

FIGURE 10.1 Importance of protein context to disease protein toxicity. External eyes and immunostained cryosections of retinal tissue of flies expressing various combinations of pathogenic and normal ataxin-3. The white arrow in cryosections indicates the depth of the retina (as revealed by Hoechst staining and epon sectioning), which reflects the degree of retinal degeneration. (A) Normal eye and (B) eye section immunostained for a full-length ataxin-3 nonpathogenic SCA3-Q27-UIM*, which is normally diffuse and highlights the normal depth of the retina in a nondegenerate situation. (C,D) The truncated pathogenic protein SCA3tr-Q78 causes severe retinal degeneration that is associated with severe collapse of the eye (white arrow) and inclusion formation by the pathogenic protein (D, anti-HA). Genotype *w; gmr-GAL4/UAS-SCA3tr-Q78(S)*. (E,F) Coexpression of normal ataxin-3 SCA3-Q27 with the truncated pathogenic SCA3tr-Q78 shows rescue of the (E) external and (F) internal eye, with the SCA3-Q27 protein now localized to the NI formed by SCA3tr-Q78 ([F], anti-HA, SCA3tr-Q78). Normally, SCA3-Q27 is diffusely expressed within the eye. Genotype *w; gmr-GAL4 UAS-SCA3tr-Q78(S)/UAS-SCA3-Q27*. (H–J) Eyes of flies expressing the normal pathogenic full-length ataxin-3 protein SCA3-Q84 (H), the pathogenic protein with ubiquitin interaction motifs UIMs mutated (I), and the pathogenic protein with a point mutation in the ubiquitin protease domain (J). The protease domain mutation generates a protein with dramatically enhanced toxicity, such that the toxicity of the full-length pathogenic protein now resembles that of strong expression of the N-terminally truncated protein lacking the N-terminal domain entirely [compare to (C)]. All flies in *trans* to *gmr-GAL4*. (Figures and legend adapted from [35, Figs. 1 and 5], with permission of Elsevier. Copyright © 2005.)

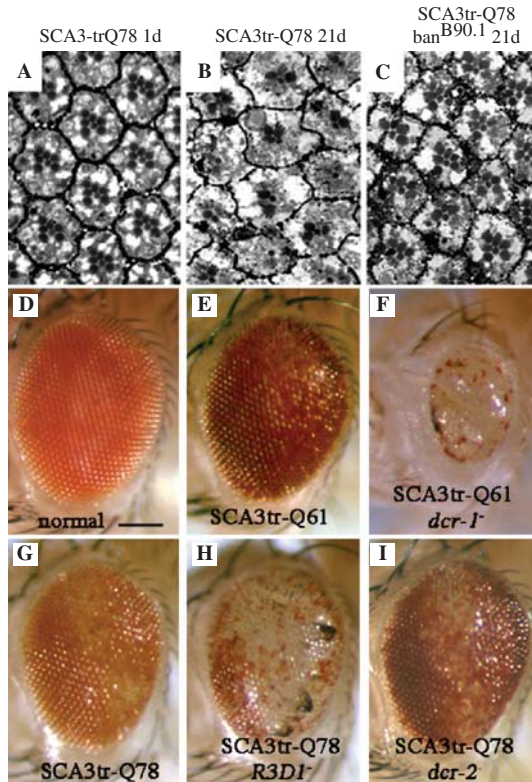


FIGURE 10.2 Modulation of ataxin-3 pathogenicity by the miRNA *bantam* and the miRNA pathway. (A–C) Tangential sections of adult fly eyes. (A) Normally, the fly eye has a highly regular pattern of seven photoreceptor neurons in each unit eye, or ommatidium, seen here in 1-day-old flies expressing the pathogenic ataxin-3, which at this time has no effect, so that the eye is normal. (B) By 21 days, ataxin-3 has induced severe degeneration, such that the regular eye structure is quite disrupted, and each unit eye has fewer than seven photoreceptor neurons. (C) Up-regulation of the miRNA *ban* with the *banB90.1* allele results in significantly reduced deterioration of the retinal structure by the pathogenic ataxin-3 at 21 days. Genotypes (A and B) *w*; *UAS-SCA3tr-Q78*/+; *rh1-gal4*/+ and (C) *w*; *UAS-SCA3tr-Q78*/+; *rh1-gal4/banB90.1*. (D–F) Compromise of miRNA processing with *dcr-1* and *R3D1* mutation result in enhanced degeneration by the ataxin-3 protein. (D) A normal fly eye. (E) *SCA3tr-Q61* normally shows weak degeneration. Genotypes (D) *gmr-gal4*/+, eye genotype: *ey-FLP*; *gmr-gal4 UAS-SCA3tr-Q61*/+; *FRT82B*. (F) *SCA3tr-Q61* degeneration in *dcr-1* is enhanced dramatically, eye genotype: *ey-FLP*; *gmr-gal4 UAS-SCA3tr-Q61*/+; *FRT82B dcr-1Q11147X*. (G–I) Expression of pathogenic ataxin-3 results in a mildly degenerate eye, reflected in disrupted pigmentation of the external eye. (G) *SCA3tr-Q61* normally shows weak degeneration. (H) Reduction of miRNA processing results in dramatically enhanced degeneration, seen here as dramatically increased loss of external pigmentation. (I) Loss of *dcr-2*, which modulates siRNA production, has no effect on polyQ toxicity. Eye genotype: (G) *ey-FLP*; *gmr-gal4 UAS-SCA3tr-Q61*/+; *FRT82B*. (I) *FRT42D dcr-2L811fsX*; *gmr-gal4 UAS-SCA3tr-Q78/ey-gal4 UAS-FLP*. Genotypes (H) *w*; *gmr-gal4 UAS-SCA3tr-Q78*/+ and (H) *w*; *R3D1f00791/R3D1f00791*; *gmr-gal4 UAS-SCA3tr-Q78*/+. Bar is 100 mm for eyes in (D–I). [(A–C) Figures and legend adapted from [68, Fig. 1], with permission of Landes Bioscience. Copyright © 2006. (D,I), Figures and legend adapted from [57, Fig. 1], with permission of Elsevier. Copyright © 2006.]

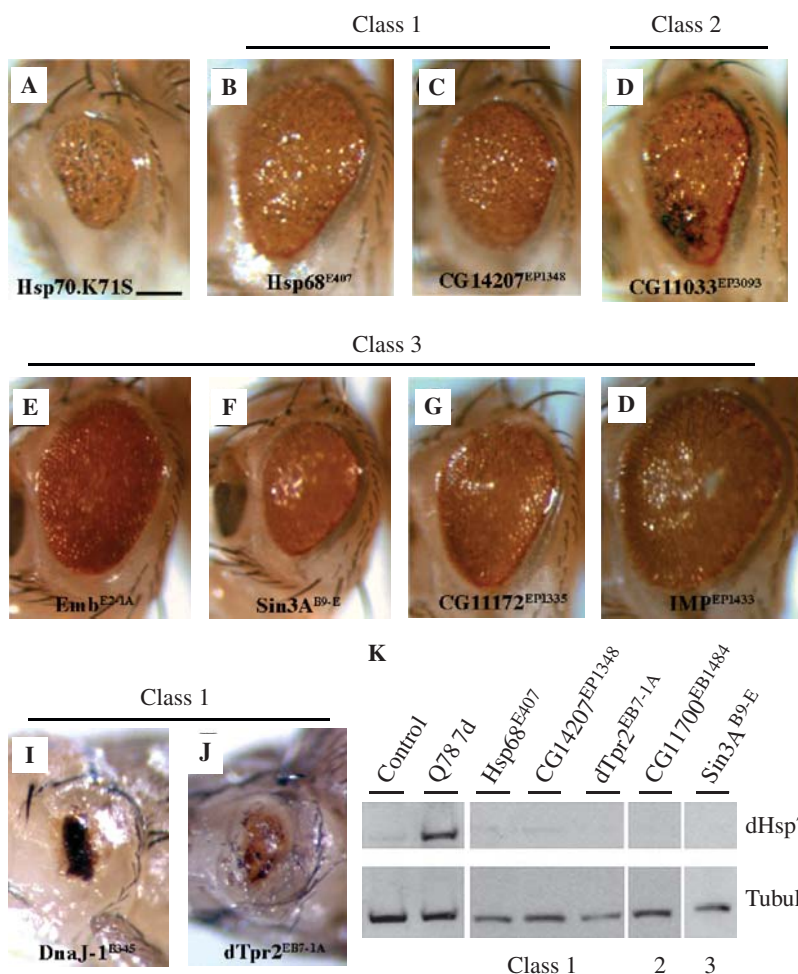
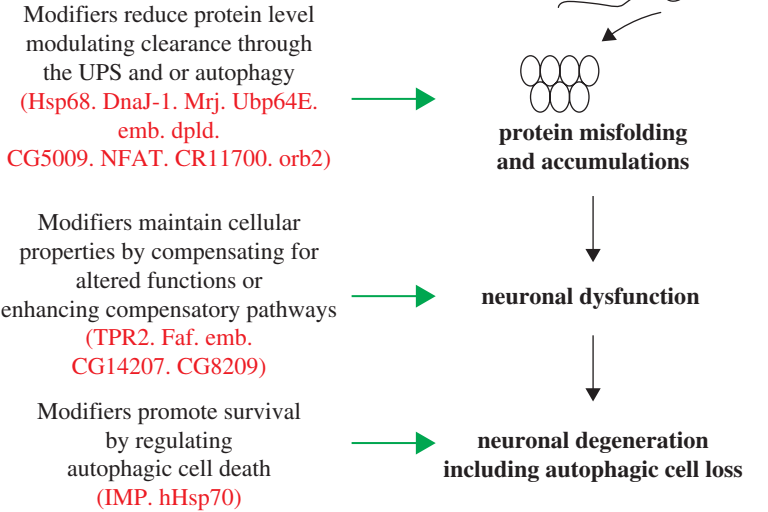


FIGURE 10.4 Genetic modifiers of ataxin-3 toxicity modulate general protein misfolding. (A) Compromised endogenous Hsp70 via expression of a dominant negative Hsp70 transgene (*UAS-Hsp70.K71E*) leads to a severely degenerate eye situation. Genotype *w; gmr-GAL4 UAS-Hsp70.K71E/+*. (B–H) The up-regulation alleles of the chaperone modifiers (B) *Hsp68*^{E407} and (C) *CG14207*^{EP1348} partially rescue Hsp70.K71E. (D) An enhancer of ataxin-3 toxicity (*CG11033*^{EP3093}) also suppresses general misfolding, suggesting that ataxin-3 toxicity and misfolding do not have identical molecular mechanisms. Up-regulation modifiers (E) *emb*^{E2-1A}, (F) *Sin3A*^{B9-E}, (G) *NFAT*^{EP1335}, and (H) *Imp*^{EP1433} also suppress the Hsp70.K71E phenotype, suggesting a role of these modifiers in protein quality control. (I and J) Co-chaperones DnaJ-1^{B345.2} and Tpr2^{EB7-1A} are pupal lethal upon expression with Hsp70.K71E, causing a more severe misfolding phenotype. Bar in (A), 100 μ m for (A–J). (K) Western blot indicates that the modifiers do not induce expression of Hsp70. Protein samples from the 1-day fly heads, with indicated genotypes, *gmr-GAL4* driver. Positive control, 7-day flies expressing the pathogenic truncated ataxin-3 protein-encoding transgene *UAS-SCA3trQ78*. (Figure and legend adapted from [75, Fig. 2], doi:10.1371/journal.pgen.0030177.g002.)

A



B

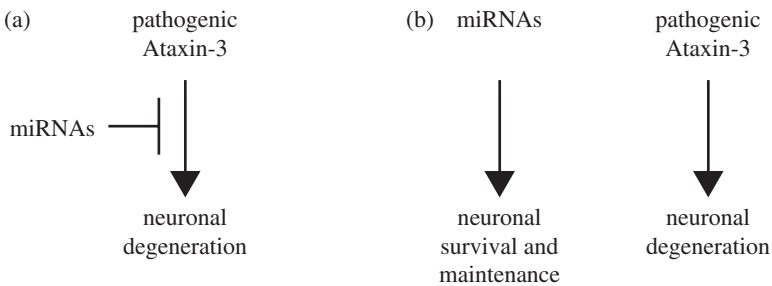


FIGURE 10.5 Overview of biological roles of modifiers of ataxin-3-associated neurodegeneration. (A) Modifiers of pathogenic ataxin-3 toxicity may (1) reduce disease protein accumulation into inclusions in a manner sensitive to proteasome activity and/or by modulating autophagy, (2) promote cellular functionality in situations of misfolded protein, and/or (3) promote neuronal survival by regulating autophagic cell loss. (B) Possible models for how the miRNA pathway influences ataxin-3-induced degeneration. (a) miRNAs may modulate mRNA target genes that affect the pathogenicity of the ataxin-3 protein, thereby mitigating neurodegeneration. One such miRNA in *Drosophila* is *ban*, which may modulate neuronal loss. (b) miRNAs, including *ban* in *Drosophila*, may modulate mRNA target genes that influence neuronal survival or maintenance. Dysregulation of miRNA targets may promote neuronal degeneration, including enhancing degeneration induced by pathogenic ataxin-3. [(A) Figure and legend adapted from [75, Fig. 6], doi:10.1371/journal.pgen.0030177.g006. (B) Figure and legend adapted from [68, Fig. 1], with permission of Landes Bioscience. Copyright © 2006.]