HUMAN NEURODEGENERATIVE DISEASE MODELING USING DROSOPHILA

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Abstract A number of approaches have been taken to recreate and to study the role of genes associated with human neurodegenerative diseases in the model organism Drosophila. These studies encompass the polyglutamine diseases, Parkinson’s disease, Alzheimer’s disease, and tau-associated pathologies. The findings highlight Drosophila as an important model system in which to study the fundamental pathways influenced by these genes and have led to new insights into aspects of pathogenesis and modifier mechanisms.

INTRODUCTION

A number of different experimental strategies utilize model organisms to investigate both the normal and aberrant functions of human disease genes. One approach that has received increasing attention for neurodegenerative disease modeling since its introduction several years ago involves expressing human disease proteins in the fruitfly Drosophila in order to analyze the resulting cellular phenotypes and uncover genetic modifier pathways. The long-term goal of this approach is to exploit the powerful genetic techniques available in Drosophila to identify candidate genes that might be relevant to human disease, but which might have escaped detection owing to well-known limitations of human genetic studies, such as complex patterns of inheritance, lack of sufficient family pedigree data, and population-based genetic heterogeneity. This directed expression approach is distinct from the classical forward genetic approach in Drosophila, in which mutants are selected and analyzed based on their initial mutant phenotype without prior...
knowledge of the molecular identity of the corresponding genes and proteins. The forward genetic approach, which typically seeks to understand gene function through loss-of-function genetic methods, has repeatedly proven itself to be extremely rewarding in model organism studies. However, this approach does not always lead to direct molecular parallels or insights that are immediately applicable to human disease. Ideally, both the loss-of-function and the directed expression approaches often can be simultaneously brought to bear on a specific biological process in Drosophila, taking full advantage of the experimental tools available for this organism to investigate the cellular and molecular basis of a disease. Many Drosophila neurodegenerative disease models utilize the fly eye as a tissue of choice for directed expression studies. The fly retina is composed of approximately 800 virtually identical unit eyes, termed ommatidia, which are arranged in a precise hexagonal array that makes up the adult eye. This highly regular structure amplifies minor perturbations in cell patterning, making them relatively easy to discern, and it also allows degenerative changes to be observed over time during the entire adult lifespan. In addition, the internal cellular architecture of the Drosophila retina has been extremely well characterized, and many imaging techniques and antibody reagents are available to probe the underlying defects associated with a particular degenerative phenotype. Finally, the fly eye is completely dispensible for survival and fertility of laboratory strains of Drosophila, facilitating genetic studies of neurodegenerative disease mechanisms.

Here we focus on a select group of fly models for specific human neurodegenerative diseases, which illustrate the use of both loss-of-function and directed expression studies in the analysis of human disease genes. Several of these Drosophila disease models have been reviewed elsewhere, with an emphasis on particular diseases or modifier genes for specific neurodegenerative disease models (Anderton 1999, Fortini & Bonini 2000, Muqit & Feany 2002, Ross 2002, Zoghbi & Botas 2002). The four human diseases that we consider here—polyglutamine repeat expansion disorders, Parkinson’s disease, Alzheimer’s disease, and frontotemporal dementia—are all characterized by prominent proteinaceous inclusions that accumulate in the extracellular milieu or intracellular compartments of affected neurons (Figure 1). These inclusions are morphologically distinct for each disease, and, in each case, their presence has both guided and confounded attempts to understand the respective diseases. The recreation of these characteristic pathological inclusions has been a driving force behind the development of most of the Drosophila neurodegenerative disease models. The remarkably similar neuronal inclusions that can be “artificially” produced in the fly emphasize the central premise of the direct modeling approach—that expression of a mutant human disease protein in the fly will reveal features of protein activity relevant to human pathogenesis. Underscoring this assumption are recent findings that over 50% of fly genes exhibit apparent homology to human genes, including conservation of entire genetic pathways (Rubin et al. 2000), and that at least 60%–70% of known human disease genes possess likely Drosophila counterparts (Fortini et al. 2000, Reiter et al. 2001). Although Drosophila genetics has classically been
applied to problems of development, it is increasingly becoming evident that the power of this approach can also be applied to late-onset biological processes such as human degenerative conditions. The successful development of several human neurodegenerative disease models in *Drosophila*, together with the high level of conservation between the fly and human genomes, offers the promise that the fruitfly will continue to serve as a valuable experimental model for learning about human disease mechanisms and exploring potential therapeutic interventions.

**MODELING HUMAN POLYGLUTAMINE DISEASE IN DROSOPHILA**

**Molecular Genetics of Polyglutamine Disease**

The polyglutamine diseases are a class of dominant human triplet repeat diseases associated with an expanded CAG repeat within the open reading frame of the respective gene. Normally, the disease proteins contain a polyglutamine repeat, which becomes expanded in the disease context to encode an abnormally long repeat. This expanded polyglutamine domain confers a dominant toxicity on the disease protein, leading to neuronal dysfunction and loss. Expanded polyglutamine domains can form polar zippers of hydrogen-bonded β-strands and amyloid fibrils in vitro and in vivo (Scherzinger et al. 1997, Perutz 1999). One current focus of structural research on such disease proteins is protofibril forms—oligomeric species rich in β-sheet structure—as toxic entities (Bucciantini et al. 2002, Lashuel et al. 2002, Walsh et al. 2002). In human disease and in transgenic mouse models, abnormal accumulations of the polyglutamine disease proteins, typically in the form of nuclear inclusions, are neuropathological signature lesions (reviewed in Zoghbi & Orr 2000, Sakahira et al. 2002, Taylor et al. 2002). Whereas the precise role of the inclusions in pathology remains unclear, they reflect structural features of the mutant protein and are characteristic of the diseases. In addition, the study of polyglutamine inclusions and colocalized proteins has revealed a number of interesting means of modulating toxicity of the proteins, as discussed below.

In humans, the polyglutamine diseases include Huntington’s disease, spinobulbar muscular atrophy (SBMA), dentatorubropallidoluysian atrophy (DRPLA), and several spinocerebellar ataxias [(SCA); types 1, 2, 3, 6, 7, and 17]. The diseases are characterized by a late-onset progressive loss of neuronal function, causing loss of proper motor control and in some cases dementia. Although the disease proteins are generally widely expressed, the diseases are associated with degeneration in specific brain regions and neuronal types (Ross 1995, Zoghbi & Orr 2000). For each disease, the longer the CAG repeat expansion is, the earlier the onset and the more severe the disease are, with most of the polyglutamine diseases showing a threshold for disease of ~40 glutamines (Gusella & MacDonald 2000). This threshold is similar to the nucleation threshold of such proteins for aggregation and amyloid fibril formation in vitro, as well as, for example, in *Caenorhabditis elegans* in vivo (Scherzinger et al. 1997, Morley et al. 2002). In some cases, such
as in SBMA, the disease phenotype, although arguably due to a gain-of-function toxic activity of the disease protein (the androgen receptor), includes features of loss-of-gene function (Pinsky et al. 1992). The diseases may reflect an interesting combination of dominant gain-of-function effects of expanded polyglutamine toxicity and select loss-of-function genetic characteristics.

**Fly Models of Polyglutamine Disease**

Human neurodegenerative disease was initially modeled by overexpression of a form of the SCA3 human disease gene, also called the Machado-Joseph disease (MJD) gene (Warrick et al. 1998), and subsequently by a form of the Huntington’s disease protein (Jackson et al. 1998). For SCA3/MJD, expression of a truncated fragment of the Ataxin-3 protein with an expanded polyglutamine domain encoding 78 glutamines (MJDtr-Q78) induces a late-onset, progressive neurodegenerative phenotype when expressed in the fly (Warrick et al. 1998). The normal control protein, with a 27-glutamine repeat within the normal allelic range (MJDtr-Q27), has no effect. The severity of the disease phenotype depends on the expression level of the MJDtr-Q78 protein, such that a more highly expressed protein causes a more severe and earlier-onset degeneration. The phenotype in flies also depends on the length of the repeat, with a longer repeat causing a more severe phenotype (Chan et al. 2000, Higashiyama et al. 2002). When expressed in the fly eye, the expanded polyglutamine protein induces a progressive loss of pigmentation and neuronal integrity (Figure 2A, B). When expressed more broadly in the entire nervous system, the expanded polyglutamine protein confers early lethality when strongly expressed or adult mortality with the flies showing tremors and a loss of coordination. Results with the Huntington’s disease protein similarly show a repeat length-dependent toxicity, with loss of photoreceptor neurons and cellular integrity when expression is directed exclusively to the eye (Jackson et al. 1998). Subsequent *Drosophila* polyglutamine disease models have been established using other human polyglutamine disease proteins, including Ataxin-1 (SCA1) and the androgen receptor protein (SBMA) (Fernandez-Funez et al. 2000, Takeyama et al. 2002). Additional studies have employed polyglutamine domains, although not in the context of a human disease gene (Kazemi-Esfarjani & Benzer 2000, Marsh et al. 2000). In addition, this research has stimulated similar approaches in other model organisms, such that models for polyglutamine toxicity have now also emerged in yeast and *C. elegans* (Faber et al. 1999, Krobitsch & Lindquist 2000, Merin et al. 2002, Muchowski et al. 2000, Satyal et al. 2000).

Polyglutamine pathogenesis is characterized by prominent nuclear inclusions in flies, as it is in human disease (Figure 2D). One exception is the *Drosophila* Huntington’s disease model, in which aggregation of protein was not detected, at least by light microscopy, although a translocation of the protein to the nucleus was noted (Jackson et al. 1998). The nuclear inclusions are visually striking, which has naturally led to the idea that they might directly promote the neurodegeneration seen in these diseases. However, despite much research guided by molecules found
in the inclusions, the significance of the inclusions per se to disease remains controversial, with some studies suggesting that they are central to pathogenesis (e.g., Chai et al. 2002) and others suggesting they are not (Cummings et al. 1999, Watase et al. 2002). Indeed, expanded polyglutamine proteins are not toxic to all cells in flies, despite formation of nuclear inclusions (Warrick et al. 1998). Moreover, some evidence suggests that inclusion formation may be protective (Cummings et al. 1999, Watase et al. 2002). More recent data in vivo suggest that proteins that colocalize to the inclusions exhibit distinct dynamics, being either loosely or tightly associated, properties that also vary according to the disease gene involved (Chai et al. 2002, Kim et al. 2002, Stenoien et al. 2002).

Studies in flies have shown that protein context around the pathogenic repeat plays a strong role in modulating the dominant toxicity of the disease proteins themselves. Pure polyglutamine tracts are highly toxic when expressed in flies, with toxicity being diminished by the addition of even a small protein tag sequence (Marsh et al. 2000, Kazemi-Esfarjani & Benzer 2002). Moreover, the human disease proteins themselves appear to differ in toxicity: Whereas a truncated form of Ataxin-3 with an expanded polyglutamine run is highly toxic when directed to the eye (Higashiyama et al. 2002), similarly directed expression of a truncated fragment of the Huntington’s disease protein yields a much milder phenotype, despite having a longer repeat (Jackson et al. 1998). The importance of protein context has also been demonstrated by artificially expanding a polyglutamine repeat within an otherwise normal fly protein, not associated with human disease, and showing that toxicity of a polyglutamine domain—highly toxic when expressed as a pure domain on its own—is strikingly mitigated (Marsh et al. 2000).

Whereas the initial fly polyglutamine disease models were performed with fragments of the human disease proteins or raw polyglutamine domains, as detailed above, subsequent models have emerged that use full-length human disease proteins. Features may be revealed in the context of the full-length protein that might not be seen with just a fragment of the protein, owing to missing functional domains. These models include one for SCA1 using the Ataxin-1 protein and another for SBMA with the androgen receptor protein. The Ataxin-1 model reveals that the protein has a similar rough eye and degenerative phenotype whether it bears a normal (Q30) or expanded (Q82) polyglutamine run (Fernandez-Funez et al. 2000). This result led to the conclusion, subsequently verified in transgenic mice, that expression levels of the Ataxin-1 protein in addition to the length of the repeat are critical in pathogenesis. The Ataxin-1-Q82 protein may be more toxic than the Q30 protein simply because it is degraded less efficiently and therefore accumulates to higher levels. The finding that normal Ataxin-1 confers a rough eye phenotype (Fernandez-Funez et al. 2000) suggests that genetic study of the fly models may reveal functional aspects of the human Ataxin-1 protein itself that are critical to SCA1 disease.

Spinobulbar muscular atrophy (SBMA) is associated with an expanded polyglutamine run in the androgen receptor protein. The fly model that expresses full-length androgen protein shows a striking dependence on steroid ingestion. Whereas
the protein with a repeat within the pathogenic range (Q52) causes no phenotype normally, when flies are fed androgen agonists or antagonists, degeneration is observed (Takeyama et al. 2002). Ligand binding is associated with translocation of the protein to the nucleus, demonstrating the importance of nuclear localization of this protein for pathology. This finding provides insight into the fact that SBMA occurs only in men in whom serum androgen levels are high (Kennedy et al. 1968). The androgen receptor protein phenotype in flies appears to not be dependent on agonist-dependent transcriptional activity, however, but simply on translocation of the protein to the nucleus: Feeding flies either agonists or antagonists induces the same phenotype (although nuclear transcriptional events may be of importance to consequent degeneration). Expression of truncated androgen receptor proteins shows constitutive degeneration, being independent of hormone treatment (Chan et al. 2002, Takeyama et al. 2002).

**Genetic and Pharmacological Modifiers of Polyglutamine Toxicity**

The fly polyglutamine models have been used in both forward and reverse genetic approaches to uncover modifiers of polyglutamine toxicity. Some of the most potent suppressors belong to the molecular chaperone family (Warrick et al. 1999, Chan et al. 2000, Fernandez-Funez et al. 2000, Kazemi-Esfarjani & Benzer 2000). These chaperone family members include Hsp70 and Hsp40 classes that aid in the proper folding of proteins and that are known to act under stress conditions to influence the processing of abnormally folded proteins (Bukau & Horwich 1998, Sakahira et al. 2002). Retinal coexpression of Hsp70 together with a polyglutamine disease protein strikingly mitigates the degenerative phenotype, restoring a normal morphology (Figure 2C) (Warrick et al. 1999). Hsp40 chaperones also strongly suppress the associated cellular toxicity, including that of raw polyglutamine domains as well as disease models involving truncated and full-length proteins (Chan et al. 2000, Fernandez-Funez et al. 2000, Kazemi-Esfarjani & Benzer 2000, Takeyama et al. 2002). The chaperones are now emerging as universal suppressors of polyglutamine toxicity; moreover, their suppression activities have recently been extended to other human neurodegenerative models (Auluck et al. 2002). Loss of Hsp70 function exacerbates neurodegeneration (Warrick et al. 1999, Fernandez-Funez et al. 2000), which suggests that chaperone activities are critical modulators of toxicity, being dosage sensitive for both upregulation and downregulation of toxicity. Simply interfering with Hsp70 levels alone, in the absence of an overexpressed disease protein, can have effects in flies reminiscent of disease-associated cellular degeneration (Elefant & Palter 1999, Bonini 2002).

A simple model for chaperone suppression would be that a change in the structure of the polyglutamine protein results in a more normal protein conformation, reduced aggregation, and a subsequent absence of protein inclusions, but the data actually reveal that in flies, at least by immunocytochemistry, the inclusions appear largely unchanged (Warrick et al. 1999, Kazemi-Esfarjani & Benzer 2000;
but see Fernandez-Funez et al. 2000). However, the inclusions immunolabel for stress-induced Hsp70 in flies, consistent with the accumulating protein being misfolded and requiring chaperone activity (Figure 2E) (Warrick et al. 1999, Chan et al. 2002). Analysis of protein aggregation by western immunoblot reveals a change in the SDS-solubility properties of the expanded polyglutamine protein in the presence of molecular chaperones (Chan et al. 2000), which is consistent with similar effects observed in vitro and in yeast models (Muchowski et al. 2000, Satyal et al. 2000). Presumably this finding reflects some change in the nature of the protein in vivo, which perhaps maintains the protein in a more native conformation or buffers the toxicity of putative protofibril forms without any discernable effect on the large nuclear inclusions (Sakahira et al. 2002). Other models for this mitigation of toxicity envision the added Hsp70 competing with other proteins for interactions with the pathogenic protein, thereby preventing key events in the degenerative process from occurring.

One current model of increasing interest is that of chaperone depletion, in which a global depletion of chaperone activity results from the excessive requirement of the abnormally folded disease protein for cellular chaperone activity (Warrick et al. 1999, Satyal et al. 2000, Bonini 2002, Sakahira et al. 2002). In the absence of a strong stress response, the disease protein may overwhelm the available cellular pool of chaperones and lead to a loss of sufficient chaperone activity for other general cellular functions, resulting in the demise of those cellular processes. This latter model is consistent with findings that interference with chaperone activity alone is able to cause phenotypes reminiscent of degeneration (Elefant & Palter 1999, Auluck et al. 2002, Bonini 2002).

The chaperones are arguably among the most promising modifiers isolated so far, owing to the strength of their toxicity mitigation and their potential applicability to multiple diseases associated with protein misfolding. Upregulation of Hsp70 has also been shown to be effective in mitigating disease pathology in transgenic mouse models of Ataxin-1 (Cummings et al. 2001), and reduction of polyglutamine toxicity in numerous vertebrate cell model systems by chaperones has now been reported (for example, Chai et al. 1999, Kobayashi et al. 2000, Zhou et al. 2001, Chuang et al. 2002). Drugs that upregulate chaperones lessen polyglutamine toxicity in mammalian cell models (Sittler et al. 2001) and also function in vivo to reduce α-synuclein toxicity in Parkinson’s disease models (Auluck et al. 2002). These recent developments illustrate the utility of large-scale genetic screening with the *Drosophila* disease models for the exploration of promising therapeutic strategies for these human neurodegenerative disorders.

In addition to protein-folding effects, clearance of the disease protein is clearly important in vivo [see Sherman & Goldberg (2001) for review], as interference with the proteasome pathway components also shows enhancement of polyglutamine toxicity in flies (Fernandez-Funez et al. 2000, Chan et al. 2002). Other cellular pathways have also been implicated by genetic screens of the fly models, including additional chaperone and stress pathways (Higashiyama et al. 2002), small ubiquitin-like modifier (SUMO) pathways (Chan et al. 2002), genes implicated
in neoplasia (Kazemi-Esfarjani & Benzer 2002), as well as pathways associated with mRNA regulation, cellular detoxification, and transcriptional regulation (Fernandez-Funez et al. 2000, Steffan et al. 2001). Overexpression of the Drosophila homologue of human myeloid leukemia factor 1 (Dmlf) mitigates polyglutamine toxicity by a mechanism that includes recruitment of the protein to the nuclear inclusions rather than to other known pathways that normally influence Dmlf activity (Kazemi-Esfarjani & Benzer 2002). Glutathione-S-transferase has been revealed as a dosage-sensitive modifier of the Ataxin-1 phenotype, which suggests that cellular detoxification pathways are of critical importance (Fernandez-Funez et al. 2000). It has not yet been established how cells die in polyglutamine-induced degeneration. Programmed cell death pathways appear not to be involved, at least to the extent that P35, a baculoviral caspase inhibitor, is a poor suppressor of polyglutamine degeneration (Jackson et al. 1998, Warrick et al. 1998). To date, apoptosis-related genes have not been reported from genetic screens.

Of special note in polyglutamine modification are transcriptional pathways, with a number of transcriptional cofactors identified as genetic modifiers in flies (Fernandez-Funez et al. 2000, Steffan et al. 2001). Some of these proteins themselves contain natural polyglutamine repeats, such as the transcriptional regulator CREB-binding protein (CBP), and they become recruited into the nuclear inclusions with the disease protein in vertebrate cells (Kazantsev et al. 2000, McCampbell et al. 2000, Nucifora et al. 2001). This finding raises the possibility that that loss of CBP or other transcriptional activities may be involved in polyglutamine-associated disease progression in humans. One of the activities of CBP is as a histone acetyltransferase (HAT), which is inhibited by the interaction of CBP with a fragment of the Huntington disease protein containing an expanded polyglutamine repeat (Steffan et al. 2001). It is remarkable that upregulating histone acetyltransferase activity by feeding histone deacetylase (HDAC) inhibitors to flies suppresses polyglutamine toxicity (Steffan et al. 2001). Moreover, HDAC inhibitors have also been shown effective in mammalian cell culture models of polyglutamine toxicity (McCampbell et al. 2001), indicating promise for this class of drugs in therapeutic treatment.

It is interesting that modulation of chaperones and histone acetylation both share the feature of also modulating lifespan in flies (Kang et al. 2002, Tatar et al. 1997, Wheeler et al. 1999). Consequently, one interpretation of their effects on polyglutamine toxicity is that they might make the nervous system “healthier” and better able to cope with stress-associated events of aging, some of which might be shared with disease pathogenesis. Aspects of the suppression, especially for Hsp70, almost certainly include modulating the structure of pathogenic proteins. It remains a challenge to determine how such biochemical effects on protein structure might manifest themselves at the organismal level as effects on neuronal survival and lifespan. Along this line, other classes of modifiers revealed in future screens and their mechanisms of action will be very informative to pursue and may touch on any number of biological areas.
MODELING PARKINSON’S DISEASE IN DROSOPHILA

Parkinson’s Disease

Parkinson’s disease is the second most common neurodegenerative disease affecting about 1% of people over 65 years of age. A movement disorder, it is characterized by tremor, rigidity, and loss of voluntary movement, primarily due to the progressive degeneration of dopaminergic neurons in a brain region termed the substantia nigra pars compacta (Lang & Lozano 1998). Although Parkinson’s disease is generally sporadic, familial forms have been characterized, permitting the identification of gene products whose altered activity is causal in disease pathogenesis. One of the first genes to be associated with rare familial forms of dominant Parkinson’s disease is \(\alpha\)-synuclein (Polymeropoulos et al. 1997, Kruger et al. 1998). \(\alpha\)-synuclein is also a major component of the cytoplasmic aggregates that accumulate as a typical feature of Parkinson’s disease called Lewy bodies and Lewy neurites (Spillantini et al. 1997). Because the pathological features of idiopathic and familial dominant forms of the disease are similar, the mechanisms underlying disease for both forms are thought likely to share mechanisms. With a defined gene product in hand, the approach of modeling the disease in a genetically tractable model organism such as Drosophila becomes feasible.

\(\alpha\)-synuclein, a small protein of only 140 amino acids, was initially identified in Torpedo california as a protein that localizes to neurons and presynaptic terminals (Maroteaux et al. 1988). The mutations in \(\alpha\)-synuclein associated with familial Parkinson’s disease, A53T and A30P, are point mutations that fall within the region encoding six degenerate N-terminal repeats, comprised of the core sequence KTKEGV. In vitro, \(\alpha\)-synuclein forms insoluble filaments with an amyloid \(\beta\)-sheet structure that are similar to the filaments of Lewy bodies in disease tissue (Conway et al. 1998, Crowther et al. 1998, El-Agnaf et al. 1998). The A53T mutation shows a faster rate of filament formation compared to wild-type \(\alpha\)-synuclein (Conway et al. 1998), whereas the A30P mutation disrupts interactions of \(\alpha\)-synuclein with vesicles (Jensen et al. 1998). Because both the familial and sporadic forms of Parkinson’s disease are associated with alterations in \(\alpha\)-synuclein, resulting in similar Lewy bodies and neurite formation, it is thought that these alterations and the subsequent loss of dopaminergic neurons might reflect a combination of mutational and nongenetic cellular events.

Modeling Parkinson’s Disease in the Fly

In Drosophila, human \(\alpha\)-synuclein expression induces selective loss of dopaminergic neurons (Feany & Bender 2000, Auluck et al. 2002). For this reason, \(\alpha\)-synuclein expression leads to a relatively subtle but highly specific phenotype, in striking contrast to disease proteins such as expanded polyglutamine and tau that produce dramatic phenotypes when expressed in the fly eye. The \(\alpha\)-synuclein-induced degeneration is also adult-onset, resulting in the gradual loss of \(\sim 50\%\)
or more of the dopaminergic neurons in select clusters in the brain over several weeks of adult life (Figure 3A,C). All three α-synuclein forms—wild type, A30P, and A53T—show similar effects in this paradigm. Global neural expression of α-synuclein is also reported to cause behavioral or locomotion defects, with the A30P form being slightly more aggressive (Feany & Bender 2000). When expressed in the fly, α-synuclein forms abnormal accumulations reminiscent of Lewy bodies and Lewy neurites (Figure 3B) (Feany & Bender 2000, Auluck et al. 2002). These structures immunolabel for ubiquitin, which is also a feature of Lewy accumulations in Parkinson’s disease, emphasizing the conservation of α-synuclein pathology between the human disease and the fly model.

**Chaperone Suppression of Parkinson’s Disease in Drosophila**

The appearance of protein inclusions and the immunolabeling of the Lewy body–like aggregates with ubiquitin suggest that pathways involved with protein misfolding may influence α-synuclein pathogenesis. Indeed, upregulation of Hsp70 in dopaminergic neurons leads to suppression of the α-synuclein toxicity in these cells, with normal numbers of dopaminergic neurons being maintained over time in the adult brain (Auluck et al. 2002). The α-synuclein toxicity is also enhanced by interference with endogenous chaperone activity (Auluck et al. 2002). In addition, simply interfering with endogenous chaperones alone can cause some dopaminergic neuron degeneration in flies, thereby phenocopying the effects of α-synuclein expression. These findings suggest that insufficient chaperone activity may itself cause degeneration, consistent with a chaperone depletion model for pathogenesis (Bonini 2002, Sakahira et al. 2002). As with the inclusions associated with polyglutamine degeneration, chaperones suppress α-synuclein toxicity without leading to the disappearance of the abnormal accumulations. Although a number of hypotheses are possible, α-synuclein, owing to an abnormal conformation, may require more chaperone activity, leading to a cellular depletion of chaperone activity and consequent impairment of other chaperone-dependent cellular processes, culminating in cell loss. Examination of Lewy bodies and neurites in Parkinson’s disease and other human neurodegenerative diseases associated with altered α-synuclein accumulation reveals the presence of chaperones in these structures (Auluck et al. 2002). Taken together, these data raise the possibility that altered chaperone activity may also contribute to Parkinson’s disease in humans.

These studies have been extended in a therapeutic direction using the compound geldanamycin. Geldanamycin is a naturally occurring antitumor molecule that binds to Hsp90, destabilizing Hsp90 complexes (Whitesell et al. 1994). Hsp90 is an inhibitor of heat shock factor, the transcription factor that upregulates the stress response (Zou et al. 1998). Treatment with geldanamycin may therefore be predicted to protect against α-synuclein toxicity by upregulating chaperone activity. Indeed, feeding adult flies geldanamycin protects against α-synuclein toxicity, resulting in long-term survival of the dopaminergic neurons (Figure 3C) (Auluck & Bonini 2002). Of potential interest to note, smoking has been negatively
correlated with Parkinson’s disease; it is tempting to speculate that smoking might augment the stress response through deleterious effects involving oxidative damage and other stressors (Helfand 2002). Agents that upregulate the stress response may provide a novel therapeutic avenue for Parkinson’s disease through treatments that influence the survival of the cells, rather than counteracting the effects of the loss of cells.

Finally, Parkinson’s disease has been linked to mutations in other genes aside from α-synuclein, including the parkin and ubiquitin C-terminal hydrolase L1 (UCHL1) genes (Kitada et al. 1998, Leroy et al. 1998). Drosophila has well-conserved homologues of these two genes, which indicates that loss-of-function approaches to investigate the role of these genes in dopaminergic neuron integrity—and potential interactions with α-synuclein—will likely be forthcoming. Another interesting avenue that could be pursued in flies is to explore the role of environmental toxins and how they affect dopaminergic neuron integrity (Lang & Lozano 1998, Betarbet et al. 2000) and to use flies to define genetic modifiers of these environmental effects.

ANALYSIS OF ALZHEIMER’S DISEASE–RELATED GENES IN DROSOPHILA

Molecular Genetics of Alzheimer’s Disease

Alzheimer’s disease is the most common cause of senile dementia in humans and is characterized neuropathologically by the accumulation of amyloid plaques and selective neuronal loss in specific brain regions (reviewed in Selkoe 1999, Sisodia & St George-Hyslop 2002). These morphological disease features typically occur before any major behavioral symptoms become evident, making accurate diagnosis of early Alzheimer’s disease problematic. However, as the disease progresses, the aberrant morphology of brain tissue becomes more severe and is accompanied by a marked decline in memory and other cognitive functions. For many years, the progressive formation of amyloid plaques containing small, 40–42 amino-acid peptide derivatives of APP in affected brain tissues has been recognized as a key feature of Alzheimer’s disease (reviewed in De Strooper & Annaert 2001, Selkoe 1998, Sisodia & St George-Hyslop 2002). APP is a type I integral membrane protein, and several different small peptides, including the neurotoxic amyloid peptides, are generated by alternative cleavages in and around the region encompassing the transmembrane domain. One of these cleavages occurs at an unusual location within the APP transmembrane domain itself and was termed the γ-secretase cleavage after the elusive protease that was postulated to perform this cut and thereby to generate secreted amyloid fragments that terminate at this carboxy-terminal γ position. Cleavage position heterogeneity for γ-secretase actually produces amyloid peptides of slightly different sizes, with 42 amino-acid peptides (Aβ42) exhibiting greater neurotoxicity than the smaller 40 amino-acid peptides (Aβ40) (De Strooper & Annaert 2001, Selkoe 1998, Sisodia & St George-Hyslop 2002).
Genetic studies of human families suffering from an early-onset form of Alzheimer’s disease provided the essential clues to the molecular identity of $\gamma$-secretase. Whereas a small proportion of these cases are caused by mutations in APP itself (Selkoe 1999), most are due to mutations in either of the two human presenilin genes PS1 and PS2 (De Strooper & Annaert 2001, Selkoe 1998, Sisodia & St George-Hyslop 2002). These disease-associated PS1 and PS2 mutants have been found to alter the ratio of $\alpha{\beta}42$ and $\alpha{\beta}40$ $\gamma$-site usage, accelerating the production of the more neurotoxic $\alpha{\beta}42$ species (De Strooper & Annaert 2001, Selkoe 1999, Sisodia & St George-Hyslop 2002). Remarkably, genetic studies in C. elegans also identified related proteins encoded by the spe-4 and sel-12 genes, which are required for sperm morphogenesis (L’Hernault & Arduengo 1992) and Notch/Lin-12 signaling (Levitan & Greenwald 1995), respectively. Whereas the SPE-4 protein appears to be a more distant relative of the family, SEL-12 is very similar to mammalian presenilins, which suggests that the presenilins are involved in a common feature of APP metabolism and Notch signaling. Presenilin gene orthologs were subsequently identified in the plant Arabidopsis thaliana (Lin et al. 1999) and several additional animal species, including Drosophila (Boulianne et al. 1997, Hong & Koo 1997, Ye & Fortini 1998).

The molecular cloning of the presenilin genes initially provided little insight into the biochemical function of the encoded proteins. The genes encode related multipass transmembrane proteins—the most widely accepted topological model includes eight membrane-spanning segments, amino- and carboxy-terminal cytosolic domains, and a large hydrophilic cytosolic loop domain between transmembrane segments 6 and 7 (Doan et al. 1996; Li & Greenwald 1996, 1998). Presenilin localizes predominantly to the endoplasmic reticulum (ER) and Golgi compartments, although a small amount is apparently present at the plasma membrane, where it would be available to participate in cell-surface events such as amyloid secretion and receptor activation (Nowotny et al. 2000, Ray et al. 1999, Ye & Fortini 1998).

**Presenilin: The Catalytic Core of $\gamma$-Secretase**

Certain structural features of presenilin led to the realization that it might be the catalytic core of the long-sought $\gamma$-secretase (Wolfe et al. 1999a,c). Presenilins, even those found in plants (Lin et al. 1999), have two conserved aspartate residues in transmembrane domains 6 and 7—positions where they would be predicted to align with the intramembranous $\gamma$-secretase cleavage site of APP. This observation, together with the fact that all aspartyl proteases possess two catalytic aspartates, and the finding that $\gamma$-secretase activity is blocked by peptide analogues based on aspartyl protease catalytic intermediates (Wolfe et al. 1999b), raised the possibility that presenilins might define a novel class of intramembranous aspartyl proteases. Several additional observations have now provided strong support for this idea. Elimination of PS1 activity in knockout mice results in reduced $\gamma$-site cleavage of APP (De Strooper et al. 1998, Naruse et al. 1998). Mutagenesis of either of the two
conserved aspartates in PS1 interferes with APP cleavage (Wolfe et al. 1999c), PS1 and \( \gamma \)-secretase activity copurify from HeLa cell membranes (Li et al. 2000a), and transition-state analogue inhibitors of \( \gamma \)-secretase label both PS1 and PS2 (Esler et al. 2000, Li et al. 2000b, Seiffert et al. 2000). Finally, a conserved motif in presenilin has been noted that has similarity to a putative catalytic site of bacterial type 4 prepilin proteases—an atypical class of aspartyl proteases (Steiner et al. 2000). Taken together, these findings argue that presenilin serves as the catalytic core of a new type of polytopic aspartyl protease, which performs the unusual intramembranous cleavage of APP and other substrate proteins.

**Using Flies and Worms to Define Additional Components of the \( \gamma \)-Secretase Complex**

Biochemical approaches in mammalian cells, along with genetic strategies in *Caenorhabditis* and *Drosophila*, have begun to identify and characterize additional components of the large (250 kDa to \( >2000 \) kDa) \( \gamma \)-secretase complex. One recently identified component, termed nicastrin, is a type I integral membrane protein (Goutte et al. 2000, Yu et al. 2000), which physically associates with presenilin and the carboxy-terminal fragments of APP and Notch (Chen et al. 2001, Esler et al. 2002, Yu et al. 2000). Nicastrin is genetically required for Notch/Lin-12 signaling in flies and worms (Chung & Struhl 2001, Goutte et al. 2000, Hu et al. 2002, Levitan et al. 2001, Lopez-Schier & St Johnston 2002), which again underscores the link between APP processing and Notch activity. In contrast to presenilin, which is largely membrane-embedded and cytosolic in topology, nicastrin has a large extracellular domain with several conserved sequence motifs and a relatively small intracellular domain with little sequence similarity across species. More recently, two additional components of the putative \( \gamma \)-secretase complex have been uncovered through genetic screens in flies and worms. The *aph-1* gene encodes a novel serpentine protein having seven transmembrane domains (Francis et al. 2002, Goutte et al. 2002), while the *pen-2* gene encodes a very small novel protein of only 101 amino acids (Francis et al. 2002). The biochemical functions of these putative \( \gamma \)-secretase components have not yet been determined.

**Involvement of Presenilin in Drosophila Notch Proteolysis and Signaling**

Given the accumulating evidence for a proteolytic role of presenilin and nicastrin in the \( \gamma \)-secretase cleavage of APP, attention quickly turned to the possibility that they also mediate the analogous intramembranous cleavage of the Notch receptor. During signaling, the intracellular domain of Notch is released from the plasma membrane and translocates to the nucleus, where it complexes with other nuclear factors and regulates downstream gene expression. This event is triggered by ligand binding to Notch at the cell surface and removal of the Notch extracellular domain by subsequent proteolysis (reviewed in Fortini 2002, Kopan & Goate...

**Genetics of Amyloid Precursor Protein in Drosophila**

As is the case with presenilin, *Drosophila* also possesses an APP homologue, encoded by the β-amyloid precursor protein-like (*Appl*) gene (Luo et al. 1990, Rosen et al. 1989). The fly APPL protein displays overall structural similarity to human APP and undergoes similar proteolytic processing but lacks homology to the short segment of human APP that gives rise to amyloid peptide. Hence, *Drosophila* does not naturally produce amyloid-like peptides or exhibit the associated changes in brain tissue morphology and function that are characteristic of human Alzheimer’s disease. Nevertheless, homozygous deletion of the fly *Appl* gene causes mild behavioral defects in locomotor responses that can be reversed by providing the flies with a functional human APP gene (Luo et al. 1992). These results are in general agreement with studies on mouse APP genes, which have found that APP is not essential for survival but that it might be required for optimal development and/or functioning of the nervous system, perhaps by regulating axonogenesis, dendritic arborization, or synaptic differentiation (reviewed in De Strooper & Annaert 2000, Mattson 2001). Several studies on mammalian APP have recently revealed that the γ-secretase-mediated cleavage of APP releases, in addition to amyloid peptide, an intracellular fragment of APP with putative signaling activities (Cao & Sudhof 2000).
It will be of interest to learn whether the APP intracellular domain is released from cell membranes in Drosophila, and, if so, what the functions are of this APP fragment in neuronal morphology and activity.

A very different view of APP biology has emerged from other studies on APP and its fly counterpart APPL. Goldstein and colleagues have proposed that APP functions as a cargo receptor for kinesin I, linking vesicles moved by fast anterograde transport to axonal microtubules (Gunawardena & Goldstein 2001, Kamal et al. 2001). In contrast to the signaling model discussed above, the cargo receptor model involves direct binding of the microtubule motor protein kinesin I to the short intracellular domain of intact, membrane-anchored APP. APP would thereby tether axonal vesicles containing APP itself, presenilin, other APP secretases, and presynaptic proteins to the fast anterograde transport machinery. In this model, γ-secretase cleavage of APP would result in release of kinesin I and uncoupling of vesicles from the transport machinery, possibly leading to vesicle stalling, retrograde transport, or synaptic delivery of the vesicles and their contents (Kamal et al. 2001). Although the association of APP with kinesin I and an anterograde-transport membrane component could simply reflect the need to deliver APP to the axonal terminus for some other APP function, the cargo receptor idea is supported by the defective transport of several other putative cargo proteins, as is seen in APP-null mice and flies (Gunawardena & Goldstein 2001, Kamal et al. 2001) and related axonal organelle accumulation phenotypes caused by kinesin mutations and APP overexpression (Torroja et al. 1999, Gunawardena & Goldstein 2001). Although further work is needed to substantiate this model, a kinesin I-binding function for the APP intracellular domain is not incompatible with the proposed signaling function of APP. Perhaps APP plays a dual role in neuronal physiology, first, by helping to deliver cargo proteins to their presynaptic destination through fast anterograde transport, and then, upon fusion of the transport vesicles with the presynaptic membrane, by transforming itself into a receptor for extracellular stimuli at the synapse.

Overexpression of Alzheimer’s Disease Genes in Drosophila

Drosophila has also proven valuable for assessing the involvement of presenilin in apoptotic cell death. Several studies in mammalian tissue culture cells have shown that overexpression of PS1 and PS2 sensitizes the cells to apoptosis induced by trophic factor withdrawal or exposure to amyloid peptide, which indicates that presenilin may promote cell death that is triggered by certain stimuli (Deng et al. 1996; Guo et al. 1996, 1997; Janicki & Monteiro 1997; Weihl et al. 1999; Wolozin et al. 1996). However, other cell culture experiments have found no evidence for such apoptotic effects of PS1 overexpression (Bursztajn et al. 1998). Removal of PS1 function in neuronal cell cultures has also been found to block neuronal differentiation and induce apoptosis (Hong et al. 1999). In PS1 knockout mice, increased apoptosis is also observed but not at early developmental stages, which
indicates that cell death might be an indirect consequence of a primary failure in Notch signaling or some other presenilin-dependent process (Handler et al. 2000, Shen et al. 1997).

Similar to the PS1 knockout mouse, fly presenilin mutants display dramatically increased levels of apoptosis during late developmental stages, when a general failure of Notch signaling is already apparent (Ye & Fortini 1999, Ye et al. 1999). However, overexpression of fly presenilin in the developing Drosophila retina also causes ectopic cell death, although the levels of apoptosis are far lower than is typically observed following overexpression of “killer genes” that function directly in the apoptotic pathway (Ye & Fortini 1999). Instead, overexpression of wild-type or Alzheimer’s disease–associated presenilins at levels several-fold higher than normal results in infrequent cell death across the fly retina (Figure 4), whereas mild overexpression at levels approximately twofold above normal produces no apparent apoptotic phenotypes. These apoptotic effects of presenilin in the fly retina are modulated by Notch pathway activity, and high levels of presenilin overexpression interfere with Notch receptor synthesis. Under these conditions, normal presenilin-dependent processes, such as Notch signaling, might be impaired by a dominant negative effect of excess presenilin within the secretory apparatus, resulting indirectly in the apoptotic elimination of developmentally aberrant cells. The results obtained using Drosophila presenilin loss-of-function mutants and overexpression transgenes thus confirm those observed in some of the mouse and human genetic and cell culture models. In this case, the Drosophila retina served as a useful in vivo assay for substantiating suspected apoptotic effects of mammalian presenilin overexpression, and it provided the opportunity to connect these effects to a previously established function of presenilin in the Notch signal transduction pathway.

Progress has also begun to be made in using overexpression strategies in Drosophila to investigate the biological properties of human APP. Transgenic flies expressing wild-type and Alzheimer’s disease mutant forms of APP have been generated and used to characterize the intracellular trafficking, proteolytic processing, and physiological effects of APP in Drosophila (Fossgreen et al. 1998, Torroja et al. 1999, Yagi et al. 2000). Overexpressed human APP is correctly transported to synaptic terminals of neurons and postsynaptic regions of the neuromuscular junction (Yagi et al. 2000), and engineered APP proteins are cleaved and secreted into the extracellular milieu (Fossgreen et al. 1998), suggesting that Drosophila is a valid model for investigating the biology of human APP. It is intriguing that overexpression of full-length forms of APP, either wild-type or Alzheimer’s disease–associated mutants, results in a blistered wing phenotype in both of the available transgenic fly APP models (Fossgreen et al. 1998, Yagi et al. 2000). Such wing blistering is typically caused by mutations in cell adhesion molecules such as integrins, owing to the fact that adult fly wings develop as two epithelial cell layers that form in close apposition to one another via adhesive interactions (reviewed in Brown 2000). Although the molecular basis for the blistered wing phenotype in the human APP–expressing flies is not yet understood, this observation is
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reminiscent of earlier findings that APP colocalizes with integrins in rat primary

Two important features of the blistered wing phenotype are that it is caused
by overexpression of full-length APP only, not amyloid peptide alone (Fossgreen
et al. 1998), and that it presumably reflects an impairment of normal cell adhesion
between the wing epithelial layers. These features raise the possibility that overex-
pressed APP is exerting a dominant interfering effect on cell adhesion or associated
signaling events, perhaps by interfering with APP-binding proteins such as prese-
nilin or other γ-secretase components. Several studies have shown that only a small
fraction of presenilin becomes functionally mature and incorporated into active
γ-secretase complexes (reviewed in De Strooper & Annaert 2001, Fortini 2002,
Sisodia & St George-Hyslop 2002), so it is likely that the overexpressed human
APP might strongly interfere with endogenous γ-secretase function in Drosophila.

Consistent with this notion, loss of PS1 activity in mouse Cajal-Retzius neurons
results in phenotypes similar to those seen in integrin mutants (Hartmann et al.
1999), and overexpression of PS1 in mammalian cells promotes the formation of
intercellular adhesive contacts that are enriched for presenilin immunoreactivity
(Singh et al. 2001). Moreover, mammalian γ-secretase has recently been shown to
be required for regulated cleavage and destruction of the cell-adhesion molecule
E-cadherin (Marambaud et al. 2002, Lammich et al. 2002), and fly and mam-

malian presenilins physically associate with the actin-crosslinking protein filamin
(Guo et al. 2000, Zhang et al. 1998). Further work will be needed to determine
if the wing blistering phenotype in transgenic APP flies might be due to inter-
ference with endogenous integrin, E-cadherin, filamin, and/or other cell adhesion
factors.

CREATING FLY MODELS OF HUMAN TAUOPATHIES

Filamentous Tau in Neurodegenerative Diseases

In the past few years, considerable progress has also been made in developing
Drosophila models of human tauopathies, a class of neurodegenerative disor-
ders typically characterized by filamentous aggregates containing the microtubule-
binding protein tau (reviewed in Goedert et al. 1998, Lee et al. 2001). Mutations
that affect the splicing pattern of tau have been found to cause some cases of inher-
ited frontotemporal dementia with parkinsonism (FTDP) (Clark et al. 1998, Hong
et al. 1998, Hutton et al. 1998). In addition, neurofibrillary tangles composed of
hyperphosphorylated tau are an important pathological feature of other neurode-
genative diseases, including progressive supranuclear palsy and Alzheimer’s
disease (reviewed in Goedert et al. 1998, Lee et al. 2001). In mammalian cells,
the serine/threonine kinase glycogen synthase kinase-3β (GSK-3β) is a major
contributor to tau phosphorylation (Hanger et al. 1992, Lovestone et al. 1994,
Lucas et al. 2001), although other kinases have also been implicated. Questions
remain, however, about whether tau hyperphosphorylation is a cause or an effect
of neurofibrillary tangle formation, whether tangle formation is obligatory for neurodegeneration, and which other proteins are involved in these events.

**Exogenous Tau Expression in Drosophila Neurons**

Flies are known to possess a single endogenous tau gene, but mutations have not yet been recovered in this locus (Cambiazo et al. 1995, Goldstein & Gunawardena 2000, Heidary & Fortini 2001). The fly tau gene is expressed in many neuronal cell types and the encoded protein accumulates in axons, as is the case with mammalian tau (Figure 5A) (Heidary & Fortini 2001). Three independent studies have used a transgenic *Drosophila* approach to investigate the biological activities of wild-type and mutant human tau proteins (Jackson et al. 2002, Williams et al. 2000, Wittmann et al. 2001), and a fourth study involved expression of bovine tau in flies (Torroja et al. 1999). In two of these studies, expression of human tau in different neuronal settings—including all neurons, cholinergic neurons, and retinal photoreceptors—was found to induce apoptotic neuronal cell death (Jackson et al. 2002, Wittmann et al. 2001). Directed tau overexpression in the retina produces a dramatically reduced and roughened eye, a phenotype that is suitable for use in modifier screens (Figure 5B,C). Both of these studies revealed that the neuronal cell loss is not accompanied by detectable levels of neurofibrillary tangle formation, which suggests that tau-linked neurodegeneration may precede or occur independently of filamentous tau deposition. This feature of the fly tau overexpression models is somewhat at odds with the prevalence of neurofibrillary tangles in human tauopathies and related rodent transgenic models, and it raises the possibility that *Drosophila* might be particularly amenable to analysis of early, pre-tangle events of tau-associated neurodegeneration. Furthermore, comparison of the patterns of neuronal cell loss in the fly tau/FTDP-17 model and the analogous fly α-synuclein/Parkinson’s disease model revealed that whereas α-synuclein overexpression selectively eliminates dopaminergic neurons (Feany & Bender 2000), tau overexpression affects a broader spectrum of neuronal cell types (Wittmann et al. 2001). The underlying cellular mechanisms of neurodegeneration are thus likely to be significantly different, and the transgenic models appear to reflect accurately the known cell-type specificities of the respective human diseases.

Although the *Drosophila* tau transgenic models display similar phenotypes (Figure 5B,C), some discrepancies between the different tau models have been noted. For example, Wittman et al. (2001) report that a mutant form of tau, namely one bearing an amino acid substitution (R406W) associated with a familial human tauopathy, is much more effective than wild-type tau in neuronal killing. In contrast, Jackson et al. (2002) report that mutant and wild-type human tau are equally potent, implying that tau overexpression per se is neurotoxic, perhaps through a dominant negative effect. Overexpression of a disease-related protein in *Drosophila* may therefore have consequences that are quite distinct from those caused by disease-inducing DNA lesions in a resident human gene, as has been noted above for transgenic flies that overexpress wild-type and Alzheimer’s disease–associated
presenilins (Ye & Fortini 1999). Furthermore, whereas Wittman et al. (2001) reported normal brain tissue volume and organization of major neuronal projection systems in adult transgenic flies, yet a different study described extensive morphological alterations of adult sensory neurons in flies expressing human tau or tau-based fusion proteins such as tau-β-galactosidase and tau-green fluorescent protein (Williams et al. 2000). These defects include abnormal axon bundling, reduced terminal arborizations, loss of axons, and axonal swelling. Likewise, overexpression of bovine tau in Drosophila neurons results in the aberrant accumulation of synaptic proteins and enhances the axonal cargo transport defects caused by APP overexpression (Torroja et al. 1999). Such phenotypes might be due to interference with normal microtubule dynamics or other cytoskeletal processes, which are known to be important for axonogenesis and neurite differentiation. Indeed, overexpression of a human tau isoform in mouse CNS neurons (Ishihara et al. 1999) or in lamprey neurons (Hall et al. 1997) results in similar axonal phenotypes as well as progressive neurodegeneration, recapitulating some key features of human tauopathies. Further analysis of the existing fly tau models must be performed to determine the extent to which the different features of each model are due to differences in expression levels, cell-type specificity of expression, and/or methods of phenotypic characterization. For both the fly and rodent models, caution must be exercised in the interpretation of overexpression phenotypes and their comparison to disease-inducing mutations in resident human genes.

Hyperphosphorylation and Aggregation of Human Tau in Drosophila

Although simple overexpression of human tau in Drosophila cells does not produce noticeable neurofibrillary tangles or other filamentous inclusions resembling those in human tauopathies, the overexpressed tau does appear to be hyperphosphorylated (Jackson et al. 2002, Wittmann et al. 2001). Aging of the transgenic tau flies leads to the progressive accumulation of hyperphosphorylated and conformationally altered human tau in brain regions of pronounced neuronal loss, whereas overall levels of human tau remain relatively constant. Moreover, overexpression of human tau along with the Drosophila GSK-3β homologue results in accelerated neurodegeneration characterized by prominent neurofibrillary tangles (Jackson et al. 2002). At the level of electron microscopy, these filamentous structures bear a striking resemblance to the filamentous tau seen in human diseases, which further validates the utility of the Drosophila tauopathy modeling approach. In contrast to the polyglutamine-induced Drosophila neurodegeneration phenotype described above, the tau phenotype is suppressed by the baculoviral protein P35, which indicates that tau-associated degeneration might involve classic programmed cell death pathways (Jackson et al. 2002). Because fly GSK-3β is also a key component of the Wnt/wingless signaling pathway, the synergistic tau/GSK-3β neurofibrillary tangle phenotype allowed the genetics of Drosophila to be exploited in assessing the potential involvement of the Wnt pathway in tau-mediated neurodegeneration.
Genetic manipulation of other Wnt pathway components produced effects opposite to those predicted if this pathway was responsible for tau/GSK-3β-induced neuronal apoptosis, reinforcing the notion that GSK-3β exerts its effect on tau independent of the Wnt pathway most likely via direct phosphorylation of tau (Jackson et al. 2002).

SUMMARY AND FUTURE DIRECTIONS

The directed expression approach has led to some notable successes in reproducing aspects of complex human diseases in experimentally tractable organisms. The four Drosophila neurodegenerative disease models considered above may be viewed as a proof-of-principle demonstration that this strategy can reproduce individual disease features, as well as broadly conserved elements of the underlying pathogenic mechanisms. For example, characteristic human-like morphological lesions can be replicated in the fly: nuclear inclusions for polyglutamine repeat diseases, Lewy body-like α-synuclein deposits for Parkinson’s disease, and filamentous tau aggregates for tauopathies. Other aspects of each model faithfully match known properties of the respective human diseases, such as their tendency to affect particular neuronal cell populations or the differential involvement of apoptosis in distinct types of neurodegeneration. The fly models have already been exploited very successfully to uncover genetic modifiers of the disease phenotypes, an approach that is difficult in humans owing to both logistical and ethical considerations. Analysis of these modifiers has provided early and important evidence that disease-associated pathological inclusions are intimately connected with protein processing, protein folding, transcriptional regulation, and other cellular pathways. The Drosophila models have thus provided key insights into molecular details of human neurodegeneration and are opening the door to a new generation of experimental and therapeutic strategies.

What are the future prospects for human disease modeling in Drosophila? Clearly, the creation of new models for other human neurodegenerative diseases remains a high priority. It is likely that these efforts will be greatly facilitated by the increased availability of genomic information and its integration with other datasets, such as family pedigree data. Human genome data will identify new candidates for modeling in the fly with both directed expression and classical loss-of-function genetic approaches. Conversely, new information from the fly genome project will spur investigations into the normal roles of identified human disease loci. For instance, homologues of polyglutamine disease genes linked to several human diseases have recently been uncovered in the fly genome (Fortini et al. 2000, Reiter et al. 2001). One of the encoded proteins, the Atrophin-1 homologue in flies, has a defined function in transcriptional repression—an activity that is lost upon expansion of the polyglutamine domain (Zhang et al. 2002). The study of these fly homologues, coupled with analyses of the pathogenic forms of the proteins, promises to provide insights into the tissue- and cell-specific mechanisms associated with particular diseases.
The future will also doubtless see an increased use of these Drosophila models for forward screens, either genetic or pharmacological, similar to the second-site modifier screens already in vogue. As informative as these genetic modifier screens have been, more systematic screens of this type will be required to define the specific molecular pathways operating in each type of neurodegeneration. Comparison of the modifiers recovered from different screens might uncover shared cellular pathways that reflect fundamental features of neuronal cells and their susceptibility to disease. In an analogous fashion, the fly models could conceivably be adapted for high-throughput testing of potential therapeutic compounds. Initial evidence for the efficacy of this approach has come from findings that HDAC inhibitors protect against polyglutamine degeneration (Steffan et al. 2001), geldanamycin blocks α-synuclein toxicity (Auluck & Bonini 2002), and a well-characterized γ-secretase inhibitor mitigates presenilin-mediated Notch cleavage in Drosophila (Hu et al. 2002). The use of an appropriate fly model to prescreen large numbers of compounds prior to testing in rodents might lead to significant reductions in the time and expense needed to check compounds for toxic side effects and help identify the most promising candidates to move into clinical trials. With this idea in mind, it is hoped that Drosophila models of neurodegenerative disease will contribute not only to the understanding of human diseases but also eventually to their treatment and prevention.

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Figure 1  Key features of human neurodegenerative diseases that have been modeled in *Drosophila*. The four classes of human neurodegenerative diseases discussed here are listed at left, with genes that are mutated in these diseases and the type of pathological lesion that is most commonly associated with each disease indicated at center and at right, respectively. This overview is meant to be illustrative and is not a comprehensive listing of all genes and specific pathological features associated with all cases of these diseases or with specific disease subtypes. The characteristic pathological feature of each disease tends to be highly variable and may be altered or absent in certain disease subtypes. Gene name abbreviations: *HD*, Huntingtin; *SCA*, spinocerebellar ataxia; *MJD*, Machado-Joseph disease; *AR*, androgen receptor; *UCHL1*, ubiquitin C-terminal hydrolase L1; *APP*, amyloid precursor protein; *PS*, presenilin.
Figure 2  Ataxin-3-induced neurodegeneration and suppression by Hsp70 in Drosophila. (a) Normal fly eye. (b) Eye of fly expressing an Ataxin-3/MJD protein with a pathogenic polyglutamine domain. The eye shows severe degeneration, characterized by loss of pigmentation and black necrotic patches (Warrick et al. 1998). (c) Coexpression of Hsp70 with Ataxin-3/MJD suppresses the degenerate eye phenotype of the polyglutamine repeat transgene and restores the normal external eye morphology (Warrick et al. 1999). (d) The pathogenic polyglutamine protein accumulates in cells, forming nuclear inclusions, which are visible as brightly fluorescing bodies. (e) The inclusions immunostain for Hsp70, indicative of misfolded protein and a stress response. Anterior at left.
Figure 3  Features of the Parkinson’s disease phenotype in *Drosophila*. (a) Dopaminergic neurons in the adult fly brain, highlighted here by immunostaining for tyrosine hydroxylase, are susceptible to α-synuclein toxicity. Normally there are ~15–20 such neurons in the dorsomedial clusters, of which 50% or more are lost upon α-synuclein expression (Auluck et al. 2002, Feany and Bender 2000). (b) α-synuclein accumulates in the brain of the fly as inclusions reminiscent of Lewy bodies and neurites in the cortex and neuropil. These accumulations also immunostain for Hsp70. (c) Treatment with the antibiotic geldanamycin completely suppresses α-synuclein toxicity to dopaminergic neurons, resulting in their long-term survival. Transgenic flies express either wild-type α-synuclein (wt-a-syn) or mutant forms of the protein (A30P, A53T). (Figure from Auluck & Bonini 2002.)
Figure 4  Apoptotic effects of presenilin overexpression in the Drosophila retinal primordium. (a–c) Transgenic flies were generated that express presenilin at several-fold higher levels than normal in defined cells within the reiterated ∼20-cell units that comprise the developing retinal epithelial monolayer, termed the eye imaginal disc. A portion of one such eye disc that was double immunolabeled using antibodies to detect the overexpressed presenilin (red in panels a, c) and TUNEL-positive apoptotic cell bodies (green in panels b, c) is shown here. White arrows denote the position of the morphogenetic furrow (mf), which marks the earliest stages of neuronal recruitment and precedes the induction of high-level presenilin overexpression posterior to the furrow. The merged image of the presenilin and TUNEL signals (c) illustrates that a small fraction of cells that overexpress presenilin are eliminated by apoptosis at a given time (Ye & Fortini 1999). (d) A higher magnification view of a small sector of this eye disc (white box in panel c), showing the close correspondence between cells overexpressing presenilin (brackets) and the sites of infrequent apoptosis. (e) Another sector of a similar transgenic eye disc, revealing several sites of apoptosis (white arrowheads) that coincide with the positions of cells overexpressing presenilin. Anterior at left.
Figure 5  Endogenous *Drosophila* tau expression and eye phenotype of transgenic flies expressing human tau. (a) Confocal analysis of a horizontal section through the head capsule of an adult fly, showing the retina and optic lobes following immunolabeling with antibodies that recognize the endogenous fly tau protein (red) and tubulin (green). Tau protein is detected throughout the cell bodies, axonal projections, and synaptic terminals of mature photoreceptor neurons (Heidary & Fortini 2001). Anterior at top; re, retina; la, lamina; me, medulla; br, central brain. (b, c) Exterior eye surfaces of a transgenic fly expressing human tau (b) (Wittman et al. 2001), showing a reduced and disorganized ommatidial field in comparison to a wild-type control eye (c). Anterior at left.