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ORIGINAL ARTICLE

Clearance of variant Creutzfeldt–Jakob disease prions *in vivo* by the Hsp70 disaggregase system

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The metazoan Hsp70 disaggregase protects neurons from proteotoxicity that arises from the accumulation of misfolded protein aggregates. Hsp70 and its co-chaperones disassemble and extract polypeptides from protein aggregates for refolding or degradation. The effectiveness of the chaperone system decreases with age and leads to accumulation rather than removal of neurotoxic protein aggregates. Therapeutic enhancement of the Hsp70 protein disassembly machinery is proposed to counter late-onset protein misfolding neurodegenerative disease that may arise. In the context of prion disease, it is not known whether stimulation of protein aggregate disassembly paradoxically leads to enhanced formation of seeding competent species of disease-specific proteins and acceleration of neurodegenerative disease. Here we have tested the hypothesis that modulation of Hsp70 disaggregase activity perturbs mammalian prion-induced neurotoxicity and prion seeding activity. To do so we used prion protein (PrP) transgenic *Drosophila* that authentically replicate mammalian prions. RNASeq identified that Hsp70, DnaJ-1 and Hsp110 gene expression was downregulated in prion-exposed PrP *Drosophila*. We demonstrated that RNAi knockdown of Hsp110 or DnaJ-1 gene expression in variant Creutzfeldt–Jakob disease prion-exposed human PrP *Drosophila* enhanced neurotoxicity, whereas overexpression mitigated toxicity. Strikingly, prion seeding activity in variant Creutzfeldt–Jakob disease prion-exposed human PrP *Drosophila* was ablated or reduced by Hsp110 or DnaJ-1 overexpression, respectively. Similar effects were seen in scrapie prion-exposed ovine PrP *Drosophila* with modified Hsp110 or DnaJ-1 gene expression. These unique observations show that the metazoan Hsp70 disaggregase facilitates the clearance of mammalian prions and that its enhanced activity is a potential therapeutic strategy for human prion disease.

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Abbreviations: GFP = green fluorescent protein; Hsps = heat shock proteins; PMCA = protein misfolding cyclic amplification; RT-QuIC = real-time quaking-induced conversion; vCJD = variant Creutzfeldt–Jakob disease

Introduction

Protein misfolding neurodegenerative diseases occur through conformational change and accumulation of disease-specific proteins. These conditions include prion diseases of humans and animals, and human-specific prion-like diseases such as Alzheimer's disease, Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis and tauopathies.[1–3] Prion diseases, which are characterized by the CNS accumulation of PrP^{Sc}, a misfolded oligomeric form of the normal host protein PrP^{C}, are unique because they are naturally transmissible within and between species. The transmissible prion agent comprises PrP^{Sc}[4,5] and prion-induced neurotoxicity arises as a consequence of prion propagation.[6,7] Prion formation is postulated to occur through nucleation-dependent polymerization, whereby oligomeric nuclei or seeds of PrP^{Sc} recruit PrP^{C} via template-directed protein misfolding into the growing assembly of aggregated misfolded PrP.[8] Subsequent prion replication is proposed to occur through their fragmentation, a process that generates new PrP^{Sc} particles with seeding activity capable of transcellular spread.[9–11] While the process of prion fragmentation remains undefined, prion accumulation occurs more rapidly than clearance, which allows for their transmissibility.[12] Prion diseases are an important paradigm for protein misfolding neurodegenerative conditions in general because human-specific conditions show prion-like cell-to-cell spread of misfolded disease-specific protein under experimental settings.[13,14]

In addition to prion-induced toxicity, neurons are vulnerable to proteotoxicity that arises through aggregation and accumulation of house-keeping proteins, which can occur during cellular stress. This may reflect that neurons have a relatively high level of protein synthesis and high energy requirements that are needed to satisfy the demands of synaptic development, transmission and plasticity.[15,16] Furthermore, neurons are post-mitotic cells and unable to divide, and effectively dilute out protein aggregation during cellular division. Neurons employ various strategies to counter misfolded protein aggregation including the role of heat shock proteins (Hsps).[17] This vital group of molecular chaperones and co-chaperones facilitate the correct folding or stabilization of nascent polypeptides or the refolding of partially unfolded or damaged proteins.[18,19] In addition, Hsps and their cofactors are the principal components of a dedicated protein disaggregation machinery that disassemble and extract polypeptides from protein aggregates for subsequent refolding or degradation.[20–23] Paradoxically, protein disaggregation machinery activity, an important component of neuronal protein homeostasis, may be responsible for the fragmentation of mammalian prions and therefore facilitate their propagation, as occurs in the case of yeast prions.[11,24]

The propagation of the yeast prions [URE^{+}] and [PSI^{+}], which are amyloid fibrillar forms of Ure2p and Sup35p proteins, respectively,[25] is regulated by an ATP-driven protein disaggregase that comprises the chaperone Hsp70 and co-chaperones comprising J proteins (Hsp40s) together with Hsp104.[26–28] Yeast prion amyloid fibrils are first recognized by Hsp70 in conjunction with DnaJ-1 and subsequently transferred to Hsp104, where individual misfolded polypeptides are removed by an ATP-driven threading action through the axial core of the hexameric chaperone protein.[29] This process causes amyloid fibril fragmentation and the formation of new prion seeds with templating activity.[30,31] Evidence to support this scheme is that ablation of Hsp104 gene expression[26] or GndHCl-induced denaturation of Hsp104 protein activity[32] in yeast cells halts prion propagation.

Metazoan cells lack Hsp104 and instead efficient protein disaggregation machinery comprises the Hsp70 and DnaJ proteins in association with the nucleotide exchange factor (NEF) Hsp110.[23,33] This potent protein aggregate disaggregation system together with other proteostatic mechanisms[17] functions to provide neuronal protein homeostasis under most conditions, including a potential impact on protein misfolding neurodegenerative diseases. Evidence in support of this view is that the Hsp70–Hsp40–Hsp110 tri-chaperone could efficiently disassemble α-synuclein fibrils *in vitro*[21] and Hsp110 overexpression affects the clinical outcome in mouse models of amyotrophic lateral sclerosis and Parkinson's disease *in vivo*.[34,35] However, as the effectiveness of the chaperone system decreases with age, it is likely that incomplete disassembly of protein aggregates predominates with the potential for accumulation rather than removal of neurotoxic and seeding-competent aggregates. In this scenario, it is proposed that therapeutic enhancement of the Hsp70 protein disassembly machinery will have the potential to suppress or reverse the accumulation of protein aggregates associated with neurodegenerative disease.[36] However, an important issue to address is whether stimulation of protein aggregate disassembly leads to enhanced formation of seeding-competent species of disease-specific proteins and acceleration of neurodegenerative disease.

Here, we tested the hypothesis that modulation of activity of the Hsp70 disaggregase system causes perturbation in prion-induced neurotoxicity and prion seeding activity in order to investigate its role in mammalian prion propagation. To do so we have used prion-exposed *Drosophila* transgenic for pan neuronal expression of PrP, a novel animal system that authentically replicates mammalian prions.[37–39] We first used RNASeq analysis to identify that gene expression of Hsp70, DnaJ-1 and Hsc70Cb, the *Drosophila* homologue of mammalian Hsp110 (hereafter referred to as dHsp110) are downregulated in prion-exposed PrP *Drosophila*. We subsequently demonstrated that RNAi-mediated knockdown of either dHsp110 or DnaJ-1 gene expression in variant Creutzfeldt–Jakob disease (vCJD) prion-exposed human PrP *Drosophila* led to enhanced prion-induced neurotoxicity, whereas transgene-mediated overexpression of either of these two co-chaperones led to amelioration of the prion-induced phenotype. Strikingly, seeding activity was fully ablated in vCJD prion-exposed human PrP *Drosophila* with overexpression of dHsp110 and was significantly reduced in the case of DnaJ-1 overexpression. These effects were not restricted to human prions as we also showed that sheep scrapie-exposed ovine PrP *Drosophila* with modified expression of dHsp110 or DnaJ-1 showed similar responses. Collectively, our novel observations are compatible with the view that the life cycle of mammalian prions interacts with the metazoan Hsp70 disaggregation

machinery and that enhanced activity of this complex can suppress experimental prion disease progression.

Materials and methods

Drosophila fly lines

The UAS-PrP fly line w; M{M129 human PrP(GPI), 3xP3-RFP.attP} ZH-51D transgenic for Met129 human prion protein will be described in detail elsewhere (A.M.T. and R.B., in preparation). The UAS-PrP fly line w; M{VRQ-PrP(GPI), 3xP3-RFP.attP}ZH-51D transgenic for ovine $V^{136}R^{154}Q^{171}$ (VRQ) prion protein was generated as previously described.[39,40] *UAS-Hsc70Cb overexpression* (Bloomington *Drosophila* stock code [BDSC] 53728); *UAS-Hsc70Cb-RNAi* (FBgn0263106) (BDSC 33742); *UAS-DnaJ-1 overexpression* (BDSC 30553); and *UAS-DnaJ-1-RNAi* (FBgn0263106) (BSC 32899) were all purchased from the Bloomington Drosophila Stock Center, Indiana University, USA. The following fly lines were obtained from the Department of Genetics, Cambridge University, UK: *UAS-luciferase-RNAi* (Luc yv, P [y+ v+ TRiP-JF 01355 (luciferase)] attp2); *UAS-mcherry overexpression* (R289, p [UAS-mcherry valium 10] attp2); *UAS-green fluorescence protein (GFP) overexpression* (R-288, p [UAS-GFP valium 10] attp2); *Elav-GAL4* (P{w[+mW.hs]=GawB}elav[C155]); 51D (w; M{3xP3-RFP.attP}ZH-51D). PrP *Drosophila* transgenic for modified co-chaperone expression, control RNAi or control overexpression transgene were generated by conventional fly crossing. Resultant *Drosophila* were crossed with the *Elav-GAL4* driver fly line to derive transgenic flies that expressed PrP with modified co-chaperone gene expression pan neuronally. 51D *Drosophila* crossed with this driver fly line were used as control flies where appropriate. All fly lines were raised on standard cornmeal media at 25°C and maintained at low to medium density and pre-mated before experimental use.

Preparation of *Drosophila* head homogenate

Whole flies in an Eppendorf tube were frozen in liquid nitrogen for 10 min and then vortexed for 2 min to cause decapitation. Individual fly heads were isolated and placed in clean Eppendorf tubes using a fine paint brush and homogenates were prepared by manual grinding of the fly heads with sterilized plastic pestles. Homogenates for protein misfolding cyclic amplification (PMCA) were prepared by processing 20 fly heads per group previously harvested at 5, 10, 20, 30 or 40 days of age. Each group of 20 fly heads was added to 20 µl of phosphate buffered saline (PBS; pH 7.4) before homogenization. Homogenates for Western blot of PrP were prepared by processing four fly heads (human PrP *Drosophila*) in 4 µl or 20 fly heads (ovine PrP *Drosophila*) in 10 µl of lysis buffer [50 mM Tris/HCl, pH 7.5, 100 mM NaCl, 0.5% Nonidet P40 and 1 mM 4-(2-aminoethyl) benzenesulfonyl fluoride] followed by 10 min of sonication on ice.

RNA extraction and cDNA synthesis

Total RNA was extracted from *Drosophila* fly heads using 0.7 ml of TRIzol reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. RNA was resuspended in 20 µl of Milli-Q water and was DNase treated using a Qiagen DNase kit (Cat. no. 79256) followed by RNA clean-up using Qiagen mini easy columns (Cat. no. 74204). RNA was quantified using a nanodrop. cDNA was generated by reverse transcription of 1 µg of RNA using a NEB Protoscript II kit according to manufacturer's instructions with reaction mixes incubated at 42°C for 1 h followed by 72°C for 20 min and subsequently stored at 4°C until further use.

Droplet digital polymerase chain reaction

Quantitation of co-chaperone gene expression was performed using a Bio-Rad QX200 droplet digital polymerase chain reaction (ddPCR) system. Reaction mixes of 20 µl were prepared in duplicate in individual wells of a 96-well plate and comprised 10 µl of 2× ddPCR supermix (Bio-Rad Cat. no. 1863025), 1 µl of 20× target primer/probe mix (PrimeTime® 5′ 6-FAM™) and 1 µl of 20× reference primer/probe mix (PrimeTime® 5′ HEX™) from Integrated DNA Technologies at concentrations of 20 µM primer/5 µM probe, respectively; 6 µl of cDNA (prepared from up to 50 ng of initial total RNA) and 2 µl H_2O. Prepared plates were loaded onto a QX200 droplet generation system and the reaction mixes subsequently transferred to a fresh plate that was sealed with a Bio-Rad PX1 PCR Plate Sealer. ddPCR was performed using the following parameters, 95°C for 10 min, 35 cycles of 94°C for 30 s and 60°C for 1 min, followed by hold at 4°C. Product amplification was carried out using a Bio-Rad QX200 droplet reader. Template concentration was calculated using the Poisson-corrected determination using QuantaSoft™ analysis pro-software (v1.0, Bio-Rad). A minimum of 10 000 acceptable droplets per reaction were analysed followed by manual selection of positive and negative droplet population. Each primer pair in the experiments also included a cDNA template-free control.

Sodium dodecyl sulphate–polyacrylamide gel electrophoreses and western blot

Fly head homogenate was mixed with an equal volume of double-strength Laemmli loading buffer, boiled for 10 min, cooled on ice and then centrifuged at 13 000*g* for 5 min at 18°C to remove debris. A volume of sample equivalent to four fly heads (human PrP *Drosophila*) or 20 fly heads (ovine PrP *Drosophila*) per track was subjected to sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) run under reducing conditions and western blot as described in detail previously[41] except that the nitrocellulose membranes were probed with a 1:2000 dilution of anti-PrP monoclonal antibody Sha31.[42]

Prion inoculation of *Drosophila*

Drosophila were exposed to mammalian prions at the larval stage of development. Met129 human PrP *Drosophila* were exposed to vCJD-infected or control MM129 human brain homogenate (supplied by the CJD Surveillance Unit Edinburgh, UK). Ovine PrP *Drosophila* were exposed to brain homogenate of cerebral cortex tissue from a confirmed PG127 (alternatively referred to as G_{338} or DAW) scrapie-positive VRQ/VRQ sheep (SE1848/0005)[41] or New Zealand-derived VRQ/VRQ scrapie-free brain tissue as control material (supplied by the APHA, Weybridge, UK). Two hundred and fifty microlitres of 1% (w/v) of the relevant brain homogenate, or a 1/100 dilution series (v/v) of these samples, prepared in PBS pH 7.4, were added to the top of the cornmeal that contained third instar *Drosophila* larvae in 3-inch plastic vials. Following eclosion (i.e. hatching) flies were transferred to fresh non-treated vials.

Transcriptome mRNA sequencing and bioinformatics analysis

Transcriptome profiling and subsequent bioinformatics analysis of prion-infected *Drosophila* was performed as previously described.[43] Raw gene-level abundance was determined through the use of Htseq-count (version 0.6.1p1). Trimmed mean of M values

normalization and differential gene expression analysis was performed using EdgeR and Limma. The normalized gene abundance is represented in $\log_2$ cpm, which stands for the base-2 logarithm of count per million. The raw sequencing data and the comparison tables are available online at Gene Expression Omnibus (accession number GSE144028). Gene annotations and pathway analysis were performed using Ingenuity Pathway Analysis (Qiagen). The statistical computing package P-heat map was used for visualization of differentially expressed genes in each genotype of prion-exposed *Drosophila*. The changes visualized for each specific gene in the heat map presentations refer to the $\log_2$-fold change between expression in scrapie-exposed versus prion-free sheep brain homogenate-exposed *Drosophila*.

Negative geotaxis climbing assay

The locomotor ability of flies was assessed in a negative geotaxis climbing assay initiated with 45 ($3 \times n = 15$) age-matched, pre-mated female flies in each treatment group.[44] *Drosophila* were placed in adapted plastic 25-ml pipettes that were used as vertical climbing columns and allowed to acclimatize for 30 min prior to assessment of their locomotor ability. Flies were tapped to the bottom of the pipette (using the same number and intensity of taps on each occasion) and then allowed to climb for 45 s. At the end of the climbing period the number of flies above the 25 ml mark, the number below the 2 ml mark and the number in between the 2 ml and 25 ml marks were recorded. This procedure was performed three times at each time point. The performance index (PI) was calculated for each group of 15 flies (average of three trials) using the formula: $PI = 0.5 \times (n\text{total} + n\text{top} - n\text{bottom}) / n\text{total}$ where *n*total is the total number of flies, *n*top is the total number of flies at the top and *n*bottom is the total number of flies at the bottom. A PI value of 1 is recorded if all flies climb to the top of the tube whereas the value is 0 if no flies climb the tube past the 2 ml mark. The mean PI ± SD at individual time points for each treatment group was plotted as a regression line.

Protein misfolding cyclic amplification

PMCA was carried out as described previously.[45] The substrate consisted of 10% (w/v) ovine VRQ PrP transgenic mouse (tg338) brain homogenate in PBS, pH 7.4, 0.1% Triton X-100 and 150 mM NaCl buffer. Fly head homogenate (5 µl) was mixed with 45 µl of substrate in 0.2 ml thin-wall PCR tubes. Sealed tubes were then placed in the horn of a Misonix 4000 sonicator for one round of 96 cycles. Each cycle consisted of a 10-s sonication step (70% of power) followed by a 14-min and 50-s incubation step. Each reaction mixture (20 µl) was subsequently treated with proteinase K (PK) (4 µg of PK per mg of protein) for 2 h at 37°C and the reaction was stopped by adding Pefabloc® (4 mM final concentration). PK-resistant PrP was detected by western blot as described previously[45] using anti-PrP monoclonal antibody Sha31.[42]

Detection of prion seeding activity by real-time quaking-induced conversion

Real-time quaking-induced conversion (RT-QuIC) was performed as described previously.[46] Substrate consisted of Syrian hamster recombinant PrP 0.1 mg/mL in RT-QuIC buffer [320 mM NaCL, 1.0 mM EDTA, 10 µM Thioflavin T (ThT)].[47] Seed was *Drosophila* head homogenate prepared as described above and diluted 1:10 in 0.1% SDS. Reaction mixes comprised 98 µl of substrate and 2 µl of seed prepared in quadruplicate on two plates by two investigators (8 replicates/sample). Reaction mixes that contained known RT-QuIC positive or negative PrP *Drosophila* head homogenates as seed were included as positive and negative controls, respectively, on all plates. Reaction mix plates were placed in a BMG Labtech Polarstar™ fluorometer and subjected to 700 rpm double-orbital shaking for 1 min every other minute for 15 min for 250 cycles at 55°C. After each cycle ThT fluorescence was read at an excitation of 450 nm and emission of 480 nm with the gain at 1700 and using orbital averaging with 20 flashes per well with a 4 mm setting. Fluorescent readings were recorded for all sample reactions for a total time of 62 h at a temperature of 55°C. Samples were considered positive if they crossed a threshold (5 SD above the mean of the initial five readings). The inverse of the time when the reaction reached the threshold (1/time to threshold) was used to determine amyloid formation rate of individual treatment groups. For statistical analysis, amyloid formation rates of sample treatment groups were compared to those of the negative control samples.

Statistical analysis

Statistical analysis of the negative geotaxis climbing assay data was performed by one-way ANOVA together with Dunnett's multiple comparisons test and the unpaired Student's *t*-test (two-tailed). Statistical analysis of the RT-QuIC data was performed by the Mann–Whitney test. All statistical analyses were performed using Prism (GraphPad Software Inc, San Diego, USA).

Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its Supplementary material.

Results

Perturbation of chaperome gene expression in prion-exposed PrP transgenic *Drosophila*

The *Drosophila* chaperome comprises at least 95 genes that encode chaperone, co-chaperone or prefoldin proteins grouped into canonical protein families.[48] We first investigated prion-induced chaperome gene expression changes in PrP transgenic *Drosophila*. This was achieved through RNASeq-based transcriptome analysis of head homogenate prepared from adult ovine PrP *Drosophila* exposed at the larval stage to either scrapie-infected or control scrapie-free sheep brain homogenate.[43] Inspection of our RNASeq analysis identified gene expression data for >75% of the *Drosophila* chaperome in each fly treatment group at each time point as shown in the Supplementary material, section 1.

Representative chaperome genes and their respective expression levels in prion-exposed *Drosophila* are shown in Fig. 1. Individual chaperone and co-chaperone gene expression levels showed a relatively wide dynamic range in prion-infected *Drosophila* (Fig. 1A), which were distinct from those seen during normal fly ageing (Fig. 1B). Notably, gene expression of Hsp70, dHsp110 and DnaJ-1, which encode principal components of the major protein disaggregation complex in metazoan cells, were all downregulated in prion-exposed adult PrP *Drosophila*. dHsp110 and DnaJ-1 showed ≥33% and ≥27% reduction in gene expression in 5- and 40-day-old prion-exposed adult PrP *Drosophila* compared to control flies, respectively (Fig. 1C).

We speculated that the decrease in the gene expression level of components of the Hsp70 disaggregase system in prion-exposed ovine PrP *Drosophila* contributed to the prion-induced neurotoxicity

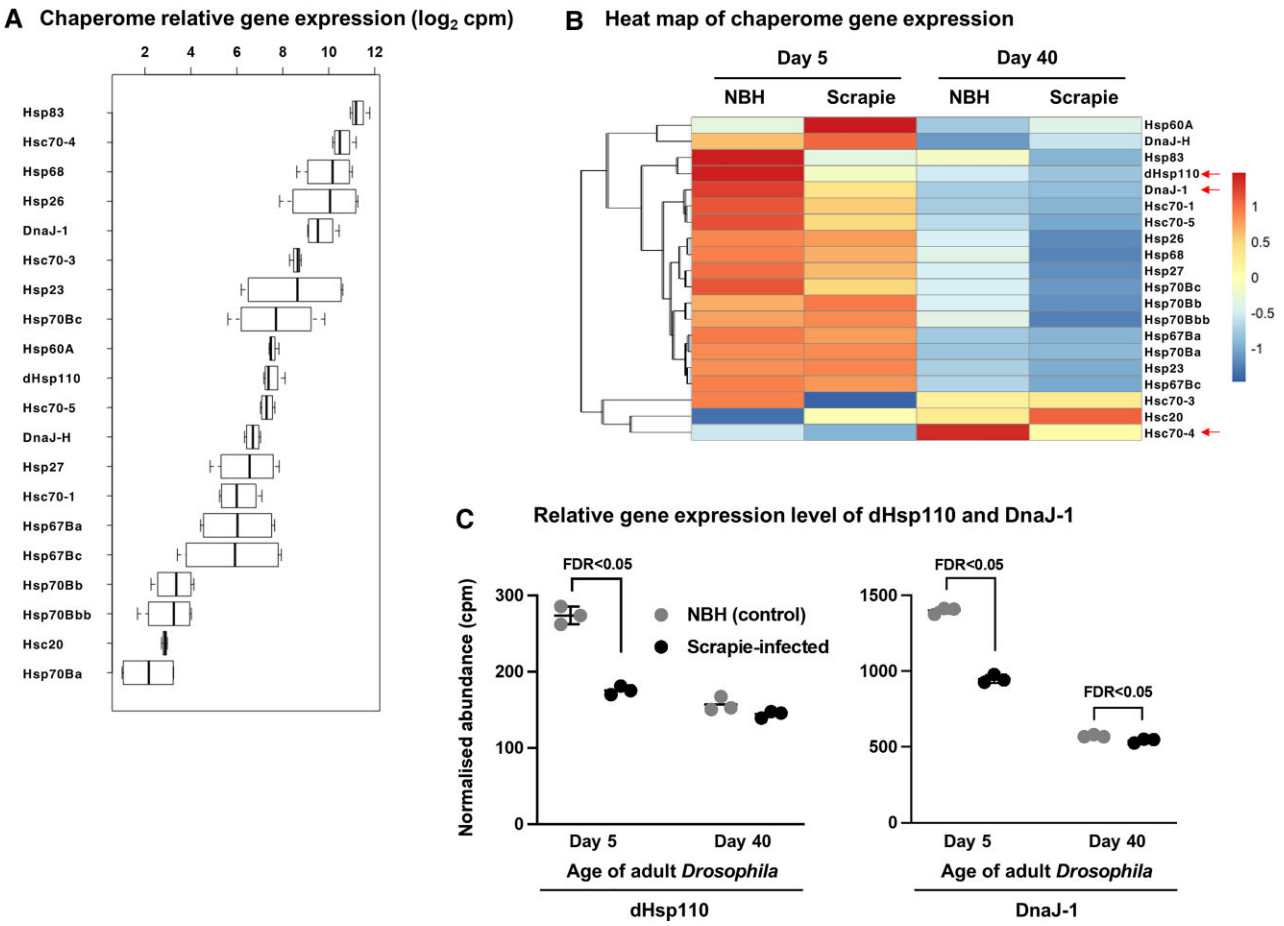

Figure 1 Chaperome gene expression level changes during prion infection in PrP *Drosophila*. Ovine PrP transgenic *Drosophila* were exposed to scrapie-infected or scrapie-free sheep brain homogenate at the larval stage. Head homogenate was prepared from adult *Drosophila* 5 and 40 days after hatching and analysed by RNASeq.[43] Data shown are (**A**) box plots showing normalized expression of Hsps detected in all PrP *Drosophila* treatment groups combined. In comparison, the normalized abundance of Act5c is 9.86 ± 0.04 $\log_2$ cpm (mean ± SEM); (**B**) hierarchical clustering analysis shown as a heat map of differentially regulated chaperome expression with DnaJ-1, dHsp110 and Hsc70-4 highlighted by red arrows; (**C**) changes in dHsp110 (Hsc70cb) and DnaJ-1 relative gene expression level determined by comparison of scrapie-free (light grey) or scrapie-infected (black) *Drosophila* ($3 \times n = 15$ flies per treatment group, data shown are mean ± SD). FDR = false discovery rate.

observed in these flies. To test this hypothesis, we assessed prion-induced toxicity and prion seeding activity in prion-exposed PrP *Drosophila* with modified (increased or decreased) dHsp110 or DnaJ-1 gene expression. We focused on dHsp110 and DnaJ-1 as these are the principal regulatory components of the Hsp70 disaggregase complex. Accordingly, we used *Drosophila* transgenic for either human PrP (Met129 PrP genotype) or ovine PrP (VRQ PrP genotype) in order to determine the effects of altered Hsp70 disaggregase activity in model systems of human and animal prion disease.

Prion-induced toxicity in PrP transgenic *Drosophila*

It was important to show that the *Drosophila* that expressed PrP used in this study were susceptible to mammalian prions. Human PrP *Drosophila* were exposed at the larval stage to vCJD-infected or prion-free human brain material. After hatching, *Drosophila* were transferred to prion-free culture tubes and at various time points (≤50 days) during their adult lifespan the locomotor ability of adult flies was assessed by a negative geotaxis climbing assay. In addition, groups of *Drosophila* were euthanized, decapitated and homogenate prepared from the isolated fly heads for use as seed in PMCA reactions to determine prion seeding activity. Human PrP *Drosophila* displayed a prion-induced phenotype and prion seeding activity after exposure to human prions as shown in Fig. 2. The data in Fig 2A show that adult human PrP *Drosophila*, after larval exposure to vCJD prions, developed a toxic phenotype evidenced by a significant ($P = 0.0006$) accelerated decrease in locomotor ability that became progressively more severe with age. The data in Fig. 2B show that prion seeding activity was detected in head homogenates prepared from vCJD-exposed human PrP *Drosophila* aged ≥20 days. We have previously shown that adult ovine PrP transgenic *Drosophila* show decreased locomotor activity and accumulation of prion seeding activity after exposure to sheep scrapie at the larval stage.[37,39,49]

Generation of PrP transgenic *Drosophila* with modified co-chaperone gene expression

We next generated *Drosophila* that were bigenic for PrP and either RNAi or an overexpression transgene specific for either dHsp110 or DnaJ-1. In addition, we also generated control fly lines that expressed Met129 human PrP or ovine PrP together with luciferase

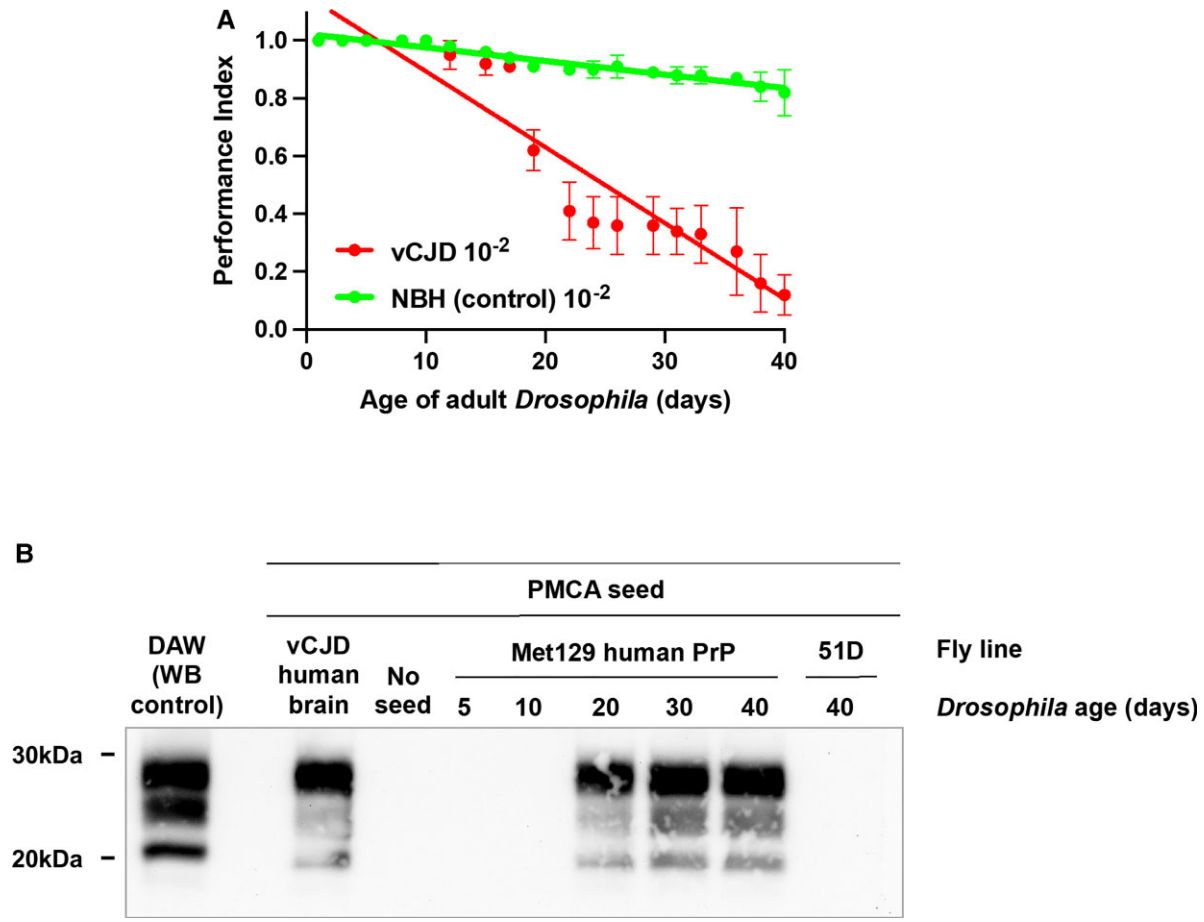

Figure 2 Prion-induced toxicity and prion seeding activity in vCJD-exposed human PrP *Drosophila*. *Elav-GAL4* × Met129 human PrP or *Elav-GAL4* × 51D (51D) *Drosophila* were exposed to a 10^{-2} dilution of vCJD-infected, or prion-free control human brain material at the larval stage. (**A**) Adult *Drosophila* were assessed for their locomotor ability by a negative geotaxis climbing assay at the time points shown. The data shown are linear regression plots of the mean performance index ± SD for three groups of flies per time point calculated as described in the 'Materials and methods' section. (**B**) At various times after hatching, head homogenate was prepared and used as seed in PMCA reactions. Western blot was used to detect PK-resistant PrP27-30 in PMCA reaction products. Molecular mass markers in kDa are shown on the *left*. WB control = western blot control comprising DAW scrapie-infected sheep brain material included to highlight the low-molecular-weight band of unglycosylated vCJD PK-resistant PrP^{Sc}. Uncropped blots are available in the Supplementary material.

RNAi (control RNAi) or a green fluorescent protein (GFP) transgene (control overexpression gene). *Drosophila* fly lines were crossed with the *elav*-GAL4 driver fly line to allow modified dHsp110, DnaJ-1 or control construct and PrP expression pan neuronally in the fly. We confirmed up- and downregulation of dHsp110 and DnaJ-1 transcript level in each appropriate fly line by ddPCR as shown in Fig. 3. We verified that modification of dHsp110 or DnaJ-1 expression did not affect the level of PrP expression in *Drosophila*. The western blot data in Fig. 4 show that similar levels of PrP were seen in flies transgenic for human or ovine PrP compared to these fly lines with co-chaperone or control construct gene expression.

Modulation of dHsp110 or DnaJ-1 gene expression perturbs prion toxicity in PrP *Drosophila*

We next determined the effect of modified dHsp110 and DnaJ-1 gene expression on prion-induced toxicity in PrP transgenic *Drosophila* by assessment of the locomotor activity of adult *Drosophila* after larval exposure to prion-infected or prion-free control inoculum.

We first analysed the effect of upregulation of dHsp110 or DnaJ-1 gene expression on prion-induced neurotoxicity in adult PrP *Drosophila*. The data in Fig. 5 (with accompanying statistical analysis in the Supplementary material, section 2) demonstrate that vCJD-exposed control adult human PrP *Drosophila* with GFP overexpression showed a significant accelerated loss of locomotor activity, which became progressively more severe with age, compared to flies exposed to control inoculum (Fig. 5A). In contrast, vCJD-exposed human PrP *Drosophila* that overexpressed dHsp110 (Fig. 5B) or DnaJ-1 (Fig. 5C) showed no significant difference in locomotor ability compared to flies exposed to control inoculum. Similarly, scrapie-exposed control adult ovine PrP *Drosophila* with GFP overexpression showed a significant progressive accelerated loss of locomotor activity (Fig. 5D), whereas scrapie-exposed adult ovine PrP *Drosophila* with overexpression of dHsp110 (Fig. 5E) or DnaJ-1 (Fig. 5F) showed no significant difference in locomotor ability compared to flies exposed to control inoculum.

We subsequently analysed the effect of downregulation of dHsp110 or DnaJ-1 gene expression on prion-induced neurotoxicity in adult PrP *Drosophila*. Supplementary material, section 3 (with accompanying statistical analysis in section 4) show that adult human PrP and ovine PrP *Drosophila* that expressed dHsp110 or DnaJ-1 RNAi,

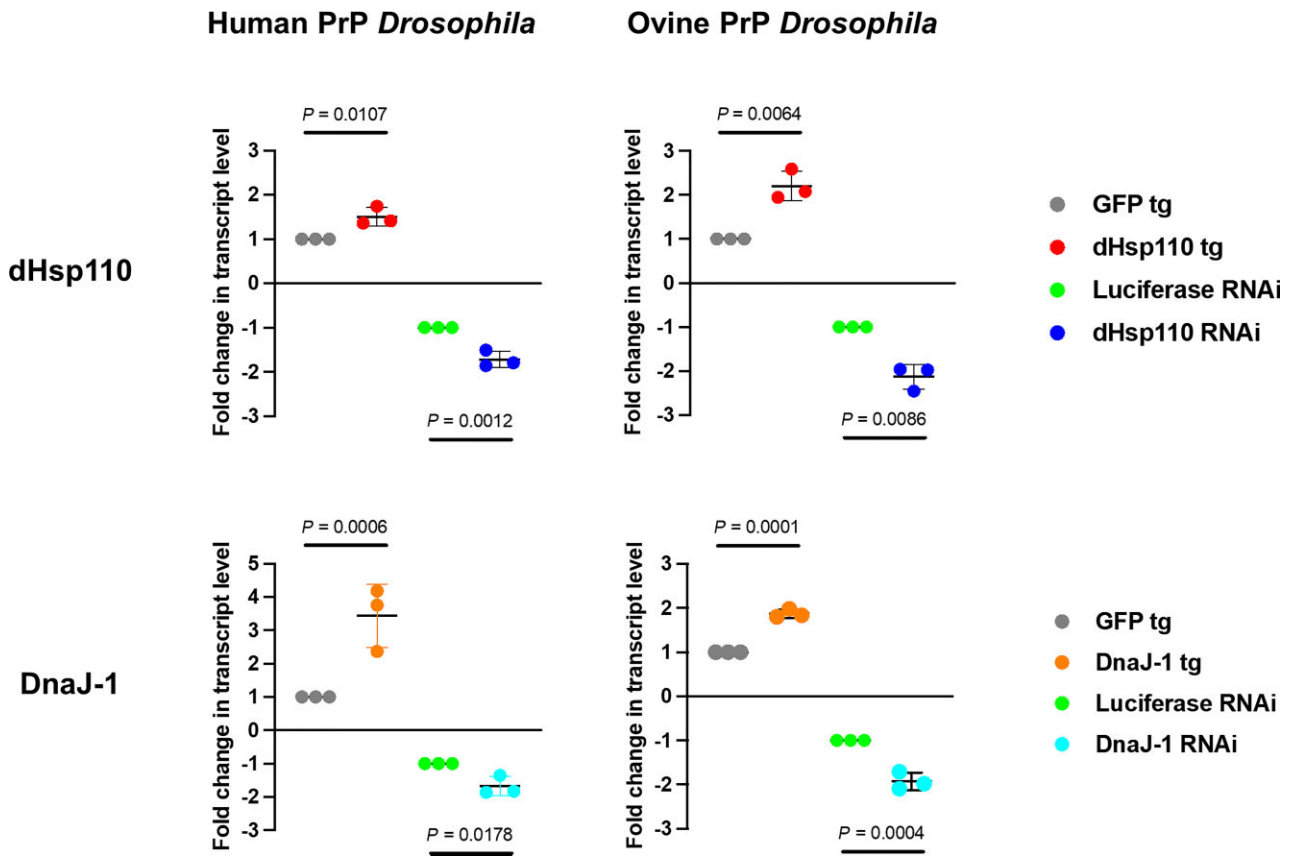

Figure 3 Modification of dHsp110 and DnaJ-1 mRNA expression in PrP *Drosophila*. *Drosophila* were generated with *Elav-GAL4*-driven pan neuronal expression of human or ovine PrP and transgene overexpression or RNAi-modified knockdown of dHsp110 or DnaJ-1. Head homogenate was prepared from adult *Drosophila* and analysed by ddPCR for dHsp110 or DnaJ-1 and control GAPDH gene expression. The data shown are fold change dHsp110 or DnaJ-1 transcript level relative to GAPDH. Statistical analysis between indicated groups was performed by the Students *t*-test.

or luciferase control RNAi showed the same level of accelerated locomotor decline after exposure at the larval stage to a 10^{-2} $\log_{10}$ dilution of vCJD or sheep scrapie inoculum compared to control inoculum, respectively. We reasoned the lack of observed effect of chaperone RNAi on prion-mediated toxicity was because the dose of inoculum used induced maximal toxic effect on the flies, thus masking any effect of dHsp110 or DnaJ-1 RNAi. Accordingly, we repeated the experiment using fresh PrP *Drosophila* exposed at the larval stage to a 10^{-2}–10^{-10} dilution series of prion inoculum to determine if chaperone RNAi expression could enhance prion-induced toxicity at low-dose inoculum. Dilutions of vCJD inoculum in human PrP *Drosophila* were toxic in the range of 10^{-2}–10^{-8} and scrapie inoculum was toxic in ovine PrP *Drosophila* in the range of 10^{-2}–10^{-6} (data not shown). With increasing dilution of prion inoculum RNAi specific for either dHsp110 or DnaJ-1 had an increasing effect on prion-induced toxicity, which was maximal at a dilution of 10^{-8} in the case of vCJD inoculum and at a dilution of 10^{-6} in the case of scrapie inoculum.

The data in Fig. 6 (with accompanying statistical analysis in the Supplementary material, section 5) show the comparison of RNAi-mediated downregulation of dHsp110 or DnaJ-1 gene expression effects on prion-induced neurotoxicity in adult human PrP *Drosophila* after exposure to 10^{-2} (Fig. 6A–C) or 10^{-8} (Fig. 6D–F) dilutions of vCJD inoculum at the larval stage. There was no significant difference in the 10^{-2} dilution of vCJD prion inoculum-induced accelerated decline in locomotor activity between the luciferase RNAi human PrP *Drosophila* and those that expressed dHsp110 or DnaJ-1 RNAi. However, with 10^{-8} dilution of vCJD prion inoculum, a significant enhancement of accelerated decline in locomotor activity was seen in *Drosophila* that expressed dHsp110 RNAi or DnaJ-1 RNAi compared to those that expressed luciferase RNAi. The data in Fig. 7 (with accompanying statistical analysis in Supplementary material, section 6) show the comparison of RNAi-mediated downregulation of dHsp110 or DnaJ-1 gene expression on prion-induced neurotoxicity in adult ovine PrP *Drosophila* after exposure to 10^{-2} (Fig. 7A–C) or 10^{-6} (Fig. 7D–F) dilutions of scrapie inoculum at the larval stage. There was no significant difference in the 10^{-2} scrapie prion inoculum-induced accelerated decline in locomotor activity between the luciferase RNAi ovine PrP *Drosophila* and those that expressed dHsp110 or DnaJ-1 RNAi. However, after exposure to 10^{-6} dilution of scrapie prion inoculum, a significant enhancement of accelerated decline in locomotor activity was seen between dHsp110 RNAi ovine PrP *Drosophila* and those that expressed luciferase RNAi. These data show that RNAi-mediated downregulation of dHsp110 had a greater effect on prion-induced toxicity than that of DnaJ-1 RNAi.

Our data presented here have been obtained through the disaggregation of human and ovine prions by *Drosophila* chaperones, which would normally be mediated by homologues of human and ovine chaperones, respectively. Supplementary material, section 7 show that significant amino acid sequence conservation exists between *Drosophila*, human and ovine DnaJ-1, Hsp110 and Hsp70 molecules considered to interact with these co-chaperones.

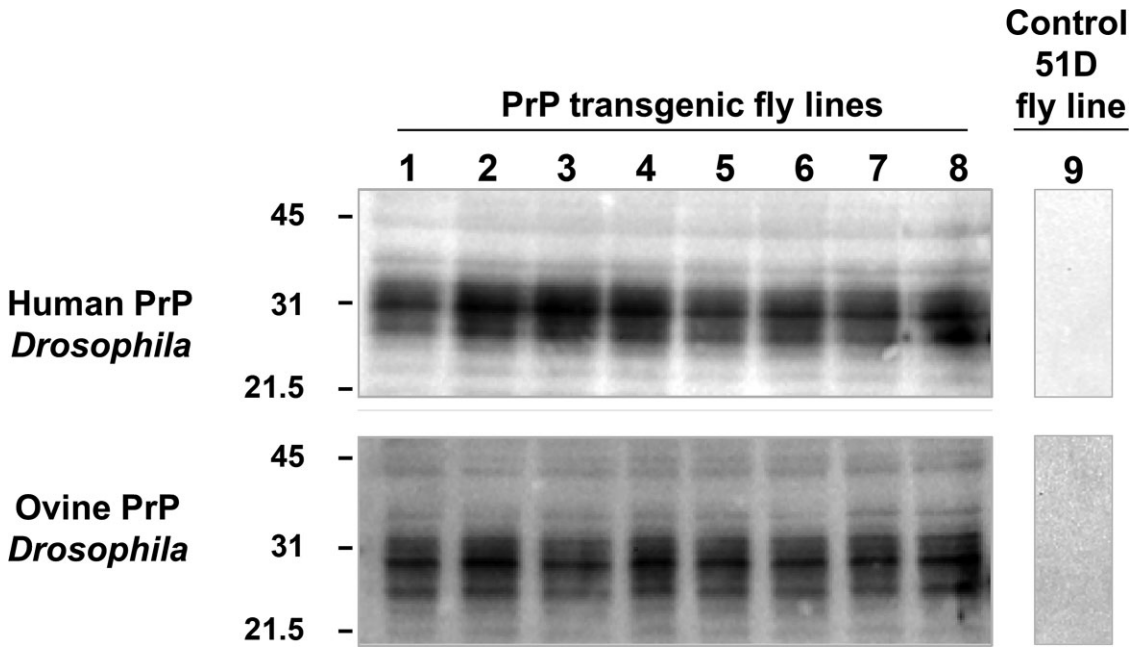

Figure 4 Western blot detection of PrP expression in *Drosophila* fly lines. Head homogenate prepared from adult *Elav-GAL4* × human (*top*) and *Elav-GAL4* × ovine (bottom) PrP *Drosophila* was analysed by western blot probed with monoclonal antibody Sha31. PrP *Drosophila* were transgenic for dHsp110 overexpression (track 1); dHsp110 RNAi (track 2); DnaJ-1 overexpression (track 3); DnaJ-1 RNAi (track 4); luciferase RNAi (track 5); mCherry expression (track 6; not used further); GFP expression (track 7); or left unmodified (track 8). Representative control *Elav-GAL4* × 51D fly head homogenates are shown in track 9. Molecular mass markers in kDa are shown on the *left*. Uncropped blots are available in the Supplementary material.

Modulation of co-chaperone perturbs prion seeding activity in PrP *Drosophila*

We next determined whether the effect of modulation of dHsp110 or DnaJ-1 expression on prion-induced toxicity in PrP *Drosophila* was accompanied by changes in prion seeding activity. To do so, head homogenate from 5- and 40-day-old adult *Drosophila*, previously exposed to prion inoculum at the larval stage, was used as seed in quantitative RT-QuIC reactions. Prion seeding activity was assessed by ThT incorporation[46,47] and the results shown in Fig. 8.

No prion seeding activity was seen in 5-day-old vCJD prion-exposed human PrP *Drosophila* harbouring control GFP, dHsp110 or DnaJ-1 overexpression genes (Fig. 8A). However, while prion seeding activity was present in head homogenate from 40-day-old vCJD-exposed human PrP *Drosophila* with control GFP expression, it was absent or reduced in flies with dHsp110 or DnaJ-1 overexpression, respectively (Fig. 8B). In a similar manner, no prion seeding activity was seen in 5-day-old scrapie-exposed ovine PrP *Drosophila* harbouring GFP, dHsp110 or DnaJ-1 overexpression genes (Fig. 8C). Prion seeding activity was detected in head homogenate from 40-day-old control GFP scrapie-exposed ovine PrP *Drosophila* but absent or reduced in similar aged scrapie-exposed ovine PrP flies with dHsp110 or DnaJ-1 overexpression, respectively (Fig. 8D).

The data in Fig. 8 also show that no prion seeding activity was seen in head homogenate from 5-day-old vCJD prion-exposed human PrP *Drosophila* that expressed luciferase, dHsp110 or DnaJ-1 RNAi (Fig. 8A) but was present in all of these fly lines at 40 days of age (Fig. 8B). In a similar manner, no prion seeding activity was seen in head homogenate from 5-day-old scrapie prion-exposed ovine PrP *Drosophila* that expressed luciferase, dHsp110 or DnaJ-1 RNAi (Fig. 8C) but was present in all of these fly lines at 40 days of age (Fig. 8D).

Supplementary material, section 8 show that no prion seeding activity was detected in head homogenate prepared from 5- or 40-day-old human PrP *Drosophila* or ovine PrP *Drosophila* exposed to control prion-free brain homogenate.

Discussion

Our studies demonstrate that the metazoan Hsp70 protein disaggregation machinery is an important determinant of mammalian prion replication and induced toxicity. Our data show that modulation of dHsp110 and DnaJ-1 gene expression perturbs mammalian prion replication in PrP transgenic *Drosophila*. Notably, overexpression of dHsp110 ablated the accumulation of vCJD or scrapie prion seeding activity in prion-exposed PrP *Drosophila*. These observations support the view that enhanced Hsp70 disaggregase activity represents a viable therapeutic strategy for human protein misfolding neurodegenerative diseases.

Mammalian prion replication only occurs in hosts that express PrP^{C}.[6,7] Accordingly, we used *Drosophila*, a normally PrP-null invertebrate species, made transgenic for mammalian PrP expression to investigate the role of the metazoan Hsp70 disaggregase in these processes. We validated the PrP *Drosophila* used by assessment of their response to prion exposure. We showed that vCJD-exposed *Drosophila* transgenic for Met129 human PrP displayed core features of mammalian prion disease, namely increasing severity of a neurotoxic phenotype that was accompanied by the progressive accumulation of prion seeding activity. PMCA products from reactions seeded with vCJD-exposed human PrP *Drosophila* head homogenate displayed PK-resistant PrP27-30 with a molecular profile of this human prion strain. Our previous observations have demonstrated that scrapie-exposed *Drosophila* transgenic for ovine PrP also display a progressive neurotoxic phenotype, one that is accompanied by the accumulation of prion seeding activity and *bona fide* prion infectivity in these flies.[37,39,49] Importantly, we have demonstrated that prion strain properties are maintained during propagation in ovine PrP

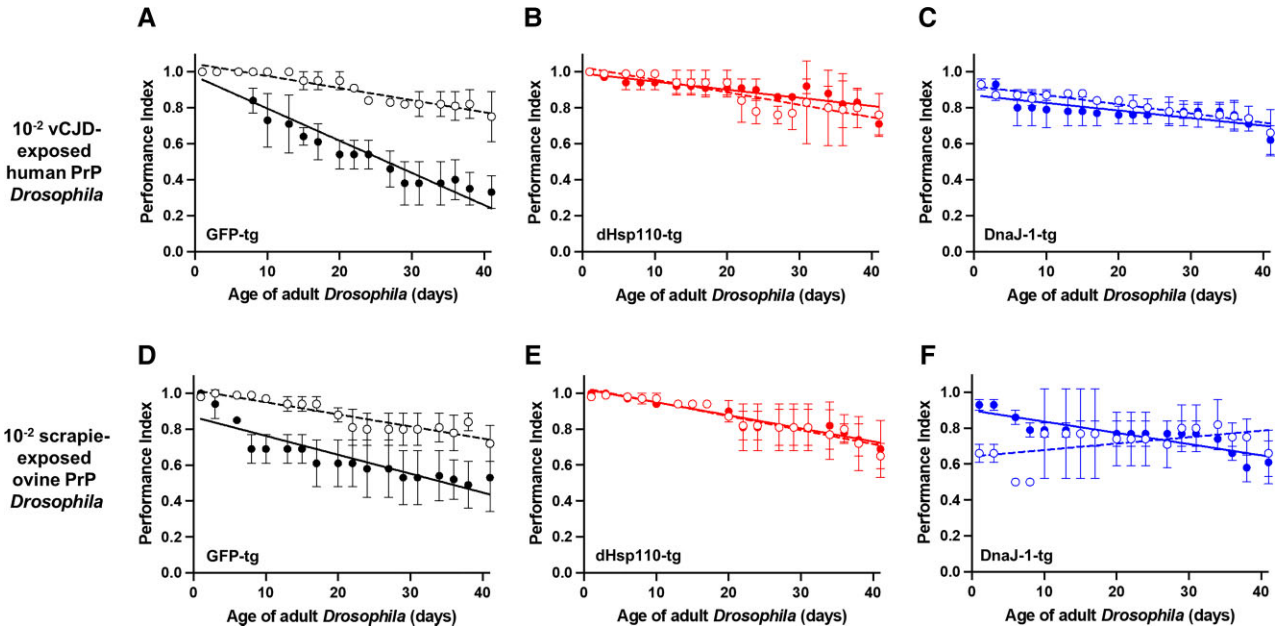

Figure 5 Overexpression of dHsp110 or DnaJ-1 ameliorates prion-induced toxicity in PrP *Drosophila*. Adult *Elav-GAL4* × human PrP *Drosophila* (**A–C**) or *Elav-GAL4* × ovine PrP *Drosophila* (**D–F**) that expressed GFP control transgene (**A** and **D**), dHsp110 transgene (**B** and **E**) or DnaJ-1 transgene (**C** and **F**) were assessed for their locomotor ability by a negative geotaxis climbing assay following exposure at the larval stage to 10^{-2} dilution of vCJD-infected human or scrapie-infected sheep brain homogenate, respectively (closed circles). Control inoculum was a 10^{-2} dilution of prion-free human or ovine brain material as appropriate (open circles). The data shown are linear regression plots of the mean performance index ± SD for 45 flies (three groups of $n = 15$) per time point calculated as described in the 'Materials and methods' section.

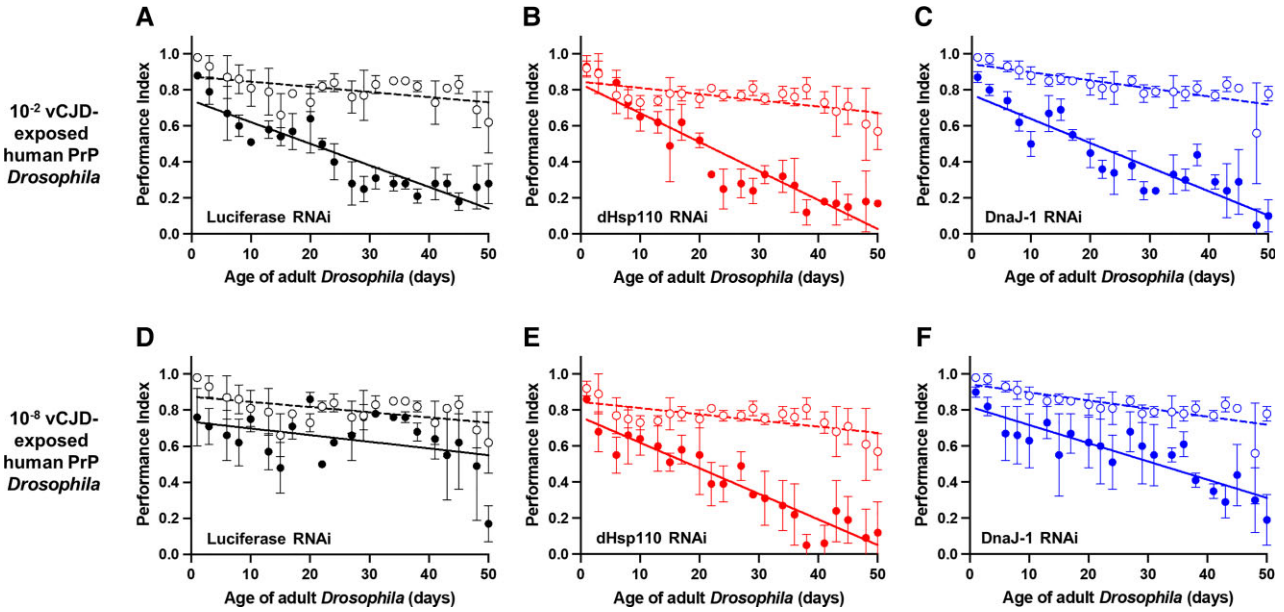

Figure 6 Downregulation of dHsp110 or DnaJ-1 enhances vCJD prion-induced toxicity in human PrP *Drosophila*. *Drosophila* with pan neuronal expression of human PrP together with luciferase control RNAi (**A** and **D**), dHsp110 RNAi (**B** and **E**) or DnaJ-1 RNAi (**C** and **F**) were assessed for their locomotor ability by a negative geotaxis climbing assay following exposure at the larval stage to a 10^{-2} dilution (**A–C**) or a 10^{-8} dilution (**D–F**) of vCJD-infected brain homogenate (closed circles). Control inoculum was a 10^{-2} dilution of prion-free human brain material (open circles). The data shown are linear regression plots of the mean performance index ± SD for 45 flies (three groups of $n = 15$) per time point calculated as described in the 'Materials and methods' section.

Drosophila.[37] Collectively, these observations demonstrate that *Drosophila* transgenic for PrP are susceptible to mammalian prions and possess the necessary molecular and cellular components required for authentic mammalian prion propagation.

Our transcriptomic analysis determined that prion-exposed PrP *Drosophila* downregulate Hsp70, DnaJ-1 and dHsp110, the principal components of the major metazoan Hsp70 protein disaggregation machinery, and presumably therefore a decrease in its functional

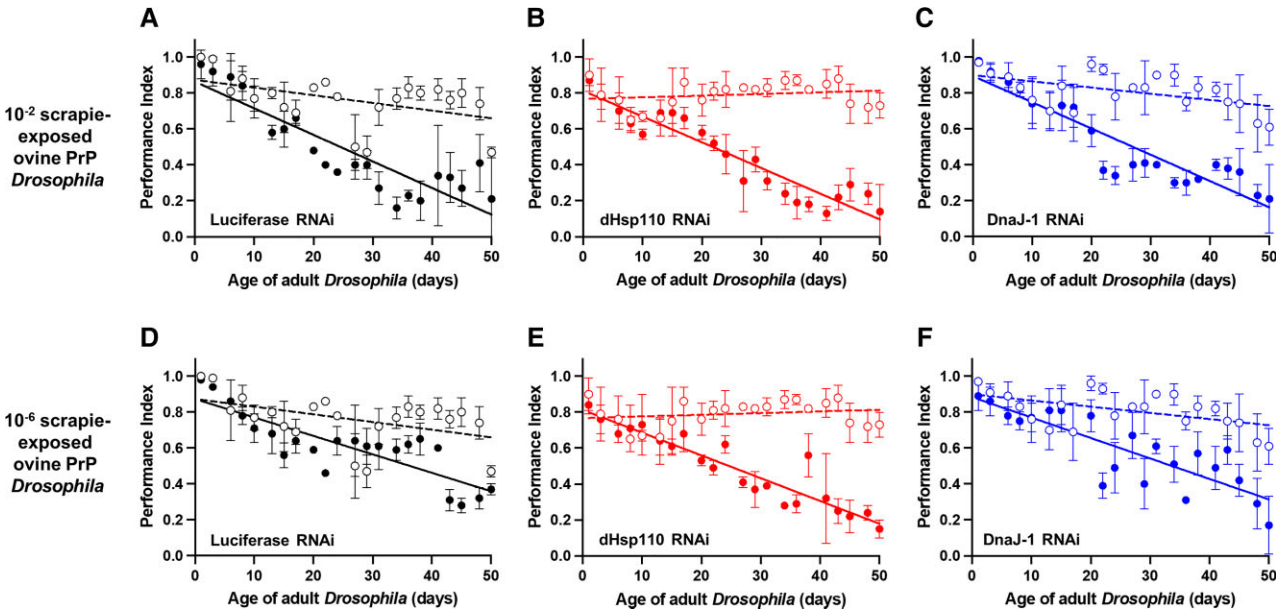

Figure 7 Downregulation of dHsp110 or DnaJ-1 enhances scrapie prion-induced toxicity in ovine PrP *Drosophila*. *Drosophila* with pan neuronal expression of ovine PrP together with luciferase control RNAi (**A** and **D**), dHsp110 RNAi (**B** and **E**) or DnaJ-1 RNAi (**C** and **F**) were assessed for their locomotor ability by a negative geotaxis climbing assay following exposure at the larval stage to a 10^{-2} dilution (**A–C**) or a 10^{-6} dilution (**D–F**) of sheep scrapie-infected brain homogenate (closed circles). Control inoculum was a 10^{-2} dilution of prion-free ovine brain material (open circles). The data shown are linear regression plots of the mean performance index ± SD for 45 flies (three groups of $n = 15$) per time point calculated as described in the 'Materials and methods' section.

activity. This downregulation of gene expression in prion-exposed PrP *Drosophila* occurred early in adult flies and was maintained when prion seeding activity and *bona fide* prion infectivity can be detected in these flies.[37,38] Other Hsp genes were also downregulated, for example *mrj*, a gene that encodes an orthologue of a brain-enriched co-chaperone, the human *MRJ* (mammalian relative of DnaJ), which has been shown to modulate poly(Q) aggregation and toxicity.[50] In addition, gene expression of the endoplasmic reticulum Hsc70-3/BiP chaperone, a modulator of prion disease in mice,[51] was downregulated. However, the phenomenon was not a general downregulation of Hsp gene expression because some were upregulated, including the mitochondrial chaperone tumour necrosis factor receptor-associated protein 1 (TRAP1), a member of the Hsp90 family[52] and a pivotal regulator of mitochondrial integrity.[53] Whatever the underlying mechanism, prion exposure of PrP *Drosophila* had a detrimental impact on proteostatic regulatory mechanisms that presumably contributed to prion accumulation and prion-induced toxicity. This view is supported by mammalian host-based studies that have shown perturbation of chaperone gene expression can lead to modification of prion disease progression. For example, mice that lack expression of heat shock factor 1 (HSF1), or cytosolic or endoplasmic reticulum Hsp70, all succumbed to prion disease faster than wild-type animals.[51,54,55]

Based on these observations, we reasoned that alteration of Hsp70 protein disaggregase activity, mediated by changes in its component gene expression levels, could induce modulation of prion propagation in PrP *Drosophila*. This was the case because overexpression of dHsp110 or DnaJ-1 in vCJD-exposed human PrP *Drosophila* strongly reduced prion-induced neurotoxicity and prion seeding activity. Strikingly, no prion seeding activity was detectable in vCJD-exposed human PrP *Drosophila* with overexpression of dHsp110. In contrast to these results, we observed that RNAi-mediated knockdown of dHsp110 or DnaJ-1 expression in vCJD-exposed human PrP *Drosophila* enhanced the prion-induced neurotoxic phenotype that was most evident at low-dose prion inoculum. These effects were not restricted to vCJD prions in human PrP *Drosophila* because modulation of dHsp110 or DnaJ-1 gene expression in scrapie-exposed ovine PrP *Drosophila* resulted in the same trends. Collectively, our observations provide a cogent argument that the metazoan Hsp70 protein disaggregase participates in the life cycle of *bona fide* transmissible mammalian prions.

Metazoan Hsp70 disaggregase activity is initiated by the DnaJ co-chaperone binding via multivalent interactions to an aggregated substrate that promotes high-affinity crowding of Hsp70 molecules to the amyloid surface, an event coupled with an ATP-driven conformational change in Hsp70.[56] Steric clashes between surface crowded Hsp70 molecules exert an entropic pulling action on substrate-bound Hsp70 molecules, which destabilizes amyloid fibrils, resulting in their fragmentation and allowing monomer extraction. This activity is enhanced by Hsp110, which accelerates ADP/ATP exchange on Hsp70 and its conformational reversion to a substrate-receptive state and recommencement of the chaperone cycle.[57,58] Additionally, the recruitment of Hsp110 to chaperone complexes on fibril surfaces contributes to steric clashes, thereby enhancing the entropic pulling forces that mediate disaggregation.[56] According to this scheme, we propose that the metazoan Hsp70 disaggregation machinery facilitates mammalian prion fragmentation with the extent of prion disassembly determined by the level of disaggregase activity. For example, in our studies here overexpression of dHsp110 or DnaJ-1 led to a significant reduction in prion-induced neurotoxicity and prion seeding activity. This we attribute to enhanced Hsp70-mediated disassembly of either vCJD or scrapie prions with full clearance of newly generated PrP^{Sc} seeds, thereby preventing their accumulation and spread. In contrast, downregulation of dHsp110 or DnaJ-1 enhanced prion-induced neurotoxicity. We propose this is due to reduced Hsp70 activity that was still sufficient to mediate prion disassembly and fragmentation but insufficient to clear newly generated PrP^{Sc} fragments,

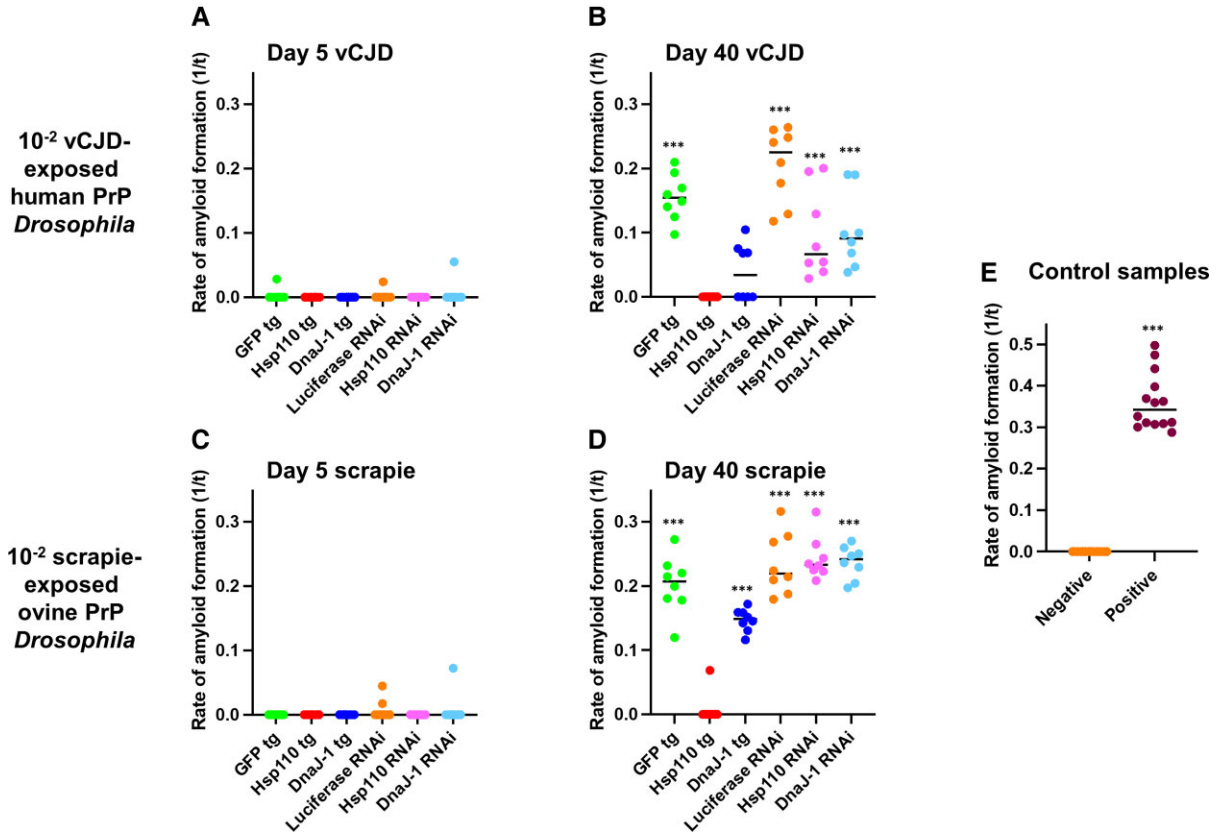

Figure 8 Modulation of co-chaperone perturbs prion seeding activity in PrP *Drosophila*. *Drosophila* with pan neuronal expression of human or ovine PrP together with modified dHsp110 or DnaJ-1 expression, or GFP transgene or luciferase control RNAi, were exposed to a 10^{-2} dilution of vCJD-infected human (**A** and **B**) or scrapie-infected sheep brain homogenate (**C** and **D**), respectively, at the larval stage. Adult *Drosophila* were collected at 5 days (**A** and **C**) and 40 days (**B** and **D**) of age after hatching and head homogenate was prepared and used as seed in RT-QuIC reactions as described in the 'Materials and methods' section. Known RT-QuIC positive and negative fly head homogenates were included as controls for the assay (**E**). The data shown are rate of amyloid formation (1/t) for each treatment group. Rates of amyloid formation for individual treatment groups that were significantly different from the negative control group are indicated. ***$P \leq 0.0005$. RT-QuIC traces are shown in Supplementary material, section 9.

thereby allowing prion accumulation and spread. This scenario may explain that in a mammalian host with wild-type levels of Hsp70 protein disaggregase activity, the ensuing progress of natural prion diseases arises as a consequence of prion propagation exceeding prion metabolism. Our studies reported here have dealt with disaggregation of human and ovine prions by *Drosophila* chaperones. Our amino acid sequence comparisons of *Drosophila*, human and ovine DnaJ-1, Hsp110 and Hsp70 molecules considered to interact with these co-chaperones, show that these canonical molecular chaperones are evolutionary well conserved between these different species.

The effects of dHsp110 and DnaJ-1 we have observed were not due to alteration of PrP expression levels in PrP *Drosophila* as these were unaffected by modulation of co-chaperone gene expression changes. This excludes the possibility that the reduced level of prion-induced toxicity and prion seeding activity in the case of overexpression of dHsp110 or DnaJ-1 was due to reduced availability of PrP^{C} for conversion into PrP^{Sc}. We do not exclude the possibility that other cellular proteostatic mechanisms cooperate with the Hsp70 disaggregase system in prion fragmentation, which may be necessary for full clearance of seeding competent oligomers.[59] Furthermore, some effects of overexpression of dHsp110 or DnaJ-1 may be due in part to an intrinsic co-chaperone action. For example, dHsp110 has been reported to display a holdase activity *in vitro* and prevent protein aggregation,[60] while DnaJ-1 can suppress poly glutamine (polyQ)-induced neurodegeneration in *Drosophila*.[61] An important issue to address is the cellular location of Hsp70-mediated disassembly of mammalian prions. PrP^{C} is tethered to the outer leaf of the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor[62] and its conversion to PrP^{Sc} is reported to occur either at the cell surface or within the endocytic pathway.[63,64] The constitutively expressed *Drosophila* heat shock protein Hsc70-4 is a candidate core chaperone of the Hsp70 protein disaggregase in the fly because it interacts with DnaJ-1, and DnaJ-1 interacts with dHsp110,[61,65] which is a conserved interaction between their orthologues in humans.[56] *Drosophila* Hsc70-4 has been identified at a variety of cellular locations including the cytoplasm and the plasma membrane.[66] In addition, Hsc70-4 is involved in vesicle-trafficking processes, including endosomal microautophagy, which it promotes at synaptic boutons by deforming membranes.[67] Hsc70-4 is also involved in endocytosis.[68] Interestingly, downregulation of Hsc70-4 expression can affect the distribution of plasma membrane proteins, which can subsequently aggregate on the cell surface and internally.[69] While Hsp110 has been viewed as principally cytoplasmic, it has been identified in exosomes under high levels of proteostatic stress, which may indicate that there is a partial redistribution of the Hsp70-machinery under certain cellular conditions.[70] It is therefore conceivable that prion infection may induce a similar redistribution of the Hsp70-machinery to sites of PrP conversion, such as multivesicular bodies from which

exosomes are formed. Collectively, these observations provide good evidence that the Hsp70 protein disaggregase can locate to the vicinity of PrP^{Sc} formation in order to mediate prion disassembly.

Our proposal that the metazoan Hsp70 disaggregase participates in mammalian prion propagation is supported by activity on prion-like amyloid proteins. *In vitro* studies have shown that the human Hsp70 complex can solubilize alpha-synuclein[21] or Tau fibrils[71] into predominantly monomeric species. However, incomplete disassembly produced material that retained toxicity or seeding competence in cell culture. *In vivo* RNAi knockdown or gene deletion of dHsp110 in *Drosophila* enhanced poly(Q)-aggregate formation,[61,72] while Hsp110 gene knockout in mice expressing mutant APP (APPβsw) leads to selective appearance of insoluble amyloid β_{42} ($A\beta_{42}$).[73] In addition, overexpression of Hsp110 in mouse models of amyotrophic lateral sclerosis[34] and Parkinson's disease[35] led to a reduction in protein aggregation and neurodegeneration. In contrast to these reports, RNAi-mediated knockdown of Hsp110 in *Caenorhabditis elegans* muscle tissue led to reduced formation of toxic spreading-alpha-synuclein species.[74] However, ubiquitous knockdown of Hsp110 in *C. elegans* has reportedly reduced[75] or increased[76] poly(Q) aggregation. While these studies all suggest a role for Hsp70 in prion-like propagation, their different outcomes may reflect differences in the state of aggregation of the substrate used or the bystander effect of Hsp110 knockdown on protein homeostasis in the different systems used.[24]

The activity of the Hsp70 disaggregation machinery promotes neuronal proteostasis and simultaneously facilitates the fragmentation of prion and prion-like proteins. The proteostatic function of chaperones declines with age contributing to the development of late-onset protein misfolding neurodegenerative diseases.[77] Our studies here demonstrate that genetic manipulation of the Hsp70 disaggregase machinery is therapeutically beneficial in an animal model of human prion disease. This suggests that enhanced Hsp70-mediated activity could be advantageous in human protein misfolding neurodegenerative diseases in general, providing it is sufficient to clear misfolded protein seeds. Therapeutic strategies aimed at enhanced Hsp70 disaggregase activity could be aided by simultaneous delivery of co-chaperones with holdase activity that sequester misfolded protein seeds until fully degraded.[78] Collectively, our studies presented here demonstrate that PrP transgenic *Drosophila* provide a novel animal system to contribute towards the development of therapeutic interventions for prion diseases and prion-like diseases in general.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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