Decreased Lifespan in the Absence of Expression of the Mitochondrial Small Heat Shock Protein Hsp22 in Drosophila*

Received for publication, July 29, 2004, and in revised form, August 20, 2004 Published, JBC Papers in Press, August 25, 2004, DOI 10.1074/jbc.C400357200 Geneviève Morrow, Sophie Battistini, Ping Zhang, and Robert M. Tanguay†‡ From the §Laboratoire de Génétique Cellulaire et Développementale, Département de Médecine, CRESFSP, Pavillon C.E.-Marchand, Université Laval, Ste-Foy, Québec G1K T4, Canada and the $Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut 06269-2131

Aging is a well regulated biological process involving oxidative stress and macromolecular damages. Three main pathways have been shown to influence lifespan, the insulin/insulin-like growth factor-1 pathway, the silent regulator pathway, and the target of rapamycin pathway. Among many proteins influencing lifespan, two transcription factors, FOXO and the heat shock factor, have been shown to be involved in the aging process and in small heat shock proteins (sHsps) expression following stress and during lifespan. We have recently shown that overexpressing the mitochondrial Hsp22 increases Drosophila melanogaster lifespan by 32% and resistance to oxidative stress. Here we show that flies that are not expressing this mitochondrial small Hsp22 have a 40% decrease in lifespan. These flies die faster than their matched control and display a decrease of 30% in locomotor activity compared with controls. The absence of Hsp22 also sensitizes flies to mild stress. These data support a key role of sHsps in aging and underline the importance of mitochondrial sHsps in this process.

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Decayed small heat shock proteins (sHsps) have also been shown to extend longevity in D. melanogaster (15–17). The overexpression of members of the small HSP family has also been shown to extend longevity in D. melanogaster (18) and in C. elegans (19). The SIR pathway also involves modulation of gene expression but by making large chromosomal regions transcriptionally inactive. Hence, overexpression of SIR proteins extends lifespan by making life-shortening genes inaccessible for the transcriptional machinery (7, 8). This pathway has been found to operate in Saccharomyces cerevisiae and in C. elegans and has been shown to promote survival particularly under conditions of scarcity (20). Recently a link between the insulin/IGF-1 and the SIR pathways has been established by demonstrating that mammalian SIRT1 deacetylase controls the cellular response to stress by regulating the transcriptional activity of FOXO3 (21) and FOXO4 (22), two of the four evolutionarily conserved FOXO family members in mammals. Finally, the TOR signaling pathway is involved in cell growth and proliferation and is conserved from Drosophila to human (reviewed in Refs. 23 and 24). This pathway mediates cell growth in response to nutrient availability by inducing ribosomal protein expression through histone deacetylation (25). Recently, Kapahi et al. (9) have shown that inhibition of the TOR pathway in D. melanogaster extends lifespan in a manner similar to dietary restriction. Multiple evidence suggests that Hsps, which are molecular chaperones, are also involved in the aging process (Refs. 18 and 26–28 and reviewed in Refs. 29–31). Expression of sHsps have been shown to be controlled by Daf-16 (14) and the insulin/IGF-1 pathway in C. elegans (28). In the same organism, a link between the heat shock factor (HSF) and Daf-16 has been demonstrated in both the insulin/IGF-1 signaling pathway and the heat shock response (32). Indeed, Hsu et al. (32) have shown that the presence of HSF is required in daf-2(−) mutants to display their increased longevity phenotype. This study has also revealed the importance of a functional HSF in determination of aging and has identified shsp genes as common targets for HSF and Daf-16 activity.

We have recently shown that overexpressing a mitochondrial sHsp (Hsp22) in all cells or specifically in motorneurons using the GAL4/UAS binary system increases D. melanogaster mean lifespan by up to 32% (18). Moreover, flies displaying an increased longevity maintain their locomotor activity for a longer period and are also more resistant to various stresses. To confirm that this mitochondrial sHsp is important in the aging process of D. melanogaster, we have now examined the longevity phenotype of a strain obtained by P-element jump-out, which does not express Hsp22 in response to stress nor during aging. Our results show that these flies display a decrease in mean lifespan of 40% and are sensitive to stress. These results confirm the key role of mitochondrial sHsps in aging determination.

MATERIALS AND METHODS

Drosophila Strains, Maintenance, and Longevity Assay—EP(3) 3583-11 (Flybase ID: FBti0023680) and EP(3)3583-18 flies have been obtained by jump-out in a study on P-element local transposition in the
male germ line cells of *D. melanogaster* (33). The genetic background of the two strains is the same except for the newly inserted P-element, which is located at −57 bp and −350 bp of the *hsp22* transcription starting site in EP(3)3583-11 and EP(3)3583-18 flies, respectively (Ref. 33, see Fig. 1). Flies were maintained at 25 °C on standard cornmeal/agar medium. Heat shock was performed by incubation for 1 h at 35 °C, the optimal temperature for induction of sHsps, followed by a recovery of 2 h at 25 °C. For longevity experiments, cohorts of male flies (15 per vial) were transferred to fresh medium every 3–4 days and scored for survivors every 2 days. The starting population for each genotype was 210. For analysis, the premortality phase (less than 10% mortality) and the mean lifespan (50% survival) were calculated. For thermal stress resistance assays, 2-day-old flies were kept at 30 or 37 °C until death.

**PCR Analysis**—Genomic DNA was prepared from flies of both genotypes as described in Huang et al. (34). PCR reactions were performed with Pp31 and Pg-A5 primers (200 ng; Ref. 33) to which have been added 100 ng of genomic DNA, 5 μl of 10× buffer number 3 (50 mM Tris-HCl, pH 9.2, 160 mM (NH₄)₂SO₄, 22.5 mM MgCl₂, 20% Me₂SO and 1% Tween 20), 10 μl of 5 mM dNTP and 2 μl of *Taq* polymerase (Amersham Biosciences). The reaction mixtures were covered with mineral oil and incubated for 5 min at 95 °C, followed by 30 cycles of 1 min at 95 °C, 1 min at 60 °C, and 1 min at 72 °C and a final elongation of 5 min at 72 °C.

**Negative Geotaxis Assay**—The negative geotaxis assay was conducted as described in Morrow et al. (18). Briefly, flies were transferred in clean empty vials (16 × 125 mm) and allowed to adapt to their environment for 8 s. Flies were then tapped gently to the bottom of the tube, and flies having climbed higher than 7 cm 8 s were scored. The assay was conducted three times on 105 flies of each cohort. The results are expressed in percentage of flies having climbed above 7 cm in 8 s.

**Protein Analysis**—Protein extracts from flies of each genotype were separated on 12% SDS-PAGE as described in Morrow et al. (18). Briefly, flies were transferred to clean empty vials (16 × 125 mm) and allowed to adapt to their environment for 8 s. Flies were then tapped gently to the bottom of the tube, and flies having climbed higher than 7 cm in 8 s were scored. The assay was conducted three times on 105 flies of each cohort. The results are expressed in percentage of flies having climbed above 7 cm in 8 s.

**Western Blotting**—Protein extracts from flies of each genotype were separated on 12% SDS-PAGE as described in Morrow et al. (35). Following transfer onto nitrocellulose membranes, Western blots were performed using antibodies specific to Hsp22 (1/5,000; Ref. 35), Hsp23 (1/100; Ref. 36), Hsp26 (1/100; Ref. 36), Hsp27 (1/100; Ref. 36), Hsp60 (1/5,000; Ref. 37), Hsp70 (1/5,000; Ref. 38) or Hsp83 (1/5,000; Ref. 39) and secondary antibodies coupled to peroxidase (1/20,000, Jackson ImmunoResearch Laboratories). Chemiluminescent detection was done with Western Lightning Chemiluminescence Reagent as described by the manufacturer (PerkinElmer Life Sciences).

**RESULTS**

The EP(3)3583-11 and EP(3)3583-18 flies were obtained from the same starting strain in a study on P-element local transposition (33). The location of the newly inserted P-element was analyzed by PCR amplification using primers specific to the P-element and *hsp22* coding sequence. In EP(3)3583-18 flies, the P-element is inserted at position −350 from the transcription starting site, and as expected, a PCR amplification with the two primers gives a product at 904 bp, while in EP(3)3583-11 flies, the P-element is inserted at position −57 and the PCR product has 611 bp (Fig. 1B).

Heat shock elements (HSEs) are DNA sequences recognized by HSF, the transcription factor responsible for Hsp90 expression following stress. Studies on Hsp22 promoter have revealed that three functional HSEs are required for the proper expression of Hsp22 following stress (40) and during aging (41). Since the P-element in EP(3)3583-11 flies is located between the three HSEs and the TATA box on hsp22 promoter (Fig. 1A), we performed a heat shock on homozygous flies to determine their level of Hsp22 expression. As shown in Fig. 2A, 10-day-old homozygous EP(3)3583-18 flies, which have the insertion upstream of the HSEs, express a large amount of Hsp22 following heat shock, while only traces of Hsp22 are detected in EP(3)3583-11 flies. In addition, during aging homozygous EP(3)3583-11 flies show no expression of Hsp22 as compared with EP(3)3583-18 flies. The expression of other Hsps such as Hsp23, Hsp26, Hsp27, Hsp60, Hsp70, and Hsp83 was the same in the two strains following heat shock and during aging thereby ruling out a possible defect in HSF activity in the two strains (Fig. 2). The distance between HSEs and the TATA box in the hsp22 promoter of EP(3)3583-11 flies could account for the absence of Hsp22 expression during aging and following stress. Indeed, due to the presence of the P-element, it is possible that the promoter architecture is not optimal thereby preventing one or more factors such as GAGA factor, TFIID, RNA polymerase II, and HSF to bind DNA (42, 43) and/or interact with each other (44).

To assess the key role of Hsp22 in the aging process, longevity experiments were conducted on homozygous EP(3)3583-18 and EP(3)3583-11 flies. While EP(3)3583-18 flies have a mean lifespan of 61.5 ± 3.4 days, EP(3)3583-11 flies that do not express Hsp22 have a mean lifespan of 43.9 ± 3.7 days (Fig. 3), which represents a decrease of 40%. In addition to a shorter life, EP(3)3583-11 flies enter rapidly in the mortality phase (<90% survival, day 3 ± 0.4 compared with day 16.9 ± 5.3 for EP(3)3583-18 flies). Flies not expressing Hsp22 also have a
reduced locomotor activity of 7.9 ± 7.8% as assessed by a negative geotaxis experiment on 20-day-old flies compared with 37.4 ± 7.7% for EP(3)3583-18 flies. These results clearly demonstrate the involvement of Hsp22 in the aging process.

The resistance to heat of flies not expressing Hsp22 was also evaluated. Homozygous EP(3)3583-11 flies have a mean lifespan of 11.9 ± 2.7 days at 30 °C compared with 20.3 ± 0.9 days for EP(3)3583-18 flies, which represents a decrease of nearly 50% (Fig. 4A). However, the survival of homozygous EP(3)3583-11 flies was not statistically different from the survival of EP(3)3583-18 flies at 37 °C (667.4 ± 89.5 h versus 442.8 ± 203.9 h, Fig. 4B).

DISCUSSION

The present results clearly demonstrate that Hsp22 is a key player in the aging process as its absence shortens flies lifespan by 40%. To our knowledge, this is the first time that such an impact is observed for an individual Hsp. In C. elegans, shsp RNAi has been shown to shorten lifespan of wild-type animals by less than 25% (32). The drastic effect obtained in the absence of Hsp22 could be due to its particular intramitochondrial localization (35). Indeed, mitochondria are known to be sensitive to stress and to aging (reviewed in Ref. 45), and it is possible that the presence of Hsp22 in this organelle helps to maintain mitochondrial integrity and function. This could be achieved by its chaperone-like activity on damaged proteins.2 The requirement of Hsp22 for good health is also visible when flies are exposed to mild thermal stress such as 30 °C, but we did not observe any effect of the absence of Hsp22 on resistance to 37 °C. This is likely due to the fact that this robust stress involves a drastic accumulation of damages to the whole cells.

Important findings have been made on the aging process recently. Indeed, it seems that specific cell types direct the longevity fate of the whole organism. In C. elegans long-lived daf-2(–) mutants, longevity was restored to normal by overexpressing wild-type Daf-2 in neurons, while there were no changes if wild-type Daf-2 was expressed in muscles or intestinal cells (46). In D. melanogaster, overexpressing dFOXO in the adult fat body was sufficient to increase lifespan and resistance to paraquat (47). Hwangbo et al. (11) have demonstrated that the activation of dFOXO in pericerebral fat body reduces the expression of an insulin-like peptide in neurons. Inhibition of the TOR signaling pathway in the fat body was also sufficient to increase lifespan (9). Moreover, HSF has been pointed out to have a major role in the regulation of longevity in C. elegans (32, 48), and sHsps have been shown to be targets of HSF and Daf-16 (32). A key role of sHsps in neurodegenerative diseases has also been suggested. Indeed, shsp RNAi accelerates the onset of polyglutamine aggregates in C. elegans, while RNAi for the other hsps did not have any effects (32). The present work reinforces the idea that sHsps are key factors in the aging process and shows that mitochondrial sHsps are particularly important as no such impact on lifespan have been obtained with other sHsps (32). Therefore, the intracellular localization of life-promoting genes appears as important as their expression in specific cell types in the determination of longevity. Altogether, these results suggest that sHsps are good targets to delay aging and perhaps the onset of neurodegenerative diseases.

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