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MOLECULAR MECHANISMS OF ANTI-AGING HORMETIC EFFECTS OF MILD HEAT STRESS ON HUMAN CELLS

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□ *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. *We have reported the hormetic effects of repeated challenge at the levels of maintenance of stress protein profile; 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. We are now undertaking a detailed analysis of the signal transduction pathways to determine alterations in the phosphorylation and dephosphorylation states of extracellular signal-related kinase, c-Jun terminal kinase and p38 MAP-kinases as a measure of cellular responsiveness to mild and severe heat stress. Furthermore, we are also undertaking comparative studies using non-aging immortal cell lines, such as SV40-transformed human fibroblasts, spontaneous osteosarcoma cells, and telomerase-immortalized human bone marrow cells for establishing differences in normal and cancerous cells with respect to their responsiveness to mild and severe stresses.*

Keywords. *aging, anti-aging, heat shock, signal transduction, proteasome*

INTRODUCTION

The possibility of modulating the process of aging through hormesis is rooted in the idea of making use of the fundamental characteristic of living systems, that is, their ability of self-maintenance and repair. Because aging is characterized by a decrease in the adaptive abilities due to progressive failure of maintenance and repair mechanisms, 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 up-regulated and the related pathways of maintenance and repair are stimulated, one should observe anti-aging, health-improving, and longevity-promoting effects. Such a phenomenon in which stimulatory responses to low doses of otherwise harmful conditions improve the functional ability of cells and organisms is known as hormesis.

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However, the harmful effects of high doses have long shadowed the hormetic effects of low-level stress (Calabrese and Baldwin, 2000, 2003). Although most of the work on hormesis has been driven by pharmacological and toxicological studies, applying hormesis in aging research and therapy is a recent development (Johnson and Bruunsgaard, 1998; Kiang and Tsokos, 1998; Rattan, 1998, 2001).

During the past few years, research done in our labs has shown hormetic effects of mild heat shock on human skin fibroblasts. Using a mild stress regime 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. These effects included the maintenance of youthful morphology, reduced accumulation of damaged proteins (Rattan, 1998; Verbeke *et al.*, 2000, 2001a, 2002), increased levels of various heat shock proteins, increased proteasomal activities, increased antioxidative abilities, and increased resistance to ethanol, hydrogen peroxide, and UV-A irradiation (Fonager *et al.*, 2002). Here we review the available data on understanding the molecular mechanisms responsible for bringing about the range of hormetic effects of repeated mild heat stress (RMHS).

HEAT SHOCK RESPONSE

Heat shock (HS) response is one of the primordial intracellular defence mechanisms against stressful conditions. Exposure of cells and organisms to stresses such as high temperature, caloric restriction, exercise, oxidative and osmotic stress, heavy metals, proteasome inhibitors, amino acids analogs, ethanol, glutathione depletion, calcium ionophores, and metabolic poisons induce the cellular stress response leading to the preferential transcription and translation of heat shock proteins (HSPs). Figure 1 gives an overview of the HS response where 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 (HSFs), DNA binding of HSF, initiation of HS gene transcription, and preferential translation of HSP, which then perform various biological functions discussed in the following.

Optimal HS response in terms of HSP synthesis and activity is essential for cell survival (Kiang and Tsokos, 1998). In contrast, inefficient and altered HS response has been implicated in abnormal growth and development, aging, and apoptosis (Söti and Csermely, 2000; 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 or "survival instinct" response for the defence and maintenance

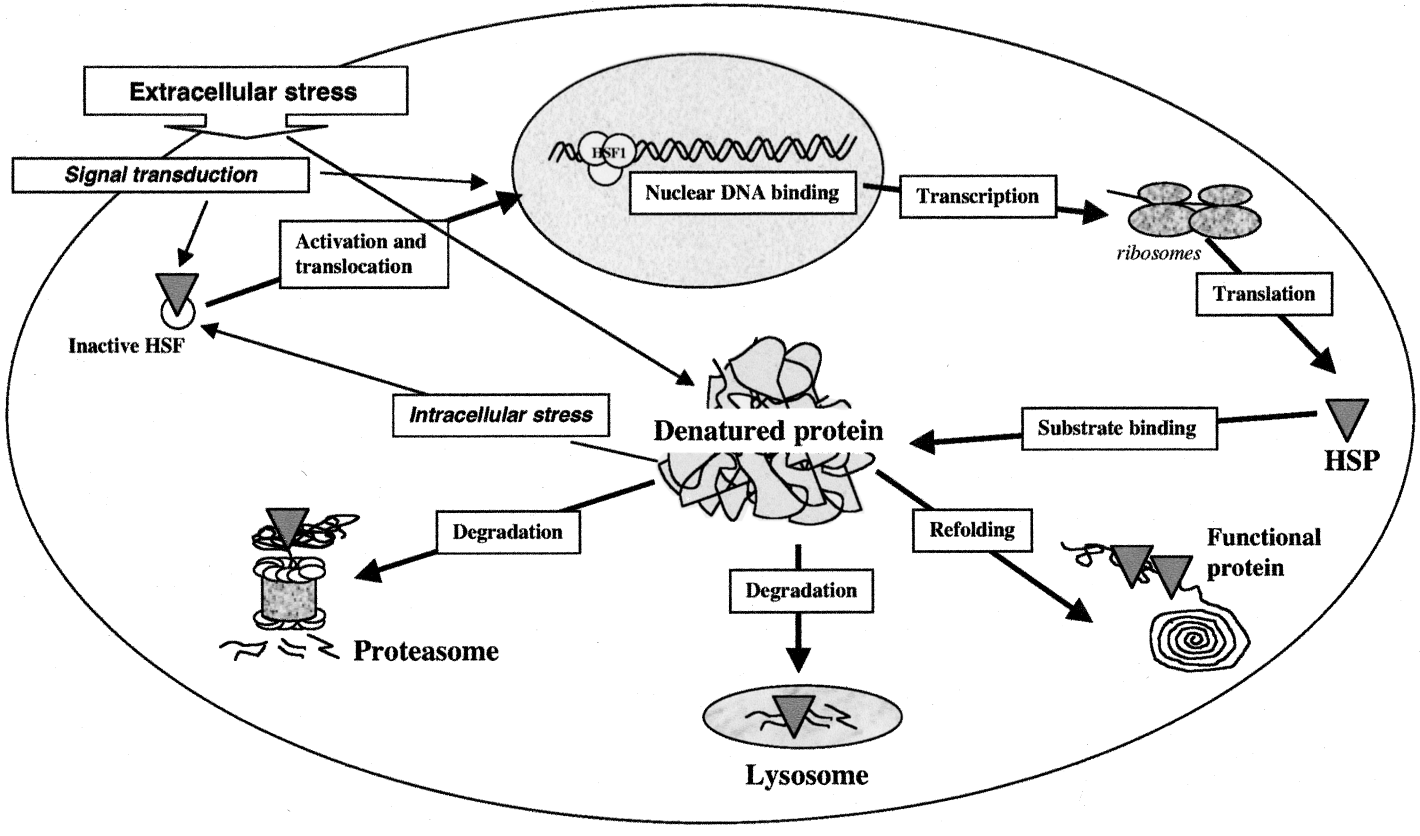

FIGURE 1 Schematic representation of heat shock response in human cells. Both extracellular and intracellular stress can initiate a series of events, starting with the activation, nuclear translocation, and DNA binding of heat shock factors, initiation of heat shock gene transcription, and preferential translation of heat shock proteins, which then perform various biological functions such as protein chaperoning, refolding, and degradation.

of its structural and functional integrity (Verbeke *et al.*, 2001b; Kiang and McClain, 2003). Therefore, any chemical, physical, or biological agent that induces this series of events can be considered a stressor.

Signal Transduction

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. Within minutes, HS activates the major signaling pathways involving mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), and SAPK (Gabai *et al.*, 1998; Dorion and Landry, 2002). 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 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 inducing 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 are now undertaking a detailed analysis of the signal transduction pathways to determine alterations in the phosphorylation and dephosphorylation states of ERK, JNK, and p38 MAP-kinases as a measure of cellular responsiveness to mild and severe heat stress. Preliminary results show that already after 5 min HS at 41°C, there is a 1.5- to 2-fold increase in ERK, p38, and JNK activation in young cells, which increases further to 3-fold activation after 60 min. Further studies are in progress to find out age-related changes in RMHS-related activation of the aforementioned signaling pathways in human cells.

Activation of Heat Shock Factors

The induction of the HS response is through the HSFs 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 for proteasome inhibitors, and is considered to be important during embryogenesis and spermatogenesis. HSF3 is also an inactive dimer and an important coregulator of HSF1 and is 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 nonstressed cells and is preferentially expressed in muscle, brain, and pancreas (Verbeke*et al.*, 2001b).

In unstressed cells, HSF1 is located both 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. 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. HSFs have a nuclear localization sequence that is both necessary for the transition of HSF from the inactive to the 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 (Fonager *et al.*, unpublished data). 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.

Heat Shock Proteins (HSPs)

Genes encoding HSPs 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 (Verbeke*et al.*, 2001b). HSPs are known to play diverse roles as chaperones and/or proteases. In unstressed cells, HSPs 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, HSPs 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 program. Chaperones and proteases can recognize the same protein substrates, and the abundance of both types of proteins suggests that HSPs are able to distinguish between those proteins that can be refolded and those fated to enter the proteolytic pathway (Söti and Csermely, 2000; Kiang and McClain, 2003; Söti *et al.*, 2003).

Some HSPs 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 nonnative conformations and are exposing hydrophobic sequences. Their role is to minimize the aggregation of nonnative 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 HSPs (sHSPs) 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, thereby preventing further aggregation. Subsequently, HSP70 and HSP60 families, helped by co-chaperones, 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 kinds of damage (Verbeke *et al.*, 2001b).

Acting as molecular chaperones, HSPs protect many different systems involved in maintenance of cellular functions. sHSPs 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 localization 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. HSP90s stabilize

damaged proteins during and after stress. HSP90s interact and 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 also play a direct role in the regulation of the HS response by modulating the HSF1 activation/deactivation process. Because 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 a 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 because 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 eliminating 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 HSPs, maintains their high levels, 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 HSPs 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 HSPs discussed earlier, 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 promote 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 HSPs.

Some HSPs 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 signaled to the HSP by the extent of unrepairable modifications, such as carbonylation (Dukan *et al.*, 2000). HSP70 and its cofactors, as well as HSC70, and HSP90, are involved in the recognition and the degradation of unnecessary and damaged proteins by the proteasome pathway (discussed in the following section). 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 polyubiquitination 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. Oxidized 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, archaebacteria, 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 categorized as 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 toward artificial peptide substrates as well as the ability to preferentially degrade oxidized proteins (Sitte *et al.*, 1998, 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. 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 and Brunk, 1998; Terman *et al.*, 1999). Other typical cellular inclusions in senescent cells contain overaggregated proteins as well as chaperones and proteasome components as if both chaperones and proteases have capitulated in the 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 favor the development of aging-linked pathologies including cataract, polyglutamine-related disorders or other neurodegenerative diseases, as well as cancer (Söti and Csermely, 2000; Verbeke *et al.*, 2001b; Söti *et al.*, 2003). Severe stress may also promote some of these pathologies more directly via 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 postmitotic 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. HSPs are involved in preventing the accumulation of highly damaged proteins during aging because 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 overexpression 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 chaperones and co-chaperones have significantly reduced the formation of aggregates (Verbeke *et al.*, 2001b). However, constitutive overexpression of individual HSPs may be toxic for organisms because a chaperone works with a complex of activatory and inhibitory co-chaperones and if some are present in large excess or without others, chaperone sequestration, protein aggregation, retardation in cell growth, and enhanced apoptosis can occur (Verbeke *et al.*, 2001b; Nardai *et al.*, 2002).

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 (Murakami and Johnson, 1998, 2001; Johnson *et al.*, 2001). Age-dependent regulation of heat shock response may implicate a common protective mechanism against deleterious effects of aging, which may be amenable to modulation through hormesis.

REFERENCES

Beedholm R, Clark BFC, Rattan SIS. Mild heat stress stimulates proteasome and its 11S activator in human fibroblasts undergoing aging in vitro. *Cell Stress Chaperon.* 2004;9:49–57.

Buchner J. Hsp90 & co.—A holding for folding. *Trends Biochem Sci.* 1999;24:136–141.

Calabrese EJ, Baldwin LA. Tales of two similar hypotheses: The rise and fall of chemical and radiation hormesis. *Hum Exp Toxicol.* 2000;19:85–97.

Calabrese EJ, Baldwin LA. Toxicology rethinks its central belief. *Nature.* 2003;421:891–892.

Cuervo AM, Dice JF. A receptor for the selective uptake and degradation of proteins by lysosomes. *Science.* 1996;273:501–503.

Cuervo AM, Dice JF. When lysosomes get old. *Exp Gerontol.* 2000;35:119–131.

Dorion S, Landry J. Activation of the mitogen-activated protein kinase pathways by heat shock. *Cell Stress Chaperon.* 2002;7:200–206.

Dukan S, Farewell A, Ballesteros M, Taddei F, Rodman M, Nyström T. Protein oxidation in response to increased transcriptional or translational errors. *Proc Nat Acad Sci USA.* 2000;97:5746–5749.

Feder ME, Hofmann GE. Heat-shock proteins, molecular chaperones, and the stress response. *Annu Rev Physiol.* 1999;61:243–282.

Fonager J, Beedholm R, Clark BFC, Rattan SIS. Mild stress-induced stimulation of heat shock protein synthesis and improved functional ability of human fibroblasts undergoing aging in vitro. *Exp Gerontol.* 2002;37:1223–1238.

Gabai VL, Sherman MY. Interplay between molecular chaperones and signaling pathways in survival of heat shock. *J Appl Physiol.* 2002;92:1743–1748.

Gabai VL, Meriin AB, Yaglom JA, Volloch VZ, Sherman MY. Role of Hsp70 in regulation of stress-kinase JNK: Implications in apoptosis and aging. *FEBS Lett.* 1998;438:1–4.

Grune T. Oxidative stress, aging and the proteasomal system. *Biogerontology* 2000;1:31–40.

Hallén A. Accumulation of insoluble protein and aging. *Biogerontology* 2002;3:307–315.

Harris N, MacLean M, Hatzianthis K, Panaretou B, Piper PW. 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.* 2001;265:258–263.

Hercus MJ, Loeschcke V, Rattan SIS. Lifespan extension of *Drosophila melanogaster* through hormesis by repeated mild heat stress. *Biogerontology.* 2003;4:149–156.

Johnson TE, Bruunsgaard H. Implications of hormesis for biomedical aging research. *Hum Exp Toxicol.* 1998;17:263–265.

Johnson TE, de Castro E, de Castro SH, Cypser J, Henderson S, Tedesco P. Relationship between increased longevity and stress resistance as assessed through gerontogene mutations in *Caenorhabditis elegans*. *Exp Gerontol.* 2001;36:1609–1617.

Kapahi P, Boulton ME, Kirkwood TBL. Positive correlation between mammalian life span and cellular resistance to stress. *Free Radical Biol Med.* 1999;26:495–500.

Kiang JG, McClain DE. Heat stress. In: Tsokos GC, Atkins JL, eds., *Combat Medicine: Basic and Clinical Research in Military, Trauma, and Emergency Medicine.* New Jersey: Humana Press; 2003;83–101.

Kiang JG, Tsokos GC. Heat shock protein 70 kDa: molecular biology, biochemistry, and physiology. *Pharmacol Therapeat.* 1998;80:183–201.

Kiang JG, McClain DE, Warke VG, Krishnan S, Tsokos GC. Constitutive NO synthase regulates the Na+/Ca2+ exchanger in human T cells: Role of [Ca2+]i and tyrosine phosphorylation. *J Cell Biochem.* 2003;89:1030–1043.

King V, Tower J. Aging-specific expression of *Drosophila hsp22. Dev Biol.* 1999;207:107–118.

Maiello M, Boeri D, Sampietro L, Pronzato MA, Odetti P, Marinari UM. Basal synthesis of heat shock protein 70 increases with age in rat kidneys. *Gerontology.* 1997;44:15–20.

Meriin AB, Yaglom JA, Gabai VL, Zon L, Ganiatsas S, Mosser DD, Sherman MY. Protein-damaging stresses activate c-Jun N-terminal kinase via inhibition of its dephosphorylation: A novel pathway controlled by HSP72. *Mol Cell Biol.* 1999;19:2547–2555.

Minois N, Vaynberg S. Fecundity and life span in transgenic *Drosophila melanogaster* overexpressing hsp70. *Biogerontology.* 2002;3:301–306.

Minois N, Khazaeli AA, Curtsinger JW. Locomotor activity as a function of age and life span in *Drosophila melanogaster* overexpressing hsp70. *Exp Gerontol.* 2001;36:1137–1153.

Murakami S, Johnson TE. Life extension and stress resistance in *Caenorhabditis elegans* modulated by the *tkr-1* gene. *Curr Biol.* 1998;8:1091–1094.

Murakami S, Johnson TE. The OLD-1 positive regulator of longevity and stress resistance is under DAF-16 regulation in *Caenorhabditis elegans*. *Curr Biol.* 2001;11:1517–1523.

Nardai G, Csermely P, Söti C. Chaperone function and chaperone overload in the aged. A preliminary analysis. *Exp Gerontol.* 2002;37:1257–1262.

Park J, Liu YC. JNK phosphorylates the HSF1 transcriptional activation domain: Role of JNK in the regulation of the heat shock response. *J Cell Biochem.* 2001;82:326–338.

Parsons PA. Life span: Does the limit to survival depend upon metabolic efficiency under stress? *Biogerontology.* 2002;3:233–241.

Préville X, Salvemini F, Giraud S, Chafour S, Paul C, Stepien G, Ursini MV, Arrigo AP. Mammalian small stress proteins protect against oxidative stress through their ability to increase glucose-6-phosphate dehydrogenease activity and by maintaining optimal cellular detoxifying machinery. *Exp Cell Res.* 1999;247:61–78.

Rattan SIS. Repeated mild heat shock delays ageing in cultured human skin fibroblasts. *Biochem Mol Biol Int.* 1998;45:753–759.

Rattan SIS. Applying hormesis in aging research and therapy. *Hum Exp Toxicol.* 2001;20:281–285.

Rivett AJ, Bose S, Pemberton AJ, Brooks P, Onion D, Shirley D, Stratford FLL, Forti K. Assay of proteasome activity in relation to aging. *Exp Gerontol.* 2002;37:1217–1222.

Rutherford SL, Lindquist S. Hsp90 as a capacitor for morphological evolution. *Nature.* 1998;396:336–342.

Shringaarpure R, Davies KJA. Protein turnover by the proteasome in aging and disease. *Free Radical Biol Med.* 2002;32:1084–1089.

Sitte N, Merker K, Grune T. Proteasome-dependent degradation of oxidized proteins in MRC-5 fibroblasts. *FEBS Lett.* 1998;440:399–402.

Sitte N, Merker K, von Zglinicki T, Grune T. Protein oxidation and degradation during proliferative senescence of human MRC-5 fibroblasts. *Free Radical Biol Med.* 2000;28:701–708.

Söti C, Csermely P. Molecular chaperones and the aging process. *Biogerontology* 2000;1:225–233.

Söti C, Sreedhar AS, Csermely P. Apoptosis, necrosis and cellular senescence: Chaperone occupancy as a potential switch. *Aging Cell.* 2003;2:39–45.

Tatar M, Khazaeli AA, Curtsinger JW. Chaperoning extended life. *Nature.* 1997;390:30.

Terman A, Brunk UT. Ceroid/lipofuscin formation in cultured human fibroblasts: The role of oxidative stress and proteolysis. *Mech Ageing Dev.* 1998;104:277–291.

Terman A, Abrahamsson N, Brunk, UT. Ceroid/lipofuscin-loaded human fibroblasts show increased susceptibility to oxidative stress. *Exp Gerontol.* 1999;34:755–770.

Verbeke P, Clark BFC, Rattan SIS. Modulating cellular aging in vitro: Hormetic effects of repeated mild heat stress on protein oxidation and glycation. *Exp Gerontol.* 2000;35:787–794.

Verbeke P, Clark BFC, Rattan SIS. Reduced levels of oxidized and glycoxidized proteins in human fibroblasts exposed to repeated mild heat shock during serial passaging in vitro. *Free Radical Biol Med.* 2001a;31:1593–1602.

Verbeke P, Fonager J, Clark BFC, Rattan SIS. Heat shock response and ageing: Mechanisms and applications. *Cell Biol Int.* 2001b;25:845–857.

Verbeke P, Deries M, Clark BFC, Rattan SIS. Hormetic action of mild heat stress decreases the inducibility of protein oxidation and glycoxidation in human fibroblasts. *Biogerontology.* 2002;3:105–108.