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REVIEW

HEAT SHOCK RESPONSE AND AGEING: MECHANISMS AND APPLICATIONS

PHILIPPE VERBEKE, JANNIK FONAGER, BRIAN F. C. CLARK and SURESH I. S. RATTAN*

Danish Centre for Molecular Gerontology, Laboratory of Cellular Ageing, Department of Molecular and Structural Biology, University of Aarhus, Denmark

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Ageing is associated with a decrease in the ability of cells to cope with environmental challenges. This is due partly to the attenuation of a primordial stress response, the so-called heat shock (HS) response, which induces the expression of heat shock proteins (HSPs), composed of chaperones and proteases. The attenuation of the HS response during ageing may be responsible for the accumulation of damaged proteins as well as abnormal regulation of cell death. Maintenance of the HS response by repeated mild heat stress causes anti-ageing hormetic effects on cells and organisms. Here, we describe the molecular mechanism and the state of the HS response as well as the role of specific HSPs during ageing, and discuss the possibility of hormetic modulation of ageing and longevity by repeated mild stress.

KEYWORDS: stress; anti-ageing; thermotolerance; hormesis.

INTRODUCTION

The so-called 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 acid analogues, 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) (Feder and Hofmann, 1999; Fehrenbach and Niess, 1999; Jolly and Morimoto, 2000). 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 (Neuer *et al.*, 2000), ageing and apoptosis (Gabai *et al.*, 1998; Söti and Csermely, 2000). In the case of ageing, it has been shown that cells isolated from aged tissues and organisms, and cells undergoing replicative senescence *in vitro*, have a reduced HS response and a higher incidence of death when submitted to stress (Lee *et al.*, 1996; Liu *et al.*, 1996). In this article, we review the literature concerning the HS response with respect to its molecular mechanisms, age-related alterations and the possibilities of its experimental manipulation for slowing down the ageing process.

MOLECULAR MECHANISMS OF HEAT SHOCK RESPONSE

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 responses. Stress activates major signalling pathways such as transcription factors HSF1, NFκb and p53, and other pathways regulated by protein kinases (Finkel and Holbrook, 2000). These

*To whom correspondence should be addressed: Dr Suresh I. S. Rattan, Department of Molecular and Structural Biology, University of Aarhus, Gustav Wieds Vej 10-C, DK-8000, Aarhus-C, Denmark. Fax: +45 86 12 31 78; E-mail: rattan@imsb.au.dk

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pathways are specifically activated depending on the nature and the intensity of the stress and the cell type. The cellular stress response can be viewed as an adaptative or 'survival instinct' response for the defence and maintenance of its structural and functional integrity (Feder and Hofmann, 1999). Therefore, any chemical, physical or biological agent that induces this series of events can be considered as a 'stressor'.

HS transcription factors

The induction of the HS response is through the heat shock transcription factors (HSFs) working as molecular links between environmental stresses and the stress response. Vertebrates express four HSFs encoded by small multigene families and regulated by a diverse array of environmental and developmental signals (Wu, 1995). The four vertebrate HSFs are expressed constitutively and co-operate functionally. HSF1 is a long-lived protein, which 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 (Morimoto *et al.*, 1994). HSF2 is a short-lived protein present as an inactive dimer refractory to typical stress stimuli except proteasome inhibitors (Mathew *et al.*, 1998), and is considered to be important during embryogenesis and spermatogenesis (Mezger *et al.*, 1994; Rallu *et al.*, 1997; Sarge *et al.*, 1994). HSF3 is also an inactive dimer and an important co-regulator of HSF1, activated by severe HS and chemical stress (Kawazoe *et al.*, 1999; Tanabe *et al.*, 1997). HSF3 may exhibit complex interactions with other transcription factors governing development, growth and apoptosis, such as c-Myb and p53 (Tanikawa *et al.*, 2000). HSF4 constitutively binds DNA even in non-stressed cells and is preferentially expressed in muscle, brain and pancreas (Santoro, 2000). Proteasome inhibition and subsequent accumulation of polyubiquitinated proteins is the common activator of HSF1, HSF2 and HSF3 (Kawazoe *et al.*, 1998; Kim *et al.*, 1999; Pirkkala *et al.*, 2000).

In unstressed vertebrate cells, HSF1 is located both in the cytoplasm and in the nucleus (Brown and Rush, 1999; Mercier *et al.*, 1999; Orosz *et al.*, 1996). 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 including HSc70, CyP40, Hdj-1, p23, and FKBPs (Morimoto, 1993; Zuo *et al.*, 1995). 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 if it was previously in the cytosol. HSFs have a nuclear localisation sequence (NLS) that is both necessary for the transition of HSF from inactive to active state and for nuclear import (Nakai and Ishikawa, 2000; Zandi *et al.*, 1997). HSF1 is activated by trimerisation and subsequent phosphorylation. Depending on the stress stimulus, several protein kinases, including ErK1-MAP kinase, glycogen synthase kinase-3, protein kinase C, stress activated protein kinase (SAPK)/jun kinase (JNK) and p38 MAP kinase have been shown to phosphorylate HSF1 (Kim *et al.*, 1999). The active HSF1 binds to the HS responsive element (HSE) present in the promoter of HS genes (Morimoto, 1998). It seems that HSF1 acquires a transcriptional activity when partially dephosphorylated by phosphatases (Kim and Li, 1999; Soncin *et al.*, 2000). Another pathway of HSP induction was recently discovered through a sensor of the cellular redox status which allows the preexisting HSP33 to respond much more quickly to stress than by regulation at the transcriptional or translational level (Jakob *et al.*, 1999). Different HSFs can bind the same HSE to induce expression of the same set of HSPs in cytosol and endoplasmic reticulum.

Attenuation of the stress response after stress may occur by repression of HSF oligomerisation and activation mediated through the recruitment of HSF-binding protein-1 (HSBP-1) or a complex of HSP, which induce the dissociation of HSF timers (Cotto and Morimoto, 1999). Trimerisation and transactivation of HSF are also affected by pH, redox status, temperature and phosphorylation (Bijur *et al.*, 1999; Zhong *et al.*, 1999; Zhong *et al.*, 1998). The attenuation of the HS response can also be related to a preferential degradation of HSP mRNA or of HSP itself (Cotto and Morimoto, 1999). Indeed, HSP70 mRNA contains a 3′UTR AU-rich element (ARE) as a tag for a quick turnover. The ARE-binding proteins (AUFI family) protect this mRNA during the acute HS response, but during the late phase, its degradation could be favoured by a higher degradation of AUF1 (Laroia *et al.*, 1999). It is also suggested that HSF could be degraded either by the ubiquitin–proteasome pathway (Bush *et al.*, 1997; Mathew *et al.*, 1998) or by caspases (Zhang *et al.*, 1999; Zhang *et al.*, 1998). Moreover, by binding constitutively to DNA, HSF4 has been thought to be a

Table 1.
Cellular localization and function of the major HSP families in mammals

Family	*HSP*	*Location*	*Function (reference)*
HSP10	Ubiquitin	Cytosol/nucleus	Tag protein for degradation (8, 9)
	HSP10	Mitochondria	Cofactor of HSP60, tolerance of ischemia (5, 7, 9, 10)
sHSP	HSP27	Cytosol/nucleus	Large oligomers, regulator of cytoskeleton, resistance to oxidative, UV and TNF stresses, tolerance of hyperthermia and ischemia, prevent aggregation, antiapoptotic (1, 4, 5, 10)
	HSP28 crystallin		
HSP30	HSP32	Cytosol/nucleus	Stress inducible, regultion of heme-protein turnover, iron metabolism and oxidative stress (3)
	HO-1		
HSP40	HSP47	Endoplasmic reticulum	Heat inducible, procollagen chaperone (1, 9)
	Hdj 1	Cytosol/nucleus	Cofactor of HSP70, increase ATPase activity and substrate release (5)
	Hdj 2		
HSP60	HSP56	Cytosol	Stress inducible, bind the steroid hormone receptor complex and FK506, rotamase function (9)
	HSP60	Mitochondria	Constitutive/inducible, weak ATPase activity, binding and folding of imported proteins, tolerance of hyperthermia and ischemia (5, 7, 10)
	HSP65		Antitumorigenic action (10)
	TCP-1	Cytosol/nucleus	Constitutive, weak ATPase activity, folding of actin and tubulin (6, 7, 9)
HSP70	HSP70/HSP72	Cytosol/nucleus	Stress inducible, ATPase activity, tolerance of hyperthermia, ischemia/hypoxia, resistance to oxidative, UV and TNF stresses, protection against protein aggregation, regulation of HS response, protection of transcription/translation, tumorigenicity, antiapoptotic (1, 4, 5, 7, 10)
	HSP73/HSC70	Cytosol/nucleus	Constitutive, ATPase activity, folding, trafficking, tolerance of hyperthermia, promote lysosomal degradation (1, 5, 7, 10)
	Grp75	Mitochondria	Constitutive/inducible, ATPase activity, transport and folding of polypeptides into matrix (2)
	Grp78 (BiP)	Endoplasmic reticulum	Constitutive/inducible, ATPase activity, protein secretion and translocation and degradation into ER (1, 2, 7, 8)
HSP90	HSP90 α/β	Cytosol/nucleus	Partly inducible, ATPase activity, autophosphorylation, tolerance of hyperthermia, ischemia, apoptosis, role in cell cycle and proliferation and in signal transduction, prevents aggregation (1, 5, 10)
	Grp94	Endoplasmic reticulum	Constitutive/inducible, ATPase activity, protein folding and secretion (1)

1. Beck *et al.*, 2000; 2. Sharp *et al.*, 1999; 3. Schipper, 2000; 4. Jaattela, 1999a; 5. Jolly and Morimoto, 2000; 6. Georgopoulos and Welch, 1993; 7. Hendrick and Hartl, 1993; 8. Sherman and Goldberg, 1996; 9. Minowada and Welch, 1995; 10. Feder and Hofmann, 1999).

negative regulator of the HS response (Nakai *et al.*, 1997).

HS proteins

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 localisation and function (Table 1). HSPs are known to play diverse roles as chaperones and/or proteases. In unstressed cells, HSPs act in successful folding, assembly, intracellular localisation, 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 damage, to solubilise aggregates to some extent, to sequester overloaded and damaged proteins into larger aggregates, to target ultimately damaged proteins to degradation and to interfere with the apoptotic programme (Jolly and Morimoto, 2000; Mathew and Morimoto, 1998). Chaperones and proteases can recognize the same protein substrates and the abundance of both types of protein suggests that HSP are able to distinguish between those proteins that can be refolded and those fated to

enter the proteolytic pathway (Söti and Csermely, 2000).

Some HSPs are known to be chaperones and are involved in the renaturation of unfolded proteins. Chaperones recognise and bind to other proteins when they are in non-native conformations and are exposing hydrophobic sequences. Their role is to minimise 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 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 preventing further aggregation (Arrigo, 1998). Subsequently, HSP70 and HSP60 families, helped by co-chaperones, bind to the stabilised unfolded proteins in the cytosol, mitochondria and endoplasmic reticulum and attempt to restore the structure of proteins in a cycle driven by ATP hydrolysis (Bruce and Churchich, 1997; Gebauer *et al*., 1997). 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 a few kinds of damage (Verbeke *et al*., 2000). PA28, a proteasome activator presenting chaperone activity, as well as the co-chaperones CHIP and BAG-1, are thought to capture the unfolded protein which is then directed to either refolding or proteasome-mediated degradation (Connell *et al*., 2001; Luders *et al*., 2000; Minami *et al*., 2000).

Acting as molecular chaperones, HSPs protect many different systems involved in maintenance of cellular functions. sHSPs induce an increase in the cellular GSH level leading to the protection of the mitochondrial membrane potential during stress (Preville *et al*., 1999). HSP70 contains a novel NLS in its C-terminal domain implying a role for HSP70 in the regulation of nuclear proteins and transcription factors such as HSF and NF-kB, as well as in repair of heat-induced damage to some nuclear and nucleolar funtions (Laszlo, 1992). Members of HSP70 and HSP90 families are associated with the centrosome, suggesting an involvement in microtuble nucleation or in centrosome assembly (Helmbrecht *et al*., 2000; Lange *et al*., 2000). 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 (Beck and De Maio, 1994). Molecular chaperones are related to the synaptic plasticity phenomena and HSP70 family members are thought to work as a shuttle protein carrier from synapse to nucleus in neurones (Ohtsuka and Suzuki, 2000). Some chaperones such as the sHSP α_2-crystallin and HSP90 could stabilise a more active conformation of the proteasome (Andersson *et al*., 1999).

Members of the HSP90 family constitute 1–2% of cytosolic proteins and have stress-related as well as housekeeping functions (Mayer and Bukau, 1999). HSP90 stabilise 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 (An *et al*., 2000; Lewis *et al*., 2000), calcineurin (Imai and Yahara, 2000), calmodulin, nitric oxide synthase (NOS) (Kone, 2000), chloride channel CFTR, telomerase, steroid receptors, oncogenes and transcription factors such as HSF1 (Mayer and Bukau, 1999; Miyata and Yahara, 2000; Pearl and Prodromou, 2000). These interactions are long lived in contrast to the interactions of protein substrates with HSP60 or HSP70. 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 (Bharadwaj *et al*., 1999). Since HSP90 exists in homeostasis with intracellular hormone receptor and HSF1, it could be hypothesised that steroid hormones activate the HSR by altering this homeostasis (Knowlton and Sun, 2001). 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 (Helmbrecht *et al*., 2000). HSP70, HSP90, HSP27 and TCP-1 are known to bind and stabilise actin, tubulin and the microtubules/microfilament network, playing a role in cellular morphology and transduction pathways (Beck *et al*., 2000). The HSP60/HSP10 chaperonin system is localised primarily in the matrix space of mitochondria, where it assists in folding, refolding and/or elimination of mitochondrial proteins (Beck *et al*., 2000).

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 signalled to the HSPs by the extent of unrepairable modifications, such as carbonylation (Dukan *et al.*, 2000). Bacteria provide good models for the study of HSPs, especially with respect to protein degradation. *Escherichia coli* possess some HSPs, either chaperones or proteases, which cooperate to form complexes which are homologues of the eukaryotic 26S proteasome, removing abnormal proteins and refolding aggregates (Hoskins *et al.*, 2000). The bacterial HtrA complex is particularly interesting since its chaperone function dominates at low temperatures while the proteolytic activity is enhanced at higher temperatures (Spiess *et al.*, 1999), suggesting that folding defects are more easily repaired at a low temperature rather than at an elevated temperature. A human homologue of HtrA carrying both proteolytic and chaperone functions has been cloned (Hu *et al.*, 1998).

The bulk of ATP-dependent proteolysis is carried out by the ubiquitin system in eucaryotes. Polypeptides to be degraded are covalently attached to ubiquitin, which is itself an extremely conserved and heat-inducible HSP (Jennissen, 1995). HSP70 and its cofactors, as well as HSP70 and HSP90, are involved in the recognition and the degradation of unnecessary and damaged proteins by the ubiquitin–proteasome pathway (Connell *et al.*, 2001; Imamura *et al.*, 1998; Jin *et al.*, 2000; Luders *et al.*, 2000). Decreased association of certain proteins with HSP90 and increased association with HSP60/HSP70 lead to their proteasome-mediated degradation (An *et al.*, 2000). Some enzymes of the ubiquitin pathway such as the ubiquitin-conjugating enzymes UBC4 and UBC5 are also heat-inducible HSPs (Hershko and Ciechanover, 1998). HSP70 has been shown to promote the polyubiquitination of damaged proteins (Sherman and Goldberg, 1996). Ubiquitination also seems 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 30% of the cytosolic protein (Cuervo and Dice, 1998).

HSP IN APOPTOSIS AND THERMOTOLERANCE

HSPs also interfere with the apoptotic programme and it is commonly thought that HSP induction and apoptosis are mutually exclusive events within the same cell. Accumulation of sHSPs, HSP60/HSP10 or HSP70/72 after mild HS or due to cell transfection renders several cell types resistant to both caspase-dependent and -independent apoptosis (Brar *et al.*, 1999; Creagh *et al.*, 2000). Moreover, in the absence of HSP27 and HSP72, lethal stresses have been shown to increase ceramide generation, which favours the degradation of the HSP70 mRNA (Kondo *et al.*, 2000). However, in nerve and immune cells, overexpression of HSF1, HSP70, as well as HSP90, generates no anti-apoptotic or pro-apoptotic effects (Galea-Lauri *et al.*, 1996; Mailhos *et al.*, 1994).

Possible mechanisms for the attenuation of apoptosis by HSPs are the inhibition of apoptosome formation, modulation of stress kinase activation and apoptotic signalling molecules, increase in glutathione levels and/or decrease of protein aggregation. HSP70 and HSP90 have been shown to inhibit apoptosis downstream of mitochondrial cytochrome-c release by forming a complex with apoptotic protease activating factor (Apaf-1). This leads to inhibition of association of Apaf-1 with procaspase 9 to form the apoptosome activating caspase 3 (Beere *et al.*, 2000; Mosser *et al.*, 2000; Panday *et al.*, 2000; Saleh *et al.*, 2000). HSP70 also inhibits the DNA-binding activity of the transcription factor NFkB involved in pro-oxidative and pro-inflammatory related apoptosis (Feinstein *et al.*, 1996; Yoo *et al.*, 2000). Under resting conditions, NFkB exists in the cytoplasm as a dimer bound to the inhibitory protein IkBα. HSP70 inhibits NFkB either by competing for access to the nuclear pore, by interacting with the NFkB–IkBα complex upon the dissociation of NFkB, by inhibiting IkBα phosphorylation or ubiquitination, or by inhibiting the proteasome responsible for the degradation of IkBα (Feinstein *et al.*, 1997; Guzhova *et al.*, 1997; Santoro, 2000; Yoo *et al.*, 2000). HSP70 also inhibits members of the stress kinase pathways involved in apoptosis such as SAPK/JNK and p38 kinase (Gabai *et al.*, 1997; Yaglom *et al.*, 1999). Interestingly, chaperone function and the substrate binding domain of HSP70 are unnecessary for the suppression of JNK activation (Mosser *et al.*, 2000). HSP90 protects the serine/treonine kinase Akt/PKB from dephosphorylation and inactivation and inhibits apoptosis (Sato *et al.*, 2000). HSC70 and HSP70 bind and can modulate the activity and/or the localization of anti-apoptotic factors such as Bcl2, while HSP70 binds and promotes the degradation or inactivates pro-apototic factors such as p53 and c-myc (Jolly and Morimoto, 2000). The chaperone function of

HSP70, regulating folding and activity of apoptotic signalling molecules, can be inhibited by other regulators (Thress *et al*., 2001).

Recently, it has been shown that the overexpression of an HSP70 homologue mortalin is correlated with a decrease of p53 function and results in the temporary escape of normal fibroblasts with senescence (Kaul *et al*., 2000). Several reports have also pointed out the anti-apoptotic role of the tripeptide glutathione, suggesting the implication of sHSPs in this process by facilitating the GSH redox cycle and modulating the cellular redox status (Arrigo, 1998). Indeed, it has been shown that the induction of sHSP expression increases the GSH content and is correlated with the inhibition of NFkB activation probably through inhibition of IkBα release that occurs only under oxidative conditions (Arrigo, 1999; Ginn-Pease and Whisler, 1998; Kondo *et al*., 1999). Moreover, HSP27 may delay poly-(ADP)-ribose)-polymerase cleavage and cytochrome c release (Garrido *et al*., 1999; Samali *et al*., 2001). It is suggested that the combined induction of both HSP70 and HSP27 should protect cells from almost all death stimuli (Jäätelä, 1999*b*).

The phenomenon of thermotolerance is defined as the capacity of cells, following a cycle of stress and recovery, to survive a second stress, which would otherwise be lethal. For instance, mild HS pretreatment prevents cell death from a variety of stresses such as hyperthermia, ethanol, heavy metals, oxidative stresses, TNF, IL1β, β-amyloid (β-APP) and energy deprivation (Feder and Hofmann, 1999). Thermotolerance is mainly due to the accurately regulated expression and accumulation of various HSPs in the endoplasmic reticulum and in the cytosol, leading to the activation of macromolecular repair mechanisms as a defensive strategy against subsequent challenges. Controversy exists as to which members of the stress protein family are the most important contributors to the phenomenon of thermotolerance. sHSP, as well as the HSP70 and HSP90 families, helped by smaller co-chaperones such as HSP40 and p23, are involved in thermotolerance (Söti and Csermely, 2000). In thermotolerant cells, sHSPs, HSP70 and HSP90 families and cyclophilins have been shown to stabilise the cytoskeletal network, limiting mitotic abnormalities in case of a severe subsequent stress (Andreeva *et al*., 1999; Georgopoulos and Welch, 1993). HSc70 protects and reactivates nuclear topoisomerase I after heat stress (Ciavarra *et al*., 1994). The ubiquitinating enzyme system is activated after stress and leads to degradation of unrepairable proteins (Fujimuro *et al*., 1997). Interestingly, the thermotolerance process can be exportable, because release and intercellular transfer of exported HSP60 and HSP70 have been reported. Transfer of a protective stress response to neighbouring cells unable to mount such a response or to activate the innate immune system after infection or cell damage may be a homeostatic mechanism (Hightower and Guidon, 1989; Kol *et al*., 2000).

Stress tolerance is not due to HSP overexpression alone, and it is suggested that there are two states of thermotolerance, namely HSP-dependent and HSP-independent states (Laszlo, 1988). Other mechanisms, including the synthesis of osmotic stress protectants, modifications of the saturation of cell membrane lipids, and expression of enzymes such as radical scavenger enzymes are also involved (Feder and Hofmann, 1999). Moreover, several proteins other than HSP contain either an HSE or a stress responsive element (STRE) in the promoter regions of their genes. HS induces the expression of two enzymes (γ-glutamylcysteine synthetase and glutathione synthetase) synthesising glutathione and the enzyme copper/zinc superoxide dismutase SOD1 (Sugiyama *et al*., 2000; Yoo *et al*., 1999). Thioredoxin, another polypeptide modulating the redox status and gene expression, whilst inhibiting apoptosis, has been found to be regulated by HSF2 and the multidrug resistance-1 gene is also activated by HSF (Leppa *et al*., 1997; Miyazaki *et al*., 1992). STRE mediates the stress induction of transcription of a set of genes coding for damage responsive proteins, such as cytosolic catalase, SOD, glyceraldehyde 3-phosphate dehydrogenase, enolase, polyubiquitin, HSP12, HSP104 and proteins involved in DNA repair (Nakagawa *et al*., 1999; Schuller *et al*., 1994). The increase of the two glycolytic enzymes may facilitate increased demand on the glycolytic pathway after metabolic stress. Heat and changes in intracellular redox homeostasis also modulate the proteasome activity (Andersson *et al*., 1999; Conconi *et al*., 1998). Even if most of its components are not heat inducible, the 20S and 26S proteasome are clearly important for stress tolerance. Indeed, the active site of the three main peptidase activities contains cysteine and threonine residues sensitive to the cellular redox status. Also, phosphorylation of the C8 subunit of the proteasome could be dependent on the cellular oxidative status (Friguet *et al*., 2000). It is also suggested that the PR500 complex could be used as a special lid regulator for the 26S proteasome during stressful situations, this regulator being associated with HSP70 under normal conditions (Peng *et al*., 2001).

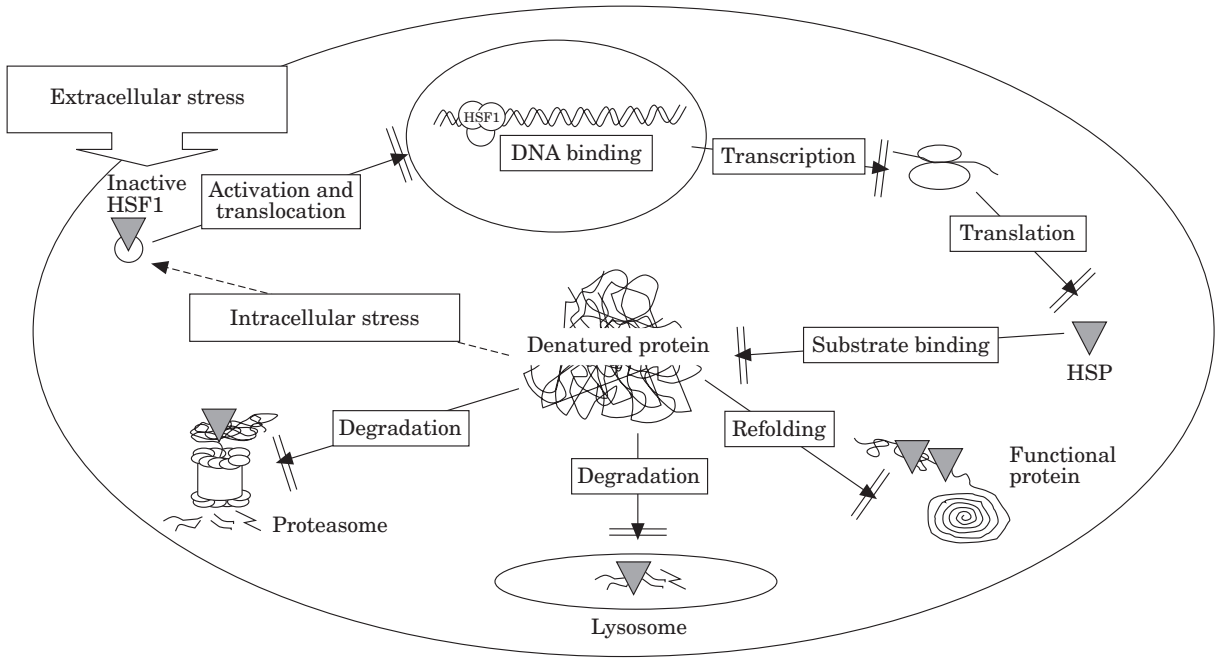

Fig. 1. Schematic representation of the intracellular heat shock (HS) response. Stress activates the expression of HS proteins through a series of steps, counteracting protein denaturation and/or facilitating the degradation of non-refoldable proteins. During ageing, the induction of the HS response and the capacity of active HSPs to act in protein refolding and protein degradation are impaired (marked with crossing on the arrowhead).

HEAT SHOCK AND AGEING

Ageing is related to a decrease in the stress response *in vitro* as well as *in vivo* (Finkel and Holbrook, 2000; Rattan, 1995). This is often related to a deterioration of the capacity of cells to produce active HSPs (Fig. 1). The reason underlying age-related attenuation of the HS response is not due to a lower level of HSF (Heydari *et al*., 2000; Söti and Csermely, 2000). However, there is some evidence for a defect in the transduction pathway leading to HSF1 activation in terms of failure of trimerisation, translocation or phosphorylation/dephosphorylation steps, a decrease in the DNA-binding activity of HSF due to post-translational modifications, and a defect in HSP mRNA maturation or translation (Heydari *et al*., 2000; Lu *et al*., 2000; Söti and Csermely, 2000, Fig. 1).

Although it is not clear whether decreased HS response is a cause or a consequence of ageing, such changes can further promote ageing since HSPs are involved in the regulation of protein turnover, of signal transduction and of gene expression and cell death (Rattan, 1995). Decreased HS response could also lead to the accumulation of damaged proteins, primarily oxidised and glycated proteins (Verbeke *et al*., 2000). It has been reported that the production of ubiquitin is modified during ageing, often leading to less free ubiquitin and more ubiquitin–protein conjugates (Cuervo and Dice, 1998; Grune, 2000). A failure in the ubiquitination process could contribute to the accumulation of highly damaged proteins, which in turn are effective inhibitors of the proteasome (Sitte *et al*., 2000). α_2-crystallin and HSP90 have been shown to modulate proteasome activity during stress, and a decrease in their production and/or activity during ageing could alter proteasome activity (Andersson *et al*., 1999; Conconi *et al*., 1998). Inhibition of proteasome activity or saturation of the cell's proteolytic capacity are unable to trigger HSF activation and expression of HS genes in old cells, leading to the cyclic accumulation of damaged and aggregated proteins. The decline of the proteasome activity during ageing could also have some implication for NFkB induction, due to the failure to activate the degradation of IkBα (Ponnappan *et al*., 1999; Rossi *et al*., 1998). However, depending on tissue and cell type, IkBα levels will either increase (Ponnappan *et al*., 1999; Trebilcock and Ponnappan, 1996), decrease (Yan *et al*., 1999) or be unaffected during ageing (Helenius *et al*., 1996; Korhonen *et al*., 1997).

Lysosome is the other major cellular proteolytic system affected by ageing. The HSC73-specific lysosomal proteolytic pathway is inhibited in

senescent fibroblasts (Cuervo and Dice, 1998). Accumulation of lipofuscin, which is an aggregate of oxidised proteins and lipids, affects the lysosomal activities (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 the face of various insults. A decline in HSF and HSP activity, if not always a decline in their expression, and a decrease in the activities of antioxidant enzymes are thought to underlie human neurodegenerative diseases (Bijur *et al*., 1999; Pappolla *et al*., 1996). This is because imbalance of the cellular redox status and lack of chaperone activity promote protein aggregation and favour the development of ageing-linked pathologies including cataract, polyglutamine-related disorders or other neurodegenerative diseases (Söti and Csermely, 2000). Severe stress may also promote some of these pathologies more directly by a transcription pathway. It has been shown that the β-APP gene involved in Alzheimer's disease is under the control of HSF and NFkB (Dewji and Do, 1996; Grilli *et al*., 1995). The expression and folding of the prion protein are activated by HS and HSP (Shyu *et al*., 2000). Furthermore, the transglutaminase gene, crucial in polyglutamine-related disorders, in under the control of a kB element (Mattson *et al*., 2000; Mirza *et al*., 1997). Accumulation of oxidised and aggregated proteins could be responsible for the increase in 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; Maiello *et al*., 1998).

The stress activation of NFkB is diminished during ageing, but in contrast to HSF activation, the basal DNA binding activity of NFkB is increased with ageing in heart, liver, kidney and brain from rodents (Finkel and Holbrook, 2000; Ginn-Pease and Whisler, 1998). This change can be due to a basal increase in oxidative stress and/or a decrease in IkBα production. It appears that the regulation of HS and NFkB responses are tightly linked during ageing, especially because IkBα is considered to be a new HSP (DeMeester *et al*., 2001; Santoro, 2000; Wong *et al*., 1999). The constitutive NFkB activity may lead to the expression of pro-inflammatory molecules that are κB-dependent, such as IL2, IL6, IL12, TNFα, interferon β, cyclooxygenase 2, inducible nitric oxide synthase, and macrophage migration inhibitory factor (Mattson *et al*., 2000; Spencer *et al*., 1997), and can contribute to the pathology of many disease states associated with ageing such as atherosclerosis and Alzheimer's disease (D Martin *et al*., 2000; Mattson and Camandola, 2001). In Alzheimer's disease particularly, the Aβ peptide and the glycated Tau protein interact with the advanced glycation end-products receptor (RAGE) to produce free radicals and activate NFkB (Christen, 2000; Grilli and Memo, 1999). Moreover, it seems that during acute neurodegenerative conditions and in chronic age-related neurodegenerative disorders, numerous cytoprotective systems under the control of NFkB are mostly turned off and the expression of proinflammatory and cytotoxic molecules (presenilin, APP) is favoured (Mattson and Camandola, 2001; O'Neill and Kaltschmidt, 1997).

ANTI-AGEING EFFECTS OF MILD HEAT SHOCK

The response to repeated mild stress allows cells to enhance their cellular defence processes, to adapt to gradual changes in their environment and to survive in otherwise lethal conditions. Such a phenomenon is known as hormesis and has been widely observed in relation to irradiation, toxins, heat shock and other stresses (Calabrese and Baldwin, 2000; Minois, 2000; Rattan, 2000). Hypergravity, low doses of ionising radiation and chloroform, moderate exercise, electric shocks, whole body hyperthermia, non-lethal ischemia and daily immersion in cold water have been shown to give hormetic effects in nematodes, fruit flies, rodents, dogs, monkeys and humans (Calabrese and Baldwin, 2000; Minois, 2000). Moderate caloric restriction, known to slow down ageing and prolong the lifespan, is suggested to have a hormetic effect because of sustained daily periods of hyperadrenocorticism and restoration of the HS response in several tissues in rodents (Masoro, 1998; Masoro, 2000). We have observed that the anti-ageing hormetic effects of repeated mild HS on human fibroblasts (Rattan, 1998) are accompanied by a reduction in the extent of oxidised and glycoxidised proteins (Verbeke *et al*., 2000). Further studies are in progress to elucidate changes in the HSP profile and proteasome activity during this treatment.

The hormetic pathways are still largely unknown but may act in delaying the breakdown of the cellular balance between pro- and anti-oxidant conditions by maintaining the level and activity of several antioxidants, such as SOD, catalase and

glutathione. The redox balance influences major functions involved in senescence and cell death, such as the activation of thiol redox-sensitive transcription factors HSF1, NFκB, AP-1, HSF1 and p53 activation of phosphatases and kinases, telomere shortening and removal of damaged proteins (Arrigo, 1999; Finkel and Holbrook, 2000; Meriin *et al*., 1999; von Zglinicki, 2000). Anti-ageing effects of hormesis could act 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 ageing since they govern both the repair of weakly damaged proteins and the catabolism of highly damaged proteins. Thus, hormetic pathways are thought to activate several key proteins involved in the stress response. Indeed, hormesis leads to maintenance of the HS response during ageing, and the concomitant transitory overexpression of HSPs in cells and organisms is greatly beneficial. Increased expression of HSP70 by genetic manipulations extends the lifespan of *Drosophila* and renders rat heart myocytes more resistant to oxidative stresses (Chong *et al*., 1998; Tatar *et al*., 1997). In neurodegenerative diseases related to polyglutamine expansion, co-transfection of several chaperones and co-chaperones have significantly reduced aggregate formation (Ohtsuka and Suzuki, 2000). However, constitutive overexpression of individual HSPs may be toxic for organisms because a chaperone works with a complex of activating and inhibitory co-chaperone and if some are present in large excess or without the others, chaperone sequestration, protein aggregation, retardation in cell growth and enhanced apoptosis can occur (Feder and Hofmann, 1999).

Cellular resistance to stress has been correlated with longevity, supporting the view that the machinery of the cellular stress response is functionally important in ageing (Kapahi *et al*., 1999). Furthermore, selected lines of long-living mutant *Drosophila* and nematodes display overexpression of HSP and of antioxidant enzymes, and have a stress-resistant phenotype, and organisms selected for stress-resistance have increased longevity (Murakami *et al*., 2000; Sampayo *et al*., 2000). Age-dependent regulation of stress-response genes in diverse species may implicate a common protective mechanism against the deleterious effects of ageing. Therefore, hormetic application of repeated mild stress in modulating ageing and longevity is a promising area for future research to elucidate the molecular pathways of maintenance and repair associated with these processes.

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