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# Chaperones come of age

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**Abstract** Chaperone function plays a key role in repairing proteotoxic damage, in the maintenance of cell architecture, and in cell survival. Here, we summarize our current knowledge about changes in chaperone expression and function in the aging process, as well as their involvement in longevity and cellular senescence.

## INTRODUCTION: CONVENTIONAL AND NONCONVENTIONAL ROLES OF MOLECULAR CHAPERONES

Chaperones are ubiquitous, highly conserved proteins, which probably played a major role in the evolution of modern enzymes (Hartl 1996; Csermely 1997, 1999; Bukau and Horwich 1998). Chaperones are vital for our cells during their whole lifetime. However, they are needed even more after environmental stress leading to proteotoxic damage. Besides the need for assistance in protein folding and refolding, cellular life also requires a constant remodeling of cell structure. In accordance with this, chaperones are ideal candidates for the long-sought constituents of a cytoplasmic meshwork, originally named microtrabecluar lattice by Keith R. Porter (Wolosewick and Porter 1979; Pratt 1997; Csermely 2001a). Various targets of molecular chaperones: (1) newly synthesized proteins, (2) ''constantly damaged'' (mutant) proteins, (3) newly damaged proteins, and (4) constituents of the cytoplasmic meshwork are competing with each other. Medical efforts of the last 2 centuries potentially enhanced our chances to be the victim of multigenic, ''civilisation diseases'' by increasing the number of chaperone-repaired, phenotypically silent mutations in the human genome and by allowing us to survive to the age when the increasing competition for the buffering capacity of chaperones by damaged proteins of the aged organism helps to expose the silent mutations phenotypically (Rutherford and Lindquist 1998; Csermely 2001b). Chaperone occupancy emerges as an integrator of cellular, organismal, and populational responses.

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## PROTEIN DAMAGE AS A CONSEQUENCE AND CAUSE IN AGING

During the life span of a stable protein, various posttranslational modifications occur. Protein stability is abrogated by numerous noxious insults, including backbone and side chain oxidation, glycation, deamidation of asparaginyl and glutaminyl residues and the subsequent formation of isopeptide bonds. (Wright 1991; Stadtman and Berlett 1998). Susceptibility to various proteotoxic damages is increased because of transcriptional and translational errors and the resulting folding defects (Dukan et al 2000). Aging is characterized by an increased rate of protein modification aggravating the folding homeostasis. As we shall describe later, chaperone function is impaired, and therefore an increase in protein degradation is required. However, activity of the major cytoplasmic proteolytic apparatus, the proteasome, also declines with aging and is compromised by glycation (Conconi et al 1996; Bulteau et al 2001). Moreover, cross-linked and glycated proteins are often effective inhibitors of proteasomal function in vitro (Friguet et al 1994; Bulteau et al 2001). Aggregation also leads to a nearly quantitative proteasomal inhibition and cell cycle arrest in vivo (Bence et al 2001). Lysosomal protein degradation is also impaired in aged rats (Cuervo and Dice 2000), probably because of the lipofuscin-mediated inhibition of autophagy (Terman et al 1999). Accumulation of misfolded proteins and attenuation of defensive mechanisms result in the buildup of protein aggregates (reviewed by Sherman and Goldberg 2001), which have a deleterious effect on cellular function, at least in postmitotic tissues, and are a causative factor in the diseases of aging.

## CHAPERONE INDUCTION IN THE AGING PROCESS

The growing number of unfolded polypeptide chains may titrate out the chaperones from the heat shock factor

**Table 1** Chaperone levels and expression in aging

| Chaperone | Change/model system | References |
|---|---|---|
| Changes in the constitutive levels of chaperones in aging | | |
| Hsp22, Hsp23, Hsp70[a] | Elevated in *Drosophila* | Wheeler et al 1995 |
| Hsp47, Hsp70[a] | Elevated in rat kidneys | Maiello et al 1998; Razzaque et al 1999 |
| Hsc70[a] | Unchanged in hepatocytes | Wu et al 1993 |
| Hsc70[a] | Decreased in testis | Krawczyk and Szymik 1989 |
| Hsc70[a] | Elevated in liver of very old rats | Cuervo and Dice 2000 |
| Hsp70[a] | Unchanged in rat skeletal muscle | Locke 2000 |
| Changes in chaperone stress inducibility in aging | | |
| Hsp27 | Heat induction is impaired in human peripheral blood lymphocytes | Rao et al 1999 |
| Heme oxygenase (Hsp32) | Oxygen damage induction is impaired in liver of senescence-accelerated mice | Nakanishi and Yasumoto 1997 |
| Hsp60 | Heat induction is impaired in human peripheral blood lymphocytes | Rao et al 1999 |
| Hsp70[a] | Heat, ischemia, restraint stress and mitogen induction are impaired in liver, adrenal cortex, and lymphocytes of humans, mice, and rats | Deguchi et al 1988; Faassen et al 1989; Blake et al 1991; Heydari et al 1994[b] |
| Hsp70[a] | Heat and exercise induction is maintained in rat skeletal muscle and liver, respectively | Kregel and Moseley 1996; Locke 2000 |

[a] Hsp70 refers to the inducible, whereas Hsc70 refers to the constitutive form of the 70-kDa heat shock protein. For the sake of better comparison and because of the lack of appropriate data in many publications, we did identify the exact genes of the Hsp70 family in each of the studies cited.

[b] The large number of reports allowed us to cite only those that were among the first or provided a review of other studies.

(HSF) complex, thereby inducing a constitutive stress response and elevated chaperone levels in aged organisms (Table 1; Sőti and Csermely 2000). Although higher chaperone levels may reflect an adaptation mechanism, the induction of various chaperones is impaired in aging (Table 1). Whereas a few reports found no significant difference and inducibility, the vast majority of the reports show that stress-induced synthesis of heat shock proteins is impaired in aged animals. However, the extent of changes may vary from chaperone to chaperone, as demonstrated in an early study of Fleming et al (1988), who showed a substantially altered pattern of heat shock protein induction in old fruit flies compared with young species.

## CHANGES IN THE MECHANISM OF CHAPERONE INDUCTION

Differential chaperone induction in aged animals makes it unlikely that a general mechanism is responsible for the impairment in chaperone transcription. Indeed, the level of HSF-1 as well as trimerization, phosphorylation, and nuclear translocation are usually unchanged during aging. However, binding of HSF-1 to the heat shock element (HSE) is decreased in aged hepatocytes as well as in myocardial cells (Locke and Tanguay 1996; Heydari et al 2000). On the contrary, HSF activation and HSE binding is preserved in rat skeletal muscle (Locke 2000). The exact mechanism of the defective activation and the tissue-specific differences are not known. In recent years several HSF-1 binding proteins were identified, all of which modulate the heat shock response (Morimoto 1998) and may well constitute the molecular mechanism of the differential impairment of chaperone induction during aging.

## CHANGES IN CHAPERONE FUNCTION

Investigations on age-induced changes in chaperone function were mostly focused on α-crystallin: this abundant lens protein is one of the longest-lived human proteins, easy to purify, prone to proteotoxic damage, and plays an important role in cataract formation. Chaperone activity of α-crystallin is markedly decreased in senile human lenses (Cherian and Abraham 1995). Intramolecular disulfide formation underlies this phenomenon, and reparation partially restores the activity (Cherian-Shaw et al 1999). As another of the sporadic examples of chaperone function in aged animals or human subjects, Hsp90 fails to protect the proteasome in aged animals (Conconi et al 1996).

## PROTEIN MISFOLDING, CHAPERONES, AND CELLULAR SENESCENCE

Peripheral cells exhibit only a limited number of replications in cell culture. Morphological and functional properties change until the cell reaches a nondividing—senescent—state. Senescing fibroblasts cannot preserve the induction of several chaperones, such as the collagen-specific Hsp47, Hsp70, and Hsp90 (Cristofalo et al 1989; Liu et al 1989; Miyaishi et al 1995). Similar to the mech-

anism found in aged animals, activation and binding of HSF-1 to the HSE is decreased in aged cells (Choi et al 1990). The exact mechanism of the defective activation is not known.

Protein misfolding and aggregation can initiate cell cycle arrest or apoptosis by 2 mechanisms, proteasomal inhibition and induction of the c-jun N-terminal kinase (JNK)-dependent pathway, respectively (Volloch et al 1998; Bence et al 2001). A robust stress response can suppress JNK activation, by Hsp70-mediated JNK-phosphatase inhibition (Meriin et al 1999). Hsp70 can overcome both caspase-dependent and -independent apoptotic stimuli and confer immortality (Nylandsted et al 2000).

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 was isolated from cytoplasmic extracts of senescing (mortal) fibroblasts, it turned out to be a member of the Hsp70 chaperone family (Wadhwa et al 1993). 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).

The previous examples may lead to the conclusion that chaperones are promoting cellular senescence. However, chaperone induction per se seems to counteract senescence because repeated mild heat shock (a kind of hormesis) delays aging in fibroblasts (Rattan 1998). Impaired Hsp90 function leads to activation of HSF-1, restoration of the heat shock response, and slower chronological aging of nondividing *Saccharomyces cerevisiae* (Harris et al 2001).

## CHAPERONES EXTEND LIFE SPAN

There are several reports showing that increased chaperone induction leads to increased longevity of both uni- and multicellular whole organisms, like *Drosophila*, *C. elegans*, or yeast (Lithgow et al 1995; Tatar et al 1997; Shama et al 1998). Hsp22, Hsp23, and Hsp70 induction correlated with increased life span in *Drosophila* and mice (Nakanishi and Yasumoto 1997; Kurapati et al 2000). This effect can be generalized: a close correlation exists between stress resistance and longevity in several long-lived *C. elegans* and *Drosophila* mutants (Lithgow and Kirkwood 1996). These examples confirm the hypothesis that a better adaptation capacity to various stresses makes a major contribution to life span extension.

## CHAPERONES AND CALORIC RESTRICTION

Caloric restriction is the only effective experimental manipulation known to retard aging in rodents and primates by diminishing oxidative protein, deoxyribonucleic acid (DNA), and membrane damage (Youngman et al 1992; Sohal and Weindruch 1996; Ramsey et al 2000). Caloric restriction (60% of ad libitum diet causing a 43% increase in life span) increased the induction of Hsp70 by hepatocytes (Heydari et al 1993), proximal gut (Ehrenfried et al 1996), alveolar macrophages (Moore et al 1998), but not by splenocytes (Pahlavani et al 1996), of aged rats compared with their aged littermates on an ad libitum diet. Similar to these findings, a reversal of the age-induced constitutive Hsp levels upon calory restriction was demonstrated in DNA microarray assays of mouse liver (Cao et al 2001) and skeletal muscle (Weindruch et al 2001).

## PERSPECTIVES

Aging leads to a decay of self-defensive mechanisms and an accumulation of damage at the molecular-cellular and organismal level. The attenuation in molecular chaperone inducibility and the simultaneous accumulation of damaged proteins raise the possibility that preservation of protein homeostasis and long-range protein organization can be major determinants in longevity. There are plenty of exciting research areas for exploring these events:

- Our knowledge about the exact mechanism of the decline in chaperone induction in aging and in senescent cells is surprisingly little.
- Chaperones preventing membrane damage or ribonucleic acid misfolding are also interesting targets of future investigations in aged organisms.
- The delicate balance and competition between various targets of chaperones deserves much greater attention, especially in aging, where protein damage becomes abundant.
- As a special case when the need for chaperone action becomes tremendously high, neurodegenerative diseases are already an exciting field of chaperone research.
- Changes in the immune response against chaperones during the aging process also provide an area worthy of further exploration.

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