Protein stress and stress proteins: implications in aging and disease

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Environmantal stress induces damage that activates an adaptive response in any organism. The cellular stress response is based on the induction of cytoprotective proteins, the so called stress or heat shock proteins. The stress response as well as stress proteins are ubiquitous, highly conserved mechanism, and genes, respectively, already present in prokaryotes. Chaperones protect the proteome against conformational damage, promoting the function of protein networks. Protein damage takes place during aging and in several degenerative diseases, and presents a threat to overload the cellular defense mechanisms. The preservation of a robust stress response and protein disposal is indispensable for health and longevity. This review summarizes the present knowledge of protein damage, turnover, and the stress response in aging and degenerative diseases.

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1. Stress, cell stress and chaperones

Stress is a sudden environmental change that induces damage at the molecular, cellular and organismal level. However, stress also induces an adaptive response that usually compensates the noxa (Calabrese 2006). The beneficial effect of stress is to enable the organism to cope with a subsequent, more severe stress. This ability is named stress tolerance. In a positive case of mild stress with no apparent damage this phenomenon is called hormesis. However, if damage overwhelms the adaptation, it results in functional decline, a so-called distress. The organismal adaptation response is mediated by the vegetative nervous system and the hypothalamo-pituitary-adrenal axis. But what is the mechanism of stress response at the cellular level?

The cellular stress response is regulated at the transcriptional, translational and post-translational levels. The major regulator of the heat shock response genes is the heat shock transcription factor 1 (HSF1) which is kept in a latent state by an inhibitory complex of stress-proteins. The major activator of HSF1 is proteotoxic insult, like heat shock. Misfolded proteins titrate out HSF1 form the inhibitory chaperone complex, HSF1 trimerizes, becomes phosphorylated and is translocated to the nucleus where it is able to bind to the heat shock element of *Hsp* genes (Morimoto 1998). Therefore the induced proteins are known as stress or heat shock proteins (Hsps). Hsps are evolutionarily conserved. are present in all cellular compartments and are classified according to their molecular weight.

Some of the major chaperones (Hsp70, Hsp90, small Hsps) are present at high concentrations in non-stressed cells reaching 1–5% of total cellular protein, which shows that a continuous intense demand is present to guard the protein conformational homeostasis. Indeed, chaperones display various activities in the cell, such as (i) proper folding of nascent polypeptide chains, (ii) facilitating protein translocation across various cellular compartments, (iii) modulating protein activity via stabilization and/or maturation to functionally-competent conformation, masking mild mutation at the conformational level (iv) promoting multiprotein complex assembly/disassembly, (v) refolding of misfolded proteins, (vi) protecting against protein aggregation, (vii) targeting ultimately damaged proteins to degradation, (viii) sequestering damaged proteins

Keywords. Aging; chaperone; protein damage; protein degradation; stress protein

Abbreviations used: Gp, glycoprotein; Grp, glucose regulated protein, the number thereafter denotes molecular weight; HSF, heat shock transcription factor; Hsp, heat shock protein, the number thereafter denotes molecular weight; PolyQ, polyglutamine.

to aggregates, (ix) solubilizing protein aggregates for refolding/degradation. (Young *et al* 2004, Sőti and Csermely 2000). Chaperones work in concert with co-chaperones and regulate local protein and signaling networks of the cell (Sőti *et al* 2005, Csermely, 2006).

2. Aging, longevity and the stress response

Though pioneering studies were done on mechanisms of stress responses in prokaryotes, and there are examples of aging and senescence in prokaryotes, this review focuses on aging and protein stress in animals. Activation of the stress response to any stimulus induces cross-tolerance to multiple stressors. Resistance to any physical stress is correlated with longevity in many, if not all species. HSF1 overexpression induces longevity, while HSF1 knock out shortens life-span in *C. elegans* (Garigan *et al* 2002). Moreover, HSF1 is indispensable to yield life-span extension in classical long-lived insulin-signaling mutants (Hsu *et al* 2003, Morley and Morimoto 2004). Thus, a robust stress response is indispensable for life-span extension in lower eukaryotes. The evolutionary advantage of such a regulatory mechanism is to spare the resources of the organism or species during unfavorable conditions (stress) to self-maintenance to survive. Interestingly, HSF1 knockout mice, though, display no heat shock response and are hypersensitive to endotoxemia, show no premature aging in laboratory conditions (Xiao *et al* 1999). This may be due to the unnatural stress-free environment served to keep transgenic mice. Additionally, there are three HSFs in mammals, and their respective functions are yet to be understood (Christians and Benjamin 2006).

Further genetic manipulations to overexpress individual chaperones, like Hsp90 and Hsp70 resulted in subtle extension of life span (Morley and Morimoto 2004), however the small chaperone Hsp16 seemed to be more efficient in worms. The mitochondrial Hsp22 overexpression induces life-span extension and oxidative stress resistance in the fruit fly *Drosophila melenogaster* (Morrow *et al* 2004). Since both *C. elegans* and *Drosophila* adults are postmitotic, effects of Hsps on cell proliferation is not reflected in these model organisms. How high level of chaperones affects cellular senescence, remains an open question. Just to give a possible outcome, it may well be that a continuous presence of high amounts of chaperones would present an increased threat of malignancy, since chaperones are intimately involved in signaling and promote cell proliferation and survival.

Beyond genetics, hormesis (proper dosing of stress) increases stress tolerance and longevity in both cellular and organismal models. Both repeated mild heat shock as well as calorie restriction are examples of hormetic interventions (Sőti and Csermely 2003; Rattan 2004; Calabrese 2006).

The heat shock response is attenuated during aging. While the activation and nuclear translocation is retained, binding of HSF1 to the heat shock element is compromised in rat hepatocytes, human lymphoblasts and peripherial mononuclear cells (Heydari *et al* 2000; Ambra *et al* 2004; Singh *et al* 2006). As in many other aspects of their successful aging phenotype, centenarians maintain the heat inducibility of the *Hsp70* gene (Ambra *et al* 2004).

Chaperone levels either increase or decrease during life, depending on the model studied (Sőti and Csermely 2003). An initial study revealed a decline in chaperone function in aged rats when compared to their young counterparts (Nardai *et al* 2002). There may be several mechanisms behind this phenomenon. Chaperones may be preferential targets of (oxidative) damage (G Kiss and C Sőti, unpublished observations). Both their extensive binding surfaces and abundance make them ideal damage scavengers (Sőti and Csermely 2003). However, this will result in functionally-incompetent "sick chaperones" (Macario and Conway de Macario 2005) that are no longer able to fulfill their roles, or even may show a dominant negative phenotype. The other possible reason for a decrease in functional chaperone complexes is chaperone overload (*see* below). However, these assumptions are yet to be tested.

3. Protein damage and proteotoxicity

Damage to macromolecules is characteristic of aging and degenerative diseases. Beyond DNA damage, protein damage may not only a consequence, but also a causal factor in cellular malfunction. Damage may induce misfolding, and the aggregating oligomeric species may gain a novel toxic property, severely compromising cellular function (Dobson 2003). Initial aggregation results in uniform globular species irrespective of the original protein (Mukai *et al* 2005), however, mature aggregates display different stuctures (Matsumoto *et al* 2006), probably as a result of the interaction of the aggregation-prone protein and the protein turnover systems (e.g. chaperones and proteolytic machineries).

The molecular basis of aggregate toxicity is the incorrect interaction with normal cellular proteins, leading to the sequestration and inhibition of key molecules, like transcription factors, cytoskeletal proteins, molecular chaperones and the degradative machineries (Bence *et al* 2001; Bennett *et al* 2005; Cuervo *et al* 2004; Matsumoto *et al* 2006; Schaffar *et al* 2004). Presence of these ensembles induces metabolic abnormalities, oxidative stress and different types of cell death, including both apoptosis and necrosis. Polyglutamine protein (polyQ) aggregation increases with age, and downregulation of chaperones and protein degradation machinery in *C. elegans* accelerates the onset of aggregation, showing an age-dependent impairment of the protein homeostasis buffer (Hsu *et al* 2003, Nollen *et al* 2004). Intriguingly, polyQ aggregation is retarded,

probably because of higher small heat shock protein levels, in long-lived insulin-signaling mutants (Morley *et al* 2002), suggesting that dysfunctional insulin-signalling may lead to disturbance in protein homeostasis and the development of neurodegenerative diseases.

Protein damage may either be inherent or induced by environmental stress. Long polyglutamine tracts present in some proteins destabilize protein structure and make the protein aggregation-prone. Other examples of genetic damage are such mutations that disrupt the native structure and induce certain conformational diseases (Dobson 2003). Cells harboring these mutants are hypersensitive to stresses, and it exhausts the regenerative capacity of the tissue. Moreover, presence of a single mutant misfolding protein disturbs general protein homeostasis in the cell (Gidalevitz *et al* 2006). These facts present a special threat for the postmitotic nervous system, liver, and muscle, and are the molecular basis of various neurodegenerative diseases (Goldberg 2003; Meriin and Sherman 2005).

Environmental stress also induces damage. The major stress of life is oxidative stress. Thus, it is not surprising that oxidized protein level increases with aging of all animal species (Stadtman, 2004; Cloos and Christgau 2004). Oxidative damage displays only a rather modest, two-to-threefold rise in aged organisms. While the majority of glycoxidative modifications to proteins are irreparable, those involving the highly susceptible sulfur-containing amino-acids are readily reversible. Cysteines are regenerated by thiol transferases, and methionines by methionine sulfoxide reductases. Overexpression of methionine sulfoxide reductases confers protection against oxidative stress in yeast, *Drosophila* and in human fibroblasts, suggesting the function of this system as a potent ROS scavenger (Stadtman 2004; Friguet 2006).

4. Cytoprotection from proteotoxic stress: the protein homeostatic buffer

Elimination of damage is essential for survival. Therefore, all the mechanisms operating to prevent proteotoxicity are considered to be part of the protein homeostatic buffer. Major elements are: (i) sequence repair by methionine sulfoxide reductases and other, yet unknown systems, (ii) conformational repair by chaperones, (iii) protein disposal and (iv) protein sequestration into solid phase aggregates. Probably all molecular machineries are present in all cell compartments, including the cytosol, nucleus, mitochondria and the vesicular system. Common features of these elements are (i) the limited overall capacity to repar/dispose the damaged proteins (ii) an age-dependent functional decline and (iii) an inhibition by misfolded/aggregated proteins. Proteins irreparable at the sequence level may serve as chaperone substrates for conformational repair (Hartl 1996). Since chaperones were summarized in several reviews (Sőti and Csermely 2003; Arslan *et al* 2006), we give a few examples from protein disposal, and the role of chaperones.

Irreparable proteins are channeled to degradation by mechanisms possessing both specific as well as overlapping and redundant elements (Cuervo 2004; Chondrogianni *et al* 2005; Friguet 2006). Both a subset of proteasomal and lysosomal autophagic (i.e. chaperone-mediated autophagy) degradation require chaperones and ATP, and are induced by oxidative stress. More importantly, both the ubiquitin-proteasome and the chaperone-mediated autophagy are inhibited by a modified, misfolded, aggregation-prone protein (Bulteau *et al* 2001; Bennett *et al* 2005; Cuervo *et al* 2004). Inhibition of any of these results in higher stress sensitivity and cell death (Bence *et al* 2001; Massey *et al* 2006) and both of them are implicated in longevity (Cuervo 2004; Chondrogianni *et al* 2005; Friguet 2006). Protein degradation declines with age, including all the proteasome, macroautophagy and chaperone-mediated autophagy machineries. There seem to be no chaperone involvement in the decline of chaperone-mediated autophagy in aging, while it was shown that the Hsp90-dependent protection of proteasome is compromised with age (Cuervo 2004; Conconi *et al* 1996).

Interestingly, a recent study reported that limiting pool of free ubiquitin in cells causes a competition of stress-related substrates with regulatory ones like histones and may influence chromatin remodeling and transcription in stressed cells (Dantuma *et al* 2006), however, studies from another laboratories argued against such a limited pool (Bence *et al* 2001; Bennett *et al* 2005).

While general protein turnover is maintained in cells under inhibition of chaperone-mediated autophagy by an upregulation of macroautophagy, this cannot confer protection against oxidative stress or aid in the degradation of the aggregation-prone mutant protein (Cuervo *et al* 2004; Massey *et al* 2006). More importantly, improper activation of macroautophagy may be a pathogenetic factor in Alzheimer's disease (Yu *et al* 2005). Similarly, while cells with proteasome inhibition may eventually escape cell cycle arrest (Bence *et al* 2001) and maintain normal protein turnover, age-dependent decrease in proteasome activity promotes Huntingtin aggregation (Zhou *et al* 2003).

Importantly, besides proteotoxic defense, there is another level of cytoprotection operated by chaperones and the proteasome. While the proteasome regulates half-lives of key proteins in the cell cycle and cell proliferation (e.g. cyclins, Hsp90-dependent clients), chaperones also promote stem (and tumor) cell proliferation and cell survival. Hsp90 ensures, amongst several hundred client proteins, Akt, Raf and cyclin-dependent kinase 4 and 6 activation and is essential for telomerase activity (Pratt and Toft 2003; Nardai

Table 1. Antiapoptotic actions of chaperones

Pathway, level	Interaction	Chaperone involved
Plasma membrane	FADD	Hsp27
	JNK	Hsp70
Mitochondria	MPT	Hsp90
	Cyt-c	Hsp70, Hsp27
	APAF-1	Hsp90, Hsp70
Execution	caspase-3	Hsp70, Hsp27
Lysozyme	granzyme B	Hsp70

Data from Sreedhar and Csermely (2004).

et al 2006). Moreover, many chaperones inhibit the apoptotic pathways at different levels by multiple interactions (table 1, Sreedhar and Csermely 2004; also *see* Arya *et al* 2007).

5. Overload of the protein homeostatic buffer: implications in aging, and degenerative civilizational diseases

Capacity of the protein homeostatic buffer declines with age (Nardai *et al* 2002, Sőti and Csermely 2003; Cuervo 2004; Chondrogianni and Gonos 2005; Friguet 2006). Even one overexpressed polyQ protein impairs general protein folding and turnover, probably by capturing chaperones and degradative machineries (Gidalevitz *et al* 2006). The same phenomenon may occur during aging or because of the living conditions of modern civilization: either increased protein damage due to detrimental post-translational modifications, or the accumulation of latent mutations in the stress-free environment would overload the cleansing ability of chaperones and protein disposal. This in turn has ample consequences by compromising many key processes, like signal transduction, protein transport, immune recognition and cellular organization, besides causing phenotypic exposure of previously buffered silent mutations (Csermely 2001; Sőti and Csermely 2003; Gidalevitz *et al* 2006; Nardai *et al* 2006). Those overloaded cells, tissues and organisms are prone to stress from any origin and may display a wide range of pathologies including cancer, diabetes, immune-problems and aging itself. Therefore any pharmacological or life-style interventions focusing to preserve the protein turnover is an attractive therapy in anti-aging research (Rattan 2004; Sőti *et al* 2005; Sőti and Csermely 2006). However, both the pathogensis and the possible therapeutic strategies await further experimental proof.

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