Biogerontology (2006) 7:383–389
DOI 10.1007/s10522-006-9053-7

REVIEW ARTICLE

Protein homeostasis and molecular chaperones in aging

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Published online: 9 September 2006

Abstract Molecular chaperones are ubiquitous, highly conserved proteins responsible for the maintenance of protein folding homeostasis in cells. Environmental stress causes proteotoxic damage, which triggers chaperone induction and the subsequent reparation of cellular damage by chaperones, including disposing irreparable protein ensembles. We summarize the current view of protein damage, turnover, the stress response and chaperone function in aging, and review novel data showing that accumulation of misfolded proteins outcompete and overload the limited resources of the protein folding, maintenance and turnover system, compromising general protein homeastasis and cellular function. Possible involvement of chaperones and proteolysis in immunosenescence is highlighted. Defects in zinc metabolism are also addressed in relation to aging and changes in chaperone levels.

Keywords Heat shock protein · Stress protein · Chaperones-chaperone overload · Aging · Neurodegenerative diseases · Protein folding · Protein damage · Zinc · Metallothionein · Immune response

Abbreviations

Grp94	94 kDa glucose regulated protein
HSF	Heat shock transcription factor
Hsp	Heat shock protein, the number thereafter denotes molecular weight
MT	Metallothionein
PolyQ	Polyglutamine
ROS	Reactive oxygen species

Presented at the ZincAge Conference, Madrid, February 10–13, 2006

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Molecular chaperones and the stress response

Chaperones are ubiquitous, highly conserved proteins playing a major role in the conformational homeostasis of cellular proteins. Diverse functions of chaperones in cells include (1) proper folding of nascent polypeptide chains, (2) facilitating protein translocation across various cellular compartments, (3) modulating protein activity via stabilization and/or maturation to functionally-competent conformation, (4) promoting multi-protein complex assembly/disassembly, (5) refolding of misfolded proteins, (6) protecting against protein aggregation, (7) targeting ultimately damaged proteins to degradation,

(8) sequestering damaged proteins to aggregates, (9) 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).

Chaperones are induced by proteotoxic environmental stress at the level of transcription, when the cytosolic level of damaged, misfolded proteins is sufficient to displace heat shock factor (HSF) from its inhibitory chaperone complex. Thus, chaperones are also known as stress or heat shock proteins (Morimoto 1998).

Protein damage and proteotoxicity in aging cells

Ageing is characterized by a continuous accumulation of macromolecular, including protein damage, thought to be mainly induced by reactive oxygen species. Oxidized protein level increases exponentially with aging of all animal species (Stadtman 2004; Cloos and Christgau 2004). 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).

Protein damage may result in the loss of the function of a single polypeptide, but as an even more aggravating result, if misfolding takes place the aggregating oligomeric species may gain a novel toxic property, severely compromising cellular function (Dobson 2003). At initial steps, relatively uniform small globular aggregates can be formed from structurally unrelated proteins, explaining the similarity of toxicity in diverse neuropathologies (Mukai et al. 2005). At later steps, however, assembly of these oligomers may result in structurally different large aggregates, which may influence disease progression (Matsumoto et al. 2006).

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). 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 of long-lived insulin-signaling mutants (Morley et al. 2002), suggesting that dysfunctional insulin-signaling may lead to disturbance in protein homeostasis and the development of neurodegenerative diseases.

A recent study reveals that co-expression of a single aggregation-prone polyQ protein with different temperature-sensitive mutant proteins in *C. elegans* induces the appearance of the mutant phenotypes at the permissive temperature, and vice versa, the presence of the unstable temperature sensitive protein promotes polyQ aggregation even at the permissive temperature (Gidalevitz et al. 2006). The authors conclude that a large number of mild folding variants present in the human genome may become phenotypically exposed when a severe mutant protein impairs protein folding and turnover, probably by capturing chaperones and degradative machineries. This, in turn may lead to dysfunction in a diverse set of cellular pathways. These results further suggest that cells have a limited overall folding capacity for their misfolded, aggregation-prone proteins and for those with reparable folding defects regardless of their structure and function and provide the first direct evidence of the chaperone overload and protein homeostasis hypotheses of aging (Csermely 2001; Sőti and Csermely 2003).

Chaperone levels and function in aging

Damaged proteins in aged organisms may displace heat shock factor-1 (HSF-1) from its chaperone

complexes, which may explain why some aged species develop constitutively elevated levels of several chaperones. This rather permanent adaptive response to stress probably ''wears out'' the mechanism to mobilize the response promptly for the next round and may predict the attenuation of the stress response in aged organisms. No significant change has been reported so far in protein levels, trimerization, phosphorylation and nuclear translocation of HSF-1 in the course of aging (Sőti and Csermely 2003). In aged hepatocytes (Heydari et al. 2000) and lymphoblasts (Ambra et al. 2004) binding of HSF-1 to the heat shock element has been shown to be decreased. However, lymphoblasts from centenarians maintain transcriptional response of the Hsp70 gene to heat stress similar to that of young subjects probably due to a preserved phosphorylation and DNA-binding activity of HSF-1 (Ambra et al. 2004).

An initial study revealed a decline in chaperone function in aged rats when compared to their young counterparts (Nardai et al. 2002). The decrease observed in chaperone activity from aged animals may partly be explained by decreased Hsp90 levels, however, there might be at least two other reasons for such an observation. 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 meet increasing folding demands of the damaged, misfolded proteins in aging cells.

Another possible reason for decrease in chaperone function with aging is chaperone overload (Csermely 2001). In aging organisms or in a cell harboring a severe mutant protein the balance between misfolded proteins and available free chaperones is severely disturbed (Sőti and Csermely 2003; Gidalevitz et al. 2006): increased protein damage due to detrimental post-translational modifications and impaired protein degradation increase the amount of misfolded proteins; while chaperone damage, insufficient transcriptional induction of chaperones, clogging up of chaperones by entrapped substrates with irreparable folding defects followed by their precipitation together with these defective proteins and sequestration into larger aggregates (Macario and Conway de Macario 2005) decrease the amount of available free chaperones. At this point where the need for chaperones overwhelmingly exceeds the available chaperone capacity, chaperone overload develops. Under such circumstances, competition for available chaperones becomes fierce and abundance of damaged proteins outrivals the normal physiological targets of chaperones, depriving them of proper folding assistance. This in turn have the potential to compromise the function of many important proteins in signal transduction, protein transport, immune recognition and cellular organization as well as lead to the phenotypical exposure of previously buffered silent mutations (Csermely 2001; Sőti and Csermely 2003; Gidalevitz et al. 2006; Nardai et al. 2006).

Indeed, the increase in the robustness of the heat shock response, chaperone overexpression and different hormetic interventions, like repeated mild heat shock lead to an increased stress-tolerance and an extension in life span (Sőti and Csermely 2003; Rattan 2004). Furthermore, HSF-1 itself induces longevity and is needed to increase the life-span of classical long-lived *C. elegans* mutants deficient in insulin-like signaling (Hsu et al. 2003; Morley and Morimoto 2004), connecting the nutritional state with stress-tolerance. Interestingly, overexpression of individual hsp genes did result only a fraction of the total increase. Moreover, the cause of death in HSF-1 down-regulated worms was bacterial infection (Garigan et al. 2002). These result imply that (1) the chaperone system is not only one of the many cellular functions that decline during aging but (2) HSF-1 is a direct regulator of longevity by switching on a program of self-maintenace and survival in periods of environmental stress and (3) the stress response besides protein metabolism plays a major role in systemic immune response. The role of the HSF-1—Hsp network in aging and longevity is summarized in Fig. 1. At present it is an open question whether HSF-1 contributes to longevity by a mechanism independent of Hsp-expression.

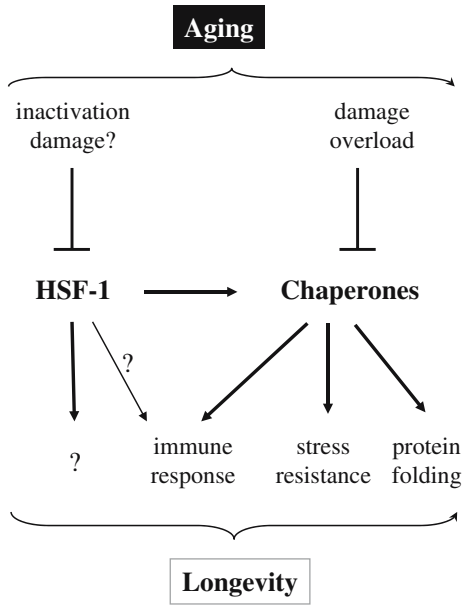

Fig. 1 The HSF-1—molecular chaperone axis as a regulator of longevity. Proven elements of the mechanisms leading to longevity, like improved chaperone-assisted protein folding (including better disposal and delayed aggregation), stress-tolerance at the cellular level and systemic immune response to pathogens are shown. Question marks denote hypothetical or unknown mechanisms

Chaperones and protein degradation in aging and in immunosenescence

Since prompt and proper elimination of waste is vital, irreversibly damaged proteins are targeted for proteasomal and lysosomal disposal by mechanisms possessing both specific as well as overlapping and redundant elements (Cuervo 2004; Chondrogianni and Gonos 2005; Friguet 2006). For instance, 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 is inhibited by a modified, misfolded, aggregation-prone protein (Bulteau et al. 2001; Bennett et al. 2005; Cuervo et al. 2004). Inhibition of any 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 and Gonos 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, while it was shown that the Hsp90-dependent protection of proteasome is compromised in aging (Cuervo 2004; Conconi et al. 1996).

Interestingly, a recent study reported that in cells there is a limiting pool of free ubiquitin, that causes the 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 lab 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, however, age-dependent decrease in proteasome activity promotes huntingtin aggregation (Zhou et al. 2003) and upon proteasome inhibition MHC-I dependent antigen production becomes compromised (Kessler et al. 2003). Since chaperones, the proteasome and the chaperone-mediated autophagy are important regulators of immune function, they may be implicated in immunosenescence. Fig. 2 depicts possible mechanisms of impaired antigen presentation in aging leading to compromised cellular immune responses, eventually causing age-dependent diseases like viral, fungal infections and cancer. The central role of chaperones in many aspects of immune function has been outlined in a recent review (Nardai et al. 2006) and the role of HSF-1 in antimicrobial defense has also been demonstrated (Garigan et al. 2002).

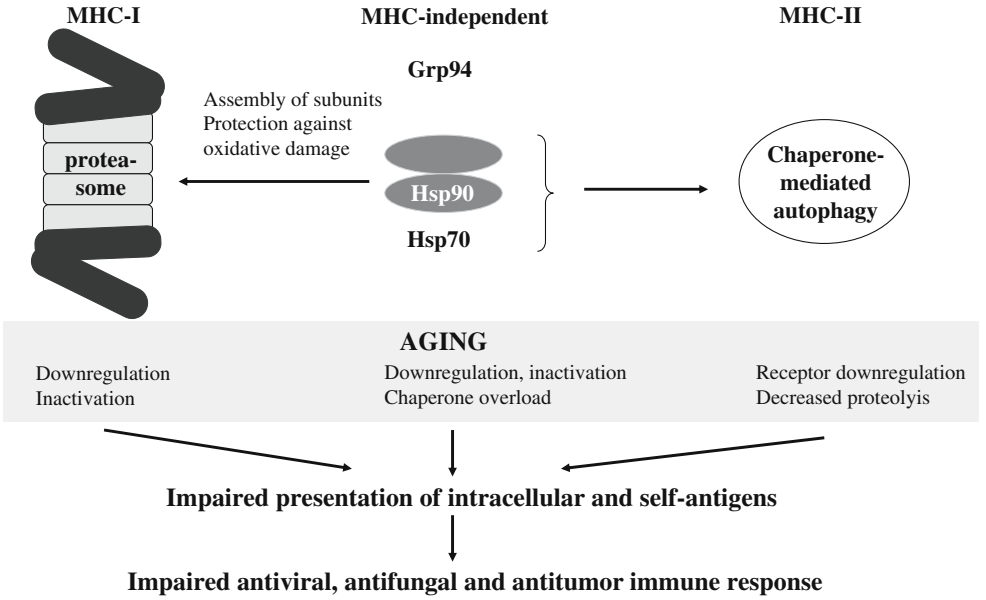

Fig. 2 Age-dependent impairment of chaperone-funtion and protein degradation as a possible cause of compromised cellular immune responses. Chaperones assist proteolytic machineries and take part in the presentation of intracellular antigens. Age-dependent decrease in all these functions may disturb immune responses against intracellular, opportunistic parasites and tumor cells, leading to an increased incidence of viral and fungal infections as well as cancer

Zinc, chaperones and aging

Zinc metabolism is implicated in immune function, immunosenescence, and solid evidence suggests that zinc is a causal agent both in neuronal injury and in neurodegenetration. Zinc bioavailability is decreased in the aged immune system, while increased neuronal synaptic zinc triggers amyloid plaque formation (Mocchegiani et al. 2004; Capasso et al. 2005).

Zinc has long been known as a potent inducer of heat shock proteins, Hsp70 in particular, both in vitro and in vivo. Moreover, zinc deprivation was found to inhibit Hsp70 expression induced by heat shock in HaCaT keratinocytes (Parat et al. 1998). Normal, non-thermotolerant HeLa cells displayed a robust Hsp70 induction upon exposure to zinc, whereas in thermotolerant cells Hsp70 induction was decreased, in part due to reduced activation of HSF-1 (Hatayama et al. 1993). Although both heat stress and zinc administration are capable of inducing Hsp70 synthesis at transcriptional level, dynamics of induction were quite different in rat ganglion cells (Qing et al. 2004). Interestingly, heat shock treatment was found to decrease levels of MT, the major zinc-responsive protein and zinc-reservoir in cells; whereas zinc was still a strong inducer of heat shock proteins in the same rat hepatocytes (Bauman et al. 1993); suggesting that there is not always a good correlation between stresses that induce heat shock and those that induce MTs.

There are very few studies investigating the effect of zinc on aging with regard to heat shock proteins. One such recent study demonstrates in vitro zinc supplementation increased the heat-induced Hsp70 expression of lymphoblasts from aged donors, the same treatments having an opposite effect in centenarians and young donors (Ambra et al. 2004). Given the facts that MTs are major determinants of zinc homeostasis in cells and they exist in two distinct forms in young and aged cells; it appears likely that there is a cross-talk mechanism between MT and Hsp70, the molecular details of which have to await future studies.

Conclusions and perspectives

Aging can be defined as a multicausal process leading to a gradual decay of self-defensive

mechanisms and an exponential accumulation of damage at the molecular, cellular and organismal level. Accumulation of damaged, misfolded proteins and aggregates as well as protein degradation defects together with the simultaneously impaired function and induction of chaperones and protein turnover in aged organisms perturb the balance between chaperone requirement and availability. The development of this imbalance in the course of aging may exhaust the folding assistance to specific chaperone targets, which play key roles in signal transduction, protein transport, immune recognition and cellular organization; as well as cause emergence of previously buffered, hidden mutations in the phenotype of the cell. These would lead to further deterioration in vital processes and accelerated decrease in the robustness of cellular networks. Future studies will have to identify changes in the aforementioned cellular phenomena and relate them to the protein folding homeostasis of aging organisms and cells.

Acknowledgements Work in the authors' laboratory was supported by research grants from the EU 6th Framework program (FP6506850, FP6518230), Hungarian Science Foundation (OTKA-F47281) and from the Hungarian National Research Initiative (1A/056/2004 and KKK-0015/3.0). C.S. is a Bolyai research Scholar of the Hungarian Academy of Sciences. The authors apologize for not citing a number of excellent publications due to space limitations.

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