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Review article

Molecular chaperones and the aging process

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Abstract

Molecular chaperones are abundant, well-conserved proteins responsible for the maintenance of the conformational homeostasis of cellular proteins and RNAs. Environmental stress is a proteotoxic insult to the cell, which leads to chaperone (heat shock protein, stress protein) induction. The protective role of chaperones is a key factor for cell survival and in repairing cellular damage. The present review summarizes our current knowledge about changes in chaperone expression and function in the aging process, as well as their possible involvement in the development of longevity and cellular senescence. We also overview their putative role in neurodegenerative diseases, such as in Alzheimer's disease and the changes in immune and autoimmune response against various chaperones in aging.

Abbreviations: Grp – glucose regulated protein; Hsc70 – the non-inducible (cognate) form of the 70 kDa heat shock protein; Hsp – heat shock protein

Introduction: cellular roles of molecular chaperones

Molecular chaperones bind to, and stabilize an otherwise unstable conformer of another protein or RNA and, by controlled binding and release, facilitate its correct fate *in vivo*: be it folding, oligomeric assembly, transport to a particular subcellular compartment, or disposal by degradation. In the molecular level chaperones (1) protect against aggregation, (2) solubilize protein aggregates, (3) assist in protein folding/refolding by partial unfolding the intermediate structures in the folding process, (3) target ultimately damaged proteins to degradation and (5) sequester overloaded damaged proteins to larger aggregates. Chaperones are ubiquitous, highly conserved proteins, which probably played a major role in the evolution of modern enzymes (Csermely 1997, 1999; Hartl 1996). Chaperones are vital for our cells during their whole lifetime. However, they are needed even more after environmental stress, that induces protein damage. Stress (especially its most studied archetype, heat shock) leads to the expression of most chaperones, which therefore are called heat-shock, or stress proteins. Lacking a settled view about their action in the molecular level, chaperones are still best classified by their molecular weights. The major chaperone families are listed in Table 1. Besides the general chaperones listed in Table 1, which have a rather large promiscuity in target-selection, there are also specialized chaperones, such as Hsp47, the special chaperone of collagen. Chaperones usually do not increase the speed of protein folding, just inversely, by binding to folding intermediates, or by their repetitive pulling attempts extend the total folding time and simultaneously increase the final yield of the native protein. Special steps of protein folding are accelerated by the so-called 'folding catalysts', such as peptidyl-prolyl isomerases (immunophilins), and protein disulfide isomerases, which promote the cis-trans isomerization

Table 1. Major molecular chaperone families.

Eukaryotic chaperone family members[a]	Recent reviews
Hsp25, Hsp27, crystallins, small heat-shock proteins	Arrigo 1998; Derham and Harding 1999
Hsp60, chaperonins	Bukau and Horwich 1998; Hartl 1996
Hsp70, Grp78	Bukau and Horwich 1998; Hartl 1996
Hsp90, Grp94	Buchner 1999; Csermely et al. 1998; Pratt and Toft 1997
Hsp104	Schirmer et al. 1996

[a]Neither the co-chaperones (chaperones which help the function of other chaperones listed, such as Hsp10, Hsp40, Hip, Hop, Hup, etc.), nor the so-called folding catalysts, the peptidyl-prolyl isomerases (immunophilins) and protein disulfide isomerases were included in this table, albeit almost all of these proteins also possess a 'traditional' chaperone activity in their own right.

of peptide bonds adjacent to proline residues and the formation of disulfide bridges in the endoplasmic reticulum, respectively.

Besides the need for assisted folding of *de novo* synthesized, nascent proteins, and the chaperone-mediated repair of protein damage, cellular life requires a constant remodelling of cell structure. Chaperones provide an essential help in all these processes. Individual chaperone proteins usually do not work alone, but either form a highly structured homo- or heterooligomeric complex, such as the Hsp60 chaperone machine, or assemble to a dynamic cohort of chaperones such as the Hsp90-containing foldosome in the cytoplasm or its counterpart in the endoplasmic reticulum. Most major chaperones are helped by several smaller co-chaperones (such as Hsp10 in case of Hsp60, Hsp40 in case of Hsp70 and p23 in case of Hsp90). The large and dynamic assemblies of individual chaperones are attached to the microfilamental and microtubular system. About twenty years ago based on high-voltage electron microscopy Porter and co-workers suggested the existence of a cellular meshwork, called as 'microtrabecular lattice' to organize cytoplasmic proteins and RNA-s (Wolosewick and Porter 1979; Schliwa et al. 1981). Some later reports questioned their original experiments and considered the lattice as an artifact of the techniques used. However, more and more data provide indirect evidence for a high-order organization of the cytoplasm. Chaperones are ideal candidates for being a major constituent of a cytoplasmic meshwork (Csermely et al. 1998; Pratt and Toft 1997).

Protein folding and the aging process

During the life-span of a stable protein various posttranslational modifications occur (Harding et al. 1989). These include deamidation of asparaginyl and glutaminyl residues and the subsequent formation of isopeptide bonds (Wright 1991), protein glycation, methionine oxidation (Sun et al. 1999), etc. Susceptibility to oxidative damage differs protein to protein, as has been demonstrated in a comparison of bovine serum albumin and glutamine synthase (Berlett et al. 1996), and of various K^+ channels (Duprat et al., 1995), suggesting a role for different tertiary-quaternary structure as well as folding. Even in early studies aging-induced inactivation of isocitrate-lyase (Reiss and Rothstein 1974), or phosphoglycerate kinase (Yuh and Gafni 1987) could be associated with the accumulation of a non-native, heat labile conformation of the enzymes. In a refolding study the increased helical content of 'old' aldolase was preserved after refolding of the enzyme, which suggested that the conformational changes were mostly induced by the various posttranslational modifications during the life of the protein (Demchenko et al. 1983).

The ocular lens is a transparent organ comprised of a highly concentrated and ordered matrix of structural proteins, called crystallins, which are probably the longest lived proteins of the body. Lens transparency is dependent upon maintenance of the short range order of the crystalline matrix. The gradual loss of this order leads to the opacification of the lens and in extreme cases to the development of cataract. Therefore crystallins became excellent substrates of widespread research activity in determining various posttranslational modification of aged proteins. Besides glycation, methionine oxidation and crystallin-crystallin crosslinking (Sharma et al. 1995; Smith et al. 1997), a loss of N-terminal amino acids, covalent modification of C-terminal lysine and photodegradation of crystallin tryptophanes were also reported (Lin et al. 1997; Kamei et al. 1997; Schauerte and Gafni 1995). Aging α-crystallin also undergoes

Table 2. Changes of chaperone expression in aging.

Chaperone	Change	References
Chaperone levels		
Hsp22, Hsp23, Hsp70	Elevated in *Drosophila*	Wheeler et al. 1995
Hsp47, Hsp70	Elevated in rat kidneys	Maiello et al. 1998; Razzaque et al. 1998
Hsc70[a]	No change in hepatocytes	Wu et al. 1993
Hsc70[a]	Decrease in testis	Krawczyk et al. 1989
Chaperone induction		
Hsp27	Heat induction is impaired	Rao et al. 1999
Heme oxygenase (Hsp32)	Oxygen damage induction is impaired	Nakanishi and Yasumoto, 1997
Superoxide dismutase	Heat induction is impaired	Niedzwiecki et al. 1992
Hsp60	Heat induction is impaired	Rao et al. 1999
Hsp70	Heat, ischemia and mitogen induction are impaired	Blake et al. 1991; Deguchi et al. 1988; Faassen et al. 1988; Fargnoli et al. 1990; Heydari et al. 1994; Liu et al. 1996; Niedzwiecki et al. 1991; Nitta et al. 1994[b]
Hsp70	Exercise induction is maintained	Kregel and Moseley 1996

[a]Hsc70 denotes the non-inducible (cognate) form of Hsp70.
[b]The large number of reports allowed us to cite only those, which were among the firsts, or provided a review of other studies.

a self-cleavage at Asn-101 following the formation of the succinimide intermediate of the deamidation reaction (Voorter et al. 1988).

Thus a large variety of posttranslational modifications accumulate in proteins having an extended life-span. Although in normal conditions intracellular protein turnover is rather fast, carbonyl content of aging proteins – an indicator of oxidative damage – increases threefold in several tissues, such as brain, heart and liver (Stadtman 1992). However, extracellular proteins, like collagen also have a longer lifetime, and their accumulating proteotoxic damage leads to increased tissue rigidity as well as impairs cell–cell communication (Monnier and Cerami 1981).

Protein degradation is mostly accomplished by the proteasome and helped by various chaperones. Aging leads to a decrease in adaptive responses and consequently to an increased occurrence of proteotoxic conditions inside the cell. Aging also attenuates both the 'cellular surveillance' of chaperones to recognize damaged proteins, and the activity of the major cytoplasmic proteolytic apparatus, the proteasome (Conconi et al. 1996; Heydari et al. 1994; Liu et al. 1996). Besides the decline in the activation of protease systems, some oxidized, crosslinked proteins are much poorer substrates, but highly effective inhibitors of the proteasome (Friguet et al. 1994). All these events cause a massive accumulation of posttranslationally modified, misfolded proteins.

Chaperones and aging

The accumulation of misfolded proteins in aged organisms would require an increased amount of chaperones to prevent protein aggregation and to assist in refolding, or degradation. This may be the reason, why some aged species develop a constitutively increased level of several chaperones, such as Hsp22, or Hsp70 (Table 2). The higher amount of chaperone proteins is especially characteristic to the adaptive response of aging kidney, where increased fibrosis requires an additional amount of the collagen-specific Hsp47 (Razzaque et al. 1998). On the other hand, there is a large number of reports demonstrating that the induction of various chaperones is impaired in aged organisms (Table 2). Interestingly, while heat-induced synthesis of Hsp70 is impaired in aged rats, exercise in the same animal is able to induce a significant amount of Hsp70 (Kregel and Moseley 1996). Different changes in the induction mechanisms of various chaperones during the aging process are further substantiated by the findings of Fleming et al. (1988), who showed a substantially altered pattern of heat shock protein induction in old fruit flies compared to young species.

Differences in chaperone induction in aged animals and human subjects (Table 2) exclude the possibility of a general impairment in the transcriptional process of molecular chaperones. Indeed, the

level of heat shock factor-1, the transcription factor responsible for the induction of most chaperones is practically unchanged during aging. However, activation and binding of heat shock factor-1 to the heat shock element, its DNA-binding site in the promoter region of molecular chaperones is decreased in aged animals (Heydari et al. 1994; Pahlavani et al. 1995; Locke and Tanguay 1996). The exact mechanism of the defective activation is not known. In recent years several heat shock factor-binding proteins were identified, which all modulate the heat shock response (Morimoto 1998), and may well constitute the molecular mechanism of the differential impairment of chaperone-induction during aging.

Interestingly, there are much less reports on the functional changes of molecular chaperones during aging than those on their level, or induction. Chaperone activity of alpha crystallin is markedly decreased in senile human lenses (Cherian et al. 1995; Derham and Harding 1997). Due to the lack of protein synthesis and degradation in the lens, crystallins are one of the longest lived proteins in the human body, and therefore they are especially prone to various proteotoxic damage (see above). Hence it is not surprising that their activity is diminished in senescent lenses. This situation makes the so far not addressed question even more exciting, whether an impaired activity of other chaperones might also add to the damage caused by their diminished induction in aged organisms. As another of the sporadic examples of chaperone function in aged animals or human subjects, Hsp90 protects the age-related decline of proteasome activity. However, the association of Hsp90 with the proteasome decreases with age which may lead to an enhanced vulnerability of the proteasome for stress-induced damage in aged organisms (Conconi et al. 1996).

Chaperones in neurodegenerative diseases

Accumulation of misfolded proteins in aged organisms is especially pronounced in postmitotic cells, such as in neurons. The threat of damaged proteins becomes even greater if the protein is protease-resistant. The difficulties of protein degradation together with an impaired protease activity and chaperone action in aging neurons, lead to a massive accumulation of these proteins, and causes neurodegeneration. The best known example of this propagating disease is Alzheimer's disease, however several other neurodegenerative diseases, such as prion disease, Hungtington disease or others may also be mentioned. Several studies showed the induction of small heat shock proteins (Hsp27, crystallin), Hsp70 and ubiquitin (a 6 kDa heat shock protein, which labels damaged proteins and directs them for proteolytic degradation) in neurons affected by Alzheimer's disease and in surrounding astrocytes. Neuronal chaperones were localized in neuritic plaques and neurofibrillary tangles and were probably participating in the heroic attempts of the affected neuron to sequester the beta-amyloid and other damaged proteins (Cisse et al. 1993; Hamos et al. 1991; Perez et al. 1991; Renkawek et al. 1993; Shinohara et al. 1993). Interestingly the endoplasmic homologue of Hsp70, Grp78 showed an increased expression within successfully surviving neurons (Hamos et al. 1991). Other, not affected cells of Alzheimer victims, such as olfactory neurons (Getchell et al. 1995) or mononuclear blood cells (Wakutani et al. 1995) showed a decreased expression of Hsp70.

Chaperones and cellular senescence

Fibroblasts and other freshly isolated cells undergo only a limited number of divisions in cell culture. During the consequent duplications these cells change many of their original properties, a process, which is called cellular senescence (Smith and Pereira-Smith 1996). Increasing cellular senescence – on one hand – is well correlated with organismal aging. On the other hand, its induction by oncogenic stimuli contributes to organismal longevity by reducing the occurrence of cancer (Serrano et al. 1997). Cellular aging of fibroblasts is known to impair the induction of several chaperones, such as the collagen-specific Hsp47 (Miyaishi et al. 1995), Hsp70 and Hsp90 (Liu et al. 1989; Cristofalo et al. 1989). Similarly to the mechanism found in aged animals, activation and binding of heat shock factor-1 to the heat shock element is decreased in aged cells (Choi et al. 1990; Effros et al. 1994). The exact mechanism of the defective activation is also not known. In some studies a decrease in the amount of heat shock factor-1 has been found (Gutssmann-Conrad et al. 1998), while other studies suggest the presence of an inhibitory compound (Choi et al. 1990). In a recent report Bonelli et al. (1999) found an impairment in the posttranslational processing of Hsp70 mRNA resulting in its impaired nuclear export.

The defect of aged cells to induce Hsp70 may lead to their death in an unexpected way. Hsp70 is known to mediate the suppression of a stress-activated kinase, JNK, an early component of stress-induced apoptotic signalling pathway. Lacking a proper activation of Hsp70 senescent cells became prone for accelerated apoptosis after various stressful stimuli, such as heat shock (Gabai et al. 1998).

Several chaperones have a direct effect on cellular senescence. Overexpression of Hsp27 in bovine arterial endothelial cells leads to an accelerated growth and senescence. Interestingly, when a mutant, nonphosphorylatable form of Hsp27 was expressed, cellular senescence was hindered (Piotrowicz et al. 1995). When a mortality factor has been isolated from cytoplasmic extracts of senescing (mortal) fibroblasts, it turned to be a member of the Hsp70 chaperone family. This Hsp70-homologue, called mortalin is able to confer cellular senescence if transfected to immortal NIH 3T3 cells, and an antibody against mortalin could transiently stimulate cell division of senescent fibroblasts. Interestingly the protein exists in two isoforms, out of which only the cytosolic form is active, and its perinuclear homologue is not (Wadhwa et al. 1993a, b). As another possible involvement of chaperones in the regulation of cellular senescence, the 90 kDa heat shock protein, Hsp90 is required for the correct assembly and function of telomerase, a major enzyme involved in determining the life-span of cells (Holt et al. 1999).

Chaperones and longevity

Despite of the above accelerating effects of Hsp27 and the Hsp70 homologue, mortalin on senescence at the *cellular level in vitro*, there are several reports suggesting that increased chaperone action may also lead to an increased longevity of uni-, or multicellular *whole organisms in vivo*. Thermally conditioned *Drosophila* (Khazaeli et al. 1997) or *Caenorhabditis elegans* (Lithgow et al. 1995) exhibit greater longevity. Heat stress also induces an increased life-span of yeast (Shama et al. 1998), a process in which both Ras1 and Ras2, known to decrease and increase yeast life-span, respectively (Sun et al. 1994), and Hsp104, the key molecular chaperone inducing yeast thermotolerance were involved. Heat-shock induction of Hsp70 (Tatar et al. 1997), or overexpression of the heat shock protein, EF-1 alpha (Shepherd et al. 1989; Shikama et al. 1994) promotes fruit fly longevity. Hsp70 induction was lower in the liver of aged mice prone to accelerated senescence than in mice that were resistant to accelerated senescence (Nakanishi et al. 1997). Moreover, a close correlation was found between stress resistance and longevity in several long-lived *Caenorhabditis elegans* and *Drosophila* mutants, which were either engineered genetically, or selected with classical population genetics (Lithgow and Kirkwood 1996). These examples confirm the hypothesis that a better adaptation capacity to various stresses make a major contribution to life span extension.

Caloric restriction is the only effective experimental manipulation known to retard aging in rodents, and this manipulation has been shown to alter a variety of processes that change with age including the oxidative damage of proteins (Sohal and Weindruch 1996; Youngman et al. 1992). Caloric restriction (60% of *ad libitum* diet causing a 43% increase in life span) increased the induction of Hsp70 of hepatocytes (Heydari et al. 1993), proximal gut (Ehrenfried et al. 1996), alveolar macrophages (Moore et al. 1998), but not of splenocytes (Pahlavani et al. 1996) of aged rats compared to their aged littermates being on *ad libitum* diet. In several cases caloric restriction parallel with an induction of an extended life span, restored the impaired chaperone-induction of aged animals to the 'young' level.

Changes in the immune response against chaperones in aging

Molecular chaperones are highly conserved proteins throughout the evolution. Bacteria and other infectious organisms experience a large stress when invade the human body. The general stress response is turned on, and they overexpress a wide variety of chaperone molecules. Several of the induced chaperones also appears on the surface of infectious bacteria and parasites. The immune system develops an immune response against these antigens during the first infection occurring in the very first days of postnatal development. This immune response becomes more and more robust, since the chaperone-antigens of various infectious organisms are highly similar to each other. In several cases, when a self-protein shares an epitop with a bacterial heat shock protein or the antibacterial immune response becomes misdirected, an autoimmune attack might develop.

How does this (auto)immune response change during the aging process? The answer is not simple. On one hand as the individual ages, the exposure to the infectious, or self-antigen becomes more manifest, the immune-memory gets stronger. This explains the findings, where anti-chaperone antibody levels were found to increase with increasing age, such as the anti-Hsp70 in human malaria infections (Alexandre et al. 1997), the anti-Hsp90 in human systemic lupus erythematosus (Faulds et al. 1995), or the rat anti-DnaK (the *Escherichia coli* homologue of Hsp70) immune response (Kimura et al. 1996). On the other hand, the general decline of immune responses in aged individuals may also impair the anti-chaperone immune response. This might be the explanation for the decline in anti-Hsp70 antibodies in dilated cardiomyopathy (Portig et al. 1997), or for the decrease in anti-synthetic peptide autoantigen antibodies (Marchalonis et al. 1993).

Perspectives and closing remarks

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. The attenuation in molecular chaperone function and the simultaneous protein oxidation, misfolding and aggregation in aged organisms, as well as the correlation between adaptation capacity and life span raise the possibility that preservation of protein homeostasis and long-range protein organization can be major determinants in longevity (Figure 1). There are a plethora of opportunities to explore the underlying mechanisms behind these events.

- The exact mechanism of the attenuation of chaperone induction in aged organisms and in senescent cells will reveal interesting elements of the regulation of the stress response.
- There is a lot to do in examining the chaperone function in aged animals or cells, preferably using more complex, *in vivo* systems, such as expressed reporter proteins for folding, like luciferase. The role of chaperones in preventing membrane damage, or RNA-misfolding also deserves a much greater attention in aged organisms.
- The role of chaperones in cellular senescence, and in longevity is an area, which also keeps a lot of surprises for the coming years.

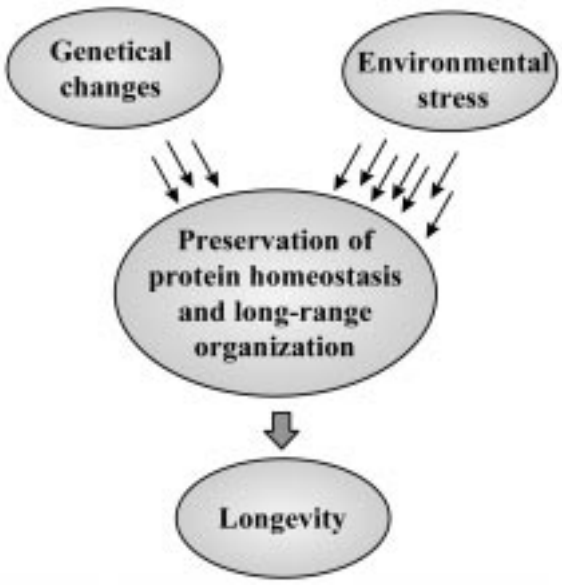

Figure 1. Hypothetical role of protein homeostasis and organization in longevity. Attenuation in molecular chaperone function and the simultaneous protein damage in aged organisms, as well as the correlation between stress adaptation capacity and life span raise the possibility that preservation of protein homeostasis and long-range protein organization can be major determinants in longevity. The putative role of chaperones in cytoplasmic protein organization is discussed in Pratt (1997) and in Csermely et al. (1998). The role of chaperones in buffering genetical changes providing a possibility for bursts in evolution has been recently demonstrated by Rutherford and Lindquist (1998).

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