Suppression of Polyglutamine Toxicity by the Yeast Sup35 Prion Domain in Drosophila

Received for publication, June 25, 2007, and in revised form, October 1, 2007. Published, JBC Papers in Press, October 23, 2007, DOI 10.1074/jbc.M705211.2

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The propensity of proteins to form β-sheet-rich amyloid fibrils is related to a variety of biological phenomena, including a number of human neurodegenerative diseases and prions. A subset of amyloidogenic proteins forms amyloid fibrils through glutamine/asparagine (Q/N)-rich domains, such as pathogenic polyglutamine (poly(Q)) proteins involved in neurodegenerative disease, as well as yeast prions. In the former, the propensity of an expanded poly(Q) tract to abnormally fold confers toxicity on the respective protein, leading to neuronal dysfunction. In the latter, Q/N-rich prion domains mediate protein aggregation important for epigenetic regulation. Here, we investigated the relationship between the pathogenic ataxin-3 protein of the human disease spinocerebellar ataxia type 3 (SCA3) and the yeast prion Sup35, using Drosophila as a model system. We found that the capacity of the Sup35 prion domain to mediate protein aggregation is conserved in Drosophila. Although select yeast prions enhance poly(Q) toxicity in yeast, the Sup35N prion domain suppressed poly(Q) toxicity in the fly. Suppression required the oligopeptide repeat of the Sup35N prion domain, which is critical for prion properties in yeast. These results suggest a trans effect of prion domains on pathogenic poly(Q) disease proteins in a multicellular environment and raise the possibility that Drosophila may allow studies of prion mechanisms.

Protein aggregation mediated by domains with a high percentage of glutamine and/or asparagine (Q/N)3-rich domain is associated with two phenomena: polyglutamine (poly(Q)) disease proteins and yeast prions. In the former, an expanded poly(Q) tract confers toxic gain-of-function properties on the host protein, leading to neuronal dysfunction (1, 2). The intrinsic propensity for expanded poly(Q) proteins to misfold is thought to underlie poly(Q) pathogenesis (3). In the latter situation with yeast, the Q/N-rich domains are critical for prion states, which are characterized by the capacity of the prion protein to pass on dominant, epigenetic effects through changes in protein conformation (4–6). The prion state of Sup35 (referred to as [PSI+]), one of the best-studied yeast prions, is characterized by aggregation of the protein which causes protein inactivation and a dominant nonsense suppression effect. [PSI+] is tightly correlated with select Sup35 conformations that can be detected as amyloid-like structures (7, 8). That is, similar to the pathogenic activities of expanded poly(Q) proteins, Sup35 prion states depend on changes of protein conformation.

Unlike the glutamine tract of expanded poly(Q) disease proteins, the Q/N-rich region of yeast prion domains is interspersed with other amino acids. However, pathogenic poly(Q) proteins and yeast prions are thought to aggregate through similar mechanisms. For example, studies in vitro suggest that hydrogen bonds formed between Q/Q or Q/N side chains can stabilize β-sheet conformations of the protein (9, 10), indicating that poly(Q) tracts and Q/N-rich prion domains may function as conserved motifs to mediate protein-protein interactions. In support of this hypothesis, studies have shown that a pure poly(Q) domain can replace the Q/N-rich region (amino acids 1–40) of the Sup35N prion domain without altering its prion capacity (11, 12). In addition, pathogenic activities of poly(Q) proteins and the activities of yeast prions are both modulated by molecular chaperones (13–15). These findings suggest that poly(Q) aggregates and yeast prion aggregates may share mechanisms and be modulated by common cellular pathways. Indeed, studies have shown that yeast prions and pathogenic poly(Q) proteins each promote the aggregation of the other in Saccharomyces cerevisiae (16–20). Furthermore, the yeast prion Rnq1 colocalizes with pathogenic poly(Q) Huntingtin protein in yeast (17, 19). These findings suggest that interactions may occur between protein accumulations formed by poly(Q) proteins and prions.

To examine potential physiological effects of a yeast prion domain on poly(Q) toxicity in the organism in vivo, we investigated interactions between pathogenic poly(Q) protein and the yeast prion domain Sup35N in Drosophila. Our findings suggest that Sup35 prion aggregation is conserved in the cellular environment of Drosophila and implicate a trans effect of the Sup35 prion conformation on the toxicity of pathogenic poly(Q) protein.

EXPERIMENTAL PROCEDURES

Constructs—Sup35N was amplified from yeast genomic DNA by PCR with primers: forward, 5′-CCGGCCGATTCCTGCAACTGTCGGATTCAAACCA-3′; reverse, 5′-CCGGCCGATCTCAGCTACGACAGAGCAGACTGTCGGATTCAAACCA-3′; and implicate a trans effect of the Sup35 prion conformation on the toxicity of pathogenic poly(Q) protein.
CACTAAGCGTAATCTGGAACATCGATGTTGGTAACCTC-CCATACCTTGAAC-3'. A hemagglutinin (HA) tag was added to the C terminus of Sup35N peptide by PCR. Constructs for S2 cell culture were as follows. Sup35N-YFP was generated by replacing NM (nucleotides 1–759) of the construct p425CpSUPeYFP (yellow fluorescent protein; gift from J. Weissman) with Sup35N (nucleotides 1–369) between restriction enzyme sites SacI and BglII and then subcloned into the fly cell culture vector pRmHa-3 (gift from R. Fedon). The Sup35N region was amplified from p425CpSUPeYFP with primers: forward, 5'-CCGGCCAGATCTATCGTTAAC-3' and reverse, 5'-CCGGCCAGATTCATACCTTGAGAC-3'.

Sup35M-YFP and Sup35NRΔ-YFP were then generated by replacing the Sup35N region of Sup35N-YFP with Sup35M (nucleotides 370–759) and Sup35NR region, respectively, which were amplified from constructs p425CpSUPeYFP and p316RΔ2–5-GFPsc (gift from S. Lindquist) (21) by PCR with primers: forward, 5'-CCGGCCATGACACAAATGTCGTGGAC-3'; reverse, 5'-CCGGCCAGATCTCTGTTACA-3'. SCA3trQ60-DsRed was generated by replacing the MJQ20 region of the construct MJQ20-GFP (gift from J. Weissman) (20) with SCA3trQ60 CDNA amplified from the fly line bearing UAS-SCA3trQ60 (22). The GFP tag was replaced by DsRed2 (Clontech) between restriction enzyme sites BglII and SacI. The New-CFP (cyan fluorescent protein) construct was a gift from J. Weissman (20).

Fly Lines—General fly lines were from the Bloomington Drosophila Stock Center. Flies were grown at 25 °C on standard medium unless otherwise indicated. SCA3Q84 fly lines are described (23). For generating new transgenic lines, appropriate constructs described above were subcloned into the pUAST transformation vector, and transgenic lines were generated by standard procedures using w1118 as the parental line, with mapping and balancing following standard procedures.

S2 Cell Culture—Fly S2 cells were maintained on Schneider's medium (Sigma) at room temperature. S2 cells were transfected according to standard calcium phosphate-DNA coprecipitation method, using 6 μg of total DNA. In transfections with a single construct, empty vector of pRmHa-3 was used to bring the total amount of DNA to 6 μg. 40 h after transfection, transient expression was induced by adding 0.7 M CuSO4 to a final concentration of 0.7 mM. Cells were analyzed by fluorescence microscopy at time points indicated for each experiment (Leica DMRBE microscope), and only cells with fluorescence were counted as transfected.

Serial Extraction Assay—40 fly heads were homogenized in 100 μl of Tris-buffered saline (TBS) solution (50 mM Tris-HCl, pH 7.4, 175 mM NaCl, 5 mM EDTA, 1:20 dilution of protease inhibitor (P8340; Sigma)). 50 μl of the above solution were analyzed by Western immunoblot as total protein. 50 μl was centrifuged at 100,000 g at 4 °C for 30 min. The supernatant was collected as the Tris-HCl soluble fraction, whereas the pellet was resuspended in 50 μl of 5% SDS TBS and re-centrifuged with the same conditions. The supernatant was collected as the SDS soluble fraction, and the pellet was resuspended in 50 μl of 8 M urea, 5% SDS TBS. All the fractions mentioned above were mixed with equal amount of 2× SDS sample buffer (Laemmli Sample Buffer (Bio-Rad), with 1:20 β-mercaptoethanol) before loading onto Tris-HCl pre-cast 12.5% gels (Bio-Rad) to analyze by Western immunoblot.

Immunohistochemistry and Western Analysis—Immunohistochemistry was performed on cryostat tissue as described (23). Primary antibodies included anti-HA (1:50, Y-11; Santa Cruz Biotechnology, Santa Cruz, CA), and anti-Myc (1:50, 9E10; Santa Cruz Biotechnology). Secondary antibodies (goat anti-mouse or anti-rabbit) were coupled to Alexa Fluor 488 or 594 (1:100; Molecular Probes). Chromatin was stained with Hoechst (1 μg/ml). For Western immunoblots, primary antibodies included anti-HA (1:500, 3F10 rat-horseradish peroxidase conjugate, Roche Applied Science), anti-Myc (1:500, 9E10; Santa Cruz Biotechnology), and anti-tubulin (1:10,000, mouse monoclonal; Developmental Studies Hybridoma Bank). Secondary antibodies included horseradish peroxidase-conjugated goat anti-mouse (1:4000; Chemicon).

RESULTS

The Sup35N Prion Domain Forms Aggregates and Is Recruited into Poly(Q) Inclusions in Drosophila S2 Cells—The yeast protein Sup35 has three distinctive domains (24).
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N-terminal region (amino acids 1–124), termed Sup35N, is the prion domain that is necessary and sufficient for prion activity of Sup35. The C-terminal region (amino acids 253–685) is the functional domain with translation-termination activity. The middle (M) region (amino acids 125–252) can modulate the solubility of the protein. In yeast, the prion states of Sup35 are reflected by the formation of fluorescent foci:

Sup35N-GFP fusion protein forms foci in yeast cells with prion proteins in prion states and remains soluble in yeast cells with prion proteins in nonprion states (21, 25–27) (although there are situations when the protein can form such aggregates, yet not contain self-perpetuating prions (12, 28, 29)). Mutations of Sup35N that eliminate propagation of prion states (11), such as deletion of the second through fifth R2–5 PQGGYQQYN oligopeptide repeats, result in loss of Sup35NM-GFP fluorescent foci (12, 21, 28). To investigate whether Sup35N can mediate foci formation related to its prion state in Drosophila, we made a series of Sup35N-YFP fusion proteins, including Sup35N (wild type) and Sup35NRΔ (deleted for the R2–5 repeats) (Fig. 1A) and expressed the constructs in Drosophila S2 cells.

Sup35N-YFP formed fluorescent foci in 28 ± 15% of transfected S2 cells, which is similar to the reported percentage of Sup35N-GFP foci in yeast cells (15 ± 8.4%) (27). In addition, Sup35N-YFP foci in S2 cells required the R2–5-repeat region, as Sup35M-YFP and Sup35NRΔ-YFP remained evenly distributed in all transfected cells over time (Fig. 1, B and C). These results suggested that the capacity of Sup35N to undergo foci formation is conserved in Drosophila S2 cells.

To further examine whether Sup35N showed characteristics reminiscent of prion states, we investigated potential interactions between Sup35N and poly(Q) protein. Studies indicate that yeast prions promote the aggregation of and, in some cases, colocalize to poly(Q) proteins in yeast (17, 19, 20). The prion state of Sup35 in particular has been shown to promote poly(Q) toxicity in yeast (18). Therefore, we asked whether Sup35N affected poly(Q) inclusion formation in S2 cells. To do this, we made ataxin-3-DsRed fusion proteins (SCA3tr, a truncated form that reflects the disease situation) (30), bearing either a pathogenic SCA3trQ60 (Q60) or a control length SCA3trQ22 (Q22) poly(Q) domain and expressed the constructs in S2 cells (Fig. 2A). Consistent with results in human cells (31, 32), ataxin-3 formed length-dependent inclusions in fly S2 cells (Fig. 2A). When Sup35N was coexpressed with Q60, Sup35N was recruited to poly(Q) inclusions in all transfected cells (Fig. 2B, and data not shown); however, the Sup35N expression pattern was not altered with coexpression of Q22 (Fig. 2C). This suggested that Sup35N selectively interacted with the pathogenic-length poly(Q) protein.

To examine whether the colocalization of Sup35N to Q60 inclusions was specific to the prion domain, we coexpressed Q60 with Sup35NRΔ and Sup35M. These data showed that Sup35NRΔ was also recruited to Q60 inclusions (Fig. 3B), whereas Sup35M was not (Fig. 3C). We further examined whether colocalization of Sup35N and Q60 was a general result of coexpressing any two proteins that form foci in S2 cells by expressing another prion domain from the New1p protein (New-CFP fusion protein) (20). However, coexpression of Sup35N or Q60 with New1p did not result in colocalization to the same foci (Fig. 3, D and E). We confirmed that the effects of Q60 on Sup35N foci did not depend on DsRed because Q60-CFP protein showed colocalization similar to Q60-DsRed (data not shown). Taken together, these results suggested a specific interaction between Sup35N and the pathogenic ataxin-3 protein.

Sup35N Forms Inclusions in Drosophila in Vivo—We then expressed Sup35N in Drosophila to examine whether Sup35N was capable of undergoing aggregation in the fly. Transgenic fly lines were generated that expressed Sup35N or Sup35NRΔ with an HA tag. Because the Sup35 prion domain has an intrinsic propensity to form amyloid-like aggregates (7, 10), we first...
examined whether this characteristic of Sup35N was conserved in the fly. Biochemical analysis revealed that when expressed in the eye photoreceptor neurons and associated cells with gmr-GAL4, both Sup35N and Sup35NRΔ proteins could be dissociated by SDS and detected as monomers at day 2, but formed SDS-insoluble complexes by day 23 (Fig. 4A). As a control, GFP remained monomeric in 23-day flies (Fig. 4A). This indicated that the Sup35N prion domain undergoes age-dependent protein complex formation in the fly.

In yeast, the prion conformation of Sup35 forms insoluble pellets, but the nonprion conformation remains in the salt-soluble supernatant upon centrifugation (8, 26). We therefore used a serial extraction assay to determine the solubility of Sup35N in the fly (Fig. 4B). These studies showed that the SDS-insoluble Sup35N complexes in 23-day flies were also unable to be dissociated to monomeric protein by urea (Fig. 4C). Moreover, although at day 2 Sup35N protein could be dissociated by SDS, it partitioned to the SDS-insoluble pellet upon high-speed centrifugation, suggesting that Sup35N may form intermediate aggregates that became enriched by centrifugation (Fig. 4C). These properties were similar to Sup35NRΔ (Fig. 4D). In contrast, the GFP control protein remained Tris-HCl-soluble (Fig. 4E). Taken together, these results suggested that the ability of Sup35N to undergo prion-like protein aggregation is conserved in the fly, but is not affected by the R2–5 repeats (see “Discussion”).

Because abnormal protein accumulation and misfolding is associated in many human diseases, we next examined whether Sup35N caused abnormalities when expressed in flies. Immunohistochemistry revealed irregular inclusions formed by both Sup35N and Sup35NRΔ in 23-day flies (Fig. 5, G–I), consistent with the results of biochemical analysis. However, such accumulations did not affect the morphology of the eye externally or internally compared with controls (Fig. 5, A–F). We also examined the effects of Sup35N when expressed in a variety of additional tissues; no obvious abnormalities were found (Fig. 5, J–L). Thus, although Sup35N formed SDS-insoluble aggregates resembling those of pathogenic poly(Q) protein, the Sup35N prion domain did not cause obvious deleterious effects in the fly.

Sup35N Prion Domain Suppresses Poly(Q) Toxicity; Suppression Requires the Oligopeptide Prion Domain Repeat—In S. cerevisiae, prions promote poly(Q) aggregation and toxicity (16–20). Given that Sup35N showed a similar interaction with poly(Q) protein in Drosophila S2 cells as in yeast studies, we examined whether Sup35N had an effect on poly(Q) protein toxicity in the fly.

In striking contrast to the yeast studies, we found that Sup35N dramatically suppressed eye degeneration induced by pathogenic poly(Q) protein. Normally, expression of full-

FIGURE 3. Interactions of ataxin-3, Sup35 domains, and New in Drosophila S2 cells. A, schematic of fusion proteins of Sup35 domains with YFP, SCA3trQ60 (Q60) with DsRed, and the yeast prion domain New with CFP. B, Sup35NRΔ (green) is diffuse on its own but is recruited to Q60 inclusions (red) with coexpression. Colocalization, yellow in overlaid image. C, distribution of Q60 (red) and Sup35M (green) proteins are not affected upon coexpression. D, patterns of fluorescent foci of New (blue) and Sup35N (green) are not affected upon coexpression. E, the pattern of fluorescent foci of New (blue) is not affected upon coexpression with Q60 (red).
We then determined whether the aggregation of the Sup35N prion domain was affected by the pathogenic poly(Q) protein. These studies showed that both Sup35N and Sup35NR\(\Delta\) were recruited to poly(Q) NIs, although more Sup35N than Sup35NR\(\Delta\) appeared recruited to NIs by immunofluorescence (Fig. 6, *H*'). Consistent with this, Western immunoblot analysis revealed that Sup35N appeared recruited into SDS-insoluble protein complexes of pathogenic SCA3 (Fig. 6K, *arrow*). In addition, SCA3Q78 increased the steady-state level of Sup35N monomeric protein and induced formation of higher molecular weight of Sup35N complexes (Fig. 6K). In contrast, SCA3Q78 had only modest effects on accumulation of Sup35NR\(\Delta\) (Fig. 6K). Deletion of the R2–5 repeats, therefore, mitigated the effect of SCA3Q78 to cause accumulation of the Sup35N prion domain.

To further examine mechanisms of Sup35N suppression, we asked whether the suppression was dependent on the ubiquitin protease activity of the ataxin-3 protein. To do this, we coexpressed Sup35N with an N-terminal truncated form of ataxin-3, which does not have the ubiquitin protease domain or the ubiquitin interaction motifs (UIMs) (23, 33–35) (Fig. 7A). In this situation suppression by Sup35N was retained, reflected by the recovery of external eye morphology (Fig. 7C) and internal retinal structure (Fig. 7F). This effect was also dependent on the Sup35 prion domain because Sup35NR\(\Delta\) had no effect (Fig. 7, D and G). These results suggested that suppression of Sup35N does not depend on the ubiquitin-associated activities of ataxin-3. We further examined the ability of Sup35N to suppress the pathogenicity of a highly toxic form of ataxin-3 in which the ubiquitin protease domain is mutated by changing the catalytic cysteine residue to alanine (SCA3Q88-C14A) (23, 33) (Fig. 7A). Sup35N still suppressed the severe degeneration caused by SCA3Q88-C14A, although the suppression was markedly weaker than that of the normal full-length protein (Fig. 7, *H*–*J*), perhaps because of the more severe toxicity of the C14A form of the protein and its deleterious interactions compared with those of the truncated protein lacking the N-terminal domain.

**DISCUSSION**

To investigate interactions between prion and poly(Q) proteins, we examined the cellular effects of the yeast prion domain
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Suppression of Poly(Q) Toxicity by Sup35N—Our studies revealed that the Sup35N prion domain is recruited into poly(Q) NIs and suppresses poly(Q) toxicity in Drosophila. The recruitment of Sup35N into poly(Q) NI is consistent with previous yeast studies showing that Q/N-rich proteins colocalize with expanded poly(Q) protein (17, 19). However, the physiological effects of such recruitment are opposite: in yeast, this leads to enhancement of poly(Q) toxicity; in the fly, this is associated with suppression of poly(Q) toxicity.

Yeast prions can exist in many different conformations in the cell, which can affect interactions with other prions and cellular factors (4, 24, 36–38). Similarly, aggregation of poly(Q) proteins is thought to involve many different intermediate products, such as oligomers, protofibrils, and fibrils (3, 39). Emerging evidence suggests that poly(Q) oligomers of select conformations may be the toxic species (40). Colocalization of yeast prions (or other Q/N-rich proteins) and poly(Q) proteins may modulate poly(Q) toxicity by altering poly(Q) protein conformation. In studies by Duennwald et al. (17), it is proposed that such colocalization may promote more toxic conformations of the poly(Q) protein. Given our findings, we suggest that Sup35N suppressed poly(Q) toxicity by promoting less toxic conformations of the pathogenic poly(Q) protein in the fly, as reflected by an increase in the solubility of the protein. Alternatively or in addition, since the ataxin-3 protein induced formation of higher molecular weight protein complexes of Sup35N, it is also possible that Sup35N underwent conformational changes or changes in interactions with other cellular factors that contribute to modulating poly(Q) toxicity.

Some of our findings suggest that the ubiquitin-protease activity of ataxin-3 may be important for Sup35N suppression. Although the activity is not required for Sup35N suppression, mutation of the ubiquitin protease domain mitigated the suppression. This reflects findings from other studies emphasizing the importance of protein context for poly(Q) toxicity and the interactions of poly(Q) proteins with other cellular factors (23, 41–43).

Sup35N May Form Prion-like Conformations in Drosophila Cells—In Drosophila S2 cells, Sup35N had different conformations from Sup35NΔ, as reflected by the formation of fluorescent foci in one situation and not the other. As foci formation in fly cells required the R2–5 oligopeptide repeat that is also critical for prion states of Sup35 in yeast (12, 21, 28), this raises the possibility that the Sup35N fluorescent foci may reflect self-propagating prion states of Sup35 in fly cells. On the other hand, in Drosophila, Sup35N and Sup35NΔ formed protein complexes with similar morphology and biochemical solubility. This may indicate that, in a multicellular environment, the R2–5 repeat of Sup35N is not critical for Sup35N accumulation. Alternatively, the R2–5 repeat may alter the formation of Sup35N in a way that is simply not reflected in the morphology.
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**FIGURE 6.** Sup35N suppresses poly(Q) toxicity and requires the oligopeptide repeats. A–C, external eye and D–F, nuclear staining of 7-day flies (arrows indicate depth of the retina which reflects poly(Q) toxicity). Expression of full-length SCA3Q78 causes loss of external eye pigmentation (A) and internal retinal tissue (D). Genotype: gmr-GAL4/+; UAS-SCA3Q78/+ . Co-expression of Sup35N suppresses ataxin-3-induced retinal degeneration, with flies now showing restored external (B) and internal retinal structure (E). Genotypes: gmr-GAL4/+; UAS-SCA3Q84/UAS-Sup35N. Co-expression of Sup35NRΔ has little effect on ataxin-3-induced degeneration, with flies showing loss of pigmentation (C) and collapse of retinal structure (F) (compare with panels A and D). Genotype: gmr-GAL4/+; UAS-SCA3Q84/UAS-Sup35NRΔ . G–I, immunostaining of flies expressing SCA3Q84 alone or with Sup35N or Sup35NRΔ . Co-expression of either Sup35N (H) or Sup35NRΔ (I) with SCA3Q84 has little effect on poly(Q) NI formation (green), compared to SCA3Q84 alone (G). Both Sup35N (red) (H) and Sup35NRΔ (red) (I) are recruited to the NIs. Genotypes: gmr-GAL4 UAS-SCA3Q84 in trans to w1118 (G), UAS-SCA3Q84 (H, H′), UAS-SCA3Q84 (I, I′). J and K, Western immunoblot of protein from 7-day flies expressing SCA3Q78 alone or with Sup35N or Sup35NRΔ . j, Co-expression of Sup35N, but not Sup35NRΔ , increases the amount of SDS-soluble polyQ protein (arrowhead). K, Co-expression of SCA3Q87 and Sup35N increases the level of Sup35 monomer (arrowhead) and induces formation of high molecular weight protein complexes. Some of the Sup35 is now retained in the gel loading well (arrow), consistent with the location of complexed SCA3Q87 poly(Q) protein. Co-expression of SCA3Q87 and Sup35NRΔ has minimal effects on Sup35NRΔ .

**FIGURE 7.** Sup35N suppresses toxicity caused by mutant and truncated forms of ataxin-3. A, schematic of ataxin-3 constructs. The C14A mutant ataxin-3 construct has a point mutation in the ubiquitin protease domain. The truncated form of ataxin-3 has the C-terminal region (amino acids 281–411) but lacks the ubiquitin protease domain (Josephin domain) and the UIMs. B–D, external eye and E–G, internal retinal nuclear staining of 1-day flies. B and E, expression of truncated SCA3tQ78 causes severe loss of eye pigmentation (B) and retinal tissue (E). C and F, co-expression of Sup35N suppresses SCA3tQ78-induced eye degeneration, with restored eye pigmentation (C) and retinal structure (F). D and G, co-expression of Sup35NRΔ has little effect on SCA3tQ78-induced degeneration. Flies still show severe loss of pigmentation (D) and collapse of internal retinal structure (G). H–J, effect of Sup35N on ataxin-3 with a mutated ubiquitin protease domain. Expression of SCA3Q88-C14A is very toxic, causing severe loss of retinal tissue (H). Genotype: gmr-GAL4 UAS-SCA3Q88-C14A/UAS-Sup35N. Co-expression of Sup35N has some effect to mitigate the toxicity, such that modest pigmentation is restored, although the ataxin-3 protein still displays significant toxicity (J). Genotype: gmr-GAL4 UAS-SCA3Q88-C14A/UAS-Sup35NRΔ . Sup35N has no effect on ataxin-3 toxicity (J). Genotype: gmr-GAL4 UAS-SCA3Q88-C14A/UAS-Sup35NRΔ.

Interestingly, yeast studies show that pathogenic huntingtin poly(Q) proteins (HttQ73 or HttQ120) can promote the self-propagating conformation of Sup35, resulting in a higher percentage of cells that are [PSI+] of the inclusions or protein solubility.
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(16). Consistent with this, our studies showed that pathogenic ataxin-3 induced Sup35N-containing protein complexes in the fly of multiple distinct molecular weights (see Fig. 6K), whereas there were milder effects on Sup35NRA. This raises the possibility that, as in yeast, pathogenic poly(Q) protein can induce conformational changes of Sup35N in the fly.

Although many studies have suggested that prions are a prevalent phenomenon throughout the animal kingdom (4, 5), it is currently not clear whether such mechanisms exist in organisms like Drosophila. Although Drosophila has orthologs of the functional domain (the elongation factor) of S. cerevisiae Sup35,4 the lack of orthologs of the Sup35 prion domain Sup35N suggests that the endogenous Drosophila prion protein (if there is one) does not share a similar protein sequence with Sup35N. Transgenic expression of the mammalian prion PrP in Drosophila failed to recreate prion states of PrP (44, 45). This could be because of lack of specific cellular factors in flies to support prion states of PrP or lack of general mechanisms for prions. Our study provides a clue that the yeast Sup35 prion domain can exist in different conformational states of Drosophila in vivo and that these conformations are promoted by pathogenic poly(Q) protein. These results suggest that Drosophila may allow both study of mechanisms underlying prion conformational transitions in a multicellular environment, as well as interactions of prion-like proteins with aggregation processes of poly(Q) and other disease proteins.

Acknowledgments—We thank J. Jung for comments and J. Weissman, R. Fehon, and S. Lindquist for reagents.

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