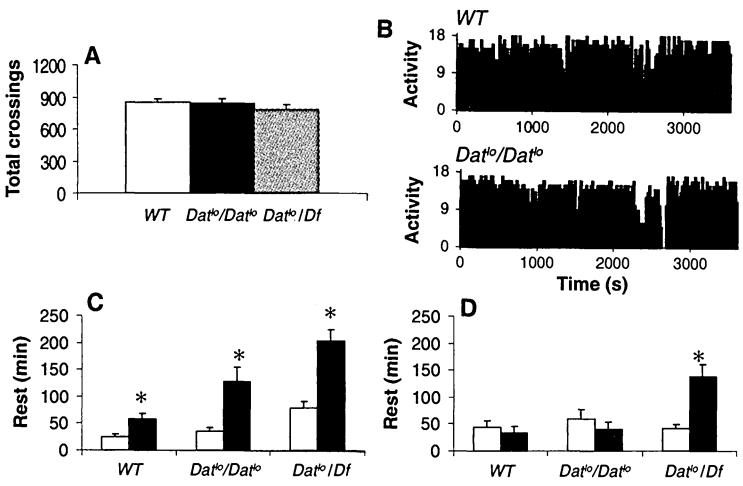

Fig. 4. (**A**) The number of infrared beam crossings per day is similar in wild-type, Dat^{lo}/Dat^{lo}, and Dat^{lo}/Df flies ($P > 0.05$, $n = 25$). (**B**) Activity patterns (ultrasound system, units as in Fig. 1A) are similar in all three *Drosophila* genotypes (two representative records for 1 hour during the light period are shown). (**C**) The amount of rest during the first 6 hours of recovery (solid bars) compared to baseline (open bars) was higher in Dat^{lo}/Dat^{lo} and Dat^{lo}/Df flies than in wild-type flies ($^*P < 0.005$, Wilcoxon test). (**D**) In Dat^{lo}/Df flies, rest rebound persists into the second 6 hours of recovery ($^*P < 0.005$).

approach for studying the phylogeny of sleep. Most important, the demonstration that a mutation modifies the homeostatic regulation of sleep-like states opens the way for gene discovery through mutant screening and validates the use of *Drosophila* as a model system for elucidating the functions of sleep.

Note added in proof: While this paper was in review, another group reported that rest in *Drosophila* is a sleep-like state (*24*).

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4. Flies were cultured at 25°C, 50 to 60% humidity, 12 hour:12 hour light/dark cycle, on yeast, dark corn syrup, and agar food. We obtained per^{01} flies from J. C. Hall (Brandeis University) and Dat^{lo} and $Df(2R)Px1/In(2LR)SM5, al^2\ Cy\ lt^v\ sn^2\ sp^2$ flies from the Bloomington Drosophila Stock Center. For details about the ultrasound monitoring system, see *Science* Online (www.sciencemag.org/feature/data/1047207.shl).
5. Five behaviors were visually scored in 2-s bins by an observer blind to the output of the ultrasound system on 18 independent trials for a total of 8 hours during the light period. The correspondence rates were as follows: locomoting, 99%; inactive, 97%; grooming anterior limbs, 94%; grooming posterior limbs, 98%; and eating, 97%.
6. Rest was defined as uninterrupted behavioral quiescence lasting for at least 5 min.
7. *Drosophila* Activity Monitoring System (Trikinetics) [M. Hamblen *et al.*, *J. Neurogenet.* **3**, 249 (1986)]. The system was validated by visual observation for 17.75 hours ($n = 7$). Flies were awake but did not cross the infrared beam in 5 of 213 bins (miss rate = 2.35%).
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12. Methods were as in (*3*), with modifications: 0.5 μg of pooled total RNA ($n = 20$) was reverse-transcribed (two independent pools per condition). Polymerase chain reactions were performed in duplicate for each pool (104 primer combinations). For RPA, 1 to 2 μg of total RNA from pooled fly heads ($n = 60$) was used. The amount of sample RNA was normalized using a riboprobe specific for ribosomal protein rp49.
13. The behavioral state was determined individually for each fly; only flies that satisfied specific criteria were selected for analysis. A fly was considered awake if it was active for at least 90% of the 3-hour light period and 100% of the hour before killing. A fly was resting if it was inactive for at least 66% of the 3-hour dark period and 100% of the hour before killing. Only about 60 to 70% of the flies examined satisfied these criteria. Failure to specifically identify rest and waking results in samples containing a mixture of behavioral states.
14. An estimated ~5000 RNA species were screened. For additional data, see *Science* Online (www.sciencemag.org/feature/data/1047207.shl).
15. The sequence matched a *Drosophila* P1 clone (AC005554). Analysis using Genescan indicated that the proposed peptide has a 49% homology with rat *Fas*.
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Genetic Suppression of Polyglutamine Toxicity in *Drosophila*

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A *Drosophila* model for Huntington's and other polyglutamine diseases was used to screen for genetic factors modifying the degeneration caused by expression of polyglutamine in the eye. Among 7000 P-element insertions, several suppressor strains were isolated, two of which led to the discovery of the suppressor genes described here. The predicted product of one, dHDJ1, is homologous to human heat shock protein 40/HDJ1. That of the second, dTPR2, is homologous to the human tetratricopeptide repeat protein 2. Each of these molecules contains a chaperone-related J domain. Their suppression of polyglutamine toxicity was verified in transgenic flies.

Expanded polyCAG tracts in the genes for Huntington's disease (HD) and at least seven other disorders are associated with hereditary neurodegeneration (*1*). The polyCAGs are translated to polyglutamines, which form cytoplasmic and/or nuclear aggregates and produce toxic effects (*1*, *2*). One approach to the identification of proteins that can modify polyglutamine aggregation and toxicity is the isolation of enhancer and suppressor genes.

For this purpose, the *Drosophila* eye offers a sensitive model system (*3*, *4*). In a candidate gene approach, a baculovirus antiapoptotic gene, *p35*, and a human heat shock protein (HSP70, encoded by the *HSPA1L* gene) suppressed polyglutamine-dependent degeneration in the eye (*3*, *5*). Here an alternative approach is described: screening the fly genome for genes that dominantly modify the toxicity of polyglutamine.

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Using a polymerase chain reaction (PCR) method, we synthesized polyCAGs of short (20 CAGs) and expanded (127 CAGs) lengths (*6*). These were placed in transgenic constructs cis to the yeast upstream activating sequence (UAS). Their expression was activated in genetic crosses trans to the yeast GAL4 transcription factor, expression of which was in turn regulated by the eye-specific promoter GMR upstream of the yeast GAL4 cDNA (*7*–*9*). GMR is composed of five tandem copies of a response element derived from the *rhodopsin 1* gene promoter, a binding site for the eye-specific transcription factor GLASS (*10*). This promoter enhances the expression of the reporter gene in all retinal cell types as they develop. Flies carrying GMR-GAL4 were crossed with three independently generated UAS-polyCAG transgenic lines carrying the short 20-CAG repeat (UAS-20Q) and with those containing the expanded 127-CAG repeat (UAS-127Q), and in all cases were tagged with a hemagglutinin (HA) epitope sequence (*11*).

In all three GMR-GAL4/UAS-20Q lines, flies eclosed as adults with eyes that were morphologically normal and had normal pigment distribution. In contrast, the three lines of GMR-GAL4/UAS-127Q had severely abnormal eyes (Fig. 1). Immunolabeling of the HA tag in cryostat sections of GMR-GAL4/UAS-127Q flies showed aggregates in the remnants of the retina (*12*). No staining was observed in GMR-GAL4/UAS-20Q flies, possibly because of a lack of aggregation or rapid turnover of the shorter protein. Heterozygous GMR-GAL4 flies expressing GAL4 alone and all three UAS-127Q lines without GMR-GAL4 had normal external and internal eye morphology and pigment distribution.

GMR-GAL4/UAS-127Q flies have severe externally visible eye abnormalities (Fig. 1) and were used to screen for dominant modifiers of the toxicity of the 127Q repeat by examining the genes in the vicinity of a series of P-element chromosomal insertion sites. This was done by crossing them with some 7000 de novo–generated autosomal P-element insertion strains (*13*) and assessing the F_1 progeny for suppression or enhancement of the eye phenotype. Thirty lines were established that suppressed the polyglutamine-dependent eye degeneration in heterozygous flies and 29 lines were made that enhanced it (*14*). Plasmid rescue of the P elements and their flanking genomic DNA was performed (*15*), and cDNA corresponding to the P-element insertion site was used to test its ability to suppress the polyglutamine toxicity. Here we report the results for the first two lines for which the suppression effects have been directly confirmed.

In the first line, EU3500, the genomic sequence, starting 98 base pairs (bp) downstream of the P element, matched an expressed sequence tag (EST) in the Berkeley Drosophila Genome Project (BDGP) database (*15*). At least three independent cDNA clones in the database had similar sequences but different lengths of 3′ UTR. For testing, GH26396 (*16*) was chosen, which is a 1711-bp cDNA that encodes dHDJ1, a predicted protein of 334 amino acids and a molecular weight of 37 kD, which has an NH_2-terminal J domain and homology to human HSP40/HDJ1 (54% identity and 72% similarity) (Fig. 2) (*17*–*19*).

For the second suppressor line, EU3220, the sequence starting 293 bp downstream of the P element matched an EST, and the cor-

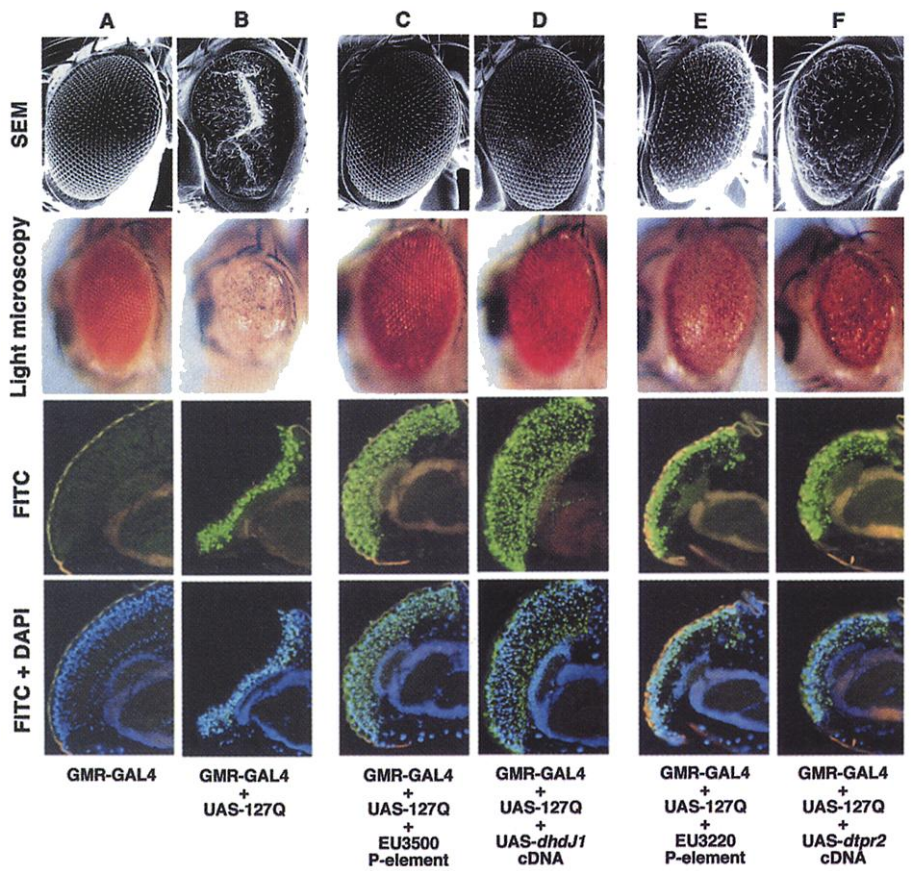

Fig. 1. Genetic suppression of the toxic effect of 127Q in the fly eye. SEM, scanning electron microscopy; FITC, frozen sections labeled with antibody to the HA tag on 127Q peptide (green); FITC+DAPI, double exposure with DAPI to stain nuclei (blue). (**A**) Control, expressing GAL4 regulated by GMR, the eye-specific enhancer/promoter, in the absence of 127Q. The red pigmentation is due to expression of the *white*$^+$ gene marker on the GMR P element. No aggregates are observed with FITC. DAPI shows a relatively normal arrangement of nuclei. (**B**) Flies expressing 127Q peptide driven by GMR-GAL4. The eye has roughly normal size but is severely malformed. Light microscopy shows the absence of pigmentation. FITC shows numerous fluorescent polyglutamine aggregates, mostly localized to nuclei, as seen by overlap with DAPI. (**C**) Suppressor P-element insertion EU3500 restores the external eye structure and pigmentation. FITC and DAPI stains show improved internal retinal structure, despite the presence of polyglutamine aggregates. (**D**) Confirmation of suppression in transgenic flies with *dhdJ1* cDNA, corresponding to the gene 3′ of the EU3500 P-element insertion. Again, the eye structure is largely restored despite the fact that polyglutamine aggregates are still present. As indicated by overlap of DAPI and FITC staining, the polyglutamine nuclear inclusions are present in the peripheral retina, whereas in the proximal retina the FITC staining alone indicates that there are cytoplasmic inclusions as well. (**E**) A second suppressor P-element insertion, EU3220, also improves the external eye structure and pigmentation, albeit less effectively than EU3500. FITC and DAPI stains show improvement of internal retinal structure with some retinal degeneration. (**F**) Confirmation of suppression in transgenic flies with *dtrp2* cDNA, corresponding to the gene 3′ of the EU3220 P-element insertion.

responding cDNA clone GH09432 (*16*) was sequenced. The P-element insertion was 649 bp 5′ of the open reading frame (ORF) of a 2239-bp cDNA, corresponding to a predicted protein of 508 amino acids and a molecular weight of 58 kD that contains 7 tetratricopeptide repeats and a COOH-terminal J domain. A protein database search revealed high homology (46% identity and 67% similarity) between this and the human tetratricopeptide repeat protein 2 (TPR2) (*20*, *21*) (Fig. 3). We have therefore named it *Drosophila* tetratricopeptide repeat protein 2 (dTPR2).

As seen by scanning electron microscopy, the abnormal eyes of GMR-GAL4/UAS-127Q flies were dramatically improved in the presence of the suppressor P-element insertion in strain EU3500 (Fig. 1C). With this insertion, the eye preserves its globular structure, pigmentation, and a uniform bristle arrangement. Although the result is weaker than in EU3500, the suppressor P-element in strain EU3220 also showed a dramatic effect (Fig. 1E).

The internal structure of the eye was examined in horizontal cryostat head sections. In unsuppressed GMR-GAL4/UAS-127Q flies, the structure was badly deformed and immunolabeling of the HA-tagged polyglutamine peptides showed numerous aggregates of fluorescein isothiocyanate (FITC) (Fig. 1B). In the presence of the suppressor insertion in strain EU3500, the retinal structure was vastly improved (Fig. 1C) even though the number of aggregates remained similar. In the presence of EU3220, the effect was similar but weaker (Fig. 1E).

To examine whether the gene immediately 3′ to the EU3500 insertion was indeed responsible for the observed suppression, the corresponding cDNA in GH26396, which contains the coding sequences for dHDJ1, was placed in the transgenic vector (*22*) and microinjected into early-stage fly embryos. All three independent transgenic lines, each carrying a heterozygous autosomal insertion of UAS-*dhdJ1* in the presence of GMR-GAL4/UAS-127Q, closely reproduced the phenotype of the EU3500 line (Fig. 1D). This confirmed that the suppression of polyglutamine-dependent degeneration of the eye by the P-element insertion and its transgenic counterparts was indeed due to the action of dHDJ1. Similarly, the transgenesis test, which uses three independent transgenic lines carrying a heterozygous insertion of UAS-*dtpr2* together with GMR-GAL4/UAS-127Q, confirmed that suppression by the EU3220 P element and its transgenic counterpart was due to the action of dTPR2 (Fig. 1F).

Drosophila dHDJ1 and dTPR2 each have a J domain, a stretch of about 70 amino acids found in J proteins that stimulates the adenosine triphosphatase activity of HSP70 (*23*), which causes the closure of its peptide-binding pocket, thus trapping protein substrates (*24*). J proteins also independently bind other proteins having secondary and tertiary structure (*25*).

Direct evidence for the role of HSPs, particularly J proteins, in preventing protein aggregation has been provided in vitro by showing that a fivefold molar excess of *Escherichia coli* DnaJ completely suppresses aggregation of a substrate protein (bovine mitochondrial rhodanese) (*26*). J proteins may also play a role in the proteasome degradation pathway because the J domain of the simian virus 40 (SV40) large T antigen (TAg) was required for proteasome-dependent degradation of p130 (related to retinoblastoma tumor suppressor protein, pRB) in human osteosarcoma cell line U-2 OS (*27*). In fact, the J domains of two other paralogs of human HSP40, HDJ2 (also known as DNAJ2), or HSJ1 could substitute for the J domain in SV40 TAg, and substitution of a glutamine for a conserved histidine in the J domains could abolish that effect.

Drosophila TPR2 may also act as a suppressor in another way. TPR domains are made of 3 to 16 degenerate repeats of a 34–amino acid stretch, each of which forms a pair of antiparallel α helices (*28*). Multiple tandem TPR units assemble into right-handed

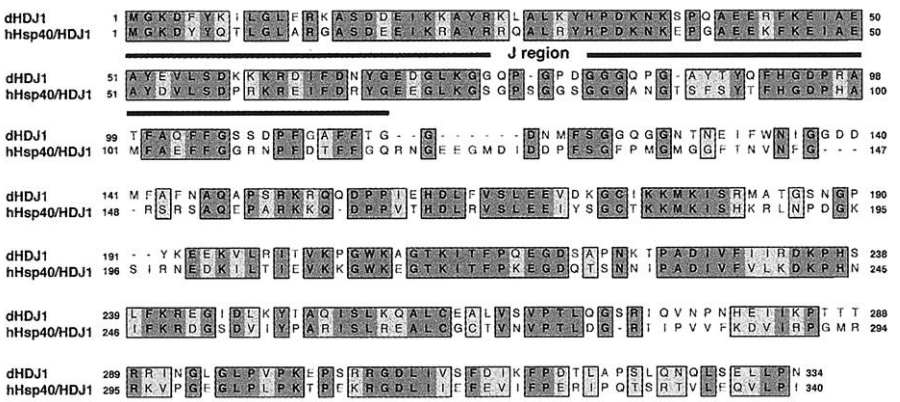

Fig. 2. Alignment of *Drosophila* (dHDJ1)and human HSP40 (hHsp40/HDJ1). The amino acid sequences are 54% identical and 72% similar (*37*). The J regions (*23*) are underlined. These are 74% identical and 88% similar. Light gray shading indicates similarity; dark gray shading indicates identity.

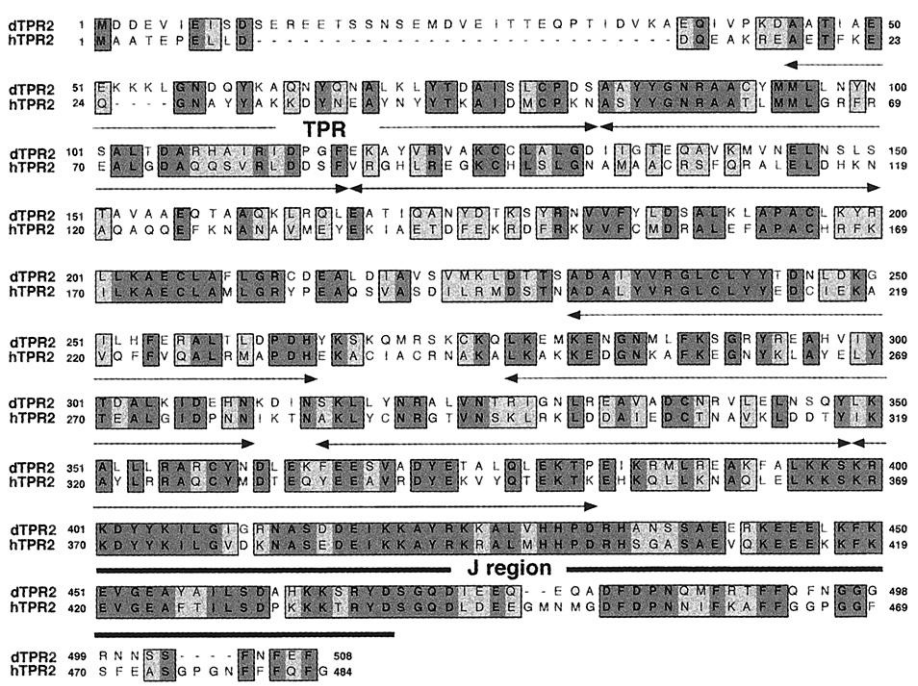

Fig. 3. Alignment of *Drosophila* (dTPR2) and the human tetratricopeptide repeat protein 2 (hTPR2) (*21*). The amino acid sequences are 46% identical and 67% similar (*37*). The J regions (underlined) are 74% identical and 93% similar. In addition, there are seven tetratricopeptide repeat motifs, indicated by arrows. Shading is the same as in Fig. 2.

superhelical structures that are suited for protein-protein interfaces. They are found in proteins involved in various functions, including protein import, neurogenesis, stress response, and chaperone action (*21*, *29*). The human TPR2 was isolated from a HeLa cell cDNA library in a two-hybrid screen, using as "bait" a 271–amino acid fragment of guanine triphosphatase (GTPase)–activating protein-related domain (GRD) of neurofibromin, the neurofibromatosis type 1 (*NF1*) gene product (*21*). Neurofibromin stimulates the GTPase activity of p21 Ras and converts it from the active form (Ras-GTP) to its inactive form (Ras-GDP) (*30*). Conceivably, overexpression of dTPR2 in the fly eye inhibits the *Drosophila* homolog of neurofibromin (dNF1) (*31*) by masking its GRD. This would increase the activity of Ras-GTP, which is known to inhibit the proapoptotic head involution defective (HID) protein (*32*) and enhance the survival of eye cells.

In cultured cells transfected with full-length ataxin-1 or the androgen receptor, each with an expanded polyglutamine, coexpression of HDJ2/HSDJ resulted in 40 to 50% reduction in the number of cells containing aggregates (*33*, *34*). Similar to the effect of *HSPA1L*, the EU3500 or EU3220 P elements or expression of their transgenic counterparts inhibited deterioration of the eye structure, yet the formation of aggregates was not suppressed. Because the GMR promoter acts early in eye development, it is possible that dHDJ1 and dTPR2 act at that early stage of differentiation by binding to 127Q and maintaining a nontoxic milieu, thus permitting eye development to proceed more normally. Conversely, these suppressor proteins, rather than directly interacting with 127Q peptide, may reduce its toxicity by a downstream effect.

The many additional suppressor strains already in hand may lead to discovery of other genes relevant to the pathogenesis of various polyglutamine disorders and their prophylactic or therapeutic treatment.

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11. Flies were maintained on a mixture of corn meal, yeast, and agar at 25°C in 70% humidity. Microinjection solutions containing the transgenic constructs were composed of the following: 13.5 μg of transgenic vector, 4.5 μg of pπ25.1 transposase vector (*38*), 0.1 M sodium phosphate buffer (pH 7.8), and 5 mM potassium chloride, in 50 μl of aqueous solution. With Transjector 5246 and Femtotips II (Eppendorf), the transgenic constructs were microinjected into 5- to 30-min-old w^{1118} embryos reared at 18°C. Several transgenic lines were established for each construct. Plasmid rescue (*39*) and sequencing of two clones of a 127Q line, and one clone for each of the four 20Q lines, confirmed the expected polyCAG and flanking sequences in the transgenic lines.
12. Whole flies were submerged in Mirsky's fixative (National Diagnostics, Atlanta, GA) for 1 to 2 min. They were then decapitated, and the heads were placed in OCT 4583 embedding medium (Tissue-Tek, Torrance, CA), frozen on dry ice, and sectioned. Slides were dried on a 50°C hot plate for 30 s, then fixed in Mirsky's fixative for 30 min at room temperature and washed three times within 10 min with phosphate-buffered saline (PBS) and Tween 20 (PBS/Tween 20, 0.1%). The sections were blocked with 1% PBS and bovine serum albumin fraction V (Sigma), then covered with primary polyclonal antibody to HA (1 μg/ml) (Y-11; Santa Cruz Biotechnology) in the block solution for 2 hours at room temperature. They were washed three times for 5 min with PBS/Tween 20 (0.1%), covered with FITC-labeled secondary antibody to rabbit (4 μg/ml) (Jackson ImmunoResearch Laboratories) in the block solution for 1 hour at room temperature, washed for 5 min with PBS/Tween 20 (0.1%), covered with 4′-6′ diamino-2-phenylindole (DAPI) (0.5 μg/ml) for 1 min, and then washed three times for 15 min with the PBS/Tween. Finally, the sections were mounted in a solution of phenylene diamine (PDA) (0.1 mg/ml), DAPI (0.5 μg/ml), and 90% glycerol and photographed on a Zeiss Axioplan fluorescent microscope.
13. This was done by de novo–generated P-element transpositions with a fly stock carrying the P[*ry*$^+$, Δ2-3](99B) transposase (*40*) and an X-linked enhancer/promoter (EP) insert containing 14 UAS sequences in tandem, followed by the *Hsp70* heat shock minimal promoter (pEP plasmid) (*41*). Transposition lines were generated by mobilizing the X-linked P element in the EP55 strain and isolating lines containing new autosomal insertions.
14. For candidate strains, the responsible chromosomes were separated from those carrying GMR-GAL4 or UAS-127Q by crossing with flies that carried the second and third balancer chromosomes CyO and TM3. Their progeny were then crossed to w^{1118} flies to separate the P elements, and the established strains were tested for suppression or enhancement.
15. Plasmid rescue (*39*) was done by purifying genomic DNA with the QIAamp Tissue kit (Qiagen, Valencia, CA) and digestion with six restriction enzymes: Bfr I, Bgl I, Eco RI, Hinc II, Sac I, and Sac II in a 100-μl reaction volume overnight. The digested fragments were purified by the QIAprep Spin Miniprep kit (Qiagen), circularized by ligation in a 50-μl reaction volume, and transformed by electroporation of 1.5 μl of ligation reaction into the DH10B strain of *E. coli* (Gibco/BRL). Bacteria were plated on agar and kanamycin (10 μg/ml). Inserts were sequenced and sequence comparison was done with the BLAST server at BDGP. The protein alignments were done with MacVector 6.0 software.
16. The clone was purchased from Research Genetics, Huntsville, AL.
17. The GenBank accession number is U34904. For consistency, we have changed the original name, DROJ1 (for *Drosophila melanogaster* DnaJ homolog) to dHDJ1 (for *Drosophila* homolog of HDJ1).
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