brother of ataxin-1 (Boat), is also reduced in Purkinje cells in mice, and this ataxin-1 association with Boat can suppress a mutant ataxin-1-mediated eye defect in flies (Mizutani et al., 2005).

Other neurodegenerative diseases have been modeled in flies, but those discussed above illustrate several recent examples of how studies in *Drosophila* can lead to the articulation of a pathogenic pathway (PD, PINK1, and parkin), can provide insight into the mechanisms of selective regional degeneration (SCA-1), and can help dissect the pathogenic contributions of selective protein processing products (tau and Aβ). One can expect continuing insights to emerge from *Drosophila* studies in other systems.

Strategies for Using *Drosophila*

Drosophila can be used to devise productive testing paradigms ranging from genetic screens to target validation (Figure 2).

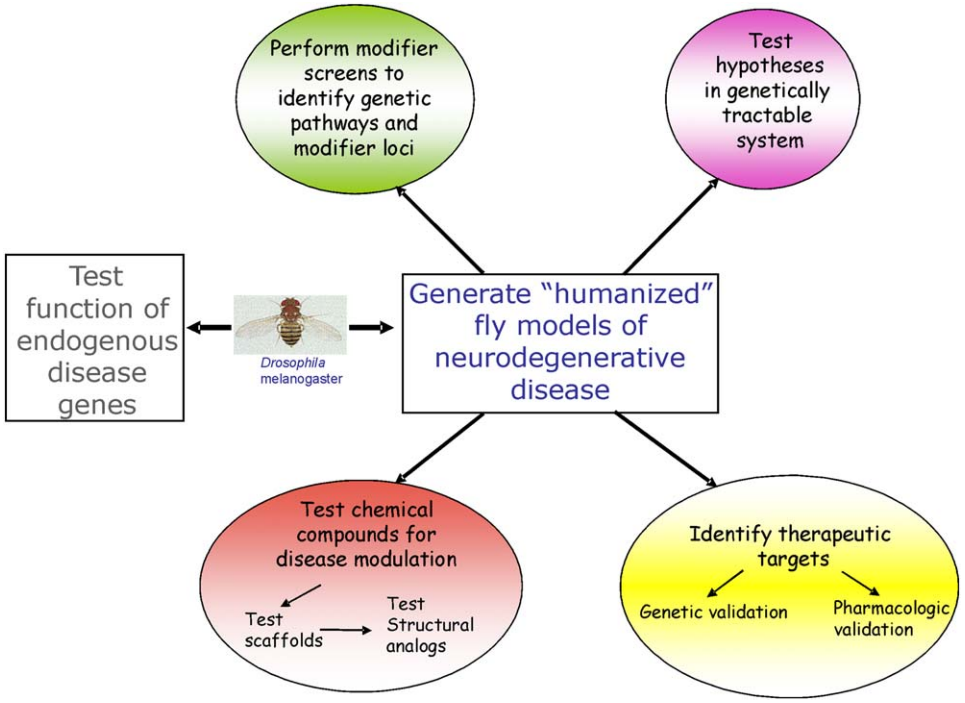

Figure 2. Schematic of Utility of *Drosophila* Models of Neurodegenerative Disease

Genetic-Modifier Screens

The power of *Drosophila* genetics can be used to conduct genetic modifier screens that will identify proteins and genetically interacting pathways that modulate pathology. These types of screens were among some of the first efforts to use fly models of neurodegenerative diseases to identify pathogenic mechanisms and highlighted the importance of heat-shock proteins/chaperones, the protein-degradation machinery, and transcriptional regulatory proteins in polyglutamine-repeat diseases (Fernandez-Funez et al., 2000; Kazemi-Esfarjani and Benzer, 2000). Similarly, kinases and phosphatases emerged as major modifiers in a genetic-modifier screen of a *Drosophila* model of tauopathy with candidate tau kinases shown to enhance tau toxicity (Shulman and Feany, 2003), as would be anticipated for diseases associated with phosphorylation of tau. Such screens can identify pathways that are not necessarily cell autonomous and that thus could be missed in screens with cultured cells. Clues to the direct or indirect nature of identified genetic interactions can then be found with colocalization studies followed by biochemical studies. Furthermore, if biochemical studies identify an interacting protein, the involvement of that protein in a particular pathway can be functionally explored in vivo by genetically manipulating the identified gene in combination with other genes in the pathway, so that multiple gene responses may be used to confirm involvement of the pathway (e.g., Steffan et al. [2001]). In addition, suspect genes can be manipulated both by reducing dose and by increasing dose via overexpression constructs. Observing the expected opposite effects on pathology provides encouraging evidence for a *bona fide* impact of that activity on pathogenesis. A recent application of using a genetic-validation screen in *Drosophila* as part of a cross-species functional-genomic approach yielded tau modifiers including puromycin-sensitive aminopeptidase (PSA/Npepps), which was protective when overexpressed and deleterious when reduced (Karsten et al., 2006). These data were further supported through studies showing that human PSA directly cleaves tau in vitro.

Hypothesis Testing

As well as being a powerful tool for identifying novel pathways, *Drosophila* can be employed in testing specific hypotheses that arise either from primary genetic or pharmacologic screening with flies or from studies in other systems. The facile genetic manipulations that are available make it possible to rapidly manipulate a variety of proteins in any given pathway and provide confirmation that the expected manipulations are relevant as hypothesized. Below, we provide some examples of hypothesis testing in flies with an emphasis on polyglutamine-repeat diseases.

Transcriptional Dysregulation. A variety of studies in mammalian cells and brain tissue have implicated transcriptional dysregulation in Huntington's disease (HD) (Cha, 2000), and subsequent studies have suggested that global transcriptional regulators such as acetyltransferases might be bound and affected by mutant Htt and other expanded polyglutamine-containing proteins (Hughes et al., 2001; McCampbell et al., 2001; Steffan et al., 2001). Through the use of Drosophila, it was possible to test the explicit hypothesis that reduction of neuronal histone acetyl transferase activity contributes to pathology in vivo by demonstrating that inhibition of the counteracting activity, HDACs, either genetically or pharmacologically, is able to suppress pathology. Subsequent studies in mice confirmed this observation (Ferrante et al., 2003; Hockly et al., 2003) and have rapidly led to human trials (Huntington's Study Group, http://www.huntington-study-group.org/). Such

trials have been facilitated by the availability of compounds already deemed appropriate for use in humans (e.g., through safety and tolerability trials) and used clinically for other disorders.

In other non-polyglutamine-repeat applications, gene-expression profiling in fly models of PD has provided insight into the temporal pattern of transcriptional dysregulation versus overt neurodegeneration (Scherzer et al., 2003). These results support a common mechanism of both PD and polyglutamine-repeat diseases whereby transcriptional changes are early events in pathogenesis prior to overt phenotypes.

Chaperones. The pathology-suppressing role that was suggested for HSPs by studies in cells, flies, and mice (for review, see Muchowski and Wacker [2005]) was nicely explored both genetically and pharmacologically in Drosophila, resulting in a potential pharmacologic strategy for PD (geldanamycin) (Auluck and Bonini, 2002; Auluck et al., 2002), and this strategy has subsequently been demonstrated to also be an excellent candidate strategy for HD (Agrawal et al., 2005). Recent application to a mouse model of SBMA further supports the translational promise of initial testing of therapeutic approaches in flies (Waza et al., 2006).

Axonal Transport. Initial studies suggested that expression of polyglutamine-repeat proteins in the cytosol and reduced Htt function cause disruption of axonal transport in flies (Gunawardena et al., 2003). Again suggesting common mechanisms among neurodegenerative diseases, deletion of the *Drosophila* homolog of APP and directed expression of hAPP cause similar effects (Gunawardena and Goldstein, 2001). To investigate nonnuclear and nuclear effects of mutant Htt expression on axonal transport, Lee et al. (Lee et al., 2004) used fly models that formed cytosolic aggregates (mutant Htt) and also showed disrupted axonal transport and accumulation of polyglutamine aggregates at synapses. In contrast, when expanded polyglutamine tracts alone or those in a SCA3 protein context were expressed, only nuclear aggregates were observed, with no disruption of axonal trafficking, suggesting that a combination of nuclear and nonnuclear events plays a role in HD pathogenesis. These results are supported by findings in mice with NLS and NES targeted Htt exon 1 polypeptides (Benn et al., 2005; Schilling et al., 2004).

Autophagy/Protein Clearance. Clearance of misfolded proteins is significantly impaired in most of the late-onset neurodegeneration diseases, and decreased proteasome activity and autophagy have been investigated (Thoreen and Sabatini, 2004). Ravikumar et al. tested the hypothesis that mTOR sequestration into polyglutamine aggregates is protective by using rapamycin (a specific mTOR inhibitor) and found that rapamycin treatment induces autophagy and suppresses neurotoxicity in both fly and mouse models of HD (Ravikumar et al., 2004). Rapamycin was found to also reduce paraquat toxicity in flies and protect cells against a range of pro-apoptotic insults (Ravikumar et al., 2006), potentially via the upregulation of autophagy and enhanced clearance of mitochondria to reduce cytochrome c release.

Protein Modification. Studies in *Drosophila* have highlighted the role of posttranslational modifications in affecting pathology and have thus opened a new area for development of potentially therapeutic interventions. For example, inhibition of SUMO modification suppresses pathology in a *Drosophila* model of HD (Steffan et al., 2004); in contrast, in an SBMA model, pathology caused by expressing an amino-terminal fragment of the human androgen receptor (hAR) containing an expanded polyglutamine repeat was enhanced by overexpression of a mutant Uba2 (activating E1 enzyme) (Auluck et al., 2002). A role for protein modification is further underscored by the observation that 14-3-3 protein binds to and stabilizes ataxin1. This association is regulated by AKT phosphorylation, and in flies, both 14-3-3 and AKT modulate neurodegeneration (Chen et al., 2003).

Protein Function and Quality Control. The influence of the normal function of the disease protein and the involvement of protein quality-control pathways, each central issues in neurodegenerative pathology, have been linked in studies of SCA3. Human ataxin-3 is a polyubiquitin-binding protein with ubiquitin protease activity. The authors show (Warrick et al., 2005) that in flies this protein suppresses polyglutamine neurodegeneration, which requires the ubiquitin-associated activities of the protein and proteasome function. These results also highlight the importance of disease-protein activity.

Ubiquitination plays a similar role in SCA1 pathogenesis, although in this case through an interaction with the ataxin-1 protein. CHIP (C-terminus of Hsc-70-interacting protein) directly interacts with ataxin-1 and promotes ubiquitination of expanded ataxin-1 in vitro and in cells (Al-Ramahi et al., 2006). Hsp70, which suppresses toxicity in several models of polyglutamine-repeat disease, increases this CHIP-mediated ubiquitination. When tested in flies, overexpression of CHIP suppresses neurotoxicity and decreases mutant ataxin-1 protein levels and thus demonstrates in vivo efficacy. CHIP was also protective in other fly models of polyglutamine-repeat disease but, significantly, did not suppress toxicity in flies expressing only a polyglutamine-repeat (127Q) polypeptide in the absence of disease-protein linkage. These observations suggest common mechanisms among the polyglutamine-repeat diseases with respect to systems regulating protein function and quality control.

Mechanisms of Neuronal Loss. Sang et al. (Sang et al., 2005) assessed the contribution of specific cell-death regulators in polyglutamine-induced cell death. Depletion of the *Drosophila* ortholog of Apaf 1 (Dark) dramatically rescues neurotoxicity elicited by expression of expanded polyglutamine repeats alone or Htt exon1 in flies. A mechanism suggested by these fly studies is that Dark facilitates accumulation of polyglutamine aggregates. Furthermore, expression of expanded polyglutamines was found to induce an increase in Dark expression. A common role for Dark/Apaf 1 across models and species was suggested by the colocalization of Apaf-1/Dark with Htt-containing aggregates in flies, mouse brain, and human brain tissues.

Other Mechanistic Studies. An extensive number of other investigators have effectively used *Drosophila* to focus on mechanisms involving a broad range of cellular processes. These include acceleration of aggregate nucleation kinetics by nonpathogenic polyglutamine

proteins (Slepko et al., 2006), p53 activation (Bae et al., 2005), EGFR signaling and glutamate transporter function (Lievens et al., 2005), and the interaction of parkin and α-synuclein (Haywood and Staveley, 2004; Yang et al., 2003) as well as the long-term effects of perturbations of ion channels and neuronal membrane excitability on the maintenance of neuronal integrity in *Drosophila* (Fergestad et al., 2006). The growing number of such studies underscores the fact that the similarities in fundamental cellular mechanisms that affect pathology between the fly and mammalian systems become more evident with every passing year.

Chemical-Compound Screens

Just as flies are good candidates for genetic-modifier screens, they are amenable to large-scale drug screening, particularly when the screening is automated and highly controlled for all variables, such as light/dark cycle, humidity, circadian effects on the assay, age, and other variables. However, flies, similar to most other intact organisms, are largely not amenable to "high-throughput" screening at levels achieved with yeast, cultured cells, or cell-free systems. However, flies have been productively employed for modestly high-throughput screening of chemical-compound libraries (e.g., EnVivo pharmaceuticals), for the testing of drugs that target a particular class of proteins, or for the testing of compounds in "secondary assays" arising from primary high-throughput small-molecule drug-discovery efforts in other systems.

The ability to use flies as a secondary screening assay for modest throughput testing of drug leads identified in other assays has proven to be a key strength of the fly model (e.g., Agrawal et al. [2005]; Apostol et al. [2003]; Ehrnhoefer et al. [2006]; and Zhang et al. [2005]; Figure 3] and may play a significant role in the drug-discovery pipeline. An example is the use of flies as a rapid means of performing preclinical testing of expanded polyglutamine-repeat-aggregation inhibitors that were first identified in yeast, cell culture, or membrane filtration assays. Although several inhibitors were found to inhibit aggregation in these studies, variables such as which compounds might be tolerated in an intact organism or might modulate a neurodegenerative phenotype in vivo could be tested in flies (Desai et al., 2006; Ehrnhoefer et al., 2006; Zhang et al., 2005). These studies led to the identification of new therapeutic targets, including several EGFR antagonists (Desai et al., 2006). Although flies possess a blood-brain barrier with several similarities (e.g., Daneman and Barres [2005]; Lane and Swales [1978], and Schwabe et al. [2005]) to the mammalian blood-brain barrier, it has proven possible to test many compounds by simply feeding them to flies. Using fly models to rapidly identify scaffolds that appear to be effective in vivo has allowed investigators to focus attention on some of the most promising leads.

Although in their infancy, other potentially powerful applications of *Drosophila* models include in vivo screening to test analogs of candidate drugs or scaffolds. Such testing in vivo has the advantage that it integrates the impact of various analogs on other cellular components to give a quick read-out of effective versus noneffective directions for further medicinal chemistry. Other types of drug testing involve overexpressing a human target protein to obtain a gain of function phenotype, which could be lethality or another readily monitored phenotype. Drugs and analogs of hits can then be screened for suppression of the overexpression phenotype as a rapid way to select high-potency analogs. Inhibitors that directly target the human protein, as well as inhibitors that block pathway elements

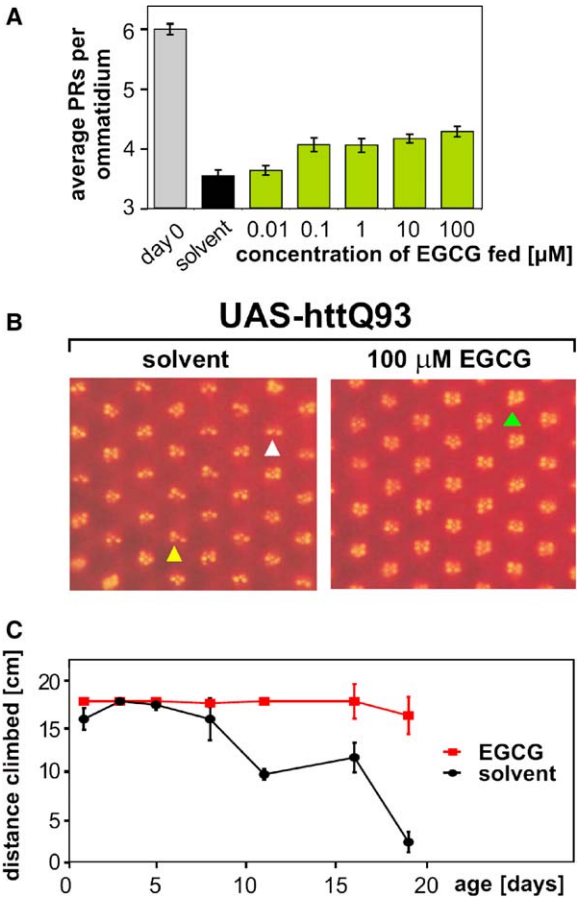

Figure 3. EGCG Diminishes the Toxicity of Mutant htt Fragments in Fly Models of HD

(A) Rescue of degeneration of photoreceptor neurons in *Drosophila* expressing mutant htt fragments with 93 glutamines. Newly eclosed flies were kept on food supplemented with different amounts of EGCG (0.01, 0.1, 1, 10, and 100 μM) or solvent only, and the average number of photoreceptor cells per ommatidium were scored 7 days post-eclosion via the pseudopupil technique. Error bars represent ± standard error. Day 0 shows the neuronal loss that has occurred during pupal development prior to drug treatment. Values for ≥0.1 μM are all significantly different ($p < 0.002$) from the no-drug control; 0.01 μM EGCG had no significant effect. Maximal rescue in the representative experiment shown was 29%.

(B) Representative deep-pseudopupil images of eyes from 7-day-old EGCG-treated and solvent-treated flies. Left panel: Solvent-treated flies show extensive degeneration with many ommatidia containing either three (white arrowhead) or four (yellow arrowhead) rhabdomeres. Right panel: Flies fed 100 μM EGCG show less degeneration; most ommatidia contain 5–6 rhabdomeres, with some retaining seven (green arrowhead).

(C) Age-dependent deterioration of climbing abilities in flies expressing mutant htt fragments with 93 glutamines (UAS-httQ93) in neurons was monitored by placement of the flies on the bottom of a vertical glass tube and measurement of the distance covered within 60 s. Flies mated and raised on food with 500 μM EGCG performed significantly better than solvent-treated controls. From Ehrnhoefer et al. [2006], by permission of Oxford University Press.

downstream of the transgene, can be detected in this type of screen (e.g., Bhandari and Shashidhara [2001]).

Target Validation

Target validation is a difficult but significant step in the effective development of a family of therapeutics for any disease. Compelling identification of the true in vivo target for any drug helps to channel efforts to develop and identify second-generation drugs that are hoped to be even more effective. Flies offer an opportunity to assist in this endeavor.

Under the term "target validation," it is beneficial to distinguish two levels of target validation. One can genetically validate a target protein by showing that reduction (or increase) in the activity of a particular gene leads to a desired affect. In that case, manipulation of the target gene or protein with drugs may be desirable [e.g., HDAC inhibitors (Steffan et al., 2001), Hsp90 Inhibitors that upregulate Hsp70 (Auluck and Bonini, 2002). Another level is pharmaceutical validation of a target protein; this can be accomplished when it can be demonstrated that several structurally distinct chemicals (scaffolds) known to biochemically target a particular protein in vitro produce the same desired phenotypic effect in living material. In one such study, the target was not validated, and the study suggests that a novel ligand-induced function of the polyglutamine hAR mutant is active in promoting neurodegeneration in SBMA (Furutani et al., 2005). However, such a resource of characterized scaffolds is not always available. In such cases, a combined genetic and pharmacological approach to validating a particular protein as the effective target of a drug in vivo can be useful.

For example, one approach to validating a drug target involves genetically deleting the target in question (either through use of characterized mutants or through RNAi approaches) and demonstrating that the drug has no effect when the target is removed. Alternatively, one can demonstrate that the level of drug necessary to achieve a particular response depends on the dose of the presumed target gene by using a series of loss- and gain-of-function mutations and transgenes (e.g., Marsh and Wright [1986]). Follow-up studies may include further "humanizing" the fly by replacing the endogenous target gene with the human homolog and testing whether the drug is still effective. Although these types of studies have not yet been highly utilized, they represent potentially useful areas for further development.

Limitations

What are some of the potential limitations of model organisms such as *Drosophila*? To the extent that a pathological response requires the interaction of the pathogenic protein with other cellular proteins, it is certainly possible that some of the proteins in *Drosophila* are different from the putatively interacting proteins in man. In this case, one could potentially obtain false positive or negative results of interaction if the human disease protein cannot interact with a diverged but genuine target protein in the fly. Remarkably, there are many cases in which a human protein is able to replace a *Drosophila* protein in a pathway as measured by the ability of an ectopically expressed human or mammalian protein to rescue mutations in fly genes. Examples of interchangeability between flies and man include those in the following references: Bhandari and Shashidhara [2001]; Grens et al. [1995]; Holley and Ferguson [1997]; McGinnis et al. [1990]; Ross et al. [2001], and Rothbächer et al. [1995]. However, some differences are apparent. For instance, secretion signals in the fly and in mammalian systems have diverged for some proteins (Ross et al., 2001), whereas other secretion signals are recognized across species.

An apparent lack of neurotrophic factors and neuregulins that are structurally related to mammalian factors, e.g., BDNF, has been described as a limitation to using flies in studying human neurodegenerative diseases. These factors are involved in functions such as axon guidance, targeting, and connectivity. However, a recent review described cellular, genetic, and functional data that demonstrate the existence of both neurotrophic and gliatrophic interactions in the *Drosophila* nervous system (Hidalgo et al., 2006) and showed that glial survival is maintained by the epidermal growth factor receptor (EGFR) signaling pathway in response to specific ligands including spitz, a transforming growth factor-α (TGF-α) signaling molecule, and vein, a neuregulin ortholog. The architecture of the fly nervous system contains separate specialized functions such as vision, learning and memory, and olfaction, and the existence of neuronal trophic factors is predicted by these similarities.

Conclusions

The range and diversity of studies highlighted here represent only a fraction of the emerging studies that use *Drosophila* to investigate fundamental cellular mechanisms that impact human pathology, from neurodegeneration to cardiac rhythm to many other disease states. These studies demonstrate that the similarities between fundamental cellular mechanisms that affect pathology in the fly and mammalian systems are becoming more apparent with every passing year. The low cost, rapid generation time, and large repertoire of genetic and developmental tools available with *Drosophila* allow fly studies to speed the progress of identifying promising therapeutic strategies for testing in mammals.

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