JNK/FOXO-mediated Neuronal Expression of Fly Homologue of Peroxiredoxin II Reduces Oxidative Stress and Extends Life Span**

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Activation of c-Jun N-terminal kinase (JNK) signaling in neurons increases stress resistance and extends life span, in part through FOXO-mediated transcription in Drosophila. However, the JNK/FOXO target genes are unknown. Here, we identified Jafrac1, a Drosophila homolog of human Peroxiredoxin II (hPrxII), as a downstream effector of JNK/FOXO signaling in neurons that enhances stress resistance and extends life span. We found that Jafrac1 was expressed in the adult brain and induced by paraquat, a reactive oxygen species-generating chemical. RNA interference-mediated neuronal knockdown of Jafrac1 enhanced, while neuronal overexpression of Jafrac1 and hPrxII suppressed, paraquat-induced lethality in flies. Neuronal expression of Jafrac1 also significantly reduced ROS levels, restored mitochondrial function, and attenuated JNK activation caused by paraquat. Activation of JNK/FOXO signaling in neurons increased the Jafrac1 expression level under both normal and oxidative stressed conditions. Moreover, neuronal knockdown of Jafrac1 shortened, while overexpression of Jafrac1 and hPrxII extended, the life span in flies. These results support the hypothesis that JNK/FOXO signaling extends life span via amelioration of oxidative damage and mitochondrial dysfunction in neurons.

FOXO transcription factors are key regulators of growth, metabolism, life span, and stress resistance in various organisms, including Drosophila (1, 2). FOXO is regulated by the insulin signaling pathway and the stress-induced JNK signaling pathway (3, 4). Oxidative stress activates the stress-responsive JNK, which promotes FOXO nuclear localization and up-regulates expression of antioxidant proteins (5, 6).

In Drosophila, neuronal activation of JNK/FOXO signaling confers resistance to oxidative stress and extends life span (4, 7). Neurons are particularly susceptible to oxidative damage because of their high levels of ROS production and relatively low levels of antioxidant enzymes (8). Thus, activation of the JNK/FOXO pathway in neurons may extend life span through up-regulation of anti-oxidative stress genes. However, little is known regarding the JNK/FOXO target genes in neurons.

Thiol-reducing systems are important reducers of many oxidative stressors, such as peroxide (9). Peroxiredoxin (Prx), also called thioredoxin peroxidase, eliminates hydroperoxide with thioredoxin as an immediate hydrogen donor and reduces ROS levels (10). Among six distinct mammalian Prxs (I–VI), Prx II is exclusively expressed in the brain (11), suggesting that Prx II may play an important role in response to oxidative stress in neurons. However, the regulation of PrxII expression in neurons has not been elucidated. In this study, we demonstrated that neuronal expression of Jafrac1, a Drosophila homologue of human Prx II (hPrxII), was regulated by JNK/FOXO signaling, promoted resistance to oxidative stress, and extended the life span of the flies.

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EXPERIMENTAL PROCEDURES

Drosophila Culture and Mutants—Drosophila melanogaster were kept at 25 °C and cultured using standard methods. Wild-type Oregon-R, w+, elav-Gal4 (pan-neuron driver), Actin5C-Gal4 (ubiquitous driver), Cha-Gal4 (cholinergic neuron driver), repo-Gal4 (glial cell driver), elavGS-Gal4 (pan-neuro-
nal GeneSwitch driver), and UAS-dFOXO.WT flies were obtained from the Bloomington Stock Center. Jafrac1G1104 mutations, which carries an EP element (12) inserted at 5′-untranslated region of the jafrac1, were purchased from GenExel, Inc. (Daejeon, Korea). The JNK signaling related bsk/4CyO (Amino acid replacement: G225E), hep/2/FM6 (mutant carries a P element inserted at a position 179 nucleotides upstream of the ATG codon), and UAS-hepCA mutants (amino acid residues, Ser-346, Thr-350, and Ser-352 in Hep were all replaced with Asp) were gifts from J. Chung. The UAS-dFOXO.TM (a triple PKB phosphorylation sites mutant of dFOXO: T4A4, S190A, and S259A), dFOXO21/TM6B, and dFOXO25/TM6B lines were gifts from E. Hafen and K. J. Min. In dFOXO21 and dFOXO25, the codons for Trp-95 and Trp-124 within the fork-head domain are mutated to stop codons, respectively (13), so they are assumed to be null alleles of dFOXO. The Drosophila Jafrac1 lines (UAS-Jafrac1 and UAS-Jafrac1-RNAi) were previously described by Ha et al. (14).

Paraquat Treatment—To investigate the effect of oxidative stress in the Drosophila model, adult flies (5 days old) were exposed in 20 mM paraquat for 24 h. The flies were kept in vials containing 1 ml of 1.3% agar for 6 h for starvation before the paraquat treatment. The flies were then transferred to vials containing a 22-mm filter paper disk soaked with 20 mM paraquat (methyl viologen, Sigma) in 5% sucrose solution. The data are presented as means ± S.E. The statistically significant differences were examined using the Student’s t test (Microsoft Excel) and p < 0.05 was accepted as statistically significant.

Measuring ROS Levels in Drosophila—To measure the intracellular ROS level in Drosophila, we used the method described by Strayer et al. (15). Non-fluorescent 2,7-dichlorofluorescein di-acetate (Molecular Probes) is a cell permeable dye and can be converted into 2,7-dichlorofluorescein by interacting with di-acetate (Molecular Probes) is a cell permeable dye and can be quantified. Three independent experiments with 50 flies in replicates were tested for each genotype. Survival, log-rank test the effect of Jafrac1 overexpression on oxidative stress, and mtDNA PCR analysis—Total ATP production was measured as previously described (17). Heads of 10 2-day-old flies were dissected and homogenized in extraction buffer (100 mM Tris and 4 mM EDTA, pH 7.8) followed by quick-freezing in liquid nitrogen and boiling for 3 min. The samples were then centrifuged to collect the supernatant and mixed with luminescent solution (Enliten kit, Promega, Madison, WI). The luminescence was measured using a luminometer (FLUOstar Optima, BMG Laboratory), and the results were compared with standards. The ATP level was measured relative to total protein concentration. For mtDNA PCR, total DNA was extracted from 2-day-old flies and subjected to PCR amplification with primer sets for various target genes (supplemental Table 1). The genomic DNA level of rp49 of each sample was used as the loading control. Results are expressed as -fold change relative to control.

Semi-quantitative Reverse Transcription-PCR Analysis—For reverse transcription-PCR analysis, 1 μg of total RNA was used with the oligo(dT) primer and avian myeloblastosis virus reverse transcriptase (Roche Biochemical) to generate first strand cDNA. Next, 1 μl of the cDNA was subjected to PCR amplification with the primer sets for various target genes (supplemental Table 1). PCR conditions were 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min using a Thermal Cycler (Applied Bioscience). PCR products were resolved in 1.5% agarose gels and visualized by ethidium bromide staining. The rp49 gene was used as a control.

Protein Analysis and Immunostaining—Western blot analyses were performed as described previously (18). The antibodies against JNK (Santa Cruz Biotechnology, Santa Cruz, CA) and phospho-JNK (Promega) were used to detect JNK activation. The antibody against dFOXO (a gift from O. Puig) was used to detect FOXO translocation to the nucleus. To detect pJNK and FOXO in vivo, the third instar larvae were fed with 20 mM paraquat for 24 h, and then cuticles were dissected and fixed in 4% paraformaldehyde in PBS for 1 h at room temperature and incubated with the pJNK antibody (1:200) or FOXO antibody (1:500) and, subsequently, with Alexa Fluor 594-conjugated anti-rabbit IgG (1:200, Molecular Probes). Fluorescence images were acquired using an Axiosvert 200M microscope (Carl Zeiss, Germany).

Life Span Assay—For longevity experiments, 1- and 2-day-old adult male or female flies were collected (10 per vial), transferred to fresh medium every 2 days, and scored for survivors. The starting population for each genotype was 100 flies. Three replicates were tested for each genotype. Survival, \( I_x \), was estimated as \( N_x / N_0 \) where \( N_x \) is the number of flies alive at the beginning of each census interval, and \( N_0 \) is the initial cohort size. Significant differences in survival between pairs of cohorts were tested using the log-rank test.

RESULTS

Drosophila Jafrac1 Is an Ortholog of Human PrxII—Based on amino acid sequence similarity, we identified a Drosophila peroxiredoxin II gene (Jafrac1, CG1633, Dpx-4783) that belongs to the 2-Cys peroxiredoxin subfamily. The amino acid sequence of the Jafrac1 protein shows significant homology to hPrxII, including conserved cysteine motifs (Fig. 1A), and the overall amino acid similarity of 83% between Jafrac1 and hPrxII (157/188). There are six Drosophila homologs of human peroxiredoxins that share at least 60% amino acid identity. Among these, the molecular characters of Jafrac1 are most similar with hPrxII, including protein size, the number of conserved cysteine residues, and subcellular localization (supplemental Table 2). Jafrac1 mRNA is expressed throughout development, and in the third instar larvae, Jafrac1 mRNA is abundant in brain, imaginal discs, and Malpighian tubules (supplemental Fig. S1).

Neuronal Expression of Jafrac1 or hPrxII ROS-induced Lethality—The expression of Jafrac1 was induced in wild-type flies treated with 20 mM paraquat for 24 h (Fig. 1B). To test the effect of Jafrac1 overexpression on oxidative stress,
Jafrac1 Promotes Stress Resistance and Life Span

A

B

C

D

Lethality in 20mM PQ for 24hr

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Jafrac1 Promotes Stress Resistance and Life Span

we generated transgenic fly lines carrying the Jafrac1 or hPrxII genes under the control of the UAS promoter. Reverse transcription-PCR (Jafrac1) and Western blot analysis (hPrxII) confirmed that both lines exhibited Gal4-dependent up-regulation of Jafrac1 transcripts and hPrxII proteins, respectively (supplemental Fig. S2). Ubiquitous expression of Jafrac1 using Actin5C-Gal4 demonstrated that Jafrac1 expression reduced oxidative stress-induced lethality (Fig. 1C). We next tested whether neuronal overexpression of Jafrac1 is sufficient to confer resistance to paraquat treatment. Using elav-Gal4, which drives expression in all postmitotic neurons (19), and Cha-Gal4, which drives expression primarily in cholinergic neurons (20), we found that elav>Jafrac1 and Cha>Jafrac1 adult flies exhibited significantly reduced paraquat-induced lethality, comparable to Actin5C>Jafrac1 flies (Fig. 1C). Interestingly, Jafrac1 overexpression in glial cells using repo-Gal4 was not protective. In contrast, neuronal knockdown of Jafrac1, but not knockdown of Jafrac1 in glial cells, sensitized flies to paraquat-induced lethality (data not shown). These results suggest that Jafrac1 plays a protective role against oxidative stress in neurons. Importantly, ROS hypersensitivity of RNAi-mediated knockdown of Jafrac1 in flies was rescued by overexpression of human PrxII (elav>Jafrac1-RshPrxII) (Fig. 1C), suggesting that human PrxII is a functional homolog of Drosophila Jafrac1.

To test the role of Jafrac1 in ROS metabolism, we measured ROS levels using a 2,7'-dichlorofluorescein-DA dependent fluorescence...
When wild-type flies were exposed to 20 mM paraquat, intracellular ROS levels were significantly increased in a time-dependent manner: 3-fold and 3.5-fold after 6- and 12-h exposures, respectively. The intracellular ROS levels were dramatically reduced by neuronal overexpression of Jafrac1 or hPrxII (elav->Jafrac1 or elav->hPrxII) (Fig. 1D). Conversely, flies with neuronal knockdown of Jafrac1 (elav->Jafrac1-Ri) showed increased ROS levels compared with wild-type control flies (Fig. 1D). These results indicate that both Jafrac1 and hPrxII can modulate intracellular ROS levels in Drosophila.

Neuronal Expression of Jafrac1 or hPrxII ROS-induced Mitochondrial Dysfunction—To test whether Jafrac1 has a protective effect on mitochondrial function under oxidative stress, we measured ATP levels in flies with neuronal overexpression of Jafrac1 or hPrxII after paraquat treatment. In the control flies, 20 mM paraquat treatment resulted in a 50% reduction in the ATP level. Neuronal overexpression of Jafrac1 or hPrxII (elav->Jafrac1, elav->hPrxII) markedly restored ATP production, whereas the reduction of ATP levels after paraquat treatment was enhanced in loss-of-function Jafrac1 mutants (Jafrac1G1104) and flies with neuronal knockdown of Jafrac1 (elav->Jafrac1-Ri) (Fig. 2A).

We next quantified mitochondrial abundance by measuring the mitochondrial DNA (mtDNA) copy number. Treatment with 20 mM paraquat caused a marked reduction in the levels of mtDNA, and this reduction in mtDNA levels induced by paraquat treatment was restored by Jafrac1 or hPrxII overexpression in neurons (Fig. 2B). The reduction in mtDNA levels after paraquat treatment was enhanced in loss-of-function Jafrac1 mutants (Jafrac1G1104) and flies with neuronal knockdown of Jafrac1 (elav->Jafrac1-Ri). These data indicate that neuronal expression of Jafrac1 or hPrxII restores oxidative stress-induced mitochondrial function.
Neuronal Expression of Jafrac1 Inhibits Oxidative Stress-induced JNK Activation—Oxidative stress can activate the stress-responsive JNK/FOXO signaling pathway (3, 4, 6, 7). To examine JNK and FOXO activation by oxidative stress in cholinergic neurons, we marked cholinergic neurons with green fluorescent protein (Cha-GFP) and immunostained with the phospho-JNK (pJNK) and FOXO antibody to detect the active form of JNK and FOXO, respectively, in the neuromuscular junctions of third instar larvae. Activated JNK was observed in the cholinergic neurons treated with 20 mM paraquat (Fig. 3B, yellow), but not in the controls (Fig. 3A). Similarly, FOXO protein was localized in the nuclei of cholinergic neurons treated with 20 mM paraquat (Fig. 3D, arrow), but not in the controls (Fig. 3C).

Jafrac1 expression (Cha->Jafrac1) reduced the number of pJNK-positive neurons (Fig. 3E). Western blot analysis following treatment with 20 mM paraquat revealed that the treatment induced activation of JNK, but over-expression of Jafrac1 in neurons (elav->Jafrac1 and Cha->Jafrac1) suppressed JNK activation (Fig. 3F). These results suggest that oxidative stress-induced JNK activation can be suppressed by Jafrac1 in neurons.

Peroxiredoxin (Prx) enzymes modulate oxidative stress via its evolutionary conserved cysteine (Cys) residues (21). Under oxidative stress condition, cysteine sulfenic acid of Prx is oxidized. To test whether a Drosophila Prx homolog, Jafrac1, is also oxidized under oxidative stress condition, we performed Western blot analysis with oxidized Prx specific antibody, anti-Prx-SO3. In wild-type flies, the level of oxidized Prx homolog was increased after 20 mM paraquat for 24 h, indicating that the oxidation of Cys residue is conserved in Jafrac1. Increase of oxidation of Jafrac1 was enhanced in a hemizygous mutation of JNKK (Jafrac1 Promotes Stress Resistance and Life Span)

FIGURE 4. Jafrac1 expression is regulated by the JNK/FOXO signaling pathway. mRNA levels of Jafrac1 in flies with genetic manipulations of the JNK pathway. A, overexpression of the constitutively active form of JNKK in neurons (elav->HepCA), or in cholinergic neurons (Cha->HepCA), increased levels of Jafrac1 mRNA, and heterozygous mutants of JNKK (Bsk1+/+) or a hemizygous mutation of JNKK (Bsk1−/−) decreased the Jafrac1 mRNA level, compared with controls (elav-Gal4 and Cha-Gal4). B, mRNA levels of Jafrac1 in flies with genetic manipulations of FOXO. Expression of wild-type dFOXO or the constitutively active form of dFOXO (dFOXO TM) was induced in adult flies using the inducible neuronal gene switch driver (elavGS) by RU486 treatment. Neuronal overexpression of dFOXO (elavGS->dFOXO WT, +RU486) and dFOXOTM (elavGS->dFOXO TM +RU486) increased expression levels of Jafrac1 compared with flies carrying driver-only constructs or flies not treated with RU486 (elavGS-Gal4, elavGS->dFOXO WT, or elavGS->dFOXO TM). In contrast, the dFOXO21/25 mutation decreased the expression of Jafrac1. C, expression of Jafrac1 in JNK/FOXO mutant flies treated with 20 mM paraquat for 24 h. Expression of Jafrac1 increased in wild-type control flies exposed to oxidative stress, but this increase was significantly reduced in a hemizygous mutation of JNKK (Hep1/Y) or transallelic dFOXO21/25 mutation. Mean ± S.E. from three independent experiments (n = 10 per each experiment; *p < 0.05; **p < 0.001; Student’s t test).

FIGURE 3. Neuronal expression of Jafrac1 inhibits oxidative stress-induced JNK activation. Cha->GFP marked cholinergic neurons in Drosophila third instar larvae (green) and 4’,6-diamidino-2-phenylindole (DAPI)-stained nuclear DNA in muscle (blue). A and B, activated JNK (p-JNK) in cholinergic neurons after paraquat treatment (yellow, white arrows). C and D, nuclear translocation of FOXO transcription factors after paraquat treatment in cholinergic neurons (arrows). E, number of p-JNK-positive neurons after paraquat treatment. 60% of cholinergic neurons were p-JNK-positive in the wild type compared with 25% in flies overexpressing Jafrac1 (Cha->Jafrac1). Mean ± S.E. from five independent samples (*, p < 0.05; **, p < 0.001; Student’s t test). F, Western blotting with anti-p-JNK (top) and anti-JNK (middle). A representative blot and the quantification of signals as the ratio of p-JNK and JNK (mean ± S.E., n = 3) are shown (bottom). Neuronal overexpression of Jafrac1 (elav->Jafrac1 and Cha->Jafrac1) suppressed the oxidative stress-induced phosphorylation of JNK. G and H, oxidation of peroxiredoxin in wild-type or JNK/FOXO mutants under oxidative stress condition. G, Western blot analysis with oxidized peroxiredoxin-specific antibody, anti-Prx-SO3. H, Coomassie Blue staining image of transferred gel used in Western blot analysis confirms equal protein loading.
Jafrac1 Promotes Stress Resistance and Life Span

![Graph showing survival rates over time for different conditions.](Image)

**FIGURE 5. Neuronal expression of Jafrac1- or hPrxII-extended life span.** A, neuronal overexpression of Jafrac1 or hPrxII in adult neurons induced by RU486 show increased average, median, and maximum life spans compared with untreated control flies (log rank test, male, \( \chi^2 = 1.50, p < 0.0001, n = 189; \) female, \( \chi^2 = 0.91, p < 0.0001, n = 197 \)). D, a model of the JNK/FOXO pathway. Oxidative stress by the paraquat treatment induces mitochondrial damage and activates the JNK/FOXO pathway. This stress induces neuronal expression of Jafrac1, which in turn suppresses oxidative stress-induced lethality and extends life span.

**DISCUSSION**

Multiple lines of evidence point to the activation of the JNK/FOXO pathway as a common cellular response to oxidative damage across animal phyla (4, 6, 7, 13, 23). In *Drosophila*, JNK confers tolerance to oxidative stress and extends life span by inducing a protective gene expression program. Increased JNK

Next, we tested the effect of genetic manipulation of FOXO on Jafrac1 gene expression. To induce FOXO expression in neurons in the adult stage, we used the RU486-inducible elav-Gal4 driver (elavGS-Gal4). Neuronal overexpression of wild-type FOXO (elavGS>dFOXO + RU) or the insulin-insensitive nuclear form of FOXO (elavGS>dFOXO.TM + RU) increased the expression level of Jafrac1 by >2-fold compared with the controls, whereas the expression of Jafrac1 was reduced in a FOXO mutant (Foxo^{21/25}) (Fig. 4B). In addition, the expression of Jafrac1 was over 3-fold increased in wild-type control flies treated with 20 mM paraquat for 24 h, but this increase was significantly reduced in a hemizygous mutation of JNKK (Hep1/Y) or transallelic mutation of dFOXO^{21/25} (Fig. 4C). These results indicate that JNK/FOXO signaling regulates Jafrac1 expression in neurons.

Neuronal Expression of Jafrac1 or hPrxII Extends Life Span in Flies—Because it is well established that activation of JNK/FOXO signaling increases life span (2, 4, 7), we examined the role of neuronal Jafrac1, a target gene of JNK/FOXO signaling, in the control of the fly life span. Neuronal overexpression of Jafrac1 or hPrxII in neurons (elav>Jafrac1 or elav>hPrxII) significantly increased life span, while neuronal knockdown of Jafrac1 (elav>Jafrac1-Ri), as well as the loss-of-function mutation (Jafrac1^{G1104}), caused a reduction in life span (Fig. 5A). To test whether neuronal expression of Jafrac1 in the adult stage is sufficient to extend life span, we used the RU486-inducible elav-Gal4 driver (elavGS-Gal4) to express Jafrac1 in adult neurons (22). It has been reported that RU486 feeding does not affect life span of flies (2). Expression of Jafrac1 in adult neurons extended life span by 26% in females (Fig. 5B) and 29% in males (Fig. 5C), compared with the control flies.

JNK/FOXO Signaling Regulates Jafrac1 Expression under Both Normal and Oxidative Stressed Conditions—JNK/FOXO signaling activates expression of several genes involved in cellular stress responses (4). To test whether JNK/FOXO signaling regulates Jafrac1 expression, we first examined the effect of genetic manipulation of JNK activity in neurons on Jafrac1 gene expression. Neuronal overexpression of the constitutively active form of hemipterus (hep), a *Drosophila* JNKK gene (elav>hep^{CA} and cha>hep^{CA}), markedly increased the expression level of Jafrac1. In contrast, *Jafrac1* mRNA levels are reduced in flies carrying one copy of a loss of function mutation of the *Drosophila* homolog of JNK, Basket (Bsk'), in the hemizygous hep' mutant (hep'/Y) background (Fig. 4A).
activity in neurons is sufficient to promote stress tolerance and extend life span in flies (4, 7). However, whether this effect is due to the specific protection of neurons against oxidative damage, or whether JNK activation in neurons may induce a humoral response that regulates longevity systemically, is unclear (1, 5).

In this study, we demonstrated that JNK/FOXO signaling is required for the expression of Jafrac1 in brains under both normal and oxidative stressed conditions (Fig. 4). There are two putative FOXO consensus binding sites (RWWAACA) in the promoter region of Jafrac1 (data not shown), suggesting that the transcription factor FOXO may bind to the Jafrac1 promoter and directly activate Jafrac1 transcription. We also demonstrated that neuronal knockdown of Jafrac1 enhances, and neuronal overexpression of Jafrac1 reduces, ROS-induced lethality. Furthermore, the neuronal knockdown of Jafrac1 shortened, while overexpression of Jafrac1 extended, the life span of the flies. These results support the hypothesis that, in Drosophila, the JNK/FOXO pathway protects neurons from oxidative stress and extends life span by induction of antioxidant genes, including Jafrac1 in neurons (Fig. 5D).

Peroxiredoxins (Prxs) are identified by their ability to neutralize cellular hydroperoxides in mammals (24). A family of five Prx genes has been identified and characterized in D. melanogaster (25). All Drosophila Prxs have peroxidase activities, and their expressions are induced by oxidative stress. Prx overexpression enhances resistance to oxidative stress by hydrogen peroxide and paraquat in cultured Drosophila cells (25, 26). In Drosophila, overexpression of Jafrac1 has been shown to counteract the enhanced susceptibility of immune-regulated catalase knockdown flies to natural infections (14). Moreover, mitochondrial peroxiredoxin (Dpx-5037, mTPx) has been reported to restore wild-type life span in a Drosophila model for Friedreich’s ataxia (27).

We have demonstrated that neuronal expression of Jafrac1 and hPrxII significantly reduces the ROS level and restores mitochondrial function in paraquat-treated flies (Figs. 1D and 2). Several studies in Drosophila show that expression of specific mitochondrial proteins can increase resistance to oxidative stress as well as extend life span (28–30), suggesting that mitochondrial function plays an important role in determining life span. Collectively, Jafrac1 or hPrxII may extend life span by acting as a guardian for neuronal mitochondria under age-associated oxidative stress conditions. Furthermore, because mitochondrial dysfunction is associated with many neurodegenerative diseases (8), induction of Jafrac1/PrxII in neurons may also be protective against age-associated neurodegenerative diseases.
Supplementary Table 1

Supplementary Table 1  PCR Primers used in this study.

<table>
<thead>
<tr>
<th>PCR primer</th>
<th>product size</th>
<th>sequences</th>
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| *Jafrac1a* | 188 bp       | forward: CCCGAAAAACCTTCTTAGACTCA  
  reverse: TTTTCAACATTTCCATCGT |
| *Jafrac1*  | 580 bp       | forward: ATGCCCCAGCTACAGAAGCC  
  reverse: TTAGGAGGTGTCCTGAAGT |
| *COX I a*  | 157 bp       | forward: GGTGCTCCTGATAGCATTCCC  
  reverse: CACCATGAGCAATTCCAGCG |
| *COX III a*| 127 bp       | forward: TTTTATAGCACCAGGATTCCACGG  
  reverse: TGCAGCTGTCTCAAAACAA |
| *Cytochrome b a* | 166 bp | forward: CACCTGCCCATATTCACCAAGA  
  reverse: GGTAAATTTGAATCCCTCGGAA |
| *rp49*     | 206 bp       | forward: AGATCGTGAAGAAGCCACCAAG  
  reverse: CACCAAGAATTTCCTCTCGG |
| *rp49 a*   | 122 bp       | forward: AGGGTATCGCAACAGAAGTG  
  reverse: CACCAGGAATTTCCTGAATC |

Note : a primers for the quantitative RT-PCR
### Supplementary Table 2. The molecular characters of the *Drosophila* peroxiredoxin family.

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<th>Gene name (amino acids)</th>
<th>CG No.</th>
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**Supplementary Fig. S1**

Supplementary Fig. S1. Pattern of *Jafrac1* expression during developmental stages (A) and in various tissues of 3rd instar larvae (B).
Supplementary Fig. S2. *Jafrac1* expression level in mutant flies. (A) The EP(X)G1104 element was inserted in the *Jafrac1* locus. (B) RT-PCR analysis of *Jafrac1* mutants. (C) Western blot analysis of *hPrxII* mutants.
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