

Suppression of polyglutamine protein toxicity by co-expression of a heat-shock protein 40 and a heat-shock protein 110

Y Kuo¹, S Ren¹, U Lao², BA Edgar³ and T Wang^{*,1}

A network of heat-shock proteins mediates cellular protein homeostasis, and has a fundamental role in preventing aggregation-associated neurodegenerative diseases. In a *Drosophila* model of polyglutamine (polyQ) disease, the HSP40 family protein, DNAJ-1, is a superior suppressor of toxicity caused by the aggregation of polyQ containing proteins. Here, we demonstrate that one specific HSP110 protein, 70 kDa heat-shock cognate protein cb (HSC70cb), interacts physically and genetically with DNAJ-1 *in vivo*, and that HSC70cb is necessary for DNAJ-1 to suppress polyglutamine-induced cell death in *Drosophila*. Expression of HSC70cb together with DNAJ-1 significantly enhanced the suppressive effects of DNAJ-1 on polyQ-induced neurodegeneration, whereas expression of HSC70cb alone did not suppress neurodegeneration in *Drosophila* models of either general polyQ disease or Huntington's disease. Furthermore, expression of a human HSP40, DNAJB1, together with a human HSP110, APG-1, protected cells from polyQ-induced neural degeneration in flies, whereas expression of either component alone had little effect. Our data provide a functional link between HSP40 and HSP110 in suppressing the cytotoxicity of aggregation-prone proteins, and suggest that HSP40 and HSP110 function together in protein homeostasis control.

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The aggregation of misfolded proteins is a hallmark of many neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and polyglutamine (polyQ) diseases.¹ The polyQ diseases constitute a class of gain-of-function neurodegenerative disorders, caused by expansion of polyQ stretches in diverse proteins.² Suppression of protein aggregation and the acceleration of misfolded protein removal by chaperones are currently viewed as promising therapeutic approaches for the treatment of neurodegenerative disorders such as polyQ disease.³

Molecular chaperones are involved in several key cellular functions, including the suppression of protein aggregation, folding of nascent proteins, refolding of denatured proteins, and translocation of proteins.^{4,5} The majority of chaperones are referred to as heat-shock proteins (HSPs), and they participate in various protein folding and refolding events through ATP-dependent binding and release cycles.⁶ Induction of HSPs by the heat-shock transcription factor, HSF1, can significantly suppress polyQ inclusion formation in cultured cells and mouse models of polyQ diseases.^{7,8}

The evolutionarily conserved J domain protein, DNAJ/HSP40, function primarily by stimulating the adenosinetriphosphatase (ATPase) activity of other chaperones, such as HSP70. Higher eukaryotes have a variety of HSP40 family

members to regulate the substrate specificity of chaperones.⁹ A few types of HSP40 proteins have been shown to dramatically suppress toxicity in polyQ disease models.^{10–15} Overexpression of HSP70 or combination of HSP70 and HSP40 reduced aggregate formation and provided cellular protection, suggesting that HSP70 and HSP40 might function together in chaperoning aggregation-prone proteins.^{14,16,17}

The essential molecular chaperones are highly efficient in selectively recognizing misfolded proteins and maintaining them in soluble states.^{18–20} HSP110 forms high-molecular-weight complexes with HSP70, and facilitates the nucleotide exchange of HSP70.^{21–24} Recent reports suggested that HSP110 associated with protein aggregation and introducing HSP110 prevented the toxicity of aggregation-prone proteins.^{25–28} As the HSP70 homolog, it is possible that HSP110 functions with HSP40 in preventing the toxicity of aggregation-prone proteins.

Here by screening the large chaperone proteins, we identified a HSP110 family protein, 70 kDa heat-shock cognate protein cb (HSC70cb), as a regulator for DNAJ-1. Co-expression of DNAJ-1 and HSC70cb had a dramatic protective effect in polyQ disease and Huntington's disease (HD) models, and knocking down of *hsc70cb* largely reduce the protective effect of DNAJ-1 on polyQ toxicity. Finally, we

¹Division of Basic Sciences, National Institute of Biological Sciences, Beijing, China; ²Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, USA and ³German Cancer Research Center-Center for Molecular Biology Heidelberg Alliance, Heidelberg, Germany

*Corresponding author: T Wang, Basic Sciences, National Institute of Biological Sciences, No. 7, Park Road, Zhongguancun Life Science Park, Beijing 102206, China. Tel: +086 010 80726675; Fax: +086 010 80727509; E-mail: wangtao@nibs.ac.cn

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Abbreviations: polyQ, polyglutamine; HD, Huntington's disease; HSP40, heat-shock protein 40; HSP70, heat-shock protein 70; HSP110, heat-shock protein 110; TEM, transmission electron microscopy; Hsc70cb, 70 kDa heat-shock cognate protein cb; ATPase, adenosinetriphosphatase; NEF, nucleotide exchange factor; UAS, upstream activator sequence; GMR, glass multiple reporter

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found that introducing human homologs of DNAJ-1 and HSC70cb, DNAJB1 and APG-1, also suppressed the cytotoxicity of polyQ proteins including mutated huntingtin. Our results also provide the basis for the development of an HSP40- and HSP110-related therapy for polyQ diseases.

Results

DNAJ-1 suppresses polyQ toxicity independently of HSP70. The cellular mechanisms of human poly-glutamine (polyQ)-related disease are conserved in invertebrates, and fly models of polyQ diseases have proven to be useful for identifying and characterizing modulators of neurodegeneration.^{29,30} HSP40s have specific functions in removing aggregation by shuffling client proteins to degradative pathways. In *Drosophila* models of polyQ disease, the HSP40 family protein DNAJ-1 was identified as a potent suppressor of aggregation and the associated toxicity of polyQ proteins.^{10,11,31} The canonical chaperone function of HSP40 is linked to HSP70 by presenting client substrates and stimulation of HSP70 ATP hydrolysis.^{9,32} Similar to DNAJ-1, direct expression of HSP70 has been shown to suppress both SCA3- and HQ-induced neurodegeneration in *Drosophila*.^{33,34} Therefore, it has been assumed that DNAJ-1 alleviates the toxicity of polyQ proteins by interacting with HSP70.

Expression of polyQ proteins containing the expanded 127 glutamine repeat in all tissues of the fly eye during the development using the upstream activator sequence (UAS)/Gal4 system with the eye-specific driver, *GMR-GAL4* (*GMR>127Q: GMR-Gal4 UAS-127Q/+*), resulted in severely abnormal eyes with an absence of pigmentation, representing degeneration of many pigment cells (Figures 1a and b).^{11,35} Complete loss of HSP70 by two deletions in *hsp70* loci, *Df(3R)hsp70* (*Df(3R)hsp70A Df(3R)hsp70B*), did not enhance this eye degeneration phenotype of *GMR>127Q* (Figures 1b and b').³⁶ Expression of DNAJ-1 did suppress the eye degeneration phenotype of *GMR>127Q*, as evidenced by the gain of pigmentation. In homozygous *Df(3R)hsp70* background, DNAJ-1 still suppressed the external eye degeneration caused by *GMR>127Q* (Figures 1c and c'), suggesting that DNAJ-1 probably does not act together with HSP70.

To verify the degeneration of photoreceptor neurons detected externally, we examined the morphology of retinae by transmission electron microscopy (TEM). The *Drosophila* compound eye consists of ~800 repetitive ommatidia. Each single ommatidium from wild-type compound eyes contains a full complement of seven intact photoreceptor cells and surrounding retinal pigment cells. Complete loss of

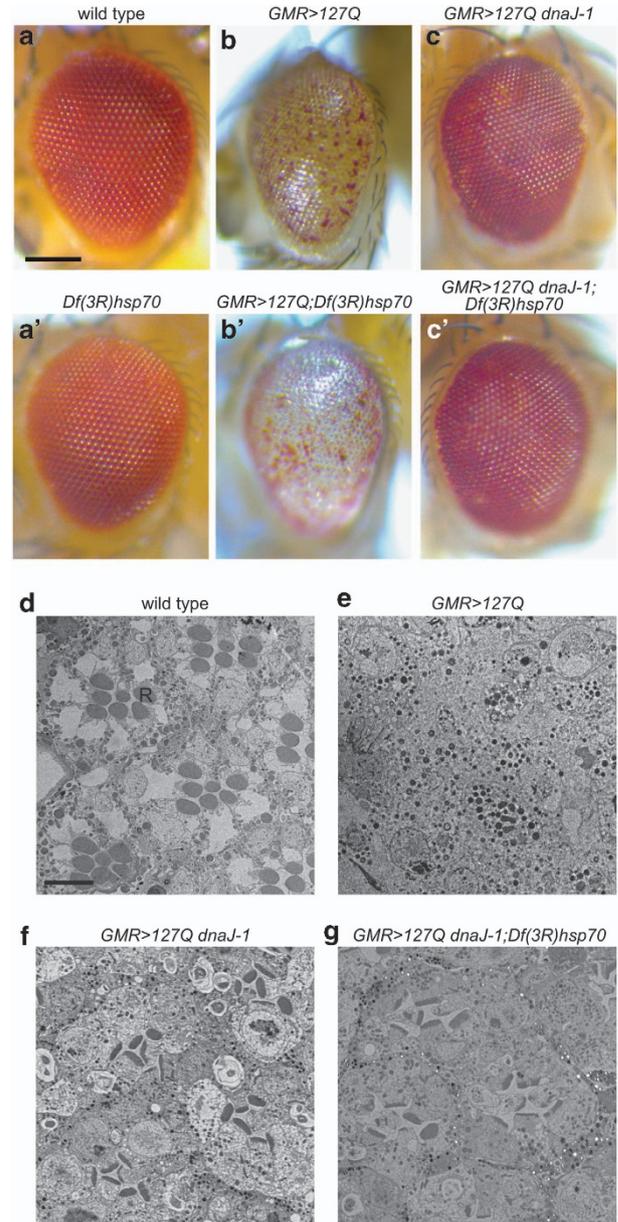


Figure 1 DNAJ-1 suppresses polyQ-induced degeneration independently of HSP70. (a–c) Photographs of the external eye of (a) wild-type, (a') *Df(3R)hsp70*, (b) *GMR>127Q: GMR-Gal4 UAS-127Q/+*, (b') *GMR>127Q; Df(3R)hsp70: GMR-Gal4 UAS-127Q/+; Df(3R)hsp70*, (c) *GMR>127Q dnaJ-1: GMR-Gal4 UAS-127Q UAS-dnaJ-1/+*, (c') *GMR>127Q dnaJ-1; Df(3R)hsp70: GMR-Gal4 UAS-127Q UAS-dnaJ-1/+; Df(3R)hsp70*. Scale bar on (a) represents 100 μm. (d–g) Examination of retinal morphology by TEM. Cross-sections were obtained from flies under 1-day old. (d) wild-type, (e) *GMR>127Q*, (f) *GMR>127Q dnaJ-1*, (g) *GMR>127Q dnaJ-1; Df(3R)hsp70*. The scale bar in d represents 5 μm, (h) Histogram of the mean number of rhabdomeres per ommatidium

photoreceptor cells was found in *GMR > 127Q* flies, whereas loss of photoreceptor cells was partially rescued in *GMR > 127Q dnaJ-1* (*GMR-Gal4 UAS-127Q UAS-dnaJ-1/+*) but not *GMR > 127Q GFP* (*GMR-Gal4 UAS-127Q/UAS-GFP*) retinæ (Figures 1 d–f, and h). Loss of *hsp70* did not have obvious effects on morphology in *GMR > 127Q dnaJ-1* retinæ (Figures 1g and h). Therefore, HSP70 is not required for suppression of the cellular toxicity of polyQ proteins by DNAJ-1.

Expression of HSC70cb enhances the cell-protective function of DNAJ-1. The action of chaperones on misfolded and aggregated proteins is ATP dependent. As HSP40 does not contain an ATPase domain, a co-chaperone with ATPase activity is likely required for HSP40-mediated anti-polyQ-induced toxicity. Our tests indicated that HSP70 is not likely to be a co-chaperone of DNAJ-1 in suppressing the cellular toxicity of polyQ proteins, and so we suspected that another large HSP might functionally interact with DNAJ-1 as a co-chaperone. In addition to HSP70, the *Drosophila* genome encodes nine other large HSPs with ATPase activity, including HSP60, HSP68, HSC70-1, HSC70-2, HSC70-3, HSC70-4, HSC70-5, HSP83 and HSC70cb (see Table 1). To find the functional partner of DNAJ-1, we expressed each of these HSPs in the retinæ of *GMR > 127Q* flies. None of them had significant suppressing effects on external eye degeneration of *GMR > 127Q*, although a little more pigmentation was detected in the eyes of several HSP expression lines including HSC70cb (Supplementary Figure 1 and Figure 2a–d). This might be because of lack of enough endogenous DNAJ-1 under normal conditions to support the chaperone activity.

Therefore, we next co-expressed each of the large HSPs together with DNAJ-1 in *GMR > 127Q* retinæ. In these co-expression tests with DNAJ-1 only HSC70cb, an HSP110 family protein, ameliorated the external degeneration of *GMR > 127Q* eyes stronger than DNAJ-1 alone (Supplementary Figures 2 and 3, Figures 2f–h and f'–h'). In young animals, *GMR > 127Q dnaJ-1* had spotted pigment loss in the retina, indicating the degeneration of retinal cells, and this phenotype was more visible in aged animals (Figures 2f and f'). This loss of pigment was not obvious in either young or aged *GMR > 127Q* eyes that co-expressed DNAJ-1 and HSC70cb (Figures 2h and h'), whereas co-expression of other HSPs and GFP did not suppress the pigment loss in eyes expressing 127Q and DNAJ-1 (Supplementary Figures 2 and 3, Figures 2g and g'). By TEM, as well as external morphology, animals expressing HSC70cb expression alone did not show any suppression the degeneration caused by *GMR > 127Q* (Figures 2k and m). However, co-expression of HSC70cb with DNAJ-1 largely ameliorated degeneration caused by *GMR > 127Q*. In 1-day old animals, all seven photoreceptor cells with normal sized rhabdomere were detected in *GMR > 127Q dnaJ-1 hsc70cb* (*GMR-Gal4 UAS-127Q UAS-dnaJ-1/UAS-hsc70cb*) ommatidia, whereas small and missing rhabdomeres were observed in *GMR > 127Q dnaJ-1* ommatidia (Figures 2k and m). In 10-day old animals, severe neuronal degeneration marked by vesicle accumulation, loss of rhabdomeres, and invasion of pigment granules was detected in *GMR > 127Q dnaJ-1* ommatidia (Figures 2l and m). Expression of HSC70cb prevented degeneration and

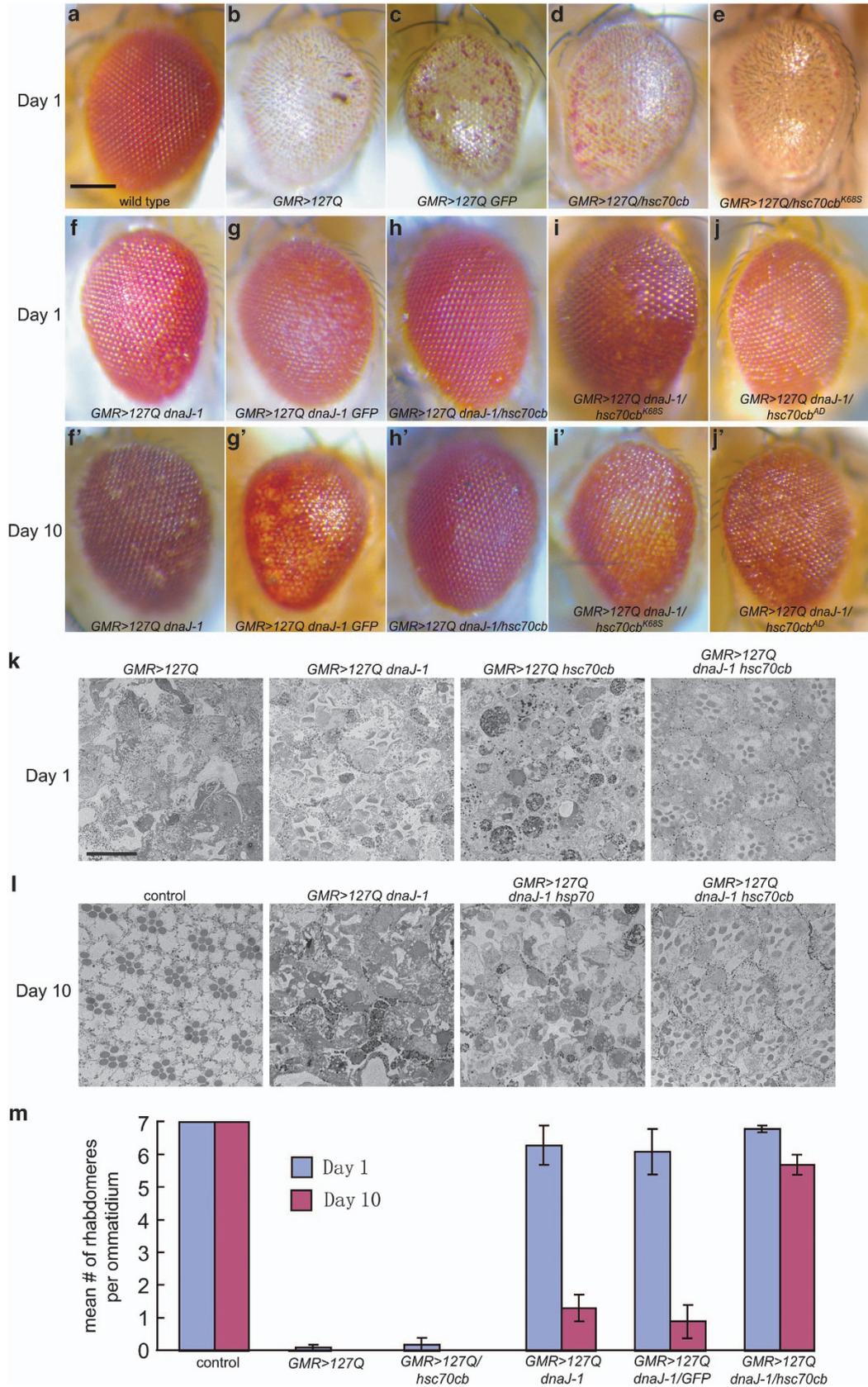
Table 1 List of heat-shock proteins used in the paper

Species	Chaperones	Molecular function	
HSP40 (DNAJ) <i>Drosophila melanogaster</i>	DnaJ-1	Unfolded protein binding	
	MRJ	Unfolded protein binding	
	DROJ2	Unfolded protein binding	
	CG5001	Unfolded protein binding	
	CG2887	Unfolded protein binding	
	DNAJB1	Interacting selectively and non-covalently with an unfolded protein	
Human	DNAJB4	Interacting selectively and non-covalently with an unfolded protein	
HSP110 <i>D. melanogaster</i>	HSC70cb	ATPase activity, NEF activity, protein folding	
	Human	APG-1	ATPase activity, NEF activity, protein folding
	HSP105	ATPase activity, NEF activity, protein folding	
HSP70 <i>D. melanogaster</i>	HSP70	ATPase activity, chaperone binding, unfolded protein binding	
	HSC70-1	ATPase activity, protein folding	
	HSC70-2	ATPase activity, protein folding	
	HSC70-3	ATPase activity, protein folding	
	HSC70-4	ATPase activity, chaperone binding	
	HSC70-5	ATPase activity, protein folding	
HSP90 <i>D. melanogaster</i>	HSP83	ATPase activity, unfolded protein binding, protein folding	

rhabdomere loss in *GMR > 127Q dnaJ-1* retinæ, whereas expression of other HSPs and GFP did not have these effects (Figures 2l and m). We further tested if the ATPase activity of HSC70cb is required for regulation of the cell-protective function of DNAJ-1, we made ATPase-dead variants of HSC70cb by either mutation of an essential amino acid in their ATP-binding domain (*UAS-hsc70cb^{K68S}*) or complete deletion of ATPase domain (*UAS-hsc70cb^{ΔD}*). Both HSC70cb^{K68S} and HSC70cb^{KD} did not further ameliorate the external degeneration of *GMR > 127Q dnaJ-1* eyes (Figures 2i, j, i' and j' and Supplementary Figure 4), which suggested that ATPase activity of HSC70cb is necessary for its function as regulator of DNAJ-1's cell-protecting function.

Next, we tested if the HSC70cb is required for functions of other HSP40 family proteins. We first checked four members of *Drosophila* HSP40 family proteins, besides DNAJ-1, transgenic expression of MRJ (mammalian relative of DnaJ) also suppressed polyQ toxicity in the retina to a less extent,^{13,37} whereas other HSP40 proteins including DROJ2, CG5001, and CG2887 lacked this function (Supplementary Figure 4). However, co-expression of HSC70cb with MRJ did not further suppress polyQ toxicity compared with MRJ alone, and suppression of polyQ by MRJ was not modified by RNAi knockdown of HSC70cb, indicating that the function of MRJ was not dependent on HSC70cb (Supplementary Figure 4).

Given this strong genetic interaction, we then checked whether DNAJ-1 physically interacted with HSC70cb *in vivo*.



We expressed DNAJ-1 and HSC70cb together in the retina under control of the UAS/GAL4 system (*GMR-Gal4/UAS-dnaJ-1 UAS-hsc70cb*), and immunoprecipitated either DNAJ-1 or HSC70cb with specific antibodies to DNAJ-1 or HSC70cb. Using either antibody, we found that DNAJ-1 efficiently co-immunoprecipitated with HSC70cb (Figure 3). In contrast, we co-expressed tagged MRJ and HSC70cb in flies with control of UAS/GAL4 system (*UAS-mrj-flag UAS-hsc70cb/GMR-Gal4*), and found that HSC70cb was not co-immunoprecipitated with MRJ (Figure 3). These results were consistent with previous results that function of DNAJ-1 but not MRJ was dependent on HSC70cb.

HSC70cb is required for the suppression of polyQ-induced cell death by DNAJ-1. We further tested the function of endogenous HSC70cb in pathogenesis of polyQ toxicity by depleting HSC70cb using RNAi derived from long double-stranded hairpin RNAs.³⁸ Expression of RNAi specific to *hsc70cb* in fly retina (*GMR>hsc70cbRi*, *GMR-Gal4/UAS-hsc70cbRi*) specifically reduced RNA level of *hsc70cb* by approximately fivefold (Supplementary Figure 5), but did not affect eye morphology (Figure 4a). Expression of *hsc70cb* RNAi in the *GMR>127Q* background, however, enhanced the external degeneration caused by *GMR>127Q*, as manifested by a complete loss of pigment (Figures 4a–c).³⁹ Importantly, expression of *hsc70cb* RNAi largely abolished the suppressive effects of DNAJ-1 on polyQ toxicity (Figures 4d and e). Moreover, in TEM sections of the retina, complete neural degeneration was observed in *GMR>127Q dnaJ-1/hsc70cbRi* (*GMR-Gal4 UAS-127Q UAS-dnaJ-1/UAS-hsc70cbRi*) retinæ, whereas most photoreceptor cells were retained in *GMR>127Q dnaJ-1* retinæ, without characteristics of cell death (Figures 4f–h). All together, these data suggest that DNAJ-1 interacts with HSC70cb in suppressing the cellular toxicity of polyQ proteins *in vivo*.

DNAJ-1 and HSC70cb function together to suppress neurodegeneration in a HD model. HD is a representative case of devastating autosomal dominant neurodegenerative disease caused by abnormal expansion of polyQ tracts. To further address the cooperative effect of DNAJ-1 and HSC70cb on polyQ-related diseases, we explored the role of DNAJ-1 and HSC70cb in a *Drosophila* model of HD. This model involves expression of an amino-terminal fragment of a human *huntingtin* protein that contains a tract of 120 glutamine residues, directly in photoreceptor neurons under the control of the *GMR* promoter.⁴⁰ Compared with control animals, which always had seven rhabdomeres per omma-

tidium regardless of age, the *GMR-huntingtin.Q120* flies (*GMR-HQ*) manifested strong age-dependent loss of rhabdomeres in an optical neutralization assay (Figures 5a and b). Expression of DNAJ-1 using the *ninaE-Gal4* driver slowed the neural degeneration caused by *GMR-HQ*, whereas expression of HSC70cb alone had no significant effect on

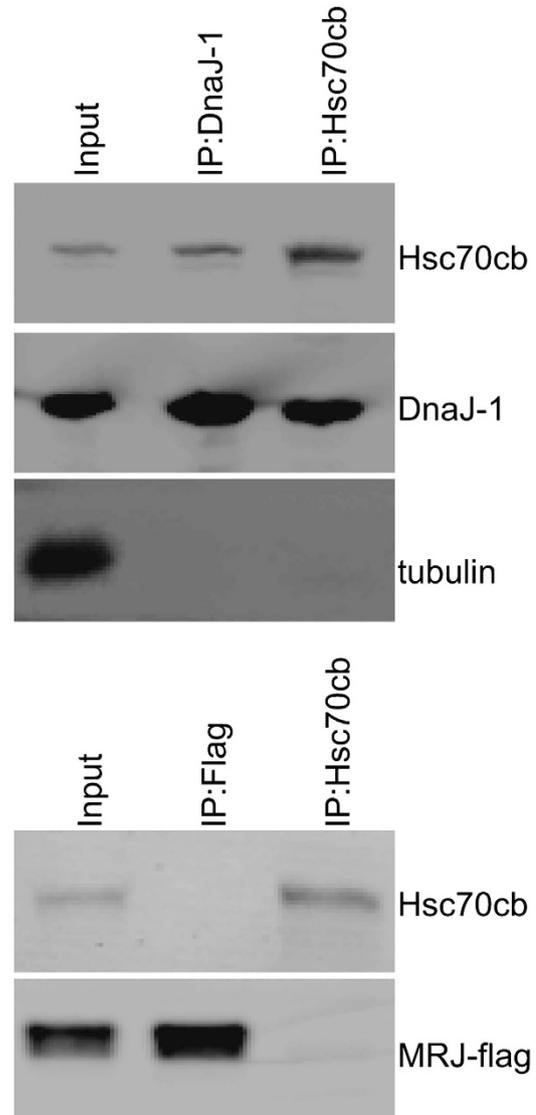


Figure 3 HSC70cb co-immunoprecipitates with DNAJ-1 from fly tissues. Protein extracts from *GMR-Gal4/UAS-dnaJ-1 UAS-hsc70cb* and *GMR-Gal4/UAS-mrj-flag UAS-hsc70cb* flies were immunoprecipitated with antibodies for DNAJ-1, HSC70cb, or Flag antibodies

Figure 2 HSC70cb interacts with DNAJ-1 and suppresses the degeneration of 127Q together with DNAJ-1. (a–e) HSC70cb did not suppress external eye degeneration caused by *GMR>127Q*. (a) Wild type, (b) *GMR>127Q: GMR-Gal4 UAS-127Q/+*, (c) *GMR>127Q GFP: GMR-Gal4 UAS-127Q UAS-GFP/+*, (d) *GMR>127Q/hsc70cb: GMR-Gal4 UAS-127Q/UAS-hsc70cb*, (e) *GMR>127Q/hsc70cb^{K68S}: GMR-Gal4 UAS-127Q/UAS-hsc70cb^{K68S}*. (f–j) HSC70cb suppressed external degeneration by *GMR>127Q* together with DNAJ-1. (f, f') *GMR>127Q dnaJ-1: GMR-Gal4 UAS-127Q UAS-dnaJ-1/+*, (g, g') *GMR>127Q dnaJ-1GFP: GMR-Gal4 UAS-127Q UAS-dnaJ-1/UAS-GFP*, (h, h') *GMR>127Q dnaJ-1/hsc70cb: GMR-Gal4 UAS-127Q UAS-dnaJ-1/UAS-hsc70cb*, (i, i') *GMR>127Q dnaJ-1/hsc70cb^{K68S}: GMR-Gal4 UAS-127Q UAS-dnaJ-1/UAS-hsc70cb^{K68S}*, (j, j') *GMR>127Q dnaJ-1/hsc70cb^{AD}: GMR-Gal4 UAS-127Q UAS-dnaJ-1/UAS-hsc70cb^{AD}*. Flies less than 1-day old were used in a–j, and 10-day-old animals were used in f'–j'. Scale bar on a represents 100 μ m. (k, l) Suppression of *GMR>127Q*-mediated photoreceptor neuron degeneration by co-expression of DNAJ-1 and HSC70cb. Cross-sections of 1-day-old retinæ from *GMR>127Q*, *GMR>127Q dnaJ-1*, *GMR>127Q/hsc70cb*, and *GMR>127Q dnaJ-1/hsc70cb* were used in k, and 10-day-old flies of control, *GMR>127Q dnaJ-1*, *GMR>127Q dnaJ-1/hsp70*, and *GMR>127Q dnaJ-1/hsc70cb* were used in l. The scale bar on k represents 5 μ m, (m) Mean number of rhabdomeres per ommatidium from 10-day-old flies

this degeneration (Figure 5b). However, co-expression of HSC70cb along with DNAJ-1 suppressed HQ-mediated rhabdomere loss more effectively than expression of DNAJ-1 alone (Figure 5b), indicating combined action.

We further examined the retinal morphology of 10-day-old and 15-day-old animals by TEM. Ommatidia from wild-type eyes contained the full complement of seven intact rhabdomeres at any age point (Figure 5c). Few rhabdomeres were detected in 10-day-old *GMR-HQ* or *GMR-HQ/ninaE>hsc70cb* (*GMR-HQ ninaE-Gal4/UAS-hsc70cb*) flies, and the rhabdomere cell bodies also showed accumulation of prominent vacuoles, a feature of degenerative cell death (Figure 5c). Expression of DNAJ-1 diminished the severity of the degeneration in *GMR-HQ* flies at 10 days, although some evidence of degeneration was observed (Figure 5c). Essentially normal morphology was restored in *GMR-HQ* flies co-expressing DNAJ-1 and HSC70cb at the 10-day age point (Figure 5c). Furthermore, in 15-day-old animals, almost complete loss of photoreceptor neurons was found in *GMR-HQ/ninaE>dnaJ-1* (*GMR-HQ ninaE-Gal4/UAS-dnaJ-1*) retinæ, whereas many normal photoreceptors still remained

in *GMR-HQ/ninaE>dnaJ-1 hsc70cb* (*GMR-HQ ninaE-Gal4/UAS-dnaJ-1 UAS-hsc70cb*) retinæ, despite of some degeneration (Figure 5d).

Human APG-1 functions together with DNAJB1 in suppressing polyQ-mediated neurodegeneration. The HSP40/DNAJ family in humans currently consists of 49 members,^{41,42} and the diversity of the HSP40/DNAJ family is thought to determine the diverse cellular function of chaperones. Among the HSP40s in *Drosophila*, DNAJ-1 has key role in relieving the toxicity of aggregated proteins, whereas other DNAJ proteins such as DROJ2, CG5001, and CG2887 lack this function (see Table 1; Supplementary Figure 4). Similarly, it has been reported that among 49 human HSP40s, several have specific functions in reducing aggregation and toxicity of polyQ proteins.¹⁵

To find the functional human analog of DNAJ-1, we queried the *human* sequence database. The closest human homologs related to *Drosophila* DNAJ-1 are DNAJB1 and DNAJB4 (see Table 1), which shared 52.4% and 54.7% identity with DNAJ-1, respectively. Hence, we tested, in flies, whether DNAJB1 or

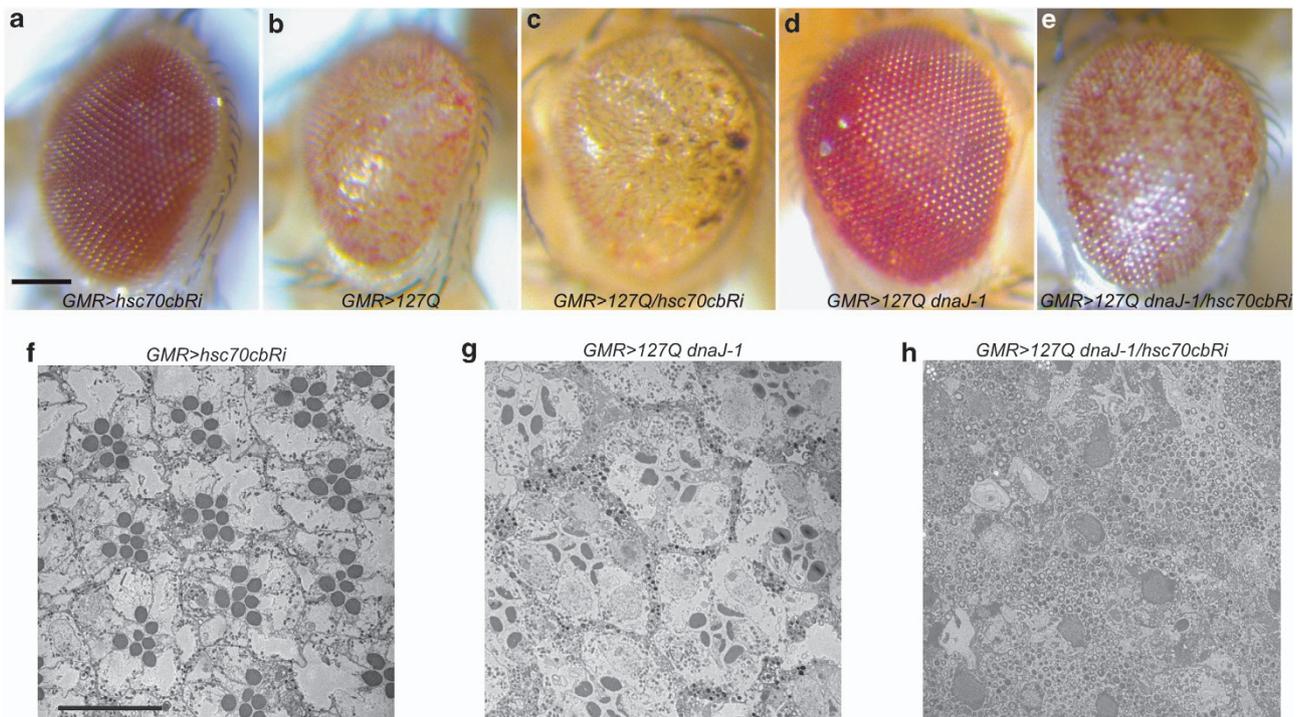
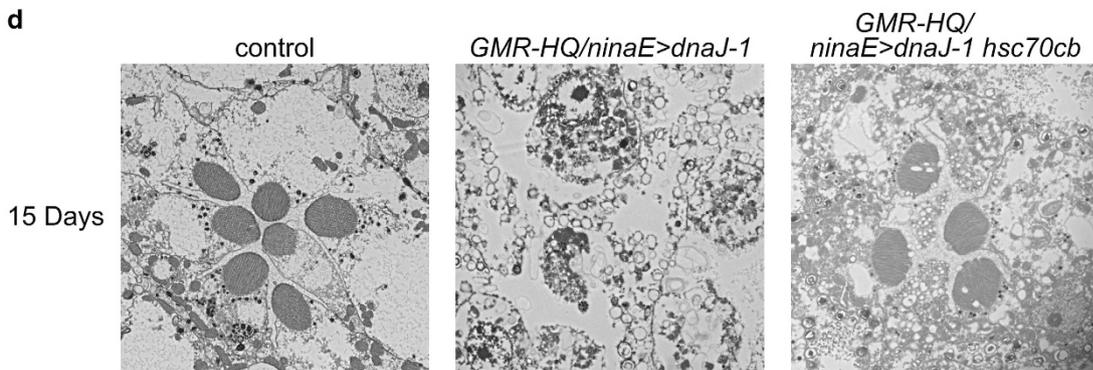
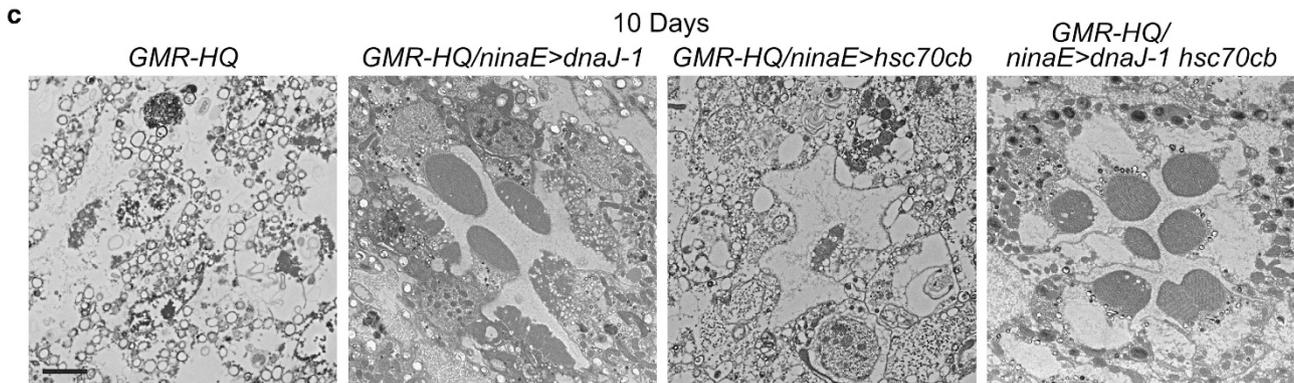
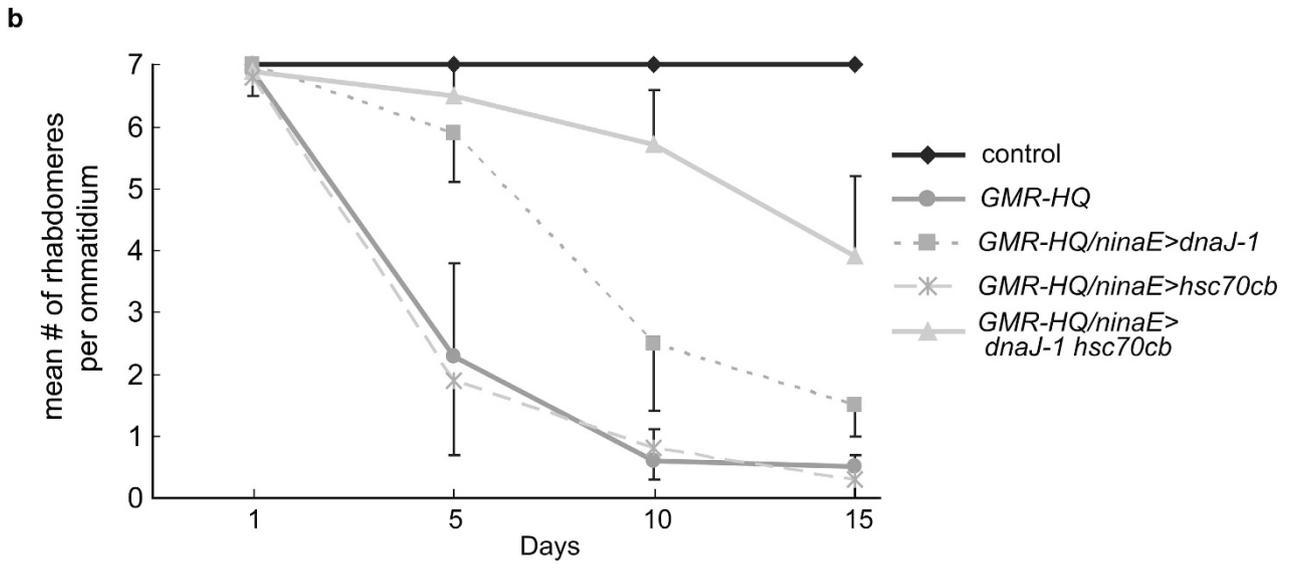
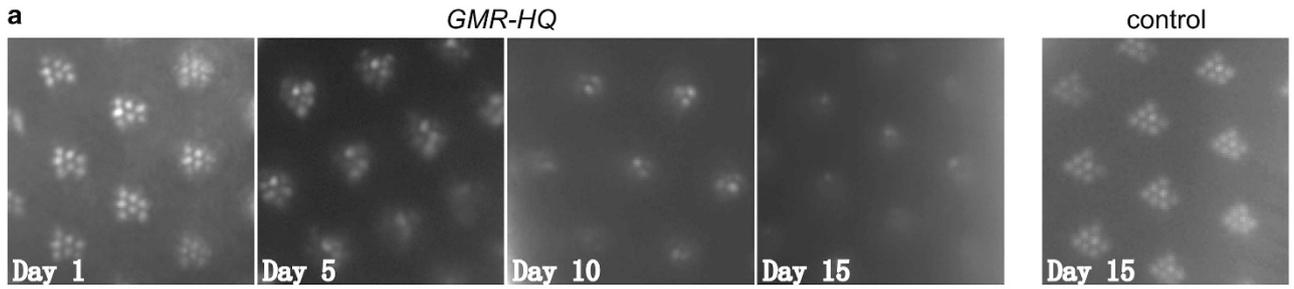


Figure 4 HSC70cb is required for DNAJ-1-mediated suppression of polyQ toxicity. (a–e) external eye morphology of (a) *GMR>hsc70cbRi*; *GMR-Gal4/UAS-hsc70cbRNAi*, (b) *GMR>127Q*; *GMR-Gal4/UAS-127Q*, (c) *GMR>127Q/hsc70cbRi*; *GMR-Gal4 UAS-127Q/UAS-hsc70cbRNAi*, (d) *GMR>127Q dnaJ-1*; *GMR-Gal4 UAS-127Q UAS-dnaJ-1/+*, and (e) *GMR>127Q dnaJ-1/hsc70cbRi*; *GMR-Gal4 UAS-127Q UAS-dnaJ-1/UAS-hsc70cbRNAi*. Scale bar on a represents 100 μ m. (f–h) Cross-sections of retina from 1-day-old animals of (f) *GMR>hsc70cbRi*, (g) *GMR>127Q dnaJ-1*, and (h) *GMR>127Q dnaJ-1/hsc70cbRi* were examined by TEM. The scale bar in f represents 5 μ m

Figure 5 Neuron degeneration in *GMR-HQ* flies is suppressed by co-expression of DNAJ-1 and HSC70cb. (a) Example of eye morphology of *GMR-HQ/ninaE>DNAJ-1* retina from 1-, 5-, 10- and 15-day-old animals by the optical neutralization technique. (b) Time course of photoreceptor degeneration was determined by the optical neutralization assay as showed in a. The flies were reared under a 12 h light/12 h dark cycle at 25 °C. Each data point was based on examination of ≥ 80 ommatidia from ≥ 5 flies. Error bars represent the SDs. (c and d) Retinal morphology was examined by TEM. (c) 10-day-old flies of *GMR-HQ* (*GMR-HQ/+*), *GMR-HQ/ninaE>hsc70cb* (*GMR-HQ ninaE-Gal4/UAS-hsc70cb*), *GMR-HQ/ninaE>dnaJ-1* (*GMR-HQ ninaE-Gal4/UAS-dnaJ-1*), and *GMR-HQ/ninaE>dnaJ-1 hsc70cb* (*GMR-HQ ninaE-Gal4/UAS-dnaJ-1 UAS-hsc70cb*) were examined. (d) Retina from 15-day-old flies of wild type, *GMR-HQ/ninaE>dnaJ-1*, and *GMR-HQ/ninaE>dnaJ-1 hsc70cb* was used. The scale bar in the left panel of c represents 2 μ m



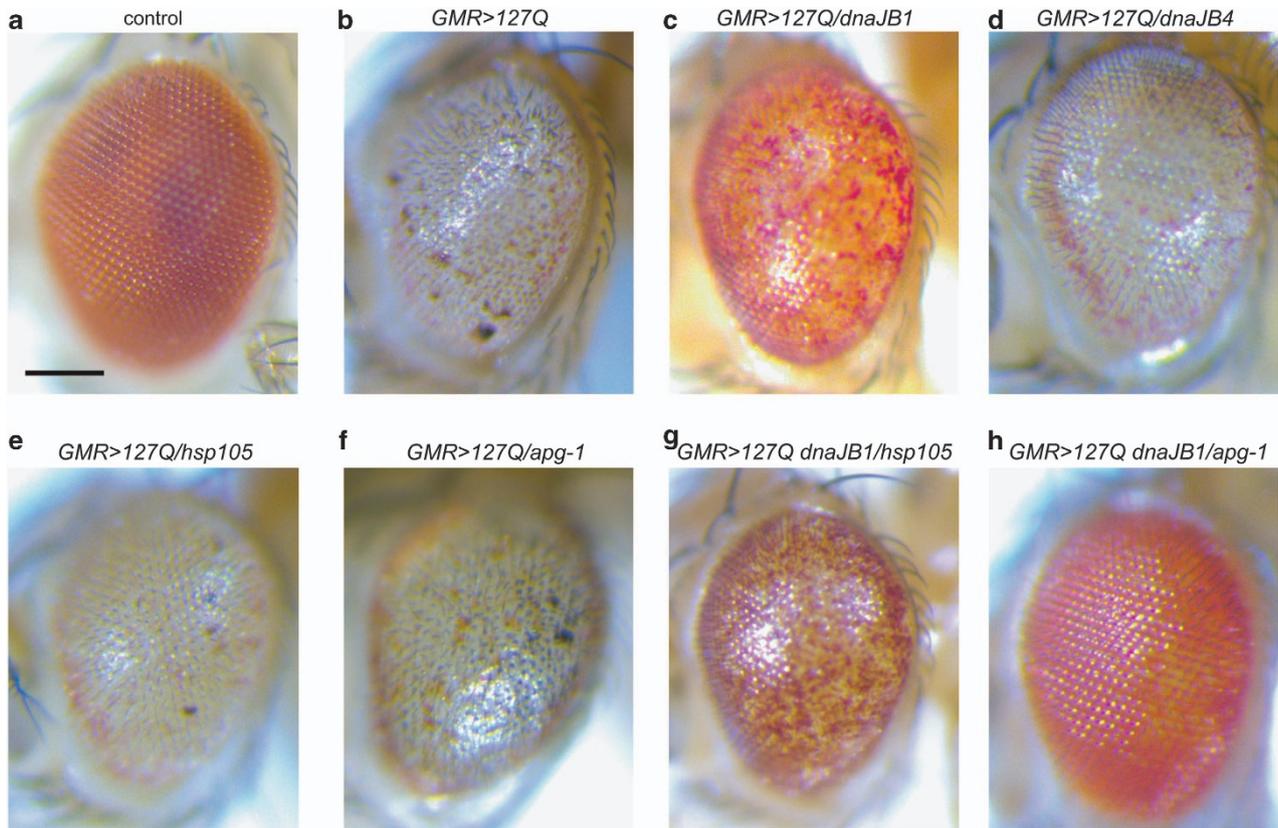


Figure 6 Suppression of *GMR>127Q*-driven external degeneration by expression of DNAJB1 and APG-1. (a) Control (*GMR-Gal4*), (b) *GMR>127Q*: *GMR-Gal4 UAS-127Q/+*, (c) *GMR>127Q/dnaJB1*: *GMR-Gal4 UAS-127Q/UAS-dnaJB1*, (d) *GMR>127Q/dnaJB4*: *GMR-Gal4 UAS-127Q/UAS-dnaJB4*, (e) *GMR>127Q/hsp105*: *GMR-Gal4 UAS-127Q/UAS-hsp105*, (f) *GMR>127Q/apg-1*: *GMR-Gal4 UAS-127Q/UAS-apg-1*, (g) *GMR>127Q dnaJB1/hsp105*: *GMR-Gal4 UAS-127Q UAS-dnaJB1/UAS-hsp105*, (h) *GMR>127Q dnaJB1/apg-1*: *GMR-Gal4 UAS-127Q UAS-dnaJB1/UAS-apg-1*

DNAJB4 could function like DNAJ-1 in suppressing the cellular toxicity of polyQ proteins. Expression of DNAJB1 but not DNAJB4 partially suppressed the loss of pigments and external degeneration caused by *GMR>127Q* (Figures 6a–d). DNAJB1 did not, however, have a significant effect on the photoreceptor degeneration rate in *GMR-HQ* animals, as shown by the optical neutralization assay (Figure 7a). We also checked retinal morphology in 7-day-old flies by TEM. Similarly to the optical neutralization results, complete loss of rhabdomeres and severe degeneration of photoreceptor cells was detected in both *GMR-HQ* and *GMR-HQ/ninaE> dnaJB1* (*GMR-HQ ninaE-Gal4/UAS-dnaJB1*) animals (Figures 7b–d).

As in flies, human HSP40s might need an HSP110 partner to relieve polyQ toxicity. Two human HSP110s, APG-1 and HSP105, the closet homologs of HSC70cb, were tested.^{43,44} APG-1 and HSP105 share 44% and 42% identity with HSC70cb, respectively (see Table 1; Supplementary Figure 6). Similar to HSC70cb, neither HSP105 nor APG-1 was able to prevent pigment loss and external degeneration in *GMR>127Q* animals. However, co-expression of APG-1 with DNAJB1 potentiated the suppression of polyQ-induced cell toxicity by DNAJB1 (Figures 6e–h). Furthermore, expression of APG-1 together with DNAJB1 significantly reduced the degeneration rate of *GMR-HQ* animals, whereas expression of APG-1 or DNAJB1 alone did not have a large effect on eye

degeneration caused by *GMR-HQ* (Figure 7a). We then selected retina from 7-day-old flies for TEM. As in *GMR-HQ* controls, both *GMR-HQ/ninaE> dnaJB1* and *GMR-HQ/ninaE> apg-1* (*GMR-HQ ninaE-Gal4/UAS-apg-1*) animals showed severe degeneration and almost complete loss of photoreceptor cells (Figures 7b–e). However, many normal photoreceptor cells remained in *GMR-HQ* retinæ when DNAJB1 and APG-1 were co-expressed (Figure 7f). These results indicate that mammalian DNAJB1 and APG-1 can function together to suppress the cellular toxicity of polyQ proteins.

Discussion

Many neurodegenerative disorders including polyQ diseases are linked to misfolding and aggregation of abnormal proteins. As misfolded, aggregated proteins accumulate, a number of molecular chaperones are produced to counter protein aggregation and its toxic consequences. Thus, induction of HSPs by active heat-shock factor can suppress polyQ inclusion formation and protect cells.^{7,8} Among the HSPs, HSP40/DNAJ family proteins have the most profound effects in protecting cells from polyQ toxicity.¹⁵ Moreover, HSP40/DNAJ proteins can be induced by increased concentrations of aggregation-prone folding intermediates, and expression of HSP40 family members positively correlates with the age of

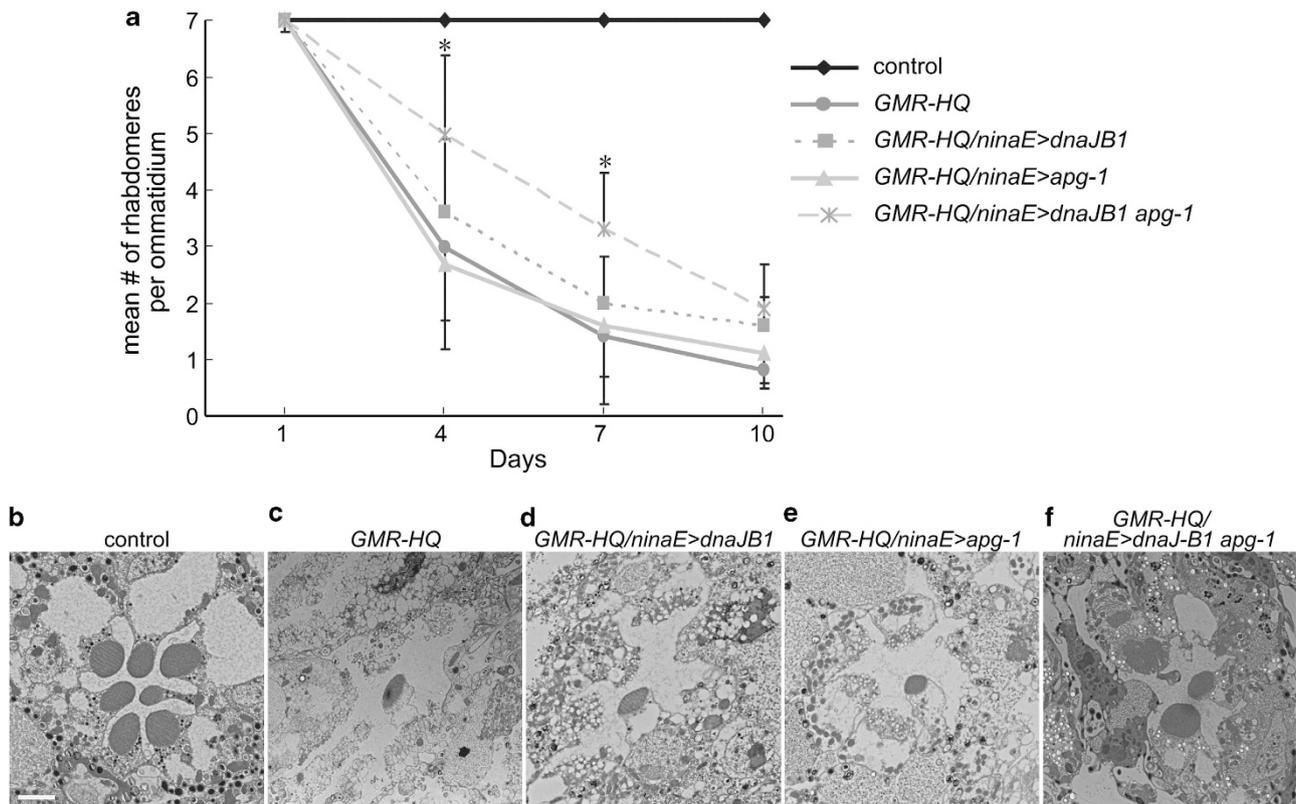


Figure 7 Suppression of neural degeneration in *GMR-HQ* retinæ by co-expression of DNAJB1 and APG-1. (a) The time course of degeneration as determined by optical neutralization. Each data point was based on examination of ≥ 100 ommatidia from ≥ 7 flies. Error bars represent the SDs. Asterisks indicated statistically significant differences (Student's unpaired *t*-test; $P < 0.05$) from *GMR-HQ*. (b–f) Examination of morphology by TEM. (b) Control, (c) *GMR-HQ* (*GMR-HQ* +), (d) *GMR-HQ/ninaE > dnaJB1* (*GMR-HQ ninaE-Gal4/UAS-dnaJB1*), (e) *GMR-HQ/ninaE > apg-1* (*GMR-HQ ninaE-Gal4/UAS-apg-1*), (f) *GMR-HQ/ninaE > dnaJB1 apg-1* (*GMR-HQ ninaE-Gal4/UAS-dnaJB1 UAS-apg-1*). Flies were reared in a 12 h light/12 h dark cycle at 25 °C, and 7-day-old flies were used

onset of polyQ diseases.⁴⁵ Several human HSP40 proteins have specific functions in reducing protein aggregation. For example, DNAJB1, DNAJB2, DNAJB6, and DNAJB8 have been identified as potent inhibitors of aggregation and the associated toxicity of polyQ-containing proteins.^{14,15,46,47} These findings suggest that it may be possible to use HSP40 proteins as therapeutic targets for treating polyQ diseases.

In the current prevalent model of HSP40/DNAJ function, a HSP40 protein initially binds unfolded client proteins and delivers them to an HSP70, and then stimulates the ATPase activity of HSP70. HSP40 proteins show a large degree of divergence, and thus have a major part in the multifunctional abilities of the protein-folding machinery. In *Drosophila* models of polyQ disease, the HSP40/DNAJ family protein DNAJ-1 was shown to have profound suppressive effects on the cellular toxicity of multiple polyQ proteins.^{10–12} We tested whether the function of DNAJ-1 was dependent on HSP70, and found that mutations in *hsp70* did not reduce suppression of polyQ toxicity by DNAJ-1. Therefore, we surmise that HSP70 and DNAJ-1 probably do not work together in inhibiting polyQ toxicity. In testing other possible co-chaperones for DNAJ-1, we found that none of the members of the HSP60, HSC70s, HSP90, or HSP110 gene families worked efficiently alone in protecting cell death caused by polyQ proteins. In co-expression assays, however, we found that the

suppressive effect of DNAJ-1 on polyQ toxicity was enhanced only by HSC70cb, an HSP110 family protein. This indicated that HSP110 has a key role in the function of HSP40/DNAJ-1. Therefore, we suggest that modulation of polyQ-induced cellular toxicity by HSP40 required HSP110, and that cell-protective function of HSP110 relies on HSP40.

HSP110 has both ATPase and substrate-binding domains similar to those found in HSP70, and is highly efficient in reducing protein aggregation *in vitro*.^{18,19} Thereby, HSP110 might serve key roles as chaperones on their own. HSP110 chaperone activity has been shown to directly contribute to prion formation and propagation,⁴⁸ endoplasmic reticulum-associated degradation,⁴⁹ and the biogenesis and quality control of CFTR.²⁷ Functioning as a core chaperone, HSP110 activities have been implicated to have protective roles in multiple disease-causing protein aggregations. For instance, in an ALS disease model, HSP105 interacted with mutated SOD1, and expression of HSP105 suppressed the aggregation of SOD1.^{25,26} Furthermore, *hsp105* knock-out mice exhibited an age-dependent accumulation of phosphorylated tau with pathological features of neurodegeneration, and the early appearance of A β 42 senile plaques in an AD transgenic mouse model.²⁸ As a HSP70-like chaperone, HSP110 might also require HSP40 protein assistant for its chaperon functions. Indeed, as we show here in flies, HSP70cb physically interacts with DNAJ-1 *in vivo*, and co-expression

of DNAJ-1 and HSP70cb affords enhanced protection against neurodegeneration caused by polyQ proteins, whereas expression of HSP70cb alone did not suppress cell death caused by polyQ proteins. Furthermore, ATPase-dead variants of HSP70cb lost its ability to protect cells with DNAJ-1. Therefore, HSP40 and HSP110 could be chaperones that denature client proteins together, and thereby prevent the cellular toxicity of protein aggregation.

In the canonical model for the function of HSP40/HSP70 chaperone complexes, a nucleotide exchange factor (NEF) serves as partner of HSP70.^{9,50} By stimulation of the dissociation of ADP from HSP70, NEF fosters dissociation of the client protein from HSP70. HSP110 has recently been suggested to form complexes with Hsp70s, and to function as the major NEF.^{23,24} Therefore, it is possible that both HSP40 and HSP110 function together in the HSP70 chaperone system. Indeed, both yeast and human HSP110 are required to synergize with HSP70 and HSP40 to drive disaggregation of luciferase, GFP and other denatured proteins.^{51,52} If this were the case, HSP70 should be necessary for the cell-protecting function of HSP40 and/or HSP110. However, we found that *hsp70* knock-out did not reduce the cellular protective role of DNAJ-1. These results suggest that HSP70 is not required for the function of HSP40 and HSP110. As HSC70s are close homologous and might be redundant in function with HSP70s, it is also possible that DNAJ-1 and HSC70cb assist HSC70 chaperones other than HSP70, in alleviating polyQ toxicity.

As one of the closest homologs of DNAJ-1, DNAJB1 was known to be able to inhibit polyQ huntingtin aggregation,^{17,53} and expression of DNAJB1 in flies weakly suppressed neurodegeneration caused by polyQ proteins. It is reasonable to expect that, as in *Drosophila*, DNAJB1 functions with an HSP110 in suppressing polyQ caused cell death in mammals. Although a role for HSP105 in protecting aggregation-associated cell degeneration has been reported in several disease models,^{25,26,28,54} in our tests HSP105 did not suppress polyQ-induced cell death in combination with DNAJB1. However, another HSP110 family protein, APG-1, did prevent neurodegeneration caused by polyQ proteins in combination with DNAJB1. These results raise the possibility that the HSP40/HSP110 chaperone system is conserved in mammals, and that DNAJB1 and APG-1 might serve as potential drug targets for neurodegenerative diseases such as polyQ diseases.

Materials and Methods

Drosophila stocks. *UAS-hsc70cbRNAi*, *UAS-hsc70-3*, *UAS-mrj-flag*, and *UAS-hsc70-4* were obtained from the Bloomington Stock Center (Bloomington, IN, USA). The *GMR-Gal4*, *ninaE-Gal4*, and *GMR-htt-Q120 (GMR-HQ)* flies were kept in Dr. Wang lab. The *UAS-127Q* flies were generated by Dr. P Kazemi-Esfarjani and *Df(3R)hsp70* flies were obtained from Dr. K Golic.³⁶ Flies of either sex were used.

Generation of transgenic flies. The cDNAs of *dnaJ-1* (EST clone SD08787), *hsp60* (EST clone AT13565), *hsp68* (EST clone RE48592), *hsp70* (EST clone RE48592), *hsc70-1* (EST clone RH49358), *hsc70-2* (EST clone AT28983), *hsc70-5* (EST clone GM13788), *hsc70cb* (EST clone GM LD32979), and *hsp83* (EST clone AT20544) were used to generate *UAS-dnaJ-1*, *UAS-hsp60*, *UAS-hsp68*, *UAS-hsp70*, *UAS-hsc70-1*, *UAS-hsc70-2*, *UAS-hsc70-5*, *UAS-hsc70cb*, and *UAS-hsp83* constructs. Mutations on *hsc70cb* were introduced using the Quick-Change (Agilent, Santa Clara, CA, USA) and PCR

method, and subsequently cloned to *pUAST-attB* vector to generate *UAS-hsc70cb^{K68S}* and *UAS-hsc70cb^{KD}*. The constructs were injected into *w¹¹¹⁸* embryos and transformants were identified on the basis of eye color. All HSPs used in the paper are indicated in Table 1.

Generation of anti-HSC70cb antibodies. A fragment encoding C-terminal region of HSC70cb (residues 700–800) was cloned into pGEX5x-1 vector (GE Healthcare, Pittsburgh, PA, USA). The glutathione S-transferase fusion protein was expressed in *Escherichia coli* BL21 codon-plus (Agilent), purified using glutathione agarose beads (GE-Healthcare), and introduced into rabbits (Covance, Beijing, China).

Optical neutralization assay. The rhabdomeres of ommatidium were examined directly without dissection of eyes by the technique of optical neutralization. Briefly, the heads of flies were cut off, and immersed into mineral oil in an orientation with eyes on side and antennae facing up. The samples were examined by a DIC light microscope (Nikon, Tokyo, Japan).

TEM. Retinae were dissected from flies reared at 25 °C, fixed in paraformaldehyde/glutaraldehyde and osmium tetroxide solutions, dehydrated with an ethanol series, and embedded in LR White resin as described.⁵⁵ 85 nm thin sections were prepared at a depth of 20 μm, and examined by transmission EM using a Zeiss (Oberkochen, Germany) FEI Tecnai 12 electron microscope. The images were acquired using a Gatan (Pleasanton, CA, USA) camera (model 794).

Co-immunoprecipitations and western blot. The co-immunoprecipitation was performed as described,⁵⁶ except that fly heads were homogenized in buffer A with 0.2% NP-40. Anti-DNAJ-1 and anti-HSC70cb argarose beads were generated by crosslinking of rabbit anti-DNAJ-1 or rabbit anti-hsc70cb antibodies to CNBr-activated agarose beads (GE-healthcare), and used for precipitation of DNAJ-1 and HSC70cb. The beads were re-suspended in SDS sample buffer, fractionated by SDS-PAGE and a western blot analysis was probed with anti-DNAJ-1,⁵⁷ anti-HSC70cb, and anti-tubulin (Developmental Studies Hybridoma Bank, Iowa City, IA, USA) antibodies.

Conflict of Interest

The authors declare no conflict of interest.

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