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Mini-review

Aging and molecular chaperones

Csaba Sőti*, Péter Csermely

Department of Medical Chemistry, Semmelweis University, P.O. Box 260, H-1444 Budapest 8, Hungary

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Abstract

Chaperone function plays a key role in sequestering damaged proteins and in repairing proteotoxic damage. Chaperones are induced by environmental stress and are called as stress or heat shock proteins. Here, we summarize the current knowledge about protein damage in aged organisms, about changes in proteolytic degradation, chaperone expression and function in the aging process, as well as the involvement of chaperones in longevity and cellular senescence. The role of chaperones in aging diseases, such as in Alzheimer's disease, Parkinson's disease, Huntington's disease and in other neurodegenerative diseases as well as in atherosclerosis and in cancer is discussed. We also describe how the balance between chaperone requirement and availability becomes disturbed in aged organisms, or in other words, how chaperone overload develops. The consequences of chaperone overload are also outlined together with several new research strategies to assess the functional status of chaperones in the aging process.

Keywords: Molecular chaperones; Heat shock proteins; Stress proteins; Hsp70; Hsp90; Protein aggregation; Protein denaturation; Chaperone overload

1. Introduction: molecular chaperones

Chaperones are ubiquitous, highly conserved proteins (Hartl, 1996), either assisting in the folding of newly synthesized or damaged proteins in an ATP-dependent active process or working in an ATP-independent passive mode sequestering damaged proteins for future refolding or digestion. Environmental stress leads to proteotoxic damage. Damaged, misfolded proteins bind to chaperones, and liberate the heat shock factor (HSF) from its chaperone complexes. HSF is activated and transcription of chaperone genes takes place (Morimoto, 2002). Most chaperones, therefore, are also called stress or (after the archetype of experimental stress) heat shock proteins (Hsp-s).

2. Aging proteins—proteins of aging organisms

During the life-span of a stable protein, various posttranslational modifications occur including backbone and side chain oxidation, glycation, etc. In aging organisms, the disturbed cellular homeostasis leads to an increased rate of protein modification: in an 80-year old human, half of all proteins may become oxidized (Stadtman and Berlett, 1998). Susceptibility to various proteotoxic damages is mainly increased due to dysfunction of mitochondrial oxidation of starving yeast cells (Aguilaniu et al., 2001). In prokaryotes, translational errors result in folding defects and subsequent protein oxidation (Dukan et al., 2000), which predominantly takes place in growth arrested cells (Ballesteros et al., 2001).

Additionally, damaged signalling networks loose their original stringency, and irregular protein phosphorylation occurs (e.g.: the Parkinson disease-related α-synuclein also becomes phosphorylated, leading to misfolding and aggregation; Neumann et al., 2002).

3. Aging protein degradation

Irreversibly damaged proteins are recognized by chaperones, and targeted for degradation. Proteasome level and function decreases with aging, and some oxidized, aggregated proteins exert a direct inhibition on

Abbreviations: HSF, heat shock transcription factor; Hsp, heat shock or stress protein; Grp, glucose regulated protein (the number refers to the molecular weight in kilodaltons).

* Corresponding author. Tel.: +36-1-266-2755x4043; fax: +36-1-266-7480.
E-mail address: csaba@puskin.sote.hu (C. Sőti).

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proteasome activity. Chaperones also aid in lysosomal degradation. The proteolytic changes are comprehensively reviewed by Szweda et al. (2002). Due to the degradation defects, damaged proteins accumulate in the cells of aged organisms, and by aggregation may cause a variety of protein folding diseases (reviewed by Sőti and Csermely, 2002a).

4. Aging chaperones I: defects in chaperone induction

Damaged proteins compete with the HSF in binding to the Hsp90-based cytosolic chaperone complex, which may contribute to the generally observed constitutively elevated chaperone levels in aged organisms (Zou et al., 1998; Sőti and Csermely, 2002b). On the contrary, the majority of the reports showed that *stress-induced synthesis* of chaperones is impaired in aged animals. While HSF activation does not change, DNA binding activity may be reduced during aging (Heydari et al., 2000). A number of signalling events use an overlapping network of chaperones not only to establish the activation-competent state of different transcription factors (e.g. steroid receptors), but also as important elements in the attenuation of respective responses. HSF transcriptional activity is also negatively influenced by higher levels of chaperones (Morimoto, 2002). Differential changes of these proteins in various organisms and tissues may lead to different extents of (dys)regulation. More importantly, the cross-talk between different signalling pathways through a shared pool of chaperones may have severe consequences during aging when the cellular conformational homeostasis is deranged (see below).

5. Aging chaperones II: defects in chaperone function

Direct studies on chaperone function in aged organisms are largely restricted to α-crystallin having a decreased activity in aged human lenses (Cherian and Abraham, 1995; Cherian-Shaw et al., 1999). In a recent study, an initial test of passive chaperone function of whole cytosols was assessed showing a decreased chaperone capacity in aged rats compared to those of young counterparts (Nardai et al., 2002).

What can be the mechanism behind these deleterious changes in chaperone function? Chaperones may also be prone to oxidative damage, as GroEL is preferentially oxidized in growth-arrested *E. coli* (Dukan and Nyström, 1999). Macario and Conway de Macario (2002) raised the idea of 'sick chaperones' in aged organisms in a recent review. Indeed, chaperones are interacting with a plethora of other proteins (Csermely, 2001a), which requires rather extensive binding surfaces. These exposed areas may make chaperones a preferential target for proteotoxic damage: chaperones may behave as 'suicide proteins' during aging, sacrificing themselves instead of 'normal' proteins. The high abundance of chaperones (which may constitute more than 5% of cellular proteins), and their increased constitutive expression in aged organisms makes them a good candidate for this 'altruistic courtesy.' It may be especially true for mitochondrial Hsp60, the role of which would deserve extensive studies.

6. Aging chaperones III: defects in capacity, the chaperone overload

Another possible reason of decreased chaperone function is chaperone overload (Csermely, 2001b). In aging organisms, the balance between misfolded proteins and available free chaperones is grossly disturbed: increased protein damage, protein degradation defects increase the amount of misfolded proteins, while chaperone damage, inadequate synthesis of molecular chaperones and irreparable folding defects (due to posttranslational changes) decrease the amount of available free chaperones. Chaperone overload occurs, where the need for chaperones may greatly exceed the available chaperone capacity (Fig. 1).

Under these conditions, the competition for available chaperones becomes fierce and the abundance of damaged proteins may disrupt the folding assistance to other

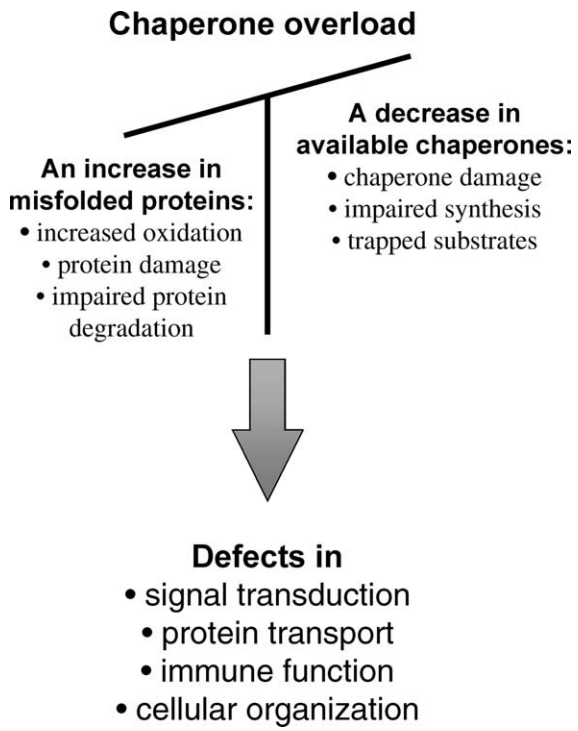

Fig. 1. Chaperone overload: a shift in the balance between misfolded proteins and available free chaperones in aging organisms. The accumulation of chaperone substrates along with an impaired chaperone function may exhaust the folding assistance to specific chaperone targets and leads to deterioration in vital processes. Chaperone overload may significantly decrease the robustness of cellular networks, and compromise the adaptative responses. See text for details.

chaperone targets, such as: (1) newly synthesized proteins; (2) 'constantly damaged' (mutant) proteins; and (3) constituents of the cytoarchitecture (Csermely, 2001a). This may cause defects in signal transduction, protein transport, immune recognition, cellular organization as well as the appearance of previously buffered, hidden mutations in the phenotype of the cell (Csermely, 2001b). Chaperone overload may significantly decrease the robustness of cellular networks, as well as shift their function towards a more stochastic behavior. As a result of this, aging cells become more disorganized, their adaptation is impaired.

7. Senescent cells and chaperones

The involvement of chaperones in aging at the cellular level is recently reviewed (Sőti et al., 2003). Non-dividing-senescent-peripheral cells tend to have increased chaperone levels (Verbeke et al., 2001), and cannot preserve the induction of several chaperones (Liu et al., 1989), similarly to cells from aged animals. Activation and binding of HSF to the heat shock element is decreased in aged cells (Choi et al., 1990). Interestingly, cellular senescence seems to unmask a proteasomal activity leading to the degradation of HSF (Bonelli et al., 2001).

Chaperone induction per se seems to counteract senescence. Repeated mild heat shock (a kind of hormesis) has been reported to delay fibroblast aging (Verbeke et al., 2001), though it does not seem to extend replicative life-span. A major chaperone, Hsp90 is required for the correct function of telomerase, an important enzyme to extend the life-span of cells (Holt et al., 1999).

Mortalin (mtHsp70/Grp75), a member of the Hsp70 family, produces opposing phenotypic effects related to its localization. In normal cells, it is pancytoplasmically distributed, and its expression causes senescence. Its upregulation and perinuclear distribution, however, is connected to transformation, probably via p53 inactivation. Mortalin also induces life-span extension in human fibroblasts or in *C. elegans* harboring extra copies of the orthologous gene (Kaul et al., 2002).

8. Aging organisms and chaperones: age-related diseases

Unbalanced chaperone requirement and chaperone capacity in aged organisms helps the accumulation of aggregated proteins, which often cause folding diseases, mostly of the nervous system, due to the very limited proliferation potential of neurons. Over expression of chaperones often delays the onset or diminishes the symptoms of the disease (Sőti and Csermely, 2002b).

Other aging diseases, such as atherosclerosis and cancer are also related to chaperone action. Here space limitation precludes a detailed description of these rapidly developing fields, however, numerous recent reviews were published on these subjects, where the interested readers may find a good summary and several hints for further readings (Ferreira and Carlos, 2002; Neckers, 2002; Sarto et al., 2000; Wick and Xu, 1999).

9. Chaperones and longevity

Increased chaperone induction leads to increased longevity (Tatar et al., 1997). Moreover, a close correlation exists between stress resistance and longevity in several long-lived *C. elegans* and *Drosophila* mutants (Lithgow and Kirkwood, 1996). As the other side of the same coin, damaged HSF has been found as an important gene to cause accelerated aging in *C. elegans* (Garigan et al., 2002). Caloric restriction, the only effective experimental manipulation known to retard aging in rodents and primates (Ramsey et al., 2000), restores age-impaired chaperone induction, while reversing the age-induced changes in constitutive Hsp levels (see Sőti and Csermely, 2002a,b). These examples confirm the hypothesis that a better adaptation capacity to various stresses greatly increases the chances to reach longevity.

10. 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. The protein oxidation, damage, misfolding and aggregation together with the simultaneously impaired function and induction of chaperones in aged organisms disturb the balance between chaperone requirement and availability. There are several important aspects for future investigation of this field:

- the measurement of active chaperone function (i.e. chaperone-assisted refolding of damaged proteins) in cellular extracts does not have a well-established method yet;
- we have no methods to measure free chaperone levels;
- among the consequences of chaperone overload, changes in signal transduction, protein transport, immune recognition and cellular organization have not been systematically measured and/or related to the protein folding homeostasis of aging organisms and cells.

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