

# Comparison of pathways controlling toxicity in the eye and brain in *Drosophila* models of human neurodegenerative diseases

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**Most human neurodegenerative diseases have a number of common features, including adult onset, progressive degeneration of selected neuronal populations and formation of abnormal protein aggregates. Although these shared characteristics raise the possibility of conserved pathogenic mechanisms, the diverse clinical and pathological features of each disorder indicate significant differences. As a number of human neurodegenerative diseases have now been modeled in *Drosophila*, and genetic modifiers identified, we have been able to perform a genetic comparison of pathways controlling toxicity in these models. By directly comparing modifiers isolated in the models of polyglutamine diseases and in a *Drosophila* model of tauopathy, we find a final common pathway of cell death involving apoptosis. Among the polyglutamine diseases, protein folding and histone acetylation are common key mediators. In addition, two novel modifiers suggest shared pathways of toxicity among all the disorders. Cell-type specificity is a salient feature of all neurodegenerative diseases; however, most work to date in the *Drosophila* models have been performed in the retina. Therefore, we determined whether similar pathways of toxicity operate in neurons of the *Drosophila* brain. Many, but not all, retinal modifiers also modify toxicity in postmitotic neurons in the brain. Analysis of polyglutamine toxicity in the adult brain facilitated the identification of nicotinamide (vitamin B3), a vitamin with histone deacetylase inhibiting activity, as a potent suppressor of polyglutamine toxicity. These findings outline common pathways of neurotoxicity, demonstrate disease- and cell-type specific pathways and identify a common vitamin as a potential therapy in polyglutamine disorders.**

## INTRODUCTION

Neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease and disorders like Huntington's disease that are caused by polyglutamine expansions have a number of common features: late adult onset, progressive degeneration of vulnerable subsets of neurons and formation of abnormal protein aggregates. These aggregates may be extracellular, as in the amyloid plaque of Alzheimer's disease, or they may occur in various intracellular compartments. Huntington's disease and related polyglutamine disorders typically have intranuclear inclusions. Cytoplasmic inclusions involving the cell soma include the neurofibrillary tangle of Alzheimer's disease and the Lewy body of Parkinson's disease. In addition, most disorders show at least some degree of neuritic inclusion pathology. These similarities imply that neurodegenerative

diseases may share important common pathogenic features. In particular, the presence of abnormal protein aggregates in all of these disorders has supported the idea that neurodegenerative disorders may be diseases of 'toxic proteins' that aggregate abnormally and thereby cause cell dysfunction and death (1).

The idea that abnormal protein folding and aggregation are key abnormalities in neurodegenerative diseases has also gained support from initial genetic modifier screens performed in *Drosophila*. The first polyglutamine disease modeled in flies was spinocerebellar ataxia type 3 (SCA3) (2). Subsequently, heat shock proteins were shown to play a critical role in ataxin 3 toxicity. Overexpression of human heat shock protein 70 (Hsp70) substantially ameliorated ataxin 3 retinal toxicity in the *Drosophila* model of SCA3, whereas the expression of a dominant negative form of *Drosophila*

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heat shock protein 40 enhanced retinal toxicity (3). Similarly, Kazemi-Esfarjani and Benzer (4) performed a forward genetic screen in a separate model of polyglutamine toxicity based on the expression of a stretch of 127 glutamines attached to a histidine tag (Q127) and found the suppression of polyglutamine toxicity in the retina by the expression of the heat shock protein Hdj1 and the treicorepeat protein Tpr-2 that contains a DNAJ domain. These studies likely have relevance to vertebrates because overexpression of human Hsp70 ameliorated toxicity in mouse model of spinocerebellar ataxia type 1 (SCA1) (5). Expression of human Hsp70 has also been reported to suppress neurotoxicity in a *Drosophila* model of Parkinson's disease (6).

Despite persuasive arguments for common underlying disease mechanisms, clinical and pathological analyses reveal striking differences among neurodegenerative disorders. For example, Alzheimer's disease is characterized by cognitive dysfunction and degeneration of cortical neurons. Pathologically, extracellular amyloid plaques and intracellular neurofibrillary tangles are seen. In contrast, patients with Huntington's disease have abnormal movements (chorea) and selective degeneration of striatal neurons accompanied by intranuclear inclusion formation. Clearly, the cellular pathways controlling cell death in these disorders must be distinct at least to some degree.

In fact, the 'polyglutamine disorders' provide a particularly salient example of the differences that must occur even among neurodegenerative disorders with similar underlying pathogenic mechanisms. Polyglutamine disorders are dominantly inherited neurodegenerative diseases that result from the expansion of a CAG repeat within the coding region of the disease gene, producing an abnormally long polyglutamine tract. To date, nine neurodegenerative disorders caused by polyglutamine expansions have been identified: Huntington's disease, dentatorubropallidolusian atrophy, spinobulbar muscular atrophy and several SCAs (7). Each of these disorders is characterized by selective neuronal loss in specific regions of the brain. The predilection of Huntington's disease for the caudate and the putamen has been mentioned earlier. In two of the diseases modeled in the current work, SCA3 and SCA1, there is some overlap in the affected areas, but SCA3 is distinguished from SCA1 by the relative preponderance of pathology in the basal ganglia. In contrast, SCA1 tends to show severe cerebellar cortical and marked spinal cord involvement, including the cholinergic anterior horn cells (8). Thus, protein context must be critical in determining the clinical and pathological differences evident in the various polyglutamine disorders.

As a number of neurodegenerative diseases have now been modeled in *Drosophila*, and genetic modifiers identified, we can use a comparative genetic approach to identify common and distinct pathways among these disorders. In addition to the effects of the heat shock proteins, previous modifier analyses have identified components of the ubiquitin/proteasome system as key mediators of polyglutamine toxicity (9,10). A number of RNA binding proteins have been implicated in SCA1 pathogenesis (9). The control of histone acetylation is important in a fly model of Huntington's disease (11), and has also been implicated in mouse models of Huntington's disease (12,13). In contrast, a modifier screen in a *Drosophila*

model of tauopathy implicated phosphorylation as a major determinant of tau neurotoxicity, but did not identify components of the protein folding or degradation machinery (14).

Specificity for particular subsets of neurons is a common feature in the polyglutamine diseases modeled in *Drosophila*, but thus far genetic modifiers have been predominantly derived from genetic screens or candidate testing in the retina. Therefore, we determined whether the modifiers could also affect defined subsets of neurons in the brain of *Drosophila*. We have thus developed robust models for studying toxicity in postmitotic neurons of the brain. In general, we find that common pathways mediate toxicity in polyglutamine diseases both in the retina and in the brain. These pathways are generally distinct from those operating in tauopathy. However, we also identify modifiers that are specific for particular cell types in the nervous system and for individual disease models as well as two modifiers that show activity in all the models tested.

## RESULTS

To determine whether common pathways control cell death in the *Drosophila* models of human neurodegenerative diseases, we tested modifiers previously described in polyglutamine diseases (3,4,9,10,11) and in tauopathy (14) in models of SCA3 (2), SCA1 (15) and a polyglutamine disease model consisting of an expanded polyglutamine tract of 127 residues attached to a histidine tag (Q127) (4). Previously described polyglutamine modifiers included transgenes capable of overexpressing human Hsp70 and a dominant negative form of *Drosophila* heat shock cognate 4 (Hsc4<sup>DN</sup>) (3), a large number of modifiers recovered in the forward genetic screen to identify modifiers of SCA1 toxicity (9) and transgenes capable of overexpressing Hdj1 and Tpr-2 (4). A complete list of polyglutamine modifiers tested is included in Materials and Methods. In addition, drugs capable of inhibiting histone deacetylase, including sodium butyrate and nicotinamide (vitamin B3), were also used. The modifiers that produced unequivocal effects in at least one of the models, but did not themselves cause a rough eye in trans to *GMR-GAL4*, are presented in Table 1 and were pursued further. Two modifiers, *UAS-Tpr* (4) and *EP(2)2417* (9) caused a rough eye in trans to *GMR-GAL4* and were not examined further. We also tested the entire group of modifiers recently identified in the laboratory as enhancers or suppressors of the rough eye produced by the expression of human tau in the fly retina (14). The entire list of these modifiers is given in Materials and Methods. None of these modifiers produced a significant rough eye in trans to *GMR-GAL4*. Only modifiers that produced a clear enhancement or suppression in at least one polyglutamine model were pursued further.

### Modifiers of retinal toxicity

The modifiers that proved generally most effective in the models tested were the heat shock proteins. Although overexpression of the heat shock proteins Hsp70 or Hdj1 did not suppress tau retinal toxicity (14), these transgenes provided the most general and robust modification of polyglutamine

Table 1. Effects of genetic modifiers on retinal and brain toxicity

Gene	Mammalian homolog/function	Mutant or transgene	Modifiers of retinal degeneration ( <i>GMR-GAL4</i> )		Modifiers of brain degeneration	
			SCA3	SCA1	SCA3 ( <i>30Y-GAL4</i> )	SCA1 ( <i>Cha-GAL4</i> )
Chaperones	<i>Hsp70</i>	<i>UAS</i>	Su	Su	Su	Su
	<i>Hdl1</i>	<i>UAS</i>	Su	Su	Su	Su
	<i>Hsc4<sup>DN</sup></i>	<i>UAS</i>	En	En	NC	NC
Apoptosis	<i>P35</i>	<i>UAS</i>	Su	Su	Su	Su
	<i>th</i>	<i>UAS</i>	Su	Su	Su	Su
Transcription factor	<i>dSir 2</i>	<i>EP(2)2300</i>	NC	En	NC	NC
	<i>tara</i>	<i>EP(3)3463</i>	NC	En	NC	NC
RNA binding	<i>pum</i>	<i>EP(3)3461</i>	NC	En	NC	NC
Miscellaneous	<i>CG5166</i>	<i>EP(3)3145/UAS</i>	En	En	NC	En*
	<i>CG7231</i>	<i>EP(2)2510</i>	En	En	En	En*

Su, suppressor; En, enhancer; En\*, lethal; NC, no change.

toxicity (Table 1, Fig. 1). When an expanded form of ataxin 3 (*UAS-SCA3-78*) was expressed in the retina (driver: *GMR-GAL4*), flies showed moderately rough and depigmented eyes at eclosion (Fig. 1A). Similarly, expression of an expanded version of ataxin 1 (*UAS-SCA1-82*) or Q127 (*UAS-Q127*) also produced roughened and depigmented eyes (Fig. 1E and I). Overexpression of either human Hsp70 or *Drosophila* Hdj1 suppressed the retinal toxicity in each of the polyglutamine models (Table 1, Fig. 1B, F and J).

Expression of antiapoptotic proteins also produced a modification in most of the models. The baculovirus protein p35 and the *Drosophila* DIAP1 homolog *thread* suppressed the retinal toxicity of expanded ataxin 3 (Table 1, Fig. 1C) (2), expanded ataxin 1 (Table 1, Fig. 1G) and tau (Table 1) (14,16). However, no suppression of retinal toxicity in flies expressing the Q127 protein was observed (Table 1, Fig. 1K). Crosses of flies expressing Q127 with flies expressing p35 or *thread* were repeated at 17°C to minimize endogenous toxicity of Q127 in an attempt to uncover modification by the antiapoptotic proteins. No modification was observed with the more modest toxicity of Q127 seen at the lower temperature (Supplementary Material).

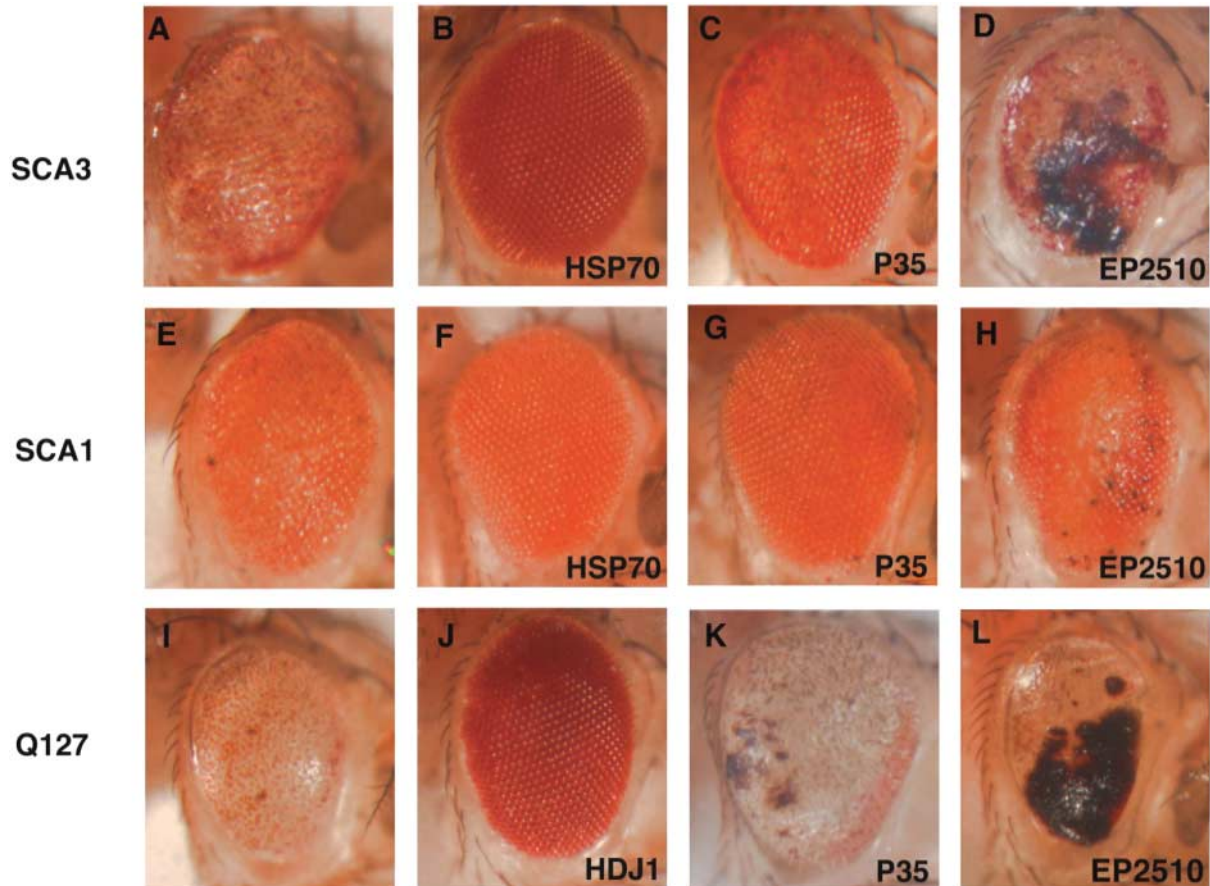
One goal of this work was to determine whether there is genetic evidence for a common pathway controlling neurodegeneration in the *Drosophila* models of human neurodegenerative diseases. Two modifiers were active in all the models tested. The EP element insertion *EP(2)2510* enhanced the retinal toxicity in all the models (Table 1, Fig. 1D, H and L). The *EP(2)2510* insertion is in the first exon of the novel gene *CG7231* in the inactivating orientation. Precise excision of this element reverts the modification in all the models tested, suggesting that the EP element is responsible for the phenotype. A second insertion in *CG7231*, *KG04307*, showed similar enhancement of tau toxicity, supporting the identification of *CG7231* as the modifying locus. Overexpression of the *Drosophila* homolog of ataxin 2 also enhanced the retinal pathology in all of the models (Table 1).

### Modifiers of neurotoxicity in the brain

Because efficient utilization of *Drosophila* as a genetic tool requires simple, robust and rapid tests for phenotypic modification, most genetic analyses in the *Drosophila* models of human neurodegenerative diseases have focused on the retina. However, patients with the majority of the diseases studied do not have prominent retinal pathology; instead, they show cell loss in specific, largely non-overlapping neuronal subtypes in the cortex, basal ganglia and spinal cord. Therefore, we determined whether modifiers of retinal toxicity also modified toxicity to neurons in the brain of *Drosophila*. We first needed to identify susceptible cell populations in the brain of the fly. We were able to identify vulnerable cell populations in the brain for mutant forms of both ataxin 3 and ataxin 1. The substantial toxicity of the Q127 protein limited the analysis of Q127 toxicity in the brain.

When mutant ataxin 3 was expressed in neurons of the mushroom body (driver: *30Y-GAL4*), neurons were present in normal numbers in 1-day-old flies, but ~50% of Kenyon cells degenerated by 30 days (Figs 2A, B and 3). Flies lived about 60 days under our culture conditions. Degeneration of





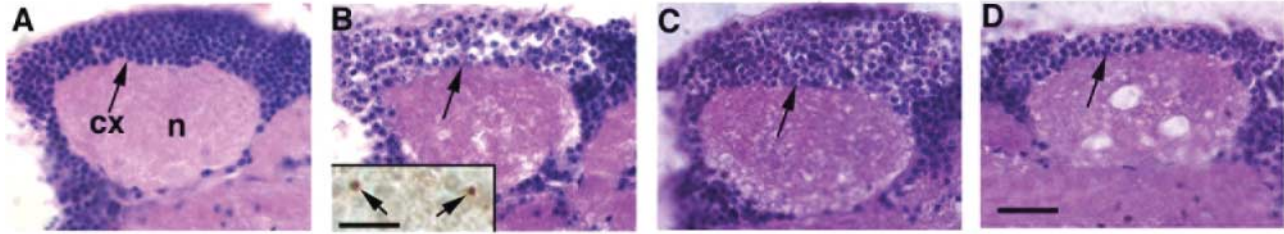
**Figure 1.** Effects of modifiers on retinal toxicity of mutant ataxin 3 (A–D), mutant ataxin 1 (E–H) or Q127 (I–L) (driver: *GMR-GAL4*). Expression of human Hsp70 (B) or the baculovirus antiapoptotic protein p35 (C) suppresses ataxin 3 toxicity. The EP element *EP(2)2510* (D) enhances ataxin 3 toxicity. Expression of human Hsp70 (F) or p35 (G) suppresses ataxin 1 toxicity. The EP element *EP(2)2510* (H) enhances ataxin 1 toxicity. Expression of *Drosophila* HDJ1 (J) suppresses Q127 toxicity, but expression of p35 (K) is ineffective. The EP element *EP(2)2510* (L) enhances Q127 toxicity.

Kenyon cells was observed as loss of cells in the cortex (compare cx in Fig. 2A and B) and rarefaction of the underlying calyx neuropil (compare n in Fig. 2A and B). In contrast, expression of expanded ataxin 1 produced no significant degeneration of Kenyon cells when expressed in the same subset of neurons (number of Kenyon cells at 30 days was  $137 \pm 3$ ,  $P > 0.05$ , compared with 30 day *30Y-GAL4/+* control).

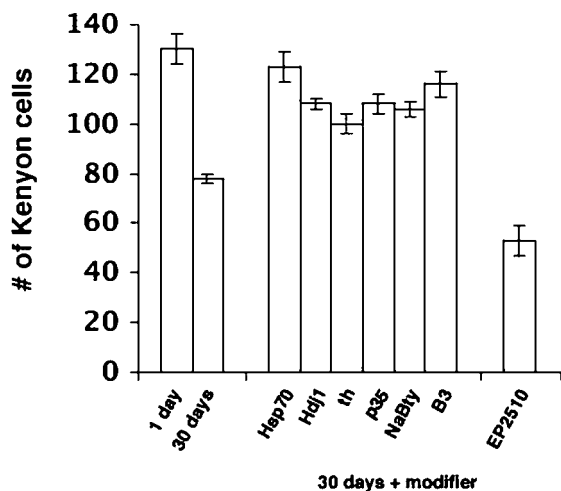
Many of the modifiers that were effective in altering toxicity in the retina also modified toxicity in the brain. In particular, expression of heat shock proteins and antiapoptotic proteins produced significant rescue of Kenyon cells (Figs 1C and 3). The role of apoptotic cell death in Kenyon cells was confirmed with TUNEL stains (Fig. 2B, inset, arrows). No TUNEL-positive cells were observed in non-transgenic controls (Supplementary Material). Protection by expression of antiapoptotic proteins was durable with significant rescue extending from 10 to 30 days (Fig. 3). Use of an adult assay of neurodegeneration facilitated administration of pharmacologic agents. Administration of the histone deacetylase inhibitors sodium butyrate and nicotinamide substantially rescued the neuronal loss (Fig. 3). The strong retinal enhancer *EP(2)2510* showed enhancement of neuronal loss with the

loss of Kenyon cells and vacuolization of the calyx neuropil (Figs 2D and 3). No toxicity to Kenyon cells was observed with the expression of *EP(2)2510* alone (number of Kenyon cells at 30 days was  $140 \pm 5$ ). The retinal enhancer ataxin 2 showed a non-significant trend towards the enhancement of the toxicity of mutant ataxin 3 in Kenyon cells (number of Kenyon cells at 30 days was  $59 \pm 4$ ,  $P > 0.05$ ).

As the expression of mutant ataxin 1 in Kenyon cells using the *30Y-GAL4* driver did not produce significant toxicity, we expressed ataxin 1 in a variety of additional neuronal subpopulations and determined that cholinergic neurons of the lamina were susceptible to ataxin 1 toxicity. When mutant ataxin 1 was expressed in cholinergic neurons (driver: *Cha-GAL4*), ~50% of these cells degenerated after 30 days (Figs 4 and 5). Figure 4A (arrow) shows well preserved lamina neurons in control flies. In the presence of ataxin 1, these neurons were markedly depleted (Fig. 4B, arrow indicates large gaps between preserved cholinergic neurons). In contrast to the toxic effects of ataxin 1, lamina cholinergic neurons were resistant to the toxicity of mutant ataxin 3 (number of lamina neurons at 30 days was  $14 \pm 1$ ,  $P = 0.6$  compared with 30 day *Cha-GAL4, UAS-GFP/+* control).



**Figure 2.** Expression of mutant ataxin 3 produces degeneration of Kenyon cells in the adult brain. Compare normal numbers of cortical neurons (A, arrow) in a 30-day-old control fly (genotype: *30Y-GAL4/+*) with substantial loss of Kenyon cells in a fly expressing mutant ataxin 3 (B, arrow). cx indicates cortex, n indicates neuropil. Expression of human Hsp70 rescues Kenyon cell loss (C, arrow), whereas the EP element *EP(2)2510* (D, arrow) enhances neurodegeneration. Scale bar is 10  $\mu$ m. Inset in (B) shows TUNEL-positive Kenyon cells (arrows). Scale bar is 5  $\mu$ m.



**Figure 3.** Quantitative analysis of effects of modifiers on toxicity of mutant ataxin 3 to Kenyon cells (driver: *30Y-GAL4*). All suppressors and the enhancer were significantly different from 30-day-old ataxin 3 transgenic flies,  $P < 0.01$ , MANOVA with supplementary Newman-Keuls test.

As with the modification of ataxin 3 toxicity in Kenyon cells, overexpression of chaperones and antiapoptotic proteins produced significant rescue of ataxin 1 toxicity in the lamina (Figs 4 and 5). The role of apoptosis was confirmed by performing TUNEL staining (Fig. 4B, inset, arrow). No TUNEL-positive cells were observed in non-transgenic controls (Supplementary Material). Oral administration of the histone deacetylase inhibitors sodium butyrate and nicotinamide also produced significant rescue (Fig. 5). Although *dSir2*, *tara* and *pum* all significantly enhanced ataxin 1 toxicity in the eye, none showed enhancement of toxicity to cholinergic neurons of the lamina (Table 1). Overexpression of *dSir2* and *pum* likely accounts for the enhancement of toxicity in the eye by the respective EP element insertions because loss of function alleles fail to show enhancement (9). *tara* likely modifies by a loss of function mechanism as enhancement is seen with independent loss of function alleles (9). Enhancement of ataxin 1 toxicity to cholinergic neurons by *Atx2* and *EP(2)2510* appeared likely because ataxin 1 flies carrying these modifiers died prior to eclosion.

Modifiers isolated as suppressors or enhancers of ataxin 1, ataxin 3 or tau have no effect on the levels of these proteins

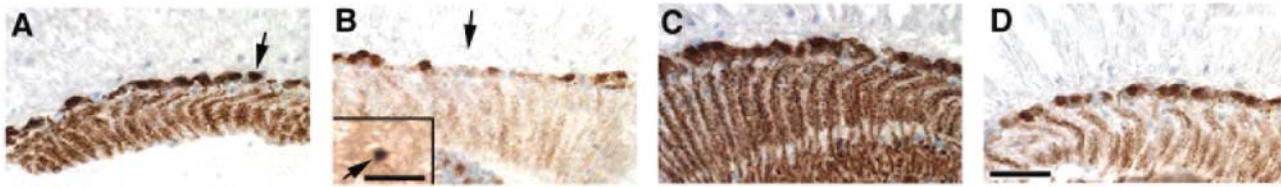
in the eye (3,9,14). To ensure that the modifiers showing effect in our brain assays (Figs 3 and 5) did not alter ataxin 1 or ataxin 3 expression in the brain, we used immunofluorescence analysis to monitor ataxin 1 and ataxin 3 levels in the presence of the genetic modifiers shown in Figures 3 and 5 and following treatment with sodium butyrate or vitamin B3. We used immunofluorescence to evaluate expression levels, because aggregation of the expanded polyglutamine proteins complicates analysis on western blots (3,9). No changes in the levels of ataxin 1 or ataxin 3 were detected, suggesting that the genetic modifiers and drug treatments did not alter toxicity by simply increasing or decreasing the levels of the polyglutamine proteins.

## DISCUSSION

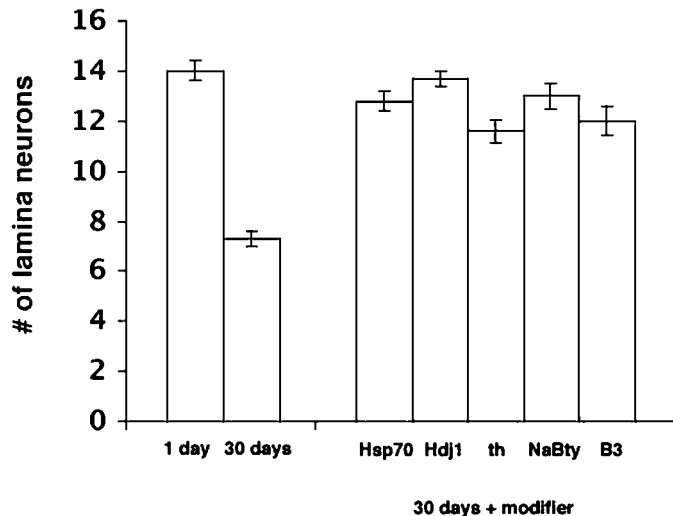
The overall goal of this study was to determine whether common pathways of toxicity govern neurodegenerative cell death in the eye and brain in the *Drosophila* models of human neurodegenerative diseases. Using a large number of previously reported genetic modifiers (3,4,9,11,14), we found that many modifiers did work in multiple models and in cells of both the retina and the brain. Overexpression of chaperone proteins was generally beneficial in polyglutamine disorders, correlating with results in the mouse model of SCA1 (5). Similarly, expression of antiapoptotic proteins protected against the toxicity of some polyglutamine proteins and against tau toxicity. We confirmed the role of apoptosis in these disorders directly with TUNEL stains (Figs 2 and 4). Apoptosis has also been implicated in human disorders (17).

Two modifiers were recovered that represent strong candidates for common mediators. Overexpression of ataxin 2 and a mutation in the novel gene *CG7231* enhanced toxicity in all models, and had similar effects in both the retina and the brain. Ataxin 2 is the *Drosophila* homolog of a protein that has an abnormal polyglutamine expansion in SCA2. The normal function of ataxin 2 is not known, but roles in regulation of the actin cytoskeleton (18) and Golgi integrity (19) have been suggested. A conserved homolog of *CG7231* is present in humans, but the sequence motifs that might suggest a possible function for the predicted protein are not present. Unlike ataxin 2, a significant stretch of polyglutamines is not predicted from *CG7231*.

In addition to potentially informative commonalities among the effects of the modifiers on the models we tested, a number



**Figure 4.** Expression of mutant ataxin 1 produces degeneration of cholinergic neurons in the optic lamina. Compare normal numbers of neurons (A, arrow) in a 30-day-old control fly (genotype: *Cha-GAL4, UAS-GFP/+*) with substantial loss of cholinergic lamina neurons in a fly expressing mutant ataxin 1 (B, arrow). Expression of *Drosophila* Hdj1 (C) or treatment with nicotinamide (D) rescues lamina degeneration. Scale bar is 10  $\mu$ m. Inset in (B) shows TUNEL-positive lamina neuron. Scale bar is 5  $\mu$ m.



**Figure 5.** Quantitative analysis of effects of modifiers on toxicity of mutant ataxin 1 to cholinergic neurons (driver: *Cha-GAL4*). All suppressors were significantly different from 30-day-old ataxin 1 flies,  $P < 0.01$ , MANOVA with supplementary Newman–Keuls test.

of differences emerged from our studies. Surprisingly, neither p35 nor thread expression suppressed retinal cell death produced by the expression of Q127. Both the mediators worked well in the ataxin 3 and ataxin 1 models, as well as in the tauopathy model (14,16). These findings suggest that a different mechanism of cell death may be operating in the case of Q127-mediated retinal toxicity. The failure of p35 and thread expression to rescue retinal cell death does not rule out an apoptotic mechanism, because there appears to be apoptotic cell death that is caspase independent. However, the distinction from the other polyglutamine models does suggest a protein context dependence of the final cell death pathway, despite what would appear to be similar triggering mechanisms and a similar sensitivity to other modifiers like the heat shock proteins. Indeed, context dependence is one of the most intriguing features of polyglutamine disorders. The proteins containing the expanded polyglutamine tracts neither share any sequence similarity nor have the same subcellular localization. The normal functions of most of the proteins are unknown. However, in all these diseases a critical expansion of a polyglutamine tract confers toxicity on the protein. As the human diseases are so different, the context of the polyglutamine expansion is clearly crucial. Genetic studies have yet to identify candidates that might

mediate this difference. This comparative study suggests that the modifiers showing ataxin 1-specific effects, *dSir2*, *tara* and *pum*, may represent such genes, at least in the retina.

We examined the sensitivity of neurons in the brain of the fly to polyglutamine proteins, because selective vulnerability of particular subsets of central neurons, not typically including the retinal cells, is a hallmark of neurodegenerative disorders. In contrast, most analysis in the *Drosophila* models of neurodegenerative diseases has been performed in the retina to harness the power of rapid genetic testing. Many of these studies have been done using an expression system that drives production of the toxic human proteins in both photoreceptors and non-neuronal cells (driver: *GMR-GAL4*), raising the possibility that some of the effects seen could be due to the toxicity in non-neuronal cells. Reassuringly, we find that the major classes of modifiers previously recovered, the molecular chaperones and antiapoptotic proteins, work well in postmitotic neurons in the fly brain. However, effects in the retina did not always predict effects in the brain. *dSir2*, *tara* and *pum* substantially enhanced ataxin 1 toxicity in the retina, but had no clear effects in the brain. In fact, decreasing the dose of *dSir2*, a histone deacetylase, enhanced ataxin 1 toxicity in the eye, whereas pharmacologic inhibitors of histone deacetylase rescued toxicity to cholinergic neurons in the brain (Fig. 5).

Our laboratory has previously shown specific cell-type vulnerability in the *Drosophila* models of Parkinson's disease (15) and tauopathy (20). We now demonstrate similar effects in ataxin 3 and ataxin 1 models. Expression of expanded ataxin 3 caused clear toxicity to Kenyon cells, the projection neurons of the mushroom bodies. These structures are implicated in learning and memory. In contrast, Kenyon cells were resistant to ataxin 1 toxicity. Conversely, cholinergic neurons of the lamina were sensitive to ataxin 1 toxicity, but not susceptible to the effects of mutant ataxin 3. These effects likely represent differences in the intrinsic vulnerability of these cell types rather than differences in levels of protein expression, given the reciprocal nature of the effects. Interestingly, cholinergic anterior horn cells show consistently severe degeneration in patients with SCA1 (8). The brain assays we describe are simple and robust and provide the basis for the investigation of cell-type vulnerability in these disorders.

In the current study, use of the brain assays facilitated testing of histone deacetylase as a common mediator of polyglutamine toxicity and identification of a common vitamin, vitamin B3, as a potent and general suppressor of polyglutamine toxicity. Effects of drugs on adult onset



retinal degeneration may be seen following the oral administration (11). However, ensuring adequate dosing during larval and pupal development can be problematic, when attempting to treat eye pathologies that develop prior to eclosion (Fig. 1). We circumvented this difficulty by using an adult brain assay. Using the brain assay, we were able to see clear effects of the histone deacetylase inhibitor sodium butyrate on degeneration of adult neurons caused by expanded ataxin 3 or ataxin 1. In addition, we demonstrated significant protective effects of nicotinamide (vitamin B3). Nicotinamide has recently been shown to be strong inhibitor of histone deacetylase (21). Other activities of nicotinamide are also known, including inhibition of poly(ADP-ribose) polymerase (22), but as the drug has previously been used in clinical trials (23,24) and has strong effects in two separate models of post-mitotic neuronal polyglutamine toxicity, it may represent a therapeutic possibility even in the absence of unequivocal identification of its molecular target.

Using a brain assay also allowed us to determine whether the prevention of apoptosis is a reliable method for rescuing neurons on a long-term basis. Previous work has suggested that blocking apoptosis may result in only a brief rescue of neuronal viability, with other mechanisms of cell death intervening eventually (25). In contrast, we find a long lasting, durable effect of blocking apoptosis by using the antiapoptotic proteins p35 and thread. Cells were rescued from 10 to 30 days of life, a significant period for an organism with a lifespan of ~60 days. These data suggest that antiapoptotic therapies may provide sustained benefit in neurodegenerative disorders.

In summary, we demonstrate that three pathways previously implicated in the *Drosophila* models of human neurodegenerative diseases, protein folding, histone acetylation and apoptosis are common features of neurotoxicity in several disease models and generalize from the retina to the brain. However, we also determine that none of these pathways operates in all the models examined, but instead suggest that the two novel modifiers are better candidates for common mediators. In addition, we demonstrate clear cell-type vulnerability in ataxin 3 and ataxin 1 models and show that some modifiers have retina-specific effects. Use of novel assays of neurotoxicity in specific postmitotic neurons in the adult brain facilitated the identification of a common dietary supplement, vitamin B3, as a potential candidate therapeutic for polyglutamine disorders, including the SCAs and Huntington's disease. Using these assays, we also demonstrate a sustained protective effect of inhibition of apoptosis on neuronal viability.

## MATERIALS AND METHODS

### Fly stocks

All stocks were maintained on standard cornmeal-based *Drosophila* medium at 25°C. The following *Drosophila* models of human neurodegenerative diseases were used: *UAS-SCA3-78* (2), *UAS-SCA1-82* (15) and *UAS-Q127* (4). The GAL4 driver lines used include: *30Y-GAL4* (26), *Cha-GAL4* (20) and *GMR-GAL4* (27). The following modifier transgenes and mutants previously identified as modifiers of polyglutamine toxicity were tested: *UAS-HSP70* and *UAS-HSC4<sup>DN</sup>* (3); *UAS-DNAJ1* and *UAS-Tpr* (4); *UAS-P35* (28); *EP(2)2231*,

*EP(2)2417*, *EP(2)2300*, *EP(3)3672*, *EP(3)3623*, *EP(3)3378*, *EP(3)3725*, *EP(3)3461*, *EP(3)3463*, *EP(3)674*, *P(3)292*, *P(3)1666*, *EP(3)411*, *EU3500*, *EP(X)1303* (9); *UAS-DTS5* (10) and *Sin3A<sup>8264</sup>* (11). The following transgenes and mutants were identified in the forward genetic screen for modifiers of tau toxicity (14) and were tested in the ataxin 3, ataxin 1 and Q127 models: *EP(X)1455*, *EP(3)1246*, *EP(3)3319*, *EP(3)3518*, *EP(3)3559*, *EP(3)3569*, *EP(3)1072*, *EP(3)3308*, *EP(3)3145*, *EP(3)3517*, *EP(3)3265*, *EP(3)581*, *EP(3)326*, *EP(3)3403*, *EP(3)1213*, *EP(2)2500*, *EP(2)613*, *EP(2)2504*, *EP(2)2208*, *EP(2)712*, *EP(2)2028*, *EP(2)2090*, *EP(2)2311*, *EP(2)2475*, *EP(2)2510*, *EP(2)2190*, *EP(2)2437*, *EP(2)899*, *UAS-Atx2*, *UAS-wun*, *UAS-dFmr1*, *UAS-th* and *UAS-par1*. In the cases where both an EP strain and a corresponding UAS transgene were available, both were tested and similar results were obtained. Control flies carried the appropriate GAL4 driver in the heterozygous state (genotypes: *30Y-GAL4/+*, *Cha-GAL4*, *UAS-GFP/+* and *GMR-GAL4/+*. Retinas were examined at 1 day following eclosion. *Drosophila* were fed 150 mM sodium butyrate or 100 mM nicotinamide (vitamin B3) in instant *Drosophila* medium (Carolina).

### Histology and immunohistochemistry

Adult flies were fixed in formalin at 1, 10 and 30 days following eclosion, dehydrated and embedded in paraffin. Sections were cut at 4 µm thickness and either stained with hematoxylin and eosin, immunostained, or used in TUNEL assays. Immunostaining on paraffin sections was performed using either the avidin–biotin–peroxidase method or immunofluorescence. An anti-GFP antibody from Chemicon was used at a dilution of 1:5000. An anti-HA antibody from Sigma was used at a concentration of 1:5000 to detect HA-tagged ataxin 3. An anti-ataxin 1 antisera (kind gift of H. Zoghbi) was used at a concentration of 1:250. TUNEL staining was performed according to the manufacturer's protocol (Oncogene research products: TdT-FragEL DNA fragmentation detection kit).

To evaluate Kenyon cell loss, the number of cells in a defined 35 µm area in well-oriented frontal sections was counted on hematoxylin and eosin stained sections. At least eight hemibrains were counted per genotype and time point. Quantitation of cholinergic neurons in the optic lamina was performed on brain sections immunostained for GFP as an independent marker. The number of lamina cholinergic neurons per 70 µm length was determined in well-oriented sections. At least eight hemibrains were examined per genotype and time point. All data shown are significant with  $P < 0.01$ , MANOVA with supplementary Newman–Keuls test.

### SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

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