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Hsp70 and aging

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Abstract. An alteration in the ability of cells to express heat shock proteins could be physiologically important in aging because all living organisms show a reduced ability to respond to stress with increasing age. Using hepatocytes freshly isolated from young adult and old rats, we have shown that the induction of hsp70 expression by heat shock is reduced approximately 50% with age. The decrease in hsp70 expression occurs at the level of transcription and appears to arise from a defect in the heat shock transcription factor. Other investigators have also shown that the induction of hsp70 expression by heat shock as well as other stresses declines significantly with age in a variety of tissues from rats as well as mononuclear cells from human subjects. In addition, a decrease in the inducibility of hsp70 is observed with cell senescence in cultured cells. Therefore, it appears that a reduced ability to express hsp70 in response to stress may be a common phenomenon underlying the aging process.

Key words. Aging; liver; transcription; heat shock transcription factor; rat; senescence.

Introduction

The reduced ability of senescent organisms to respond to stress and to maintain homeostasis is a phenomenon that is observed with senescene in essentially all living organisms[37]. It is generally believed that the age-related loss in the ability of an organism to maintain homeostasis is at least partially responsible for the increase in morbidity/mortality that is observed as an organism ages. The dramatic increase in the incidence of heat stroke with age[6,14–16,27,39] is an example of the inability of senescent organisms to maintain homeostasis in response to a stress. Figure 1 shows data from a study by Jones et al.[14], in which the rate of the incidence of heat stroke was compared as a function of age. The rate of heat stroke was more than 10-fold higher for persons of 65 years or older as compared to younger individuals. Interestingly, heat stroke was rare for subjects under 44 years of age. Although Jones et al.[14] also showed that the rate of heat stroke was greater in poor and in non-whites, the parameter that had the greatest impact on the incidence of heat stroke was age.

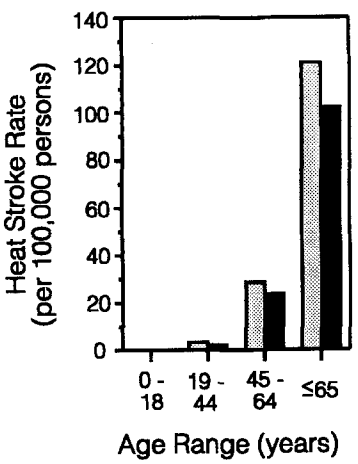

Figure 1. Effect of age on heat stroke rates in humans. The rate of heat stroke in St. Louis (shaded bars) and in Kansas City (solid bars) during the 1980 heat wave in Missouri was determined by a retrospective study. Heat stroke was defined as severe heat illness with documented hyperthermia. These data were taken from Jones et al.[14].

It is often assumed that the increased incidence of heat stroke in the elderly is due to underlying disease. However, the data in figure 1 show that the incidence of heat stroke increases significantly between 44 and 64 years of age, well before major changes in underlying disease would be expected to occur. Therefore, it appears that basic, physiological changes in the organism might be important in the age-related increase in heat stroke. For example, it has been proposed that a decline in sweating efficiency or changes in cardiac output and systemic vascular resistance might be physiological changes that increase the vulnerability of older individuals to heat stroke[14].

Data over the past decade have shown that heat shock proteins play an important role in protecting cells against the adverse effects of hyperthermia[18,19]. For example, several studies have shown that the thermosensitivity of cells is altered if the expression of hsp70 is enhanced or reduced[2,13,17,30]. Therefore, it is possible that an age-related decline in the ability of cells to express heat shock proteins might be a contributing factor in the increased incidence of death from heat stroke that is observed in the elderly. In this paper, we describe our studies on the effect of age on the induction

of hsp70 expression in rat hepatocytes. In addition, we review data obtained by other laboratories on the effect of age on the induction of hsp70 expression by hyperthermia in other tissues/cells and organisms.

Effect of age on hsp70 expression in hepatocytes

It is clear from studies conducted over the past two decades that the expression of heat shock proteins, e.g., hsp70, is regulated primarily at the transcriptional level[18,19]. The increased transcription of hsp70 following heat shock has been shown to require the binding of a protein, the heat shock transcription factor (HSF), to a conserved DNA sequence, the heat shock element (HSE), which is located within the promoter region of all heat inducible genes[1,20,24,25,38]. In *Drosophila* and mammalian cell lines, HSF is found in an inactive (i.e., non-DNA binding) form in the cytoplasm in non-stressed cells. An increase in temperature results in the activation of HSF to a form that binds the HSE through oligomerization and the translocation of the active HSF into the nucleus of the stressed cell[3,20,24,25]. Therefore, it is generally believed that the conversion of HSF from an inactive (i.e., non-HSE binding) monomer to an oligomer that binds the HSE is essential for the induction of hsp70 transcription in mammalian cells.

Over the past three years, our laboratory has characterized the induction of hsp70 expression by hepatocytes freshly isolated from male rats[12,42]. The synthesis of hsp70 is induced significantly when hepatocytes are exposed to a temperature of 42 to 43 °C for 30 minutes, and this heat shock has very little effect on the viability of the hepatocytes[42]. A heat shock of 42 to 43 °C for 30 minutes also induces dramatically the levels of the hsp70 transcript and the nuclear transcription of hsp70[12]. More recently, we have studied the relationship between the induction of hsp70 transcription and the activation of HSF to a form that binds the HSE. Using a gel-shift assay to measure HSF binding activity, we have found that in response to heat shock the induction of hsp70 transcription and HSF binding activity are tightly correlated, as shown in figure 2. HSF binding activity was rapidly induced when hepatocytes are incubated at 41 or 43 °C, e.g., HSF binding activity is maximum within 10 to 15 minutes. The induction of hsp70 transcription follows the induction of HSF binding activity and was maximum 20 to 30 minutes after the heat shock. The data in figure 2 also show that at a temperature of 39 °C or lower, neither hsp70 transcription nor HSF binding activity was detectable in the hepatocytes. The transcription of hsp70 and HSF binding activity were maximum when hepatocytes were incubated at 41 °C and decreased slightly when hepatocytes were incubated at 43 °C. The transcription of hsp70 was undetectable when hepatocytes were incubated at 45 °C, and HSF binding activity was only briefly observed at a very low level at this temperature; the viability of hepatocytes is reduced significantly at this temperature[42]. Thus, the data in figure 2 are consistent with the view that hsp70 transcription is regulated in hepatocytes by the transformation of HSF to a form that binds the HSE.

To determine if aging alters the ability of cells to express heat shock proteins, we measured the induction of hsp70 expression by a mild heat shock (42.5 °C for 30 minutes) in hepatocytes isolated from either young adult (4- to 6-month old) or old (26- to 28-month old) rats. The data presented in figure 3 show that hepatocytes isolated from old rats have a reduced ability to express hsp70 in response to heat shock. The synthesis of hsp70 was approximately 50% less for hepatocytes isolated from the old rats compared to hepatocytes isolated from young adult rats. The induction of hsp70 mRNA transcripts was also reduced in the hepatocytes isolated from the old rats. Using in situ hybridization, we have found that the decreased expression of hsp70 is not due to a reduced number of old cells responding to the heat shock and expressing hsp70 mRNA tran-

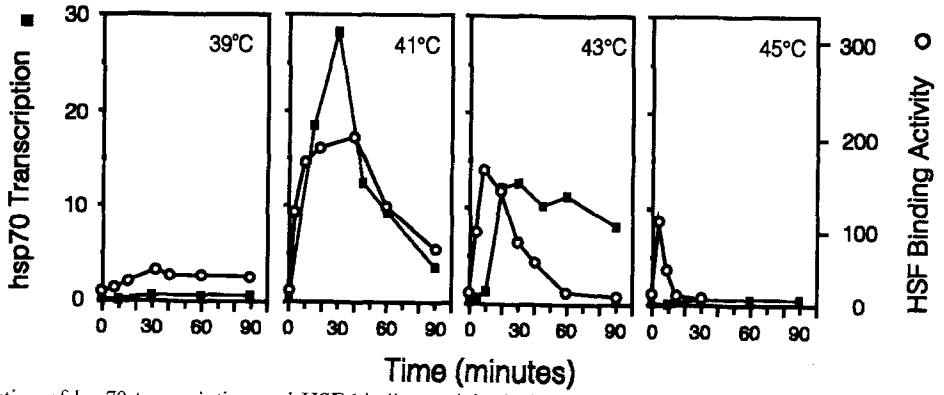

Figure 2. Induction of hsp70 transcription and HSF binding activity in hepatocytes. Hepatocytes isolated from young adult rats were incubated at four different temperatures (39, 41, 43 and 45 °C) for various time. HSF binding to the HSE (○) was determined by a gel shift assay using radiolabeled HSE[12], and the data are expressed as the amount of radioactivity bound (machine counts) per microgram of whole cell extract. The transcription of hsp70 transcription (■) was determined as described previously[12], and the data are expressed as machine counts per 20×10^6 nuclei.

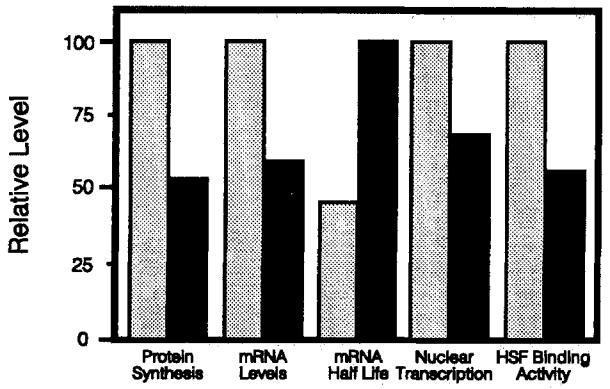

Figure 3. Effect of age on the induction of hsp70 expression. Hepatocytes were isolated from 4- to 6-month old (shaded bars) and 26- to 28-month old (solid bars) male F344 rats. The hepatocytes were heat shocked at 42.5 °C for 30 minutes and then incubated at 37 °C. The induction of hsp70 synthesis, mRNA levels, nuclear transcription, and HSF binding to HSE are shown as well as the half life of the hsp70 mRNA. The values are expressed as the percentage of values obtained for young adult rats except for the half life of hsp70 mRNA, which is expressed as the percentage of value obtained for old rats. The data were taken from Heydari et al.[12].

scripts[12]; essentially all of the hepatocytes isolated from either young adult or old rats express hsp70 mRNA in response to the heat shock. In other words, the age-related decrease in the induction of hsp70 expression is not due to a subpopulation of cells in the old animals that cannot respond to heat shock.

Two lines of evidence show that the reduced expression of hsp70 with age arises at the level of transcription. First, the age-related decrease in the induction of hsp70 mRNA is not due to increased degradation of hsp70 mRNA as shown by the measurement of the half life of hsp70 mRNA transcripts. In fact, it would appear that hsp70 mRNA is degraded more slowly with increasing age because the half-life of hsp70 mRNA was greater for hepatocytes isolated from old rats compared to hepatocytes isolated from young adult rats. Second, the transcription of hsp70 by nuclei isolated from hepatocytes obtained from old rats was reduced significantly compared to the nuclear transcription of hsp70 by nuclei isolated from young adult rats. Because HSF plays a critical role in the induction of hsp70 transcription, the effect of age on the induction of HSF binding activity in cell extracts isolated from hepatocytes obtained from young adult and old rats was studied using a gel-shift mobility assay. The data in figure 3 demonstrate that the level of HSF binding activity induced by heat shock in hepatocytes isolated from the old rats was approximately 50% lower than the level observed in heat shocked hepatocytes isolated from young rats.

In summary, the data in figure 3 show that hepatocytes isolated from old rats have a reduced ability to express hsp70 in response to heat shock. More importantly, these data also show that the decreased expression of hsp70 arises at the level of transcription and that the decreased hsp70 transcription appears to occur because of reduced levels of HSF binding activity. In other words, in response to hyperthermia, the old cells have less 'active' HSF, which results in a decrease in the amount of HSF bound to the HSE on the promoter of the hsp70 gene. The decreased binding of HSF to the promoter of the hsp70 gene would be predicted to result in decreased hsp70 transcription and, therefore, decreased expression of hsp70. Based on our knowledge of the HSF, the age-related decrease in HSF binding activity could occur by two mechanisms:

1) The decreased HSF binding activity could arise from a decrease in the expression of HSF. In other words, less HSF binding activity is observed in hepatocytes isolated from the old rats because less of the HSF protein is present in the hepatocytes isolated from the old rats.

2) The decreased HSF binding activity arises from a decrease in the post-translational activation (oligomerization) of HSF by heat shock. In other words, similar levels of HSF are present in non-stressed hepatocytes isolated from young and old rats; however, in response to heat shock, the conversion of HSF from its inactive, non-DNA binding form to its HSE-binding form is reduced in the hepatocytes isolated from the old rats.

In 1991, the human and mouse cDNAs for HSF were cloned[29,35,36]. Two HSFs have been isolated and characterized: HSF1 and HSF2, and HSF1 has been identified as the transcription factor that is involved in the regulation of heat shock gene expression in response to heat shock. Using a polyclonal antisera raised against mouse HSF1[34], we have conducted a series of preliminary experiments in which the level of HSF1 was measured by western blots in hepatocytes before and after heat shock. Our preliminary data indicate that the level of HSF1 protein is not reduced in hepatocytes isolated from old rats. In fact, the data suggest that HSF1 levels might be higher in cell extracts isolated from hepatocytes obtained from old rats. Thus, it appears that the age-related decrease in HSF binding activity is not due to a decrease in the level of HSF1 in the hepatocytes isolated from old rats. Rather, it appears that the decrease in HSF binding activity arises from a decline in ability of hepatocytes from old rats to convert HSF1 from its inactive (non-DNA binding) monomeric-form to its active (HSE binding) oligomeric-form.

Effect of age on hsp70 expression in other tissues and animals

Since 1988, a number of laboratories have studied how aging alters the ability of a variety of cells to express

Table 1. Effect of age on the induction of hsp70 expression by heat shock.

Organism/tissue	Assay for hsp70 expression	Ages studied	Change with age	References
Insects				
Drosophila Whole body	synthesis	10 and 45 days	increase	Fleming et al.[11]
Drosophila Whole body	synthesis	1 to 50 days	180% increase	Niedzwiecki et al.[26]
Animals				
Male Wistar Lung, skin	synthesis mRNA level	5 and 24 months	decrease 55% decrease	Fargnoli et al.[10]
Male Wistar Brain, lung, skin	mRNA level	5–6 and 24–25 months	50–75% decrease	Blake et al.[4]
Male F344 Rat Hippocampus	mRNA level	4 and 30 months	85% decrease	Pardue et al.[28]
Male F344 Rat Hepatocytes	synthesis	5–7 and 25–27 months	37% decrease	Wu et al.[42]
Male F344 Rat Hepatocytes	synthesis mRNA level transcription	4–6 and 26–28 months	45% decrease 40% decrease 31% decrease	Heydari et al.[12]
Human subjects Mononuclear cells	transcription	23–29 and 75–89 years	30% decrease	Deguchi et al.[7]
Cultured cells (cell senescence)				
Human (IMR-90) Lung diploid fibroblasts	synthesis mRNA level transcription	17 to 51 population doubling levels	65% decrease 80% decrease 50% decrease	Liu et al.[22]
Human (IMR-90) Lung diploid fibroblasts	mRNA level	21 and 45 population doubling levels	70% decrease	Liu et al.[21]
Human (WI-38) Diploid fibroblasts	synthesis mRNA level	50 and 90% of in vitro life span	decrease decrease	Luce and Cristofalo[23]
Human T-lymphocytes	synthesis mRNA level	11 to 80% of in vitro life span	50% decrease 50% decrease	Effros et al.[8]

hsp70 in response to hyperthermia, and these studies are listed in table 1. In addition to our studies on rat hepatocytes, Holbrook's laboratory has shown that the induction of hsp70 expression by heat shock is reduced with increasing age in several tissues/cells from rats. For example, the induction of hsp70 synthesis and mRNA levels by hyperthermia was lower in primary cultures of fibroblasts isolated from either skin or lung of old rats compared to young rats[10]. In addition, Holbrook's laboratory also showed that the induction of hsp70 mRNA in vivo decreased with age in lung and skin, as well as brain, when rats were exposed to elevated temperatures.[4] Pardue et al.[28] also showed that the induction of hsp70 mRNA was less in the hippocampus of old rats when the rats were exposed to hyperthermia in vivo. Our studies with freshly isolated hepatocytes also show that a decrease in hsp70 expression occurs with age in liver. Thus, an age-related decline in the induction of hsp70 expression by hyperthermia appears to be a common phenomenon in most tissues of a rat. Peripheral mononuclear cells isolated from human subjects also appear to show a decline in hsp70 expression with age. Deguchi et al.[7] showed that the induction of hsp70 transcription by heat shock was lower in mononuclear cells isolated from elderly human subjects compared to young adult humans.

The induction of hsp70 has also been reported to decline in cultured cells when they reach the end of their replicative life span and are unable to proliferate, a phenomenon termed 'cell sensecence'. In 1989, Liu et al.[22] showed that the induction of hsp70 expression by heat shock was significantly reduced in late passage human fibroblasts compared to early passage human fibroblasts. Luce and Cristofalo[23] also showed that the induction of hsp70 expression by heat shock was significantly reduced in late passage human fibroblasts compared to early passage human fibroblasts. More recently, Effros et al.[8] showed that the induction of hsp70 expression decreased with passage number in human T cells.

Thus, the current data indicate that the induction of hsp70 expression declines with age in animals as well as in cell senescence in culture. It also appears that an age-related decrease occurs in the induction of hsp70 by stresses other than heat shock. For example, Holbrook's laboratory has shown that the induction of hsp70 expression (hsp70 synthesis and mRNA levels) by restraint stress decreased approximately 80% with increasing age in the adrenal cortex[5] and aortic smooth muscle[41] of rats. Faassen et al.[9] has shown that the induction of hsp70 synthesis by mitogens decreased with age in lymphocytes isolated from young and elderly human subjects. In cultured human fibroblasts, the induction of hsp70 expression by other stresses has also been observed to decline as cells senesce. Liu et al.[22] observed that the induction of hsp70 synthesis by the amino acid analog canavanine was 50% less for late passage human fibroblasts than for early passage human fibroblasts. More recently, Luce and Cristofalo[23] showed that the induction of hsp70 expression by arsinite was reduced in late passage human fibroblasts. Therefore, it appears that the induction of hsp70 by a variety of stresses declines with age. The only example in the literature in which no age-related decline in the induction of hsp70 expression has been observed was reported by Fleming's laboratory in *Drosphila melanogaster*[11,26]. They actually observed an age-related increase in the expression of hsp70 in response to hyperthermia.

Our studies with freshly isolated hepatocytes indicate that the age-related decline in hsp70 expression occurs at the level of transcription. Liu et al.[22] also found that the induction of hsp70 expression by heat shock was reduced in late passage human fibroblasts at the transcriptional level. Using a nuclear runoff assay, they showed that hsp70 transcription was lower in late passage human fibroblasts after a heat shock. In addition, when early and late passage human fibroblasts were transfected with a plasmid containing the human hsp70 promoter fused to the bacterial chloramphenicol acetyltransferase gene, Liu et al.[21,22] observed that the late passage cells expressed less of the bacterial enzyme in response to heat shock. More recently, Liu et al.[21] showed that the decrease in hsp70 transcription with cell senescence was correlated to a decrease in HSF binding activity. Cell extracts from late passage fibroblasts that were heat shocked showed a decrease in the binding of HSF to HSE in a gel-shift assay when compared to cell extracts from early passage fibroblasts. Thus, the changes we have observed in the induction of hsp70 expression by heat shock with age in hepatocytes is similar to that reported for human fibroblasts as they senesce in culture and are unable to proliferate, i.e., the transcription of hsp70 and the binding of HSF to HSE are reduced.

Conclusions

The regulation of hsp70 expression is an excellent example of a cellular mechanism that has evolved to protect all living organisms from hyperthermia and other types of stress. Therefore, changes in this system could seriously compromise the capacity of an organism to respond to changes in its environment. Because aging and senescence are characterized by a reduced ability of an organism to maintain homeostasis in response to stress, a large number of investigators have compared the ability of young and old cells to respond to hyperthermia and express hsp70. At the present time it appears that aging, either at the organismic level or at the cellular level, is associated with a decreased ability to express hsp70 in response to hyperthermia as well as a variety of other stresses. This defect occurs at the transcriptional level and appears to involve the transcription factor HSF. In response to stress, cells from old animals or cells that have senesced in culture have decreased 'active' HSF, i.e., less HSE binding activity is found in cell/nuclear extracts from the old cells. Our preliminary data indicate that the decrease in HSF binding activity is not due to a decrease in the amount of HSF present in the cell but is most likely due to a defect in the conversion of HSF from its inactive monomeric form to the active oligomer. One possible mechanism for the age-related decline in the binding activity of HSF is the accumulation of 'abnormal' HSF. Research over the past two decades has shown that 'altered' enzymes accumulate with age[31–33]. These alterations have been shown to arise from conformational changes rather than from errors in translation, and the conformational changes in proteins of old organisms occur through post-synthetic modifications, e.g., protein oxidation, amino acid racemization, deamination, glycation, conformational drift, etc.[31–33,40]. These conformational changes give rise to subtle changes in protein conformation, which usually have a small effect on enzymatic activity. An accumulation of 'abnormal' HSF molecules, which have a decreased ability to oligomerize in response to stress, is an attractive explanation for why cells from old organisms lose their ability to respond to stress and express hsp70.

One would predict that an age-related defect in the ability of cells to express hsp70 in response to hyperthermia would make senescent organisms more vulnerable to hyperthermia because it has been shown that cells become more thermosensitive when the expression of hsp70 is inhibited[13,30]. Although there is a great deal of research showing that the expression of hsp70 is reduced with age and it is well documented that heat stroke increases dramatically in the elderly, it is ironic that there is very little information on the effect of aging on thermotolerance at the cellular level. The only study in this area is by Luce and Cristofalo[23]. They found that

late passage human diploid fibroblasts, which show reduced levels of hsp70 expression, were more sensitive to a temperature of 49 °C than early passage cells. Thus, the decline in hsp70 expression that occurs during cell senescence in cultured cells is associated with a decrease in the ability of the cells to withstand hyperthermic stress. At the present time, however, there is no study in which the thermosensitivity of cells from animals of various ages has been compared. We would predict that cells from old organisms, which show a reduced capacity to express hsp70 in response to hyperthermia, would also be more thermosensitive than cells isolated from young organisms.

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1 Ang, D., Liberek, K., Showyra, D., Zylicz, M., and Georgopoulos, C., Biological role and regulation of the universally conserved heat shock proteins. J. biol. Chem. *266* (1991) 24233–24236.
2 Angelidis, C. E., Lazaridis, I., and Pagoulatos, G. N., Constitutive expression of heat-shock protein 70 in mammalian cells confers thermoresistance. Eur. J. Biochem. *199* (1991) 35–39.
3 Baler, R., Dahl, G., and Voellmy, R., Activation of human heat shock genes is accompanied by oligomerization, modification, and rapid translocation of heat shock transcription factor HSF1. Molec. cell. Biol. *13* (1993) 2486–2496.
4 Blake, M. J., Fargnoli, J., Gershon, D., and Holbrook, N. J., Concomitant decline in heat-induced hyperthermia and HSP70 mRNA expression in aged rats. Am. J. Physiol. *260* (1991) 663–667.
5 Blake, M. J., Udelsman, R., Feulner, G. J., Norton, D. D., and Holbrook, N. J., Stress-induced heat shock protein 70 expression in adrenal cortex: an adrenocorticotropic hormone-sensitive, age-dependent response. Proc. natl Acad. Sci. USA *88* (1991) 9873–9877.
6 Clowes, C. H. A., and O'Donnell, T. F., Heat stroke. N. Eng. J. Med. *291* (1974) 564–567.
7 Deguchi, Y., Negoro, S., and Kishimoto, S., Age-related changes of heat shock protein gene transcription in human peripheral blood mononuclear cells. Biochem. biophys. Res. Commun. *157* (1988) 580–584.
8 Effros, R. B., Zhu, X., and Walford, R. L., Stress response of senescent T lymphocytes: Reduced hsp70 is independent of the proliferative block. J. Geront. *49* (1994) B65–B70.
9 Faassen, A. E., O'Leary. J. J., Rodysill, K. J., Bergh, N., and Hallgren, H. M., Diminished heat-shock protein synthesis following mitogen stimulation of lymphocytes from aged donors. Expl. Cell Res. *183* (1989) 326–334.
10 Fargnoli, J., Kunisada, T., Fornace, A. J., Schneider, E. L., and Holbrook, N. J., Decreased expression of heat shock protein 70 mRNA and protein after heat treatment in cells of aged rats. Proc. natl Acad. Sci. USA *87* (1990) 846–850.
11 Fleming, J. E., Walton, J. K., Dubitsky, R., and Bensch, K. G., Aging results in an unusual expression of Drosophila heat shock proteins. Proc. natl Acad. Sci. USA *85* (1988) 4099–4103.
12 Heydari, A. R., Wu, B., Takahashi, R., Strong, R., and Richardson, A., Expression of heat shock protein 70 is altered by age and diet at the level of transcription. Molec. cell. Biol. *13* (1993) 2909–2918.
13 Johnston, R. N., and Kucey, B. L., Competitive inhibition of hsp70 gene expression causes thermosensitivity. Science *242* (1988) 1551–1554.
14 Jones, T. S., Liang, A. P., Kilbourne, E. M., Griffin, M. R., Patriarca, P. A., Wassilak, S. G. F., Mullan, R. J., Herrick, R. F., Donnell, H. D., Choi, K., and Thacker, S. B., Morbidity and mortality associated with the July 1980 heat wave in St Louis and Kansas City, Mo. J. Am. med. Assoc. *247* (1982) 3327–3355.
15 Kilbourne, E. M., Choi, K., Jones, T. S., and Thacker, S. B., Risk factors for heatstroke. J. Am. med. Assoc. *247* (1982) 3332–3336.
16 Levine, J. A., Heat stroke in the aged. Am. J. Med. *47* (1969) 251–258.
17 Li, G. C., Li, L., Liu, Y. K., Mak, J. Y., Chen, L., and Lee, W. M., Thermal response of rat fibroblasts stably transfected with the human 70-kDa heat shock protein-encoding gene. Proc. natl Acad. Sci. USA *88* (1991) 1681–1685.
18 Lindquist, S., The heat-shock response. A. Rev. Biochem. *55* (1986) 1151–1191.
19 Lindquist, S., and Craig, E. A., The heat-shock proteins. A. Rev. Genet. *22* (1988) 631–677.
20 Lis, J., and Wu, C., Protein traffic on the heat shock promoter: Parking, stalling, and trucking along. Cell *74* (1993) 1–4.
21 Liu, A. Y., Choi, H., Lee, Y., and Chen, K. Y., Molecular events involved in transcriptional activation of heat shock genes become progressively refractory to heat stimulation during aging of human diploid fibroblasts. J. cell. Physiol. *149* (1991) 560–566.
22 Liu, A. Y., Lin, Z., Choi, H., Sorhage, F., and Li, B., Attenuated induction of heat shock gene expression in aging diploid fibroblasts. J. biol. Chem. *264* (1989) 12037–12045.
23 Luce, M. C., and Cristofalo, V. J., Reduction in heat shock gene expression correlates with increased thermosensitivity in senescent human fibroblasts. Expl. Cell Res. *202* (1992) 9–16.
24 Morimoto, R. I., Cells in stress: Transcriptional activation of heat shock genes. Science *259* (1993) 1409-1410.
25 Morimoto, R. I., Sarge, K. D., and Abravaya, K., Transcriptional regulation of heat shock genes. A paradigm for inducible genomic responses. J. biol. Chem. *267* (1992) 21987–21990.
26 Niedzwiecki, A., Kongpachith, A. M., and Fleming, J. E., Aging affects expression of 70-kDa heat shock proteins in *Drosophila*. J. biol. Chem. *266* (1991) 9332–9338.
27 Oechsli, F. W., and Buechley, R. W., Excess mortality associated with three Los Angeles September hot spells. Envir. Res. *3* (1970) 277–284.
28 Pardue, S., Groshan, K., Raese, J. D., and Morrison-Bogorad, M., Hsp70 mRNA induction is reduced in neurons of aged rat hippocampus after thermal stress. Neurobiol. Aging *13* (1992) 61–672.
29 Rabindran, S. K., Giorgi, G., Clos, J., and Wu, C., Molecular cloning and expression of a human heat shock factor, HSF1. Proc. natl Acad. Sci. USA *88* (1991) 6906–6910.
30 Riabowol, K. T., Mizzen, L. A., and Welch, W. J., Heat shock is lethal to fibroblasts microinjected with antibodies against hsp70. Science *242* (1988) 433–436.
31 Rothstein, M., The formation of altered enzymes in ageing animals. Mech. Ageing Dev. *9* (1979) 197–202.
32 Rothstein, M., Posttranslational alteration of proteins, in: Handbook of Biochemistry in Aging, pp. 103–111. Ed. J. R. Florini. CRC Press, Boca Raton, FL 1981.
33 Rothstein, M., Enzymes, enzyme alteration, and protein turnover, in: Review of Biological Research in Aging, pp. 305–314. Ed. M. Rothstein. Alan R. Liss, Inc., New York 1983.
34 Sarge, K. D., Murphy, S. P., and Morimoto, R. I., Activation of heat shock gene transcription by heat shock factor 1 involves oligomerization, acquisition of DNA-binding activity, and nuclear localization and can occur in the absence of stress. Molec. cell. Biol. *13* (1993) 1392–1407.
35 Sarge, K. D., Zimarino, V., Holm, K., Wu, C., and Morimoto, R. I., Cloning and characterization of two mouse heat shock factors with distinct inducible and constitutive DNA-binding ability. Genes Dev. *5* (1991) 1902–1911.

36 Schuetz, T. J., Gallo, G. J., Sheldon, L., Tempst, P., and Kingston, R. E., Isolation of a cDNA for HSF2: Evidence for two heat shock factor genes in humans. Proc. natl Acad. Sci. USA *88* (1991) 6911–6915.
37 Shock, N. W., Systems integration, in: Handbook of the Biology of Aging, pp. 639–665. Eds C. E. Finch and L. Hayflick. Van Nostrand Reinhold Company, New York 1977.
38 Sorger, P. K., Heat shock factor and the heat shock response. Cell *65* (1991) 363–366.
39 Sprung, C. L., Hemodynamic alterations of heat stroke in the elderly. Chest *75* (1979) 362–366.
40 Stadtman, E. R., Protein oxidation and aging. Science *257* (1992) 1220–1224.
41 Udelsman, R., Blake, M. J., Stagg, C. A., Li, D., Putney, D. J., and Holbrook, N. J., Vascular heat shock protein expression in response to stress. Endocrine and autonomic regulation of this age-dependent response. J. clin. Invest. *91* (1993) 465–473.
42 Wu, B., Gu, M. J., Heydari, A. R., and Richardson, A., The effect of age on the synthesis of two heat shock proteins in the HSP70 family. J. Geront. *48* (1993) B50–B56.