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Review

Chaperones and aging: role in neurodegeneration and in other civilizational diseases

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Abstract

Chaperones are highly conserved proteins responsible for the preservation and repair of the correct conformation of cellular macromolecules, such as proteins, RNAs, etc. Environmental stress leads to chaperone (heat-shock protein, stress protein) induction reflecting the protective role of chaperones as a key factor for cell survival and in repairing cellular damage after stress. The present review summarizes our current knowledge about the chaperone-deficiency in the aging process, as well as the possible involvement of chaperones in neurodegenerative diseases, such as in Alzheimer's, Parkinson's, Huntington- and prion-related diseases. We also summarize a recent theory implying chaperones as "buffers" of variations in the human genome, which role probably increased during the last 200 years of successful medical practice minimizing natural selection. Chaperone-buffered, silent mutations may be activated during the aging process, which leads to the phenotypic exposure of previously hidden features and might contribute to the onset of polygenic diseases, such as atherosclerosis, cancer, diabetes and several neurodegenerative diseases.

1. Introduction: general functions of chaperone proteins

Molecular chaperones (1) protect against protein aggregation; (2) solubilize initial, loose protein aggregates; (3) assist in folding of nascent proteins or in refolding of damaged proteins; (4) target severely damaged proteins to degradation; and (5) in the case of excessive damage, sequester damaged proteins to larger aggregates. Chaperones are ubiquitous, highly conserved proteins, which utilize a cycle of ATP-driven conformational changes to refold their targets and which probably played a major role in the molecular evolution of modern enzymes (Csermely, 1997, 1999; Hartl, 1996).

Environmental stress (a sudden change in the cellular environment, to which the cell is not prepared to respond, such as 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. Besides the major classes of chaperones listed in Table 1, which generally target all misfolded proteins with hydrophobic surfaces, there are also specialized chaperones, like Hsp47, which is the procollagen–chaperone (Nagata, 1998). Chaperones usually increase only the yield but not the speed of protein folding. However, special chaperones, called "folding catalysts" may accelerate certain steps of protein folding, such as the isomerization of peptide bonds besides prolyl residues (peptidyl-prolyl cis/trans isomerases, or immunophilins), or the formation of disulfide bridges (protein disulfide isomerases) (Bukau and Horwich, 1998; Hartl, 1996).

Besides the conformational homeostasis of individual macromolecules, higher levels of cellular organization also need a constant remodeling. Chaperones are obvious candidates to provide help in these processes. Most major chaperones are helped by several smaller co-chaperones (e.g. Hsp60 with Hsp10, Hsp70 with members of the DnaJ and GrpE families, Hsp90 with Hsp70, DnaJ homologues, p23, etc.; Bukau and Horwich, 1998; Csermely et al., 1998; Hartl, 1996). These chaperones form highly dynamic, low-affinity complexes. These large assemblies of individual chaperones are attached to the microfilamental and microtubular system. About 20 years ago based on high-voltage electron microscopy Keith A. Porter and co-workers suggested the existence of a cellular meshwork, called as "microtrabecular lattice" to organize cytoplasmic proteins and RNAs (Schliwa et al., 1981). Almost instantly a fierce debate arose considering the lattice as an artifact of the techniques

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Table 1
Major classes of molecular chaperones

Most important eukaryotic representatives[a]	Recent reviews
Hsp25[b], Hsp27, crystallins, small heat-shock proteins	Arrigo (1998), Head and Goldman (2000)
Hsp60, chaperonins	Bukau and Horwich (1998), Hartl (1996), Thirumalai and Lorimer (2001)
Hsp70, Hsc70, Grp78	Bukau and Horwich (1998), Hartl (1996), Ohtsuka and Suzuki (2000)
Hsp90, Grp94	Csermely et al. (1998), Pratt et al. (1999), Richter and Buchner (2001), Young et al. (2001)
Hsp104	Porankiewicz et al. (1999)

[a] Neither the co-chaperones (chaperones which help the function of other chaperones listed), 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. Several chaperones of the endoplasmic reticulum (e.g. calreticulin, calnexin, etc.), which do not belong to any of the major chaperone families, as well as some heat-shock proteins (e.g. ubiquitin), which do not possess chaperone activity were also not mentioned.

[b] "Hsp" and "Grp" refer to heat-shock proteins and glucose regulated proteins, chaperones induced by heat-shock or glucose deprivation, respectively. Numbers refer to their molecular weight in kDa.

used. However, as time passes more and more data provide indirect evidence for a high-order organization of the cytoplasm (Ovadi and Srere, 2000; Verkman, 2002). Chaperones are ideal candidates for being a major constituent of a cytoplasmic meshwork: they are highly abundant, form a complex with all the elements of the cytoskeleton and also attach to a plethora of other proteins. Several lines of initial evidence shows that disruption of chaperone/protein complexes disturbs the organization of cytoplasmic traffic of several proteins, such as the steroid receptors and accelerates cell lysis (Csermely, 2001a; Pato et al., 2001; Pratt et al., 1999).

2. Protein folding and the aging process

During the life-span of a stable protein various post-translational 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. In several cases age-related post-translational modifications induce conformational changes and impaired protein function: 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).

Due to their vulnerability for aggregation accumulating misfolded proteins pose a great danger to the aging cell. Since the reason of the folding anomaly is mostly a post-translational modification, the change becomes irreversible and can not be reversed by molecular chaperones. Chaperones may only accompany these proteins and by a stable association with their hydrophobic surfaces, prevent their aggregation. Thus, the only solution to protect the cell from these misfolded proteins is their elimination and not their repair. Protein degradation is mostly accomplished by the proteasome and helped by various chaperones. Aging leads to a decrease in the activity of the major cytoplasmic proteolytic apparatus, the proteasome (Conconi et al., 1996; Heydari et al., 1994). Besides the decline in the activation of protease systems, some oxidized, glycated and aggregated proteins are much poorer substrates, but highly effective inhibitors of the proteasome (Bence et al., 2001; Bulteau et al., 2001; Friguet et al., 1994). Autophagic lysosomal protein degradation is also impaired in aged rats (Cuervo and Dice, 2000), probably due to the lipofuscin-mediated inhibition of autophagy (Terman et al., 1999). All these events cause a massive accumulation of posttranslationally modified, misfolded proteins.

3. Chaperones in aged organisms and brain

Accumulation of misfolded proteins in aged organisms requires an increased amount of chaperones to prevent protein aggregation. This may be the reason, why some aged species develop a constitutively increased level of several chaperones, such as small heat-shock proteins or Hsc70 (Söti and Csermely, 2000). On the other hand, there is a large number of reports demonstrating that the induction of various chaperones is impaired in aged organisms (Söti and Csermely, 2000). 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).

The above general statements can be applied to chaperone levels and chaperone inducibility in the brain of aged organisms. Level of several chaperones, such as small heat-shock proteins and Hsc70 is elevated, while the inducibility of Hsp70 is impaired (Table 2). In contrast to ad libitum fed rats, Hsc70 elevation could not be observed in food restricted rats (Unno et al., 2000). Moreover, the brain of aged, food-restricted rats does not display a loss of capacity to accumulate Hsp70 in response to heat stress

Table 2
Changes of chaperone expression in the aging nerve system

Chaperone	Change	Reference
Chaperone levels		
Ubiquitin, Hsp27, alpha B-crystallin	Elevated in pallido-nigral spheroid bodies	Schultz et al. (2001)
Hsc70[a]	Elevated in pons, medulla, striatum and thalamus	Unno et al. (2000)
Chaperone induction		
Hsp70	Heat induction is impaired	Rogue et al. (1993)
Hsp70	Heat induction is maintained in food-restricted rats	Walters et al. (2001)

[a] Hsc70 denotes the non-inducible (cognate) form of Hsp70.

(Walters et al., 2001). This shows that calorie-restriction, a well-known method to increase longevity (Hall et al., 2000; Ramsey et al., 2000), maintains the brain chaperone system in a "young-state". On the other hand, rats maintained on a dietary restriction schedule exhibit increased resistance of hippocampal neurons and striatal neurons to excitotoxic and metabolic stress (Bruce-Keller et al., 1999). Calorie-restriction also attenuates the degeneration of dopaminergic neurones in mouse Parkinson models (Duan and Mattson, 1999).

4. 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 cause neurodegeneration (Macario and Conway de Macario, 2001).

Oxidative damage and inflammatory processes are more prevalent during aging, accompany and aggravate neurodegeneration (Gibson et al., 2000; Goodman and Mattson, 1994; Hemmer et al., 2001). Several molecular chaperones are involved in the maintenance of cellular redox status (Arrigo, 1998) and protect neurones against oxidative stress (Lee et al., 1999; Yu et al., 1999). However, a direct effect of chaperones on aging-, or neurodegeneration-induced redox changes has not been demonstrated yet.

4.1. Chaperones and Alzheimer's disease

The best known example of folding-related neurodegenerative diseases is Alzheimer's disease. 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 (Cisse et al., 1993; Hamos et al., 1991; Perez et al., 1991; Renkawek et al., 1993; Shinohara et al., 1993).

Accumulated chaperones are participating in the heroic attempts of the affected neuron to sequester the β-amyloid and other damaged proteins in Alzheimer's disease (Hamos et al., 1991; Kouchi et al., 1999). However, the small heat-shock protein, αB-crystallin enhanced the neurotoxicity of the amyolid-β 1–40 peptide probably by keeping it in a nonfibrillar, highly toxic form (Stege et al., 1999). Cytoplasmic Hsp60, a specific chaperone for actin and tubulin is decreased in Alzheimer's disease-affected neurons leaving the cytoskeletal proteins deficient and aggregated (Schuller et al., 2001). Non-affected nerve cells of Alzheimer victims, such as olfactory neurons (Getchell et al., 1995) showed also a decreased expression of Hsp70.

Since the amyloid precursor is an integral protein of the plasma membrane, which should be processed in the endoplasmic reticulum (ER), the ER might be an especially important scene for the fight for cell survival. Indeed, calreticulin, an abundant ER chaperone was shown to participate in the quality control of the amyloid precursor protein (Johnson et al., 2001) and the ER-homologue of Hsp70, Grp78 had an increased expression in successfully surviving neurons (Hamos et al., 1991). There are reports to show that mutant presenilin-1, an ER transmembrane protein being the most prevalent cause of early-onset familial Alzheimer's disease, impairs the ER chaperone response and thus sensitizes the affected neuron to apoptosis. However, this latter finding could not be confirmed in other systems (Lee, 2001).

4.2. Chaperones and Parkinson's disease

Parkinson's disease is an age-related disorder characterized by a progressive degeneration of dopaminergic neurons in the substantia nigra and showing a corresponding motor deficit. An increasing number of evidence shows that besides oxidative stress and mitochondrial dysfunction protein folding defects are also key elements of Parkinson's disease etiology. Similarly to Alzheimer's disease, glial and astroglial cells of Parkinson's disease victims showed the expression of αB-crystallin and similarly to neurofibrillary tangles aggregated proteins in Lewy bodies had a large

content of various heat-shock proteins (Jellinger, 2000). Dietary restriction induced an expression of Hsp70 and Grp78 parallel with a protection in a Parkinson's disease model (Duan and Mattson, 1999). Interestingly, parkin, the protein whose mutations cause the autosomal recessive juvenile parkinsonism was identified as an ubiquitin-ligase playing a key role in the degradation of ER misfolded proteins, such as a G-protein coupled membrane receptor, called Pael and synphilin, an α-synuclein interacting protein (Chung et al., 2001; Imai et al., 2001). This gives us one more example for the similarities of the protein folding homeostasis in Parkinson's and Alzheimer's diseases.

4.3. Chaperones in polyglutamine diseases such as Hungtington's disease

Polyglutamine repeats make proteins vulnerable for aggregation. Diseases, such as Hungtington's disease, Kennedy spinal bulbar muscular atrophy, spinocerebral ataxia, Machado-Joseph disease, etc. all develop due to an expansion of polyglutamine segments in the respective proteins. Chaperones co-localize with the aggregates of these polyglutamine-containing proteins and increased chaperone levels, such as that of Hsp40, Hsp60, Hsp70, Hsc70, Hsp100 inhibit polyglutamine-containing protein aggregation and slow down the progress of the disease (Carmichael et al., 2000; Cummings et al., 1998; Hughes and Olson, 2001; Krobitsch and Lindquist, 2000).

4.4. Chaperones in prion-related diseases

Prions are proteins, which have a rather extraordinary structure trapped in a high-energy state containing α-helices. These "normal" prions are present in our brain, can not form large aggregates and are sensitive to proteolysis. If their conformation switches to the low-energy state, characterized by β-sheet formation, they become resistant for proteolysis and prone to excessive aggregation (Baskakov et al., 2001). The in vivo functions of prions are not entirely clear. They probably play a role in signaling, in copper metabolism, in redox regulation and in the protection of neurons (Prusiner, 2001). Prion aggregation, which is a cause of Creutzfeld-Jakob disease, involves many chaperones. Chaperones try to block the contact surfaces of "transformed" prion molecules. Therefore, it is not surprising, that many chaperones, such as Hsp60, Hsp70, or its co-chaperone, Hsp40 were found to fight against prion aggregation (DebBurman et al., 1997; Newnam et al., 1999). Similarly a proteolytic attack is tried to be achieved, therefore the labeling for proteasomal degradation, the ubiquitinylation is also present (Laszlo et al., 1992). Since prions are also extracellular proteins attached to the plasma membrane, the fight against their transformed (mutant) forms begins already in ER. Grp78, the ER homologue of Hsp70 binds to these deadly prions and mediates their degradation by the proteasome (Jin et al., 2000).

An even more interesting role can be assigned to the Hsp100 chaperone. Prions can be cured by both the deletion and overexpression of Hsp100 in yeast, whose primary function in prion propagation is to disassemble prion aggregates and generate the small prion seeds that initiate new rounds of prion propagation (Chernoff et al., 1995; DebBurman et al., 1997; Wegrzyn et al., 2001). This situation, where the "help" of the chaperone may actually promote an even more deadly form of the disease, resembles to the αB-crystallin-enhanced neurotoxicity of amyolid-β 1–40 (Stege et al., 1999) mentioned previously. Thus, chaperones may both neutralize and activate aggregation processes depending on the exact chaperone-target ratio. This vastly different final outcome of minor changes in chaperone availability warns us to focus on chaperone occupancy much more seriously than previously thought. The next chapter will elaborate this idea further.

5. Chaperone overload: a possible contribution to the onset of several polygenetic diseases

Besides their protective role against protein aggregation chaperones may participate in the prevention of various polygenic diseases in a rather unexpected manner. Recently, one of the major cytoplasmic chaperones, Hsp90 was shown to act as posttranslational "silencer" of several genetical changes by assisting in an efficient repair of folding defects (Rutherford and Lindquist, 1998). However, severe stress leads to the accumulation of damaged, misfolded proteins. Stress-induced, new chaperone-targets compete with existing malfolded proteins, which leads to a decline in chaperone-mediated repair of conformational defects.

According to a recent hypothesis (Csermely, 2001b) the development of modern medical practice depressed natural selection by its groundbreaking achievements to reduce prenatal and infant mortality leading to a rise of phenotypically silent mutations in the genome. As a consequence we carry more and more chaperone-buffered, silent mutations from generation to generation. The chance of the phenotypic manifestation of these mutations becomes especially large in aged subjects, where protein damage is abundant and chaperone induction is impaired. The background of misfolded proteins increases and by competition prevents the chaperone-mediated buffering of silent mutations. Phenotypically exposed mutations contribute to a more abundant manifestation of multigene-diseases, such as atherosclerosis, autoimmune-type diseases, cancer, diabetes, hypertensive cardiovascular disease and several psychiatric illnesses (Alzheimer's disease, schizophrenia, etc.). The "chaperone overload" hypothesis emphasises the need for efficient ways to enhance chaperone-capacity in ageing subjects and calls for the identification and future "repair" of silent mutations (Csermely, 2001b).

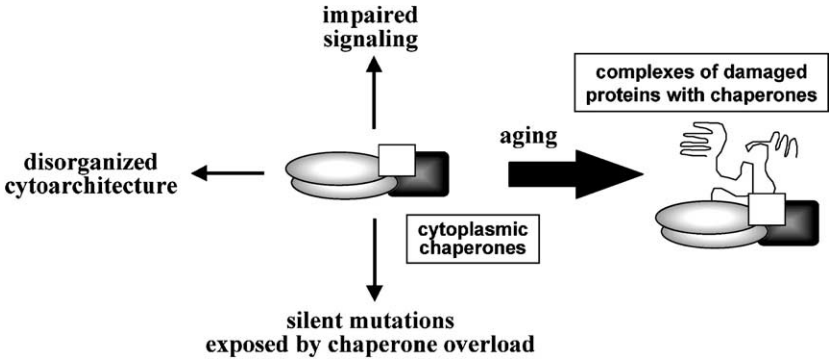

Fig. 1. Competition for chaperone occupancy and its changes in the aging process. Clockwise from bottom: (a) phenotypically buffered, silent mutations require the assistance of chaperones to rescue them from folding traps (Rutherford and Lindquist, 1998; Csermely, 2001b). (b) Chaperones form low affinity and highly dynamic extensions of the cytoskeleton participating in cellular traffic and in the organisation of the cytoarchitecture (Csermely, 2001a; Pratt et al., 1999). (c) Cytoplasmic chaperones of eukaryotic cells participate in the maintenance of the conformation of some, selected protein substrates. Most of these unstable proteins are parts of various signalling cascades (Csermely et al., 1998; Pratt et al., 1999). (d) During the aging process, chaperones become more and more occupied by damaged proteins. As a consequence of this: (1) silent mutations escape and contribute to the onset of polygenic diseases; (2) cell architecture becomes disorganized; (3) signaling is impaired. The verification of these, presently largely hypothetical, changes requires further experimentation.

6. 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 inducibility and availability and the simultaneous protein oxidation, misfolding and aggregation in aged organisms raise the gap between the amount of tasks and the available help as aging proceeds. Since numerous key elements of cellular life are competing with each other for the maintenance and repair function of molecular chaperones (damaged proteins, signaling proteins, silent mutations and cytoarchitecture, see Fig. 1) chaperones emerge as a central switchboard of the integration of cellular homeostasis. Aging shifts the "normal" functions of chaperones from the maintenance of cytoarchitecture, signaling and silent mutations to their struggle with the increasing amount of damaged proteins. Cells become disorganized, non-responsive and silent mutations escape causing unexpected disturbances and the development of various polygenic diseases. There are a plethora of opportunities to explore the complexity of these competitive events:

- Exposure of silent mutations can be manipulated by the overexpression of chaperones, administration of chaperone-specific antisense oligonucleotides, or overexpression of damaged proteins. The incidence of polygenic diseases can be monitored.
- Chaperone-sensitive signaling events (such as those of nuclear hormone receptors, the MAP kinase cascade, apoptosis, etc.) as well as the organization of the cytoarchitecture could be assessed after similar manipulations of chaperone and/or damaged protein levels.
- All these experiments could be tested in aged organisms and the aggravation, or prevention of aging-induced damage can be monitored.

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