

HORMETIC MODULATION OF AGING AND LONGEVITY BY MILD HEAT STRESS

Suresh I. S. Rattan □ Laboratory of Cellular Ageing, Danish Centre for Molecular Gerontology, Department of Molecular Biology, University of Aarhus

□ Aging is characterized by a stochastic accumulation of molecular damage, progressive failure of maintenance and repair, and consequent onset of age-related diseases. Applying hormesis in aging research and therapy is based on the principle of stimulation of maintenance and repair pathways by repeated exposure to mild stress. In a series of experimental studies we have shown that repetitive mild heat stress has anti-aging hormetic effects on growth and various other cellular and biochemical characteristics of human skin fibroblasts undergoing aging in vitro. These effects include the maintenance of stress protein profiles, reduction in the accumulation of oxidatively and glycoxidatively damaged proteins, stimulation of the proteasomal activities for the degradation of abnormal proteins, improved cellular resistance to ethanol, hydrogenperoxide and ultraviolet-B rays, and enhanced levels of various antioxidant enzymes. Anti-aging hormetic effects of mild heat shock appear to be facilitated by reducing protein damage and protein aggregation by activating internal antioxidant, repair and degradation processes.

Keywords: Aging, anti-aging, heat shock, signal transduction, proteasome

INTRODUCTION

Application of hormesis as an anti-aging approach is based on the understanding of biological aging as a progressive failure of homeodynamics. Aging is characterized by a progressive accumulation of molecular damage in nucleic acids, proteins and lipids. The inefficiency and failure of maintenance, repair and turnover pathways is the main cause of age-related accumulation of damage. Therefore, it has been hypothesized that if cells and organisms are exposed to brief periods of stress so that their stress response-induced gene expression is upregulated and the related pathways of maintenance and repair are stimulated, one should observe anti-aging and longevity-promoting hormetic effects. Various chemical, physical and biological treatments have been used to unravel the pathways of maintenance and repair whose sustained activities can improve the physiological performance and survival of cells and organisms. Stresses that have been reported to delay aging and prolong longevity in various systems (for example, yeast, *Drosophila*, nematodes, rodents and human cells) include temperature shock, irradiation (UV-, gamma- and X-rays),

Address correspondence to Dr. Suresh I.S. Rattan, Department of Molecular Biology, University of Aarhus, Gustav Wieds Vej 10C, DK-8000 Aarhus – C, Denmark. Email: rattan@mb.au.dk. Telephone: +45-8942-5034. Fax: +45-8612-3178.

heavy metals, pro-oxidants, acetaldehyde, alcohols, hypergravity, caloric restriction (CR), and exercise (Rattan, 2004; 2005). Hormesis-like beneficial effects of chronic but mild undernutrition and intermittent fasting have been reported (Anson et al., 2003; Raji et al., 1998).

TEMPERATURE AS A HORMETIC AGENT

High temperature stress is a widely used horemptic agent, not only because it is easy to implement and gives consistent results, but also because heat stress mainly acts through an evolutionarily highly conserved stress response pathway known as the heat shock (HS) response. HS response is one of the primordial intracellular defence mechanisms against stressful conditions in which extracellular stress (and intracellular stress from denatured proteins) initiates a series of events starting with signal transduction, activation and nuclear translocation of heat shock factors (HSF), DNA binding of heat shock factor (HSF), initiation of HS gene transcription, and preferential translation of heat shock proteins (HSP) which then perform various biological functions (Kiang and McClain, 2003; Kiang and Tsokos, 1998; Park et al., 2005; Verbeke et al., 2001b).

Studies performed with the nematode *Caenorhabditis elegans* have shown that the wild-type and *age-1* mutant hermaphrodite worms exposed for 3 to 24 hr to 30°C had a significant increase in mean lifespan compared with controls (Lithgow et al., 1994; Lithgow et al., 1995). Similarly, a 6 hr exposure at 30°C of wild-type worms induced a 12.5% increase in lifespan, but no effect was found after exposures of 2 or 4 hr (Yokoyama et al., 2002). In a series of studies (Butov et al., 2001; Michalski et al., 2001; Yashin et al., 2001), *C. elegans* worms subjected to 35°C HS of different durations showed that HS not longer than 2 hr produced an extension of lifespan of animals. In contrast, longer HS had either no effect (3-hr HS) or deleterious effects (exposures longer than 3 hr). In a study of multiple stresses in *C. elegans* an extension of lifespan after 1 and 2 hr HS at 35°C was reported (Cypser and Johnson, 2002). Longer HS had either no effect (3 hr) or deleterious effects (4 hr and more). The same effects of different HS were observed on thermotolerance (Cypser and Johnson, 2002; Johnson, 2002; Johnson et al., 2001).

In studies performed on testing the hormetic effects of heat stress on survival and longevity of fruitflies, virgin males of inbred lines of *Drosophila melanogaster* exhibited a 2-day increase in mean lifespan and lower mortality rates during several weeks after a heat treatment of 36°C for 70 min (Khazaeli et al., 1997). No beneficial effect of HS was reported in females or in mated flies. It has also been shown that wild-type *D. melanogaster* exposed to 37°C for 5 min a day, 5 days a week, live on average 2 days longer than the control flies (Le Bourg et al., 2001). Longer exposures had either no effect or negative effect on lifespan. In our stud-

ies on *D. melanogaster*, exposure of young flies to four rounds of mild HS at 34°C significantly increased the average and maximum lifespan of female flies and increased their resistance to potentially lethal heat stress (Hercus et al., 2003).

Studies have also been performed on the effect of subjecting transgenic *D. melanogaster* overexpressing the inducible HSP70, to 20 min at 36°C in an incubator under saturated humidity (Minois et al., 2001). In the control “parental” line, such an exposure significantly increased the lifespan of both virgin flies kept in groups and of mated flies. The effect was more pronounced in males than in females. In individually kept flies, the same trend was observed but was statistically not significant. No beneficial effect of this HS has been seen in the transgenic lines (Minois *et al.*, 2001).

Other examples of the effects of thermal stress on longevity include longevity extending effects of 2-hr HS at 37°C on the yeast *Saccharomyces cerevisiae* (Shama et al., 1998). The same HS had no effect if applied later in life as well as if applied everyday. In the case of mice, irradiated and non-irradiated mice intermittently cold-shocked showed lower rates of mortality in non-irradiated males as well as in both sexes in irradiated mice (Minois, 2000; Rattan, 2005). Longer lifespans were observed in thermally stressed non-irradiated males and irradiated females. Finally, rats kept in water set at 23°C, 4 hr a day, 5 days a week, had a 5% increase in average lifespan. In addition, this treatment seemed to diminish the occurrence of certain age-related diseases (Minois, 2000; Rattan, 2005).

A summary of results of our studies: We have demonstrated the hormetic effects of mild HS on human skin fibroblasts. The mild HS conditions were selected from a series of pilot studies performed on testing the effects of 1 hr HS at different temperatures, ranging from 37°C to 45°C, on the synthesis of HSP70 protein in the following 3 hr period. Maximum HSP70 synthesis (more than 8-fold synthesis as compared with that at 37°C) was observed at 43°C. However, at 41°C, HS response was about one-third of the maximum response, and so this temperature was selected for long term studies.

Using a mild stress regimen of exposing serially passaged human fibroblasts to 41°C for 1 hr twice a week throughout their replicative lifespan in vitro, we have reported several beneficial anti-aging effects (Table 1). These effects included the maintenance of youthful morphology, reduced accumulation of damaged proteins increased levels of various heat shock proteins, increased proteasomal activities, increased antioxidative abilities, and increased resistance to ethanol, hydrogen peroxide and UV-A irradiation.

We have also observed that a pre-exposure of human skin fibroblasts to 1 hr HS at 41°C and 42°C protects them from glucose-, fructose- and glyoxal-induced premature senescence and apoptosis. Recently, we have also completed studies on the hormetic effects of repeated mild HS on human

TABLE 1 Hormetic effects of repeated mild heat shock on human fibroblasts undergoing ageing *in vitro*

Characteristic	Hormetic effect	Reference
Cell size	reduced enlargement	(Rattan, 1998)
Cellular morphology	reduced irregularisation	(Rattan, 1998)
Glycation, furasine level	50-80% reduction	(Verbeke <i>et al.</i> , 2001a)
Glycooxidation level	10-30% reduction	(Verbeke <i>et al.</i> , 2001a)
CML-rich protein level	20-85% reduction	(Verbeke <i>et al.</i> , 2001a)
Lipofuscin pigment level	6-29% reduction	(Verbeke <i>et al.</i> , 2001a)
Protein carbonyl levels	5-40% reduction	(Verbeke <i>et al.</i> , 2001a)
Reduced glutathione level	3-fold increase	(Verbeke <i>et al.</i> , 2001a)
Oxidised glutathione level	2-fold reduction	(Verbeke <i>et al.</i> , 2001a)
Induction of sugar-induced protein damage	10-fold reduction	(Verbeke <i>et al.</i> , 2002)
H ₂ O ₂ decomposing ability	50-140% increase	(Fonager <i>et al.</i> , 2002)
Survival after H ₂ O ₂ exposure	10-18% increase	(Fonager <i>et al.</i> , 2002)
Survival after ethanol exposure	10-40% increase	(Fonager <i>et al.</i> , 2002)
Survival after UVA exposure	5-17% increase	(Fonager <i>et al.</i> , 2002)
Hsp27 level	20-40% increase	(Fonager <i>et al.</i> , 2002)
Hsc70 level	20% increase	(Fonager <i>et al.</i> , 2002)
Hsp70 level	7-20-fold increase	(Fonager <i>et al.</i> , 2002)
Hsp90 level	50-80% reduction	(Fonager <i>et al.</i> , 2002)
Proteasome activities	40-90% increase	(Beedholm <i>et al.</i> , 2004)
20S proteasome content	no change	(Beedholm <i>et al.</i> , 2004)
19S activator content	no change	(Beedholm <i>et al.</i> , 2004)
11S activator content	increase	(Beedholm <i>et al.</i> , 2004)
11S activator binding	increase	(Beedholm <i>et al.</i> , 2004)

epidermal keratinocytes, and the results obtained are very much similar to those for dermal fibroblasts (*Ali, Rattan – manuscript in preparation*). Additionally, we have also observed that vitamin-D-induced differentiation of bone marrow stem cells into osteoblasts can be enhanced by pre-exposure to 1 hr HS at 42.5°C (*Nørgaard, Rattan – manuscript in preparation*).

MECHANISMS OF HORMETIC EFFECTS OF HEAT SHOCK

Optimal HS response in terms of HSP synthesis and activity is essential for cell survival. In contrast, inefficient and altered HS response has been implicated in abnormal growth and development, aging and apoptosis (Kiang and Tsokos, 1998; Söti and Csermely, 2000; Söti *et al.*, 2005; Verbeke *et al.*, 2001b). When a cell encounters a “stressor”, modifications of the cytoskeleton, cytoplasmic structures, cell surface morphology, cellular redox status, DNA synthesis, protein metabolism and protein stability occur. Stress generates molecular damage, especially abnormally folded proteins, which can aggregate and initiate a sequence of stress response. The cellular stress response can be viewed as an adaptative response for the defence and maintenance of its structural and functional integrity (Rattan *et al.*, 2003).

Signaling pathways involved in HS response are still largely unknown. However, some kinases in the stress pathways, such as stress activated protein kinase (SAPK) c-Jun terminal kinase (JNK or SAPK1) and p38 (SAPK2), are suggested to play an important role. HS activates within minutes the major signaling pathways involving mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK) and SAPK (Dorion and Landry, 2002; Gabai et al., 1998). These kinases are involved in both survival and death pathways in response to other stresses and may, therefore, contribute significantly to the HS response (Gabai and Sherman, 2002). Activation of p38 occurs very early during stress and leads to the phosphorylation of HSP27. It is triggered by a highly specific HS sensing pathway and requires the activation of upstream kinases such as the MAPKK MKK3/6 and the MAPKKK apoptosis signal-regulating kinase-1 (ASK1) (Meriin et al., 1999). HS is also thought to activate (and thus phosphorylate) the epidermal growth factor (EGF) receptor in an agonist-independent way (Dorion and Landry, 2002). HS has also been shown to phosphorylate constitutive nitric oxide synthase at tyrosine residues and increase fas/CD95 expression on the cell membrane (Kiang et al., 2003), in addition to induce increased $[Ca^{2+}]_i$, $[Na^+]_i$, pHi, cAMP cellular levels, and inositol 1,4,5-trisphosphate (Kiang and Tsokos, 1998).

It is suggested that JNK is preferentially associated with the protective effects of HS against severe stress (Park and Liu, 2001). A major mechanism for HS-induced JNK appears to be the direct inhibition of the JNK phosphatase that normally inactivates JNK. In the absence of this phosphatase, the basal activity of MAPKK4 (MKK4) is sufficient to activate JNK (Meriin *et al.*, 1999). An early and transient activation of the JNK and p38 pathways is usually associated with survival and differentiation, whereas a late and sustained activation might point to apoptosis (Dorion and Landry, 2002). Therefore a balance between the JNK and p38 pathways (apoptotic) and ERK pathways (survival), and their interplay, determine whether a cell exposed to HS will die or survive and become stress tolerant (Gabai and Sherman, 2002). We have observed a rapid activation of MAP-kinases in terms of phosphorylation after 41°C or 42°C HS, and cells exposed to multiple rounds of 41°C or 42°C HS seem to have an increased amount of total JNK and p38 as compared with unstressed cells (Nielsen, Rattan – *manuscript in preparation*).

Activation of heat shock factors

The induction of the HS response is through the heat shock transcription factors (HSF) working as molecular links between environmental stresses and the stress response (Kiang and Tsokos, 1998; Verbeke *et al.*, 2001b). The four vertebrate HSF are expressed constitutively and cooperate functionally. HSF1 is a long-lived protein, it is an inactive

monomer considered to be a general stress responsive factor which is expressed ubiquitously and is activated by mild HS as well as multiple environmental or physiological stresses. HSF2 is a short-lived protein present as an inactive dimer refractory to typical stress stimuli except proteasome inhibitors and is considered to be important during embryogenesis and spermatogenesis. HSF3 is also an inactive dimer and an important co-regulator of HSF1, activated by severe HS and chemical stress. HSF3 may exhibit complex interactions with other transcription factors governing development, growth and apoptosis, such as c-Myc and p53. HSF4 constitutively binds DNA even in non-stressed cells and is preferentially expressed in muscle, brain and pancreas (Verbeke *et al.*, 2001b).

In unstressed cells, HSF1 is both located in the cytoplasm and in the nucleus. It is maintained as a non DNA-binding inactive complex both by internal coiled-coil interactions and by stoichiometric binding with HSP90, HSP70 and other chaperones. The synergistic interaction between these chaperones modulates HSF1 activity by feedback repression (Shamovsky and Gershon, 2004). During and after stress, the cellular proteins undergo denaturation and/or polyubiquitination and sequester the chaperones capping HSF1. The inactive HSF1 becomes free and translocates into the nucleus. HSF have a nuclear localization sequence that is both necessary for the transition of HSF from inactive to active state and for nuclear import. HSF1 is activated by trimerization and subsequent phosphorylation (Kiang and Tsokos, 1998). Using electrophoretic mobility shift assay, we have demonstrated that RMHS at 41°C activates HSF1 and facilitates its nuclear translocation and DNA binding in human skin fibroblasts, thus initiating the HS response. No studies have yet been performed on other HSF, and also it is not known whether mild stress activates HSF to the same extent as a severe stress at higher temperatures (Shamovsky and Gershon, 2004).

Heat shock proteins (HSP)

Genes encoding HSP are highly conserved. Many of their products can be assigned to families on the basis of sequence homology and molecular weight. In mammals, many HSP families comprise multiple members that differ in inducibility, intracellular localization and function (Park *et al.*, 2005; Sørensen *et al.*, 2003; Verbeke *et al.*, 2001b). HSP are known to play diverse roles as chaperones and/or proteases. In unstressed cells, HSP act in successful folding, assembly, intracellular localization, secretion, regulation and degradation of other proteins. Under conditions in which protein folding is perturbed or proteins begin to unfold and denature, HSP have been shown to assist in protein refolding, to protect cellular systems against protein damages, to dissolve protein aggregates to some extent, to sequester overloaded and damaged proteins into larger aggregates, to target damaged proteins to degradation, and to interfere

with the apoptotic programme. Chaperones and proteases can recognise the same protein substrates and the abundance of both types of proteins suggests that HSP are able to distinguish between those proteins that can be refolded and those fated to enter the proteolytic pathway (Kiang and McClain, 2003; Söti and Csermely, 2000; Söti et al., 2003).

Some HSP are known to be chaperones and are involved in the renaturation of unfolded proteins. Chaperones recognize and bind to other proteins when they are in non-native conformations and are exposing hydrophobic sequences. Their role is to minimize the aggregation of non-native proteins formed during stress. Typically, chaperones function as oligomers, if not as a complex of several different chaperones, co-chaperones and/or nucleotide exchange factors (Feder and Hofmann, 1999). In response to heat and oxidative stresses, different small HSP (sHSP) either become phosphorylated or dephosphorylated. Depending on their phosphorylation status, sHSPs form large (300-800 kDa) and active oligomers having an ATP-independent chaperone activity. sHSPs and HSP90 families capture unfolded proteins and create a reservoir of folding intermediates preventing further aggregation. Subsequently, HSP70 and HSP60 families, helped by cochaperones, bind to the stabilized unfolded proteins in the cytosol, mitochondria and endoplasmic reticulum and attempt to restore the structure of proteins in a cycle driven by ATP-hydrolysis. If the target protein is damaged by post-translational modifications, it could be repaired by specific cellular systems before refolding, but such systems exist for only few kind of damages (Verbeke *et al.*, 2001b).

Acting as a molecular chaperones, HSP protect many different systems involved in maintenance of cellular functions. sHSP induce an increase of the cellular GSH level leading to the protection of the mitochondrial membrane potential during stress (Préville et al., 1999). HSP70 contains a novel nuclear localisation signal in its C-terminal domain implying a role for HSP70 in the regulation of nuclear proteins and transcription factors such as HSF. Members of HSP70 and HSP90 families are associated with the centrosome, suggesting an involvement in microtubule nucleation or in centrosome assembly. The protection of protein synthesis during stress, called translational thermotolerance, is due to the association of HSP72 with ribosomal subunits in polysomes of thermotolerant cells. Some chaperones such as the sHSP α_2 -crystallin and HSP90 could stabilize a more active conformation of the proteasome (Verbeke *et al.*, 2001b).

Members of the HSP90 family constitute 1-2% of cytosolic proteins and have stress-related as well as housekeeping functions. HSP90 stabilize damaged proteins during and after stress. HSP90 interact and either modulate the assembly, the stability and/or the activity of particular cellular proteins such as protein kinases, calcineurin, calmodulin, nitric oxide synthase, telomerase, steroid receptors, oncogenes and transcription factors (Verbeke *et al.*, 2001b). HSP90 is presented as a suppressor of

cryptic genetic variations by assisting mutant proteins to maintain a wild type structure and function (Rutherford and Lindquist, 1998). HSP90 and p23 play also a direct role in the regulation of the HS response by modulating the HSF1 activation/deactivation process. Since HSP90 exists in homeostasis with intracellular hormone receptor and HSF1, it could be hypothesized that steroid hormones activate the HSF by altering this homeostasis. HSP90, HSP70, HSP60 and p23 make heterocomplex with a variety of transcription factors and protein kinases involved in mitogenic signal transduction. The major function of this complex may be to fold the client protein and to keep it inactive until it reaches its ultimate location. There is also a potential involvement of HSP70 and HSP90 in DNA replication since members of these families interact with components of the eukaryotic cell cycle. HSP70, HSP90, HSP27 and TCP-1 are known to bind and stabilize actin, tubulin and the microtubules/ microfilament network playing a role in cellular morphology and signal transduction pathways. The HSP60/HSP10 chaperonin system is localized primarily in the matrix space of mitochondria where it assists in folding, refolding and/or elimination of mitochondrial proteins (Kiang and Tsokos, 1998; Verbeke *et al.*, 2001b).

Our studies show that the basal levels of both the constitutive HSC70 and stress-inducible HSP70 and HSP27 proteins increase during cellular aging of human skin fibroblasts even without any HS (Fonager *et al.*, 2002). A similar increase in the basal level of HSP22 in aged *Drosophila* (King and Tower, 1999), and HSP70 in rat kidneys (Maiello *et al.*, 1997) has been reported previously and is taken as the cells' adaptive response to increased intracellular stress during aging. Therefore, it appears that increased levels of HSP27, HSC70 and HSP70 in senescent cells are indicative of their failed attempt to maintain structural and functional ability and to survive for as long as possible. In comparison, exposing these cells to repeated bouts of mild stress stimulates the synthesis of these HSP, maintains their levels high and helps to improve the functional ability and survival of cells without interfering with their replicative lifespan (Fonager *et al.*, 2002). Further analysis of the activities and different modes of action of these HSP and the molecular significance of their increased levels during cellular aging and RMHS treatment is yet to be performed.

In contrast to the increase in the basal level of some HSP discussed above, the basal levels of HSP90 decreased significantly during cellular aging with and without RMHS treatment (Fonager *et al.*, 2002). Although the exact mechanism for the disappearance of HSP90 is not fully understood, it has been proposed that HSP90 during stress binds to partially unfolded proteins and is degraded together with them in a manner similar to what can be observed for HSP70 after HS (Buchner, 1999). Furthermore, HSP90 is a powerful modulator of the HS transcription factor HSF1 activation, and the deletion of HSP90 has been shown to pro-

mote yeast cells' ability to launch a stress response (Harris et al., 2001). Therefore, it is possible that a decrease in the level of HSP90 during cellular aging and after RMHS treatment is also an adaptive response resulting in the activation of HSF1, which then stimulates the transcription and translation of other HSP.

Some HSP are known to be proteases or to make up the components of a protease system involved in the degradation of the damaged proteins. The unrepairable state of a protein could be signalled to the HSP by the extent of unrepairable modifications, such as carbonylation (Dukan et al., 2000). HSP70 and its cofactors as well as HSC70, HSP90 are involved in the recognition and the degradation of unnecessary and damaged proteins by the proteasome pathway (*discussed below*). Decreased association of certain proteins with HSP90 and increased association with HSP60/HSP70 lead to their 20S proteasome-mediated degradation. HSP70 has been shown to promote the poly-ubiquitination of damaged proteins. Ubiquitination seems also to be involved in the degradation of unfolded polypeptide by the lysosome. One major mechanism of the lysosomal degradation of proteins is dependent on HSC73 and is responsible for the degradation of a significant amount of the cytosolic protein (Cuervo and Dice, 2000).

Protein degradation

One of the main effects of RMHS on human cells is the reduction in the extent of accumulation of oxidatively and glycoxidatively damaged proteins (Verbeke et al., 2000; 2001a). Although this may be due to an increase in cellular resistance of RMHS-treated cells to glucose and other protein damaging agents (Verbeke et al., 2002), another possibility is the enhanced removal of abnormal proteins by increased turnover. The bulk of proteolysis is carried out by the ubiquitin-proteasome system in eukaryotes. The proteasome is a multisubunit, multicatalytic proteinase complex, also known as multicatalytic proteinase (MCP). Oxidised proteins are preferentially degraded by the 20S proteasome in an ATP-independent manner, whereas the proteins marked by covalently attached ubiquitin are degraded in an ATP-dependent way by the 26S proteasome, which is ubiquitous among eukaryota, archaeobacteria, eubacteria, and prokaryota (Grune, 2000; Rivett et al., 2002; Shringaarpure and Davies, 2002). The eukaryotic proteasome is present both in the nucleus and in the cytoplasm and constitutes approximately 1% of the total content of cytosolic protein. Polypeptides to be degraded are covalently attached to ubiquitin, which is itself an extremely conserved and heat-inducible HSP. The substrates for the proteasome can be categorised as either misfolded, denatured and otherwise damaged proteins, or perfectly healthy proteins, which have to be removed for normal functioning of the cell, such as cell cycle control, protein quality control, apoptosis and antigen presentation.

During aging, there is a decline in the activities of the proteasome, including a decreased activity of the proteasome towards artificial peptide substrates as well as the ability to preferentially degrade oxidized proteins (Sitte et al., 1998; Sitte et al., 2000).

We have found that human skin fibroblast cells exposed to RMHS had 20-100% increased proteasome activities, without any accompanied increase in the 20S proteasomal content. However, these hormetic effects of proteasome stimulation by mild heat stress can be dependent on the cell cycle status of the cells. Furthermore, we have observed that this increase in proteasomal activities was related to a significant increase in the amount of the proteasome activator 11S, which is an adaptor between the 20S proteasome and some of the chaperones in the cytosol. The increase of the 20S may be due to an increase in its transcription and translation of 11S activator, an increase in its binding to the 20S proteasome, and a higher level of HSPs in RMHS-treated cells. Although we have not yet determined the extent of transcription, it has been observed that the amount of 11S activator bound to the 20S proteasome was significantly higher in RMHS-treated cells (Beedholm et al., 2004). Such an increased binding makes it possible for the RMHS-treated cells to activate the proteasome faster than the unstressed cells.

Lysosome is the other major cellular proteolytic system affected by aging. The HSC73-specific lysosomal-proteolytic-pathway is inhibited in senescent fibroblasts (Cuervo and Dice, 1996; 2000; Hallén, 2002). Accumulation of lipofuscin, which is an aggregate of oxidized proteins and lipids, affects the lysosomal activities (Terman et al., 1999; Terman and Brunk, 1998). Other typical cellular inclusions in senescent cells contain over-aggregated proteins as well as chaperones and proteasome components as if both chaperones and proteases have capitulated in face of various insults. A decline in HSF and HSP activity, if not always a decline in their expression, and decrease in the activities of antioxidant enzymes are thought to underlie human neurodegenerative diseases. This is because imbalances of the cellular redox status and lack of chaperone activity promote protein aggregation and favour the development of aging-linked pathologies including cataract, polyglutamine-related-disorders or other neurodegenerative diseases as well as cancer (Söti and Csermely, 2000; Söti *et al.*, 2003; Verbeke *et al.*, 2001b). Severe stress may also promote some of these pathologies more directly by a transcription pathway. Accumulation of oxidized and aggregated proteins could be responsible for the increase in the constitutive expression of some HSPs such as HSP22, HSC70 and HSP70 observed in aged animals, especially in tissues formed by post-mitotic cells exposed to stress for a long period of time (King and Tower, 1999). However, no studies have yet been done on the effects of RMHS on lysosome-mediated protein degradation.

CONCLUSIONS

Anti-aging hormetic effects of mild heat shock appear to be facilitated by reducing protein damage and protein aggregation by activating internal antioxidant, repair and degradation processes. HSP are involved in preventing the accumulation of highly damaged proteins during aging since they govern both the repair of weakly damaged proteins and the catabolism of highly damaged proteins. Thus, hormetic pathways are suggested to activate several key proteins involved in the stress response. Indeed, hormesis leads to the maintenance of the HS response during aging and the concomitant transitory and moderate over-expression of HSP in cells and organisms is greatly beneficial.

Increased expression of HSP70 by genetic manipulations may (Tatar et al., 1997) or may not extend the lifespan in *Drosophila* (Minois et al., 2001; Minois and Vaynberg, 2002), but repeated exposure to mild heat stress increases their lifespan (Hercus et al., 2003). In neurodegenerative diseases related to polyglutamine expansion, cotransfection of several chaperone and cochaperone have significantly reduced the formation of aggregates (Verbeke et al., 2001b). However, constitutive over-expression of individual HSP may be toxic for organisms because a chaperone works with a complex of activatory and inhibitory cochaperones and if some are present in large excess or without others, chaperone sequestration, protein aggregation, retardation in cell growth and enhanced apoptosis can occur (Nardai et al., 2002; Verbeke et al., 2001b).

Cellular resistance to stress has been correlated with longevity, supporting the view that the machinery of the cellular stress response is functionally important in aging (Kapahi et al., 1999; Parsons, 2002). Furthermore, selected lines of long living mutant *Drosophila* and nematodes display an overexpression of HSP and antioxidant enzymes and have a stress-resistant phenotype, and organisms selected for stress-resistance have increased longevity (Johnson et al., 2001; Murakami and Johnson, 1998; 2001). Age-dependent regulation of heat shock response may implicate a common protective mechanism against deleterious effect of aging, which may be amenable to modulation through hormesis.

ACKNOWLEDGEMENTS

Research grants from the Danish Medical Council (FSS), Danish Research Council (FNU), and Senetek PLC are acknowledged.

REFERENCES

- Anson RM, Guo Z, de Cabo R, Lyun T, Rios M, Hagepanos A, Ingram DK, Lane MA, and Mattson MP. 2003. Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from calorie restriction. Proc Natl. Acad. Sci. USA 100: 6216-6220

- Beedholm R, Clark BFC, and Rattan SIS. 2004. Mild heat stress stimulates proteasome and its 11S activator in human fibroblasts undergoing aging in vitro. *Cell Stress & Chaperones* 9: 49-57
- Buchner J. 1999. Hsp90 & co. - a holding for folding. *TIBS* 24: 136-141
- Butov A, Johnson TE, Cypser J, Sannikov I, Volkov M, Sehl M, and Yashin AI. 2001. Hormesis and debilitation effects in stress experiments using the nematode worm *Caenorhabditis elegans*: the model of balance between cell damage and HSP level. *Exp. Gerontol.* 37: 57-66
- Cuervo AM, and Dice JF. 1996. A receptor for the selective uptake and degradation of proteins by lysosomes. *Science* 273: 501-503
- Cuervo AM, and Dice JF. 2000. When lysosomes get old. *Exp. Gerontol.* 35: 119-131
- Cypser JR, and Johnson TE. 2002. Multiple stressors in *Caenorhabditis elegans* induce stress hormesis and extended longevity. *J. Gerontol. Biol. Sci.* 57A: B109-B114
- Dorion S, and Landry J. 2002. Activation of the mitogen-activated protein kinase pathways by heat shock. *Cell Stress & Chaperones* 7: 200-206
- Dukan S, Farewell A, Ballesteros M, Taddei F, Rodman M, and Nyström T. 2000. Protein oxidation in response to increased transcriptional or translational errors. *Proc. Natl. Acad. Sci. USA* 97: 5746-5749
- Feder ME, and Hofmann GE. 1999. Heat-shock proteins, molecular chaperones, and the stress response. *Annu. Rev. Physiol.* 61: 243-282
- Fonager J, Beedholm R, Clark BFC, and Rattan SIS. 2002. Mild stress-induced stimulation of heat shock protein synthesis and improved functional ability of human fibroblasts undergoing aging in vitro. *Exp. Gerontol.* 37: 1223-1238
- Gabai VL, Meriin AB, Yaglom JA, Volloch VZ, and Sherman MY. 1998. Role of Hsp70 in regulation of stress-kinase JNK: implications in apoptosis and aging. *FEBS Lett.* 438: 1-4
- Gabai VL, and Sherman MY. 2002. Interplay between molecular chaperones and signaling pathways in survival of heat shock. *J. Appl. Physiol.* 92: 1743-1748
- Grune T. 2000. Oxidative stress, aging and the proteasomal system. *Biogerontology* 1: 31-40
- Hallén A. 2002. Accumulation of insoluble protein and aging. *Biogerontology* 3: 307-315
- Harris N, MacLean M, Hatzianthis K, Panaretou B, and Piper PW. 2001. Increasing *Saccharomyces cerevisiae* stress resistance, through the overactivation of the heat shock response resulting from defects in the Hsp90 chaperone, does not extend replicative life span but can be associated with slower chronological ageing of nondividing cells. *Mol Genet Genomics* 265: 258-263.
- Hercus MJ, Loeschke V, and Rattan SIS. 2003. Lifespan extension of *Drosophila melanogaster* through hormesis by repeated mild heat stress. *Biogerontology* 4: 149-156
- Johnson TE, de Castro E, de Castro SH, Cypser J, Henderson S, and Tedesco P. 2001. Relationship between increased longevity and stress resistance as assessed through gerontogene mutations in *Caenorhabditis elegans*. *Exp. Gerontol.* 36: 1609-1617
- Johnson TE. 2002. A personal retrospective on the genetics of aging. *Biogerontology* 3: 7-12
- Kapahi P, Boulton ME, and Kirkwood TBL. 1999. Positive correlation between mammalian life span and cellular resistance to stress. *Free Radic. Biol. Med.* 26: 495-500
- Khazaeli AA, Tatar M, Pletcher SD, and Curtsinger JW. 1997. Heat-induced longevity extension in *Drosophila*. I. Heat treatment, mortality, and thermotolerance. *J. Gerontol. Biol. Sci.* 52A: B48-B52
- Kiang JG, and Tsokos GC. 1998. Heat shock protein 70 kDa: molecular biology, biochemistry, and physiology. *Pharmacol. Ther.* 80: 183-201
- Kiang JG, and McClain DE. 2003. Heat stress. In: Tsokos GC, and Atkins JL (ed), *Combat Medicine: Basic and clinical research in military, trauma, and emergency medicine.* 83-101 pp. Humana Press. New Jersey
- Kiang JG, McClain DE, Warke VG, Krishnan S, and Tsokos GC. 2003. Constitutive NO synthase regulates the Na⁺/Ca²⁺ exchanger in human T cells: role of [Ca²⁺]_i and tyrosine phosphorylation. *J. Cell. Biochem.* 89: 1030-1043
- King V, and Tower J. 1999. Aging-specific expression of *Drosophila hsp22*. *Dev. Biol.* 207: 107-118
- Le Bourg E, Valenti P, Lucchetta P, and Payre F. 2001. Effects of mild heat shocks at young age on aging and longevity in *Drosophila melanogaster*. *Biogerontology* 2: 155-164
- Lithgow GJ, White TF, Hinerfeld DA, and Johnson TE. 1994. Thermotolerance of a long-lived mutant of *Caenorhabditis elegans*. *J. Gerontol.* 49: B270-B276
- Lithgow GJ, White TM, Melov S, and Johnson TE. 1995. Thermotolerance and extended life-span conferred by single-gene mutations and induced by thermal stress. *Proc. Natl. Acad. Sci. USA* 92: 7540-7544

Heat shock mediated anti-aging hormesis

- Maiello M, Boeri D, Sampietro L, Pronzato MA, Odetti P, and Marinari UM. 1997. Basal synthesis of heat shock protein 70 increases with age in rat kidneys. *Gerontol.* 44: 15-20
- Meriin AB, Yaglom JA, Gabai VL, Zon L, Ganiatsas S, Mosser DD, and Sherman MY. 1999. Protein-damaging stresses activate c-Jun N-terminal kinase via inhibition of its dephosphorylation: a novel pathway controlled by HSP72. *Mol. Cell. Biol.* 19: 2547-2555
- Michalski AI, Johnson TE, Cypser JR, and Yashin AI. 2001. Heating stress patterns in *Caenorhabditis elegans* longevity and survivorship. *Biogerontology* 2: 35-44
- Minois N. 2000. Longevity and aging: beneficial effects of exposure to mild stress. *Biogerontology* 1: 15-29
- Minois N, Khazaeli AA, and Curtsinger JW. 2001. Locomotor activity as a function of age and life span in *Drosophila melanogaster* overexpressing hsp70. *Exp. Gerontol.* 36: 1137-1153
- Minois N, and Vaynberg S. 2002. Fecundity and life span in transgenic *Drosophila melanogaster* overexpressing hsp70. *Biogerontology* 3: 301-306
- Murakami S, and Johnson TE. 1998. Life extension and stress resistance in *Caenorhabditis elegans* modulated by the *thr-1* gene. *Curr. Biol.* 8: 1091-1094
- Murakami S, and Johnson TE. 2001. The OLD-1 positive regulator of longevity and stress resistance is under DAF-16 regulation in *Caenorhabditis elegans*. *Curr. Biol.* 11: 1517-1523
- Nardai G, Csermely P, and Söti C. 2002. Chaperone function and chaperone overload in the aged. A preliminary analysis. *Exp. Gerontol.* 37: 1257-1262
- Park HG, Han SI, Oh SY, and Kang HS. 2005. Cellular responses to mild heat stress. *Cell. Mol. Life Sci.* 62: 10-23
- Park J, and Liu YC. 2001. JNK phosphorylates the HSF1 transcriptional activation domain: role of JNK in the regulation of the heat shock response. *J. Cell. Biochem.* 82: 326-338
- Parsons PA. 2002. Life span: does the limit to survival depend upon metabolic efficiency under stress? *Biogerontology* 3: 233-241
- Prévile X, Salvemini F, Giraud S, Chafour S, Paul C, Stepien G, Ursini MV, and Arrigo AP. 1999. Mammalian small stress proteins protect against oxidative stress through their ability to increase glucose-6-phosphate dehydrogenase activity and by maintaining optimal cellular detoxifying machinery. *Exp. Cell Res.* 247: 61-78
- Raji NS, Surekha A, and Subba Rao K. 1998. Improved DNA-repair parameters in PHA-stimulated peripheral blood lymphocytes of human subjects with low body mass index. *Mech. Ageing Dev.* 104: 133-148
- Rattan SIS. 1998. Repeated mild heat shock delays ageing in cultured human skin fibroblasts. *Biochem. Mol. Biol. Int.* 45: 753-759
- Rattan SIS, Eskildsen-Helmond YEG, and Beedholm R. 2003. Molecular mechanisms of anti-aging hormetic effects of mild heat stress on human cells. *Nonlinear. Biol. Toxicol. Med.* 2: 105-116
- Rattan SIS. 2004. Aging intervention, prevention, and therapy through hormesis. *J. Gerontol. Biol. Sci.* 59A: 705-709
- Rattan SIS. 2005. Principles and practice of hormesis as an aging intervention. In: Rattan SIS (ed), *Aging Interventions and Therapies*. 365-378 pp. World Scientific Publishers. Singapore
- Rivett AJ, Bose S, Pemberton AJ, Brooks P, Onion D, Shirley D, Stratford FLL, and Forti K. 2002. Assay of proteasome activity in relation to aging. *Exp. Gerontol.* 37: 1217-1222
- Rutherford SL, and Lindquist S. 1998. Hsp90 as a capacitor for morphological evolution. *Nature* 396: 336-342
- Shama S, Lai C-Y, Antoniazzi JM, Jiang JC, and Jazwinski SM. 1998. Heat stress-induced life span extension in yeast. *Exp. Cell Res.* 245: 379-388
- Shamovsky I, and Gershon D. 2004. Novel regulatory factors of HSF-1 activation: facts and perspectives regarding their involvement in the age-associated attenuation of the heat shock response. *Mech. Age. Dev.* 125: 767-775
- Shringarpure R, and Davies KJA. 2002. Protein turnover by the proteasome in aging and disease. *Free Rad. Biol. Med.* 32: 1084-1089
- Sitte N, Merker K, and Grune T. 1998. Proteasome-dependent degradation of oxidized proteins in MRC-5 fibroblasts. *FEBS Lett.* 440: 399-402
- Sitte N, Merker K, von Zglinicki T, and Grune T. 2000. Protein oxidation and degradation during proliferative senescence of human MRC-5 fibroblasts. *Free Rad. Biol. Med.* 28: 701-708
- Sørensen JG, Kristensen TN, and Loeschcke V. 2003. The evolutionary and ecological role of heat shock proteins. *Ecology Letters* 6: 1025-1037

- Söti C, and Csermely P. 2000. Molecular chaperones and the aging process. *Biogerontology* 1: 225-233
- Söti C, Sreedhar AS, and Csermely P. 2003. Apoptosis, necrosis and cellular senescence: chaperone occupancy as a potential switch. *Aging Cell* 2: 39-45
- Söti C, Pál C, Papp B, and Csermely P. 2005. Molecular chaperones as regulatory elements of cellular networks. *Curr. Opin. Cell. Biol.* 17: 210-215
- Tatar M, Khazaeli AA, and Curtsinger JW. 1997. Chaperoning extended life. *Nature* 390: 30
- Terman A, and Brunk UT. 1998. Ceroid/lipofuscin formation in cultured human fibroblasts: the role of oxidative stress and proteolysis. *Mech. Ageing Dev.* 104: 277-291
- Terman A, Abrahamsson N, and Brunk UT. 1999. Ceroid/lipofuscin-loaded human fibroblasts show increased susceptibility to oxidative stress. *Exp. Gerontol.* 34: 755-770
- Verbeke P, Clark BFC, and Rattan SIS. 2000. Modulating cellular aging in vitro: hormetic effects of repeated mild heat stress on protein oxidation and glycation. *Exp. Gerontol.* 35: 787-794
- Verbeke P, Clark BFC, and Rattan SIS. 2001a. Reduced levels of oxidized and glycoxidized proteins in human fibroblasts exposed to repeated mild heat shock during serial passaging in vitro. *Free Rad. Biol. Med.* 31: 1593-1602
- Verbeke P, Fonager J, Clark BFC, and Rattan SIS. 2001b. Heat shock response and ageing: mechanisms and applications. *Cell Biol. Int.* 25: 845-857
- Verbeke P, Deries M, Clark BFC, and Rattan SIS. 2002. Hormetic action of mild heat stress decreases the inducibility of protein oxidation and glycooxidation in human fibroblasts. *Biogerontology* 3: 105-108
- Yashin AI, Cypser JR, Johnson TE, Michalski AI, Boyko SI, and Novoseltsev VN. 2001. Ageing and survival after different doses of heat shock: the results of analysis of data from stress experiments with the nematode worm *Caenorhabditis elegans*. *Mech. Ageing Dev.* 122: 1477-1495
- Yokoyama K, Fukumoto K, Murakami T, Harada S, Hosono R, Wadhwa R, Mitsui Y, and Ohkuma S. 2002. Extended longevity of *Caenorhabditis elegans* by knocking in extra copies of hsp70F, a homolog of mot-2 (mortalin)/mthsp70/Grp75. *FEBS Lett.* 516: 53-57