Aging-Specific Expression of Drosophila hsp22

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hsp22 is among the least abundantly expressed Drosophila heat shock (hs) genes during both development and heat stress. In contrast, hsp22 was found to be the most abundantly expressed hs gene during Drosophila aging. During aging, hsp22 RNA was induced 60-fold in the head, with somewhat lower level induction in abdomen and thorax. Induction of the other hs gene RNAs was <3-fold, except for hsp23, which was induced ~5-fold in thorax. hsp22 protein was detected using rat anti-hsp22 polyclonal antisera and was induced >150-fold, with particularly abundant expression in eye tissue. Aging-specific induction of hsp22 was reproduced by hsp22: lacZ fusion reporter constructs in transgenic flies. Analysis of specific promoter mutations in transgenic flies indicated that functional heat shock response elements are required for hsp22 induction during aging. Finally, comparison of hsp22 RNA and protein expression patterns suggests that aging-specific expression of hsp22 is regulated at both the transcriptional and the posttranscriptional levels. Aging-specific induction of hsp22 is discussed with regard to current evolutionary theories of aging. © 1999 Academic Press

Key Words: molecular chaperone; heat stress; heat shock; transgenic.

INTRODUCTION

Current evolutionary theory suggests that aging results from the decreasing force of natural selection with increasing age (Charlesworth, 1994; Partridge and Barton, 1993; Rose, 1991). The decreasing force of natural selection ultimately results in the failure to indefinitely maintain somatic tissue and the deterioration of tissue structure and function characteristic of aging. Aging-associated changes can be observed at the organismal level and at the molecular level as well. In virtually every aging organism examined, aging has been found to be associated with the accumulation of inactive enzymes, including proteins which are partially denatured and/or oxidatively damaged (Finch, 1990; Gershon and Gershon, 1970; Stadtman, 1992). The accumulation of abnormal proteins with age led us to examine the expression of the heat shock proteins (hsps), which are induced in response to protein damage caused by heat and other stresses (Ananthan et al., 1986; Lindquist and Craig, 1988; Morimoto et al., 1994; Parsell and Lindquist, 1993).

The hsps include both highly conserved and more variable members and are induced in all cells in response to heat stress. The hsps are members of a larger group of proteins called molecular chaperones, characterized by their ability to affect the structure or folded state of other proteins. In Drosophila six major hsps are induced in response to heat stress, -70, -27, -26, -23, and -22. hsp83 is constitutively expressed and upregulated several fold during heat stress. hsp83 is a member of the hsp90 gene family, which is highly conserved through evolution. hsp83 functions include regulating the activity of other proteins such as specific steroid hormone receptor molecules (Parsell and Lindquist, 1993; Picard et al., 1990). hsp70 is the most highly conserved through evolution and, in Drosophila, is induced over 1000-fold during heat stress. Based on studies of hsp70 family members in Drosophila and other organisms, hsp70 is thought to decrease protein denaturation and aggregation, facilitate refolding of partly denatured proteins, facilitate entry of damaged proteins into proteolytic pathways, and protect the organism from additional stress (Morimoto et al., 1994; Parsell and Lindquist, 1993). The Drosophila small hsps (-22, -23, -26, and -27) are related to each other and to small hsps from other organisms by a conserved α-crystallin protein domain and also appear to be molecular chaperones involved in stress resistance (Arrigo et al., 1988).
and Landry, 1994; Horwitz, 1992; Jakob et al., 1993; Merck et al., 1993). When Drosophila cells are heat stressed the small hsps accumulate to lower levels than hsp70, with hsp22 being among the least abundant (Lindquist, 1980).

The mechanism of Drosophila hsp gene induction by heat stress has been studied in detail (Lis and Wu, 1993; Yost et al., 1990). Binding sites for the GAGA transcription factor and other promoter sequences are required to generate an “accessible” promoter structure in unstressed cells, which involves a transcriptionally engaged RNA polymerase paused ~25 nucleotides downstream of the start site for transcription (Gilmour and Lis, 1986; Lee et al., 1992; Rougvie and Lis, 1988). Heat stress causes trimerization and activation of the constitutively expressed heat shock transcription factor (HSF), which is required for hsp gene induction (Jedlicka et al., 1997; Westwood et al., 1991). HSF binds to the heat shock response elements (HSEs), which are evolutionarily conserved, well-studied promoter elements essential for transcriptional induction during heat stress (Amin et al., 1988; Dudler and Travers, 1984; Holmgren et al., 1981; Topol et al., 1985; Xiao and Lis, 1988). HSF binding to the HSEs results in release of paused polymerase and high-level transcription of the hsp genes. In addition, hsp gene RNAs are also preferentially translated in heat stressed cells and are more stable during heat stress (Yost et al., 1990).

In addition to their induction in response to heat and other stresses, the small hsps each have distinct developmental expression profiles (Arrigo and Landry, 1994). Each small hsp promoter contains matches to the consensus sequence for the ecdysone response element (Cheney and Shearn, 1983; Haass et al., 1990; Mason et al., 1984; Michaud et al., 1997; Sirotkin and Davidson, 1982) and hsp26 and hsp27 are expressed in germ-line cells in the adult (Frank et al., 1992; Glaser and Lis, 1990; Zimmerman et al., 1983). In contrast to the other small hsps, hsp22 RNA and protein levels remain low throughout development (Arrigo and Tanguay, 1991; Klemenz and Gehring, 1986; Mason et al., 1984; Sirotkin and Davidson, 1982). While Drosophila hsp22 is the least abundantly expressed hsp gene during heat stress and development, we report here a dramatic and preferential induction of hsp22 RNA and protein during aging. The high-level, aging-specific expression pattern for hsp22 is unique among the Drosophila hsp genes.

**MATERIALS AND METHODS**

**Drosophila stocks.** Drosophila melanogaster strains are as described (Lindsay and Zimm, 1992). All transgenic lines were generated by P-element germ-line transformation using standard methods (Rubin and Spradling, 1982) and using a w	extsuperscript{118} recipient strain. All experiments presented are with w	extsuperscript{118} strain flies or white° transformants. Essentially identical patterns of hsp22 RNA and protein induction, including preferential induction in eye tissue, were obtained with white and wild-type (white°) strains.

**Drosophila culture.** All stocks were grown on cornmeal/agar medium (Ashburner, 1989). Flies were cultured at 25ºC until eclosion. After eclosion males were transferred to 29ºC, unless otherwise indicated in figure legends. A temperature of 29ºC was not sufficient to cause detectable induction of Drosophila hsp genes (Wheeler et al., 1995) (this study, see Figs. 2, 4, and 5) and was used because the shorter adult life span facilitated more rapid experiments. The adult males were maintained for the duration of their lives in groups of 40 per vial and transferred to new vials every other day. “Young” flies and heat-shocked flies were 6 days posteclosion, whereas “old” flies were 30 days posteclosion. Heat shock was performed as described (Simon and Lis, 1987). Briefly, flies in culture vials were incubated in a water bath at 29ºC for 20 min followed immediately by 36ºC for 100 min.

Female flies were usually not used for aging experiments because the larvae produced partially liquidify the surface of the food, which in turn often traps and kills the adult females. hsp22:lacZ reporter induction with age was also observed in females (data not shown), suggesting that the results on hsp22 induction with age are generally applicable to females.

**Northern analysis.** Fifty males were dissected into head, thorax, and abdomen segments at 6, 12, 25, 30, and 35 days posteclosion. Total RNA was isolated and Northern analysis performed as described (Chirgwin et al., 1979; Chomczynski and Sacchi, 1987). Northern analyses were quantitated by phosphoimager (ImageQuant; Molecular Dynamics). All Northern hybridization probes were as previously described (Wheeler et al., 1995). Probes specific for hsp22, -23, -26, and -27 were generated from genomic subclones. The probe for hsp70 was the unique hsp70 5'UTR. The loading control was rp49 (O'Connell and Rosbash, 1984).

**Western analysis.** Male flies were dissected as for the Northern analyses. SDS-PAGE was according to standard procedures (Laemmli, 1970). Whole Drosophila or dissected Drosophila tissues were homogenized directly into SDS-gel loading buffer, boiled, and cleared by brief microcentrifugation. Following electrophoresis, proteins were transferred to nitrocellulose, and the membranes were incubated with polyclonal antiseria specific for hsp22. Primary antibody binding was detected with HRP-conjugated mouse anti-rat IgG (Amersham ECL chemiluminescence kit). The Western blots were then stripped according to the manufacturer's instructions and incubated with monoclonal antibody specific for β-tubulin (Chu and Klymkowsky, 1989). The anti-β-tubulin antibody was obtained from the Developmental Studies Hybridoma Bank, Department of Biological Sciences, University of Iowa, NICHD Contract NO1-HD-7-3263. Protein concentration of parallel fly extracts was determined using Bradford reagent (Bio-Rad).

**Generation of anti-hsp22 polyclonal antiseria.** hsp22 protein was expressed in Escherichia coli as a fusion with glutathione S-transferase (GST) (Smith and Johnson, 1988). The hsp22 coding region was cloned into pGEX-4T-1 (Pharmacia) into BamiHI and XhoI sites. The hsp22 fragment was generated by PCR with engineered BamHI and XhoI sites at the 5' and 3' ends, respectively, using the following primers: 5'-GATCGGATCCATGCGTTCCTTACCGATGTTT-3' and 22Ab3'-5'-GATTCCTGAGCTGACTGGCGGTTTTGTCTTT-3'. Bacterial expression of the hsp22-GST fusion protein was induced by the addition of β-mercaptoethanol (0.1 mM) and the bacterial
FIG. 1. Design of transgenic hsp22::lacZ fusion reporter constructs. Numbering is relative to the +1 start site for transcription. (A) The “wild-type” promoter construct hsp22 5’Δ(−314), containing hsp22 sequences from −314 to +275 fused to E. coli lacZ, is diagrammed. The numbered boxes indicate the three heat shock response elements (HSEs). The arrow indicates the start site for transcription, and AUG marks the start site for translation. The location of the 5’ promoter deletion at residue −114 in construct hsp22 5’Δ(−114) is also indicated. (B) The quadruple point mutation in the promoter of construct hsp22 5’Δ(−314)PointMutation is presented. The wild-type bases are underlined, with the substituted bases indicated under the lines. Arrows indicate the NGAA(N motifs altered within each HSE (each heat shock gene HSE is composed of multiple inverted repeats of the NGAA(N motif. Additional partial matches to the NGAA(N motif present in the promoter are not indicated) (Amin et al., 1988; Xiao and Lis, 1988). Generation of the constructs is described in detail under Materials and Methods. The diagram is not to scale.

Aging Induces hsp22

Aging induces hsp22 expression in rats. To study the role of hsp22 in aging, transgenic rats were generated using an hsp22::lacZ fusion reporter construct. The construct contains four point mutations generated by site-directed mutagenesis to create a reporter construct with a altered HSE. The reporter construct was introduced into the genome of transgenic rats, and the expression of hsp22 was monitored over time.

DNA constructs. hsp22::lacZ fusion reporter constructs are diagrammed in Fig. 1 and were generated using the following PCR primers: primer 1, 5'-GATCCCTGCAATGTCTCTGTCCGT-3'; primer 2, 5'-GATCCCTGCAATGTCTCTGTCCGT-3'; and primer 3, 5'-GATCCCTGCAATGTCTCTGTCCGT-3'. These primers were used to amplify the hsp22::lacZ construct using PCR. The amplified fragment was then cloned into the genome of transgenic rats to create the reporter construct.

β-Galactosidase assays. β-Galactosidase (β-gal) enzymatic activity was quantified in tissue extracts using a commercial assay kit. The activity was determined by measuring the amount of β-galactoside produced over time.

Confocal microscopy. Whole-mount immunofluorescence staining of eye tissues was performed as described. Whole-mount sections of eye tissues were stained with anti-hsp22 antibodies and visualized using confocal microscopy. The staining was used to analyze the expression pattern of hsp22 in different tissues.

The results presented in this study indicate that hsp22 expression is induced in aging rats, and that this induction is mediated by point mutations within the HSE. The expression pattern of hsp22 in different tissues was studied using confocal microscopy, and the results showed that hsp22 expression is upregulated in aging rats.
Immunofluorescence was visualized using a Bio-Rad MRC600 confocal microscope. Tissue from old flies was found to be more fragile during isolation and staining than was tissue from young and young heat-shocked flies. While highly specific signals were obtained with whole-mount preparations, we have so far been unable to obtain satisfactory results with immunohistochemistry of sections (data not shown).

RESULTS

Preferential induction of hsp22 RNA during aging. hsp22 induction with age was first characterized by Northern analysis (Fig. 2). Total RNA was isolated from the head, thorax, and abdomen of flies of increasing age. Ribosomal protein 49 (rp49) RNA is expressed at a constant level throughout aging relative to several other housekeeping genes (Wheeler et al., 1995) and was used as a loading control. hsp22 expression was detected throughout the body and increased with age. The most dramatic induction occurred in the head, where a 60-fold increase was observed between day 6 and day 30. Expression in the abdomen also increased with age, and the induction was 16-fold. In contrast to the head and abdomen, hsp22 RNA expression in the thorax was abundant throughout the adult life span, with a 2.5-fold induction between day 6 and day 30. The same Northern blot was hybridized successively with probes specific for four other heat shock genes, hsp70, -27, -26, and -23. These other hsp genes were expressed at much lower levels than hsp22, requiring significantly longer autoradiographic exposures to detect expression (Fig. 2). Consistent with our previous observations (Wheeler et al., 1995), a very low level expression of hsp70 RNA was

FIG. 2. Northern analysis of heat shock gene RNA expression during aging. Each lane contains total RNA isolated from 20 each of heads, thoraces, and abdomens of flies of the indicated ages, as well as from 2 each of 6-day-old heat-shocked fly heads (H), thoraces (T), and abdomens (A), as indicated. The Northern blot was hybridized successively with probes specific for rp49 and the indicated heat shock genes. Length of time of autoradiographic exposures: hsp22 and rp49, 5 h; hsp70, 10 days; hsp23, 3 days; hsp26, 5 days; and hsp27, 4 days. The same result was obtained with independent Northern blots hybridized to the probes in different order (data not shown). The hsp70 signal is not readily visible above background in the 10-day autoradiographic exposure presented. hsp70 RNA induction during aging was consistently quantitated by phosphoimager to be ≤threefold in this and additional experiments (data not shown), consistent with our previously published analysis of hsp70 expression during aging (Wheeler et al., 1995).

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detected, which increased with age ≤3-fold. hsp23 RNA was barely detectable in head, exhibited an ~5-fold induction in thorax and decreased with age in abdomen. hsp-26 and -27 exhibited low level expression with ≤3-fold induction in head and thorax and a decrease with age in abdomen. Thus, relative to the other hs genes, hsp22 exhibits a dramatic and preferential induction with age.

**Preferential induction of hsp22 protein during aging.**
To allow analysis of hsp22 protein expression, rat anti-hsp22 polyclonal antiserum was raised against an hsp22:GST fusion protein. The resultant antisera was characterized by Western analysis (Fig. 3) and was found to react with a single protein of 22 kDa in heat-shocked flies and yield no signal with total protein isolated from young, non-heat-shocked flies.

To assay hsp22 protein expression during aging, two age-synchronized cohorts of 400 adult male Drosophila were cultured at 25 and 29°C. Life span varies as a function of culture temperature in Drosophila, and as expected the 50% survival time for the cohort maintained at 25°C (52 days) was 20 days longer than the 50% survival time for the cohort maintained at 29°C (32 days) (Fig. 4). Total protein was isolated from samples of flies at various time points for both aging cohorts, and hsp22 protein was detected by Western analysis (Fig. 4). hsp22 protein was first detected at 12 days of age in the 29°C cohort, whereas hsp22 was not detected until 40 days in the 25°C cohort. Thus the onset of hsp22 protein induction with age is shifted in proportion to the change in life span as altered by culture temperature. It is of interest to note that the point in the survival curve at which hsp22 is first detected coincides approximately with the beginning of rapid death of the cohort.

Western analysis was used to determine the time course of hsp22 protein induction in different segments of the fly. Total protein was isolated from the head, thorax, and abdomen of flies from 6 to 35 days of age, and expression of hsp22 and the loading control β-tubulin was analyzed (Fig. 5). β-Tubulin protein levels were essentially constant with age in each body segment. In the head and abdomen, hsp22 protein was first detected at 12 days of age and increased with age. In thorax hsp22 was barely detectable at 12 days of age and was abundantly expressed from 25 to 35 days of age. In each body segment hsp22 expression in old flies was generally greater than expression in heat-shocked flies. Because hsp22 RNA is abundantly expressed throughout the adult life span in thorax (Fig. 2), the lag in onset of hsp22 protein expression in thorax suggests posttranscriptional regulation of hsp22 induction during aging.

Comparison of hsp22 protein levels in young and old heads yielded an estimate of hsp22 protein induction of >150-fold (Fig. 6). This is greater than the 60-fold increase in RNA (Fig. 2) and is likely due to the fact that the hsp22 protein was highly stable: Western analysis of hsp22 protein levels after induction by heat shock in 3-day-old flies suggested a half-life of 3-5 days (data not shown).
In the head, where hsp22 induction was the greatest, hsp22 protein could be readily detected in whole-mount preparations of heat-shocked and old eye tissue (Fig. 7). In a recent study hsp23 protein was found to have a striking tissue-specific expression pattern in the eye of heat-shocked flies: hsp23 protein was abundantly induced in heat-stressed cone cells, but was absent in pigment cells and photoreceptor cells (Marin et al., 1996). hsp22 protein expression was examined in whole-mount preparations of eye tissue, and hsp22 protein was found to be induced in both heat shocked and old flies (Fig. 7). hsp22 protein induction was detected in all cell types of the ommatidia: cone, pigment, and photoreceptor cells (Figs. 7B and 7C). The red fluorescence marks the F-actin of the rhabdomeres of the photoreceptor cells, and the green fluorescence marks hsp22 protein expression. The yellow fluorescence indicates coincident expression of the two proteins, and thus hsp22 protein was detected in some, but not all, photoreceptor cells during both heat shock and aging. The cone and pigment cells are arranged in rings around the photoreceptor cells in the view presented, and thus the continuous rings of green hsp22 fluorescence around groups of rhabdomeres indicate abundant hsp22 expression in both cell types.

**hsp22: lacZ fusion reporter constructs are induced during aging in transgenic Drosophila.** To further characterize the induction of the hsp22 gene with age, an hsp22: lacZ fusion reporter gene was constructed (Fig. 1) and introduced into transgenic flies. The “wild-type” promoter construct, called hsp22 5′Δ(−314), contained all of the hsp22 gene sequences previously shown to be required for expression in response to heat shock and during development (Klemenz and Gehring, 1986). Induction of the reporter was assayed by staining 20-μm sections of young, heat-shocked, and old flies for β-gal activity (Fig. 8). In young flies, a low level β-gal staining was detected specifically in flight muscle of the thorax (Fig. 8A). Thus the hsp22: lacZ fusion reporter protein does not detectably exhibit the delay in expression that was observed with the endogenous hsp22 protein (Fig. 5). As discussed below, this difference between expression of the reporter and the endogenous hsp22 protein may be due to the lack of more 3′ hsp22 sequences in the reporter construct. Aside from this low-level expression in young flight muscle, the hsp22: lacZ reporter reproduced the expression pattern of the endogenous hsp22 protein. Reporter expression was abundantly induced in all tissues in both heat-shocked flies (Fig. 8B) and old flies (Fig. 8C). Relatively even expression was observed throughout the brain, and old flies exhibited additional intense reporter expression in eye tissue (Fig. 8C).

Expression of the hsp22: lacZ reporter construct was quantitated by spectrophotometric assay of β-gal activity in extracts of young, old, and heat-shocked flies (Fig. 9). Multiple independent transgenic lines containing the wild-type promoter construct hsp22 5′Δ(−314) were assayed. Expression of the wild-type promoter construct was induced both during heat shock and during aging, in each...

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**FIG. 5.** Time course of hsp22 protein induction during aging in head, thorax, and abdomen. Total protein was isolated from samples of 20 heads (A), thoraces (B), or abdomens (C), of flies of the indicated ages, which were cultured at 29°C as adults. The two indicated amounts of protein were assayed for each time point in duplicate Western analyses for expression of hsp22 and β-tubulin, respectively. The two indicated amounts of protein are equivalent to ½ and 1 body part (head, thorax, or abdomen) at each time point. 6 + HS, 6-day-old heat-shocked flies.

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**FIG. 6.** Estimation of fold induction of hsp22 protein during aging. Protein was isolated from heads of flies of the indicated ages, and the indicated amounts of protein were assayed for hsp22 by Western analysis.
segment of the fly. Induction during aging was generally greater than or equal to induction during heat shock and ranged from 2- to 50-fold, depending on the particular transgenic line.

To begin to characterize the promoter requirements for hsp22 induction during aging, expression was quantitated in multiple independent transformant lines containing a 5′ promoter deletion to residue −114, construct hsp22 5′Δ(−114). The −114 deletion was found to virtually eliminate both the heat shock response and the aging-specific expression (Fig. 9). Thus one or more elements required for aging-specific expression are located between or overlap-

**FIG. 7.** Whole-mount immunolocalization of hsp22 protein in ommatidia. Photoreceptor cell rhabdomeres (red fluorescence) and hsp22 protein (green fluorescence) were visualized in the ommatidia of manually isolated retinas by confocal microscopy. (A) Young. (B) Young, heat shocked. (C) Old. Retinas from old flies were found to be more fragile and to retain less structural integrity during isolation and staining than retinas from young and young heat-shocked flies.

**FIG. 8.** Expression of hsp22: lacZ fusion reporter constructs in transgenic flies. β-Gal expression was assayed in situ in cryostat sections of flies transgenic for wild-type promoter construct hsp22 5′Δ(−314). The head, thorax, and abdomen are indicated, with head to the right. Flight muscle is labeled with an asterisk. (A) Young. (B) Young, heat shocked. (C) Old. The region of intense blue β-gal staining in the old fly head is the eye tissue. In this section the head is rotated clockwise −45° relative to its position in the sections shown in A and B. Essentially identical results were obtained with multiple independent transgenic lines. Tissue-general induction of β-gal, with the highest level induction in the eye, was also observed by staining of manually dissected tissues from old flies (data not shown).
ping residues −314 and −114. This region includes the distal HSE 3, which is required for heat shock induction, as well as sequences required for developmental expression. To determine if the HSEs are required for aging-specific induction of hsp22, multiple independent transformant lines were generated for construct hsp22 5′Δ(−314)Point-Mutation. This construct contains specific point mutations disrupting each of the three HSEs (Fig. 1). As a result of these point mutations, both the heat shock induction and the aging-specific induction were virtually eliminated (Fig. 9), indicating that functional HSEs are required for hsp22 induction during aging.

DISCUSSION

Drosophila hsp22 RNA and protein were found to be induced during aging throughout the fly, with particularly high levels of induction in eye tissue. The onset of hsp22 protein induction corresponded approximately with the beginning of the period of rapid death of the population, sometimes called the “dying phase” of the survival curve. The dramatic induction of hsp22 is unique, as the other hsp gene RNAs and proteins assayed exhibited either no increase or markedly smaller increases with age. Induction of the other hsp genes during aging appears to be limited to the following: An ~fivefold induction of hsp23 RNA in thorax (Fig. 2) (Wheeler et al., 1995), which is not accompanied by a detectable induction of hsp23 protein as assayed in whole-fly extracts (Marín et al., 1993), and a severalfold increase in hsp70 expression specifically in thorax (Wheeler et al., 1995).

hsp22 gene induction during aging appears to be primarily transcriptional: hsp22 RNA levels increased up to 60-fold in particular tissues, and this induction was reproduced by hsp22: lacZ fusion reporter constructs in transgenic flies. Deletion of 5′ promoter sequences to residue −114, or point mutation of specific promoter elements, the HSEs, virtually
eliminated this induction. The hsp22 HSEs may function during aging by binding HSF or another protein with similar sequence requirements for binding. The hsp22 promoter appears most similar in size and structure to the hsp70 promoter. For both the hsp22 and the hsp70 promoters all of the sequences required to support the known expression patterns are located 3′ of residue −200. Both promoters contain three HSE elements required for hsp induction: a pair more proximal and one more distal. Despite these similarities only the hsp22 promoter exhibits dramatic induction during aging. Therefore, we suggest the existence of a specific trans-acting factor which is responsible for aging-specific induction of hsp22. This hypothetical factor might bind to the HSEs with a sequence specificity overlapping that of HSF or might bind to a different promoter element and act in concert with the HSEs and HSF.

Aging-specific induction of hsp22 also appears to be regulated at the posttranscriptional level. In the thorax, the appearance of hsp22 protein during aging is delayed relative to abundant expression of hsp22 RNA by about 2 weeks. The dramatic delay in protein accumulation suggests regulation of either hsp22 translation or protein stability as a function of age in thorax. The hsp22:lacZ fusion reporter construct containing hsp22 sequences from −314 to +275 did not appear to exhibit this delay in protein expression, suggesting that more 3′ coding region or 3′ untranslated sequences might be required. The posttranscriptional regulation of hsp22 expression in thorax may be related to the thorax-specific, posttranscriptional induction of hsp70 and specific hsp70:lacZ fusion proteins during aging (Wheeler et al., 1995).

The dramatic induction of Drosophila hsp22 RNA during aging so far appears to be a novel pattern of gene expression in higher eukaryotes. Where specific gene expression patterns have been analyzed, they have most often been found to be constant or to decrease with age (Finch, 1990). In Drosophila, RNA levels for several non-heat-shock genes were found to be constant or to decrease slightly during aging (Wheeler et al., 1995). Helfand and co-workers have used P-element "enhancer traps" as a measure of gene expression during aging in the Drosophila antenna (Helfand et al., 1995). β-Gal activity in antenna resulting from enhancer traps inserted at the engrailed and wingless genes decreased dramatically in the first third of the adult life span (Rogina and Helfand, 1997). However, β-gal activity in antenna increased during aging with enhancer traps inserted at several unknown loci, suggesting that additional Drosophila genes which are transcriptionally upregulated during aging might yet be identified.

A small number of specific genes have been shown to be induced at the RNA level during aging in mammals, but the degree of induction is generally less than that identified for Drosophila hsp22. Examples of RNAs induced during mammalian aging include the twofold induction in rat liver of the mRNAs for apolipoprotein A1 and c-myc (Matocha et al., 1987; Waggoner et al., 1990), and α1-antitrypsin and albumin (Richardson et al., 1987), and the fivefold increase of an mRNA of unknown function, SMP-2 (Chatterjee et al., 1989; 1987). The mRNAs for the rodent acute-phase response proteins, ceruloplasmin, haptoglobin, and T-kininogen, which are normally induced in liver in response to inflammation, are also induced severalfold during aging (Bowman et al., 1990; Sierra et al., 1989). Finally, the structural protein GFAP RNA is induced severalfold in healthy aging rodents and humans, and this increase was shown to be at the transcriptional level in rat brain glia (Goss et al., 1991; Morgan et al., 1998; Yoshida et al., 1996).

Current evolutionary theory suggests that aging exists because of the decrease in the force of natural selection with increasing age (Charlesworth, 1994; Partridge and Barton, 1993; Rose, 1991). A logical extension of this theory is that there should be no mechanism for the evolution of a gene or gene expression mechanism which is specific for senescence (Tower, 1996). We hypothesize that the dramatic and preferential induction of hsp22 during aging is a response to a stress which is relatively minor and/or rare early in the life cycle, but which becomes especially severe during aging. Induction of hsp22 may then help ameliorate the damage caused by this stress, and perhaps prolong the functional life span of the fly. The small hsps in general appear to be molecular chaperones, and thus, likely functions for hsp22 are the prevention, repair, and/or turnover of protein damage. In several organisms the small hsps have been shown to be specialized for functions in different subcellular compartments (Arrigo and Landry, 1994). Perhaps the stress causing aging-specific induction of Drosophila hsp22 is damage at a subcellular organelle which is especially subject to deterioration during aging, such as the mitochondria (Takahashi et al., 1970a, b). Similarly, the stress might be abnormalities in a specific subset of cellular proteins which are particularly subject to damage during aging and which are targets for hsp22 function.

While the time course of hsp22 induction correlates and scales with aging, the current data do not directly address hsp22 function. However, experimental manipulations which increase life span have consistently been found to be associated with increased stress responses and increased stress resistance in several organisms (Lithgow, 1996; Tower, 1996). Drosophila populations can be genetically selected for increased life span, and these populations exhibit increased resistance to various stresses, including desiccation, starvation and oxidative damage (Arking et al., 1991; Force et al., 1995; Graves et al., 1992; Rose et al., 1992; Service, 1987). In Caenorhabditis elegans several specific gene mutations which increase life span have been identified, and in each case tested these mutations also increase resistance to heat stress, UV irradiation, and oxidative stress (Larsen, 1993; Lithgow et al., 1994, 1995; Murakami and Johnson, 1996; Vanfleteren, 1993). Finally, mild heat stress can increase the life span of Drosophila and C. elegans (Khazaeli et al., 1997; Lithgow et al., 1995; Maynard Smith, 1958a, b). Larger heat-induced increases in life span were achieved in Drosophila transgenic for extra copies of the hsp70 gene, thus demonstrating that an hsp...
can have positive effects on survival (Tatar et al., 1997). Identification of the stress response pathways most relevant to aging, such as hsp22 induction, promises to facilitate the analysis of the link between stress resistance and life span.

Finally, hsp22 appears to be a biomarker of aging in Drosophila and may provide a measure of aging that can be used in addition to life span. The dramatic induction of the hsp22 promoter during aging may be useful as a means to drive aging-specific expression of various transgenes in other studies of aging. It will be of interest to determine if any small hsps exhibit a similar aging-specific expression pattern in other organisms, such as C. elegans and mammals. Detailed analysis of the induction and function of Drosophila hsp22 during aging should increase our understanding of the mechanisms of aging in Drosophila and perhaps in other species.

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