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Dynamic regulation of molecular chaperone gene expression in polyglutamine disease ☆

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Abstract

Expanded polyglutamine disease proteins cause adult-onset progressive neurodegeneration. Constitutive overexpression of the Hsp70 molecular chaperone is capable of suppressing polyglutamine neurodegeneration. We showed that endogenous Hsp70 expression was induced, at both transcriptional and translational levels, in *Drosophila* models of polyglutamine disease. Soon after the endogenous Hsp70 induction reached a maximum level at larval stage, its expression declined progressively with age. We further showed that cellular heat shock response remained intact in aged flies, indicating the decline of Hsp70 levels observed in polyglutamine-expressing flies is not due to normal ageing. In contrast to the well-documented polyglutamine suppression caused by constitutive Hsp70 overexpression, no suppression of degeneration was observed when inducible copies of *hsp70* transgenes were instead coexpressed. This supports a transcriptional dysregulation of endogenous *hsp70* gene induction in polyglutamine flies. Altogether, we propose that transcriptional malfunctioning of molecular chaperone gene expression contributes to the late-onset and progressive nature of polyglutamine toxicity.
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Most, if not all, cellular processes are mediated through the actions of proteins. To ensure the proper functioning of a cell, parallel surveillance mechanisms are evolved to safeguard proteins from denaturation and to channel irreversibly damaged proteins for degradation [1]. The heat shock protein (Hsp) 70/40 molecular chaperone system is one such protective mechanism [2]. Hsp70 is an ATPase and its activity can be modulated by co-chaperones such as Hsp40 [1,2]. Expression of some molecular chaperones, including certain types of Hsp70 and 40, is induced under various stress conditions, such as hypoxia and tissue injury [1]. Upon stress stimulation, heat shock transcription factor (HSF) becomes trimerized and binds to heat shock response element (HSE) in the *hsp* gene promoter region to initiate gene transcription [3]. Other transcription regulators, such as the TAC1 histone modification complex, which is composed of Trithorax (Trx), CREB-binding protein (CBP), and SET domain binding factor 1 (Sbf1) [4], are also involved in *hsp* gene expression [5].

Many human neurological disorders, including polyglutamine (polyQ) disease, are caused by alteration of protein conformations. PolyQ disease is caused by CAG trinucleotide repeat expansion in the coding regions of disease genes [6]. When translated, an expanded polyQ domain is produced which confers toxic gain-of-

☆ *Abbreviations:* AR, androgen receptor; CBP, CREB-binding protein; dHdj, DNAJ-like; dpe, days post-eclosion; GFP, green fluorescent protein; HSE, heat shock element; HSF, heat shock transcription factor; Hsc70, heat shock cognate 70; Hsp70, heat shock protein 70; MJD, Machado Joseph Disease; dmrj, *Drosophila* homology of mammalian relative of DnaJ; polyQ, polyglutamine; Sbf1, SET domain binding factor 1; TAC1, trithorax acetylation complex 1; Trx, trithorax.

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function properties to the disease protein and subsequently causes neuronal degeneration [6]. Expanded polyQ proteins are aggregation-prone and the formation of intracellular insoluble polyQ-containing protein aggregates are found to be associated with polyQ disease [6].

Molecular chaperones are responsible for protein folding and have been implicated in protein conformational diseases [7]. In support of this notion, Hsp70/40 molecular chaperone proteins are found to be recruited to intracellular insoluble protein aggregates in polyQ patients [8,9]. The modifying effects of molecular chaperones on polyQ diseases are recently reviewed [10,11]. In particular, constitutive overexpression of Hsp70 protein is able to suppress degeneration in various polyQ transgenic models whereas compromising cellular molecular chaperone activities enhances polyQ degeneration [12,13].

It is interesting to note that both mRNA and protein expression levels of the endogenous inducible Hsp70 appear to vary from one polyQ disease model to another [12,14–18]. Although a progressive down-regulation of inducible Hsp70 protein has recently been reported in a HD transgenic mouse model [19], this however still could not explain the alteration of *hsp* transcript levels [17]. Nevertheless, these findings would reflect a complex and dynamic regulation of hsp gene expression in polyQ diseases. In view of this, we examined the endogenous cellular heat shock response in *Drosophila* polyQ disease models. We demonstrated that expanded, but not unexpanded, polyQ proteins induce heat shock response. Further, we showed sequential up- and down-regulation of the inducible Hsp70 expression at both transcription and translation levels in expanded polyQ-expressing flies. Our findings propose a transcriptional dysregulation of *hsp* gene expression in polyQ degeneration, and also highlight the importance of maintaining a critical level of molecular chaperone activities in degenerating neurons.

Materials and methods

Drosophila genetics. Fly strains were grown at 25 °C on standard corn meal medium supplemented with dry yeast. The following fly lines were used in this study: *w*, *gmr-GAL4*, *UAS-GFP::lacZ.nls*, *UAS-h-HSPA1L* (human Hsp70), *UAS-MJDtrQ27*, *UAS-MJDtrQ61*, *UAS-MJDtrQ78(W)*, *UAS-MJDtrQ78(S)*, *UAS-ARtrQ16* and *UAS-ARtrQ112(W)*, *UAS-Hsc4.K71S*, nej^{EP1410} and the OE^{+} strain which carries 12 additional transgenic inducible copies of the *hsp70* gene [13,20–25].

Light microscopy. Adult fly eyes of appropriate age and genotype were examined under an Olympus SZX-12 stereomicroscope. Eye images were captured using a SPOT Insight CCD camera (Diagnostic Instruments).

Immunostaining. Immunostaining of adult eye sections was performed as described [22]. Stained sections were visualized under an Olympus BX51 fluorescence microscope. Primary antibodies used were rat anti-Hsp70 7FB (1:250; [26]), mouse anti-HA (1:333; Zymed). Secondary antibodies used were goat anti-rat IgG (H + L) Rhodamine conjugated (1:100, Pierce) and goat anti-mouse IgG (H + L) FITC conjugated (1:250; Zymed).

Heat shock treatment. Flies were aged to the appropriate time points at 25 °C and then transferred to a 37 °C water bath, with all surfaces of the fly vial (except for the cotton plug) kept under water. Flies were heat shocked at 37 °C for 30 min followed by a 30-min recovery period, and this procedure was repeated twice. Fly heads were then homogenized for RNA extraction or Western blot analysis immediately after the last heat shock treatment.

Semi-quantitative RT-PCRs. RT-PCRs were performed as previously described [27]. In brief, 15 fly heads were used for total RNA isolation using TRIZOL reagent (Invitrogen), and reverse transcription was performed using oligo(dT) primer. The *β-actin* gene was used as loading control. Primers used in RT-PCRs were: *hsp70*F 5′ aat cct gaa cgt cag cgc caa g 3′; *hsp70*R 5′ gtg ttg ctg tcc agc cac cg 3′; *dhdj1*F 5′ cga gca tga tct gtt cgt gtc g 3′; *dhdj1*R 5′ ctg acc agt gcc tcg cac aag 3′; *dhdj2*F 5′ cga cag tgg aga tct cta cat g 3′; *dhdj2*R 5′ tca aag tga gac gac tgt ccg 3′; mrjF 5′ gcg atc gtg gcc aga gtc gc 3′; mrjR 5′ gac tgc cac tcg agc cgc ttg 3′; *actin*F 5′ atg tgc aag gcc ggt ttc gc 3′; and *actin*R 5′ cga cac gca gct cat tgt ag 3′.

Western blot analysis. Fifteen adult fly heads were homogenized in 75 μl of 6× SDS sample buffer. For larvae, four third instar larvae were homogenized in 60 μl of 1× PBS supplemented with DNaseI (Promega) and protease inhibitors (Sigma). The homogenate was first incubated at 37 °C for 15 min, and 240 μl of SDS sample buffer was then added. SDS–PAGE separation and Western blotting were performed as described [22,23]. Primary antibodies used were rat anti-Hsp70 7FB (1:500; [26]), mouse anti-HA (1:250; Zymed), mouse anti-androgen receptor N20 (1:1000; Santa Cruz), rabbit anti-human Hsp70 SPA812 (1:1000; Stressgen), mouse anti-GFP JL-8 (1:1000; BD Biosciences), and mouse anti-β-tubulin E7 (1:2000; Developmental Studies Hybridoma Bank, Iowa City, IA, with funding from the NICHD). Secondary antibodies used were affinity purified goat anti-rat, goat anti-mouse, and goat anti-rabbit IgG (H + L) peroxidase conjugated (1:2500; Chemicon).

Results

Inducible Hsp70 protein co-localized with MJDtrQ78 protein in protein aggregates of adult polyQ transgenic flies

Overexpression of the expanded form of a truncated Machado Joseph Disease protein (MJDtrQ78) in the *Drosophila* eye was toxic (Fig. 1B) and caused progressive degeneration (Figs. 1D and E; [24]). Overexpression of the human Hsp70 protein, HSPA1L, suppressed polyQ toxicity as shown by the restoration of external pigment cell degeneration (Fig. 1C; [13,23]). Overexpression of GFP, a control protein, did not result in suppression of MJDtrQ78 degeneration confirming the specificity of HSPA1L suppression (Fig. 1G).

Hsp70 and heat shock cognate 70 (Hsc70) are two different classes of molecular chaperone proteins. Hsp70 expression is stress-inducible, whereas Hsc70 is constitutively expressed in the cell. The *Drosophila* genome carries 10 copies of *hsp70* genes [28], and 5 copies of *hsc70* genes [29] including *Hsc4* [30]. Endogenous Hsp70 protein was previously found to co-localize with polyQ aggregates in larval eye imaginal disc tissues [13]. Because of the late-onset and progressive nature of polyQ

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