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Review

Sick chaperones and ageing: a perspective

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Abstract

Intra- and extra-cellular protein deposits characterize many pathologic disorders (proteinopathies). These deposits contain different proteins, some of which are distinctive of a given disorder or type of disease, whereas others are not. The disorder-specific proteins are as a rule defective and have a tendency to misfold, aggregate, and precipitate—thus deposits are formed. The abnormal proteins typical of the better-characterized proteinopathies are not molecular chaperones, and the chaperoning systems in patients with these diseases are apparently normal. In this article, disorders in which abnormal chaperones seem to be the main pathogenetic factor are presented as a subset of the proteinopathies and termed chaperonepathies. A defective chaperone molecule (sick chaperone) will impact on the chaperoning system and pathway in which it normally participates. Since chaperones are ubiquitous their malfunctioning will have universal deleterious effects. In fact, the few known disorders in which a sick chaperone seems to be the main etiology, are clinically heterogeneous with many tissues affected. Senescence is characterized by a progressive decline of vigor and function throughout the body reflecting the failure of many molecules due to wear and tear, accumulation of mutations, and perhaps other factors. It is likely that this age-associated decline is due in part to a progressive failure of the chaperoning systems. Senescence would proceed even faster when a defective chaperone appears early in life due to a genetic defect. Furthermore, sick chaperones will make the individual vulnerable to stressors since the chaperoning systems are key anti-stress mechanisms. In this context, a combination of sick chaperones with stress appears as a driving force in the process of ageing.

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1. Stress and molecular chaperones: a unifying view

Study of stress is part of several disciplines some of which are far apart from each other in subject matter, methods, and purpose (McQuade and Aikman, 1974; Selye, 1974; McEwen, 2000; Macario and Conway de Macario, 2000a). Although there is overlap among all disciplines that include study of stress in living beings, cross-fertilization among them has not yet reached the level that would produce the benefits one can reasonably expect from such interaction. For example, study of psychological stress in humans and research on *Escherichia coli* proteases could benefit from each other's progress in areas pertaining to the role of protein degradation in gene regulation during stress. Likewise, study of heat shock in bacteria deals with questions and problems that are very similar to those pertinent to the study of stress in archaea, and the latter studies have relevance to stress in eukaryotes including humans (Conway de Macario and Macario, 2000). However, there is relatively little interaction among these fields despite the fact that bacteria and archaea are prokaryotes very similar in a number of characteristics, and archaea share with eukaryotes various key features, whose elucidation in archaea will help significantly in the understanding of stress and anti-stress mechanisms in eukaryotes (see for example Section 4.2).

It may be said that all types of stress in living organisms have the same central effect: protein denaturation inside the cell. It is known that stress also affects nucleic acids, lipids, and membranes. However, some of these latter structures are affected by stress via prior protein damage (denaturation). Due to this, and for the purpose of this article, protein denaturation will occupy the central stage in the drama brought about by stressors, the stress response, and the attempts by the cell to survive. This drama has unavoidable consequences, some of which are manifestations of senescence. Thus stress has to be considered a major factor in ageing.

The degree of protein denaturation varies among the multiple protein species and families in any given cell and organism, and for a single type of protein it varies depending upon the molecules present in the cell at the time the stressor impacts on it. These variations depend on: (i) the folding stage at which any given molecule happens to be at the time of stressor impact; (ii) its location in the cell with respect to membranes, ribosomes, and other multi-molecular assemblies; and (iii) its degree of association with other proteins (Fig. 1). More importantly, however, the degree of denaturation will be determined by the built-in resistance of a protein that depends on its primary and secondary structures and their integrity at the time of stressor impact. Mutations that alter these structures may have profound effects on the protein's foldability and resistance to denaturation by stressors. Pathological disorders arise as a consequence of these mutations, or posttranscriptional or posttranslational aberrations, that endow the protein with a tendency to misfold and aggregate (for review see Dobson, 1999; Kopito, 2000; Chapple et al., 2001;

Macario and Conway de Macario, 2001a; McNaught et al., 2001; Prusiner, 2001; Selkoe, 2001). Aggregated proteins can form precipitates (deposits) inside and around the cell, which are a cause of cell damage (Fig. 1). Although not a universal rule, it may be said that the larger the aggregate-deposit, the higher the probability that the cell will become ill and die. Due to this tendency to misfold and aggregate, mutant or otherwise abnormal proteins make the cell highly susceptible to stress and its consequences. Stress will accelerate the progression of a disease due to an abnormal protein with intra- or extracellular precipitates. Stressors may even start the development of lesions when a mutation for example does not by itself cause marked misfolding and aggregation but results in a protein with less stability in the face of stress. Similarly, stress would accelerate senescence by causing aggregation of defective proteins that appear during ageing due to mutation and wear and tear.

2. Anti-stress mechanisms

Nature has evolved anti-stress mechanisms many of which involve proteins (for review see Macario and Conway de Macario, 2001b). Some of these proteins are molecular chaperones (Ellis and van der Vies, 1990; Hartl and Martin, 1995; Netzer and Hartl, 1998; Buchner, 1999; Bukau et al., 2000; Lee, 2001). Integrity and functionality of anti-stress mechanisms, including the molecular chaperones and their coding genes, are key elements in the maintenance of all cellular proteins within functional, efficient, ranges of concentration and configuration (Fig. 1). Readiness and functionality of the whole protein complement in any given cell depends to a considerable extent on optimal functioning of molecular chaperones. The need for this readiness is especially critical when a stressor impacts on a cell. Diseases may be caused and ageing may be enhanced by deficiencies of anti-stress mechanisms. This article focuses on deficiencies in molecular chaperones as potential primary or secondary cause of pathologic disorders and as enhancers of the ageing process.

Proteins denatured beyond repair due to stress, or misfolded to a degree beyond that which can be rescued by chaperones due to a mutation or structural defect with profound effects on folding, are degraded before they aggregate and precipitate, Fig. 1 (Suzuki et al., 1997; Tamura et al., 1998; Geier et al., 1999; Ciechanover et al., 2000; Maupin-Furlow et al., 2000; Zwickl et al., 2000; Selkoe, 2001; Tomoyasu et al., 2001). The protein-degrading systems exemplified by the proteasome and other proteases, which act in collaboration with chaperones in certain instances, are, therefore, also important anti-stress mechanisms. In addition, the cell has mechanisms capable of dissolving protein aggregates and precipitates, at least before they become firm and irreversible, and in this process chaperones also play a role, Fig. 1 (Glover and Lindquist, 1998; Horwich et al., 1999; Ben-Zi and Goloubinoff, 2001; Glover and Tkach, 2001). Failure of the systems that eliminate peptides before they aggregate to form cell-damaging precipitates, and failure of the aggregate-dissolving mechanisms are also factors contributing to disease development and cellular ageing.

3. Proteinopathies and chaperonepathies

Anti-stress mechanisms are varied but one of the most studied involves the chaperoning systems. These systems are essentially composed of proteins that interact with each other as members of teams through defined pathways (Hartl and Martin, 1995; Netzer and Hartl, 1998; Bukau et al., 2000; Dunn et al., 2001). Interaction among pathways also occurs in what amounts to chaperoning networks, some of which are very complex. These systems, pathways, and networks are important not only as anti-stress mechanisms when the cell needs to respond to the consequences of a stressor impacting on it, but also in normal life.

Fig. 1. Schematic representation (not to scale) of the life of a protein from gene to fully folded molecule to degradation in a eukaryotic cell. Crucial steps that need chaperone assistance and places of stress impact with adverse effects that might enhance senescence are pointed out. (1) Protein-coding gene (brown rectangle) with two introns (black); (2) transcription; (3) primary transcript (pre-mRNA) still with the two introns; (4) pre-mRNA processing including intron removal and exon splicing; (5) mature mRNA (brown rectangle); (6) mRNA transport from nucleus to cytosol via nuclear pore complex; (7) mRNA translation at the ribosome (light blue); (8) nascent polypeptide (orange) emerging from the ribosome; (9) newly-made polypeptide released by the ribosome on its way to folding; (10) the same polypeptide farther along the folding pathway; (11) new protein, correctly folded (native, functional configuration); (12) some proteins are translocated into organelles (e.g. mitochondria) through their membranes—translocation occurs while folding is in progress; (13) aggregate formed by unfolded, or misfolded polypeptides; (14) precipitate of aggregated polypeptides (protein deposit); (15) unfolded or partially folded polypeptides freed from the aggregates by the action of aggregate-dissolving mechanisms involving chaperones; (16) protein-degradation products drawn inside a shaded area that symbolizes a dynamic 'graveyard' where degradation proceeds from oligopeptides of decreasing lengths down to amino acids, which will ultimately be recycled as building blocks for new proteins in step 8; some of the known proteolytic machineries found in prokaryotic and eukaryotic cells are also outlined: proteasome-ubiquitin system (magenta), tricorn protease (light green), and other proteases (dark blue)—aminopeptidases are not shown. Black solid arrows: physiological route from gene to functional protein. Black waving arrow: translocation pathway from cytosol into an organelle, for example a mitochondrion. Green solid arrows indicate critical steps that require chaperone assistance, whereas green discontinuous arrows point to critical steps that most likely need chaperone assistance but demonstration of this assistance is still incomplete. Red arrowheads point to molecule (or structure, function, pathway) known to be adversely affected by stress. Red arrows: route from unfolded, partially folded, or misfolded proteins toward aggregation; this route is followed by abnormal proteins even in the absence of stress, and by normal and abnormal proteins denatured by stress. Blue solid arrow: route from protein aggregation toward precipitation of aggregate (protein deposit formation). Violet arrows: route to protein degradation followed by normal proteins in physiological cellular processes (e.g. in gene regulation via activator or repressor degradation) and by abnormal proteins that cannot be folded or rescued (re-folded) by chaperones—there should also be a similar arrow from 9 to 16, which was omitted for the sake of clarity. Green waving arrows indicate that chaperones participate in some protein-degradation pathways. Black discontinuous (square dots) arrows: recovery route from aggregate to free polypeptides to re-folding; possibly this route is very actively utilized in proteinopathies as the chaperoning systems attempt to free the organism from pathologic aggregates and avoid protein deposition—a similar course of events may occur during senescence with the caveat that the required chaperoning systems probably become less and less efficient with age. Black discontinuous (squares alternating with solid circles) arrow from 14 to 15: possible but improbable recovery route from protein deposit toward the release of free, re-foldable polypeptides. Note that the need for chaperone assistance and the stress-sensitive points are widespread. See text for references.

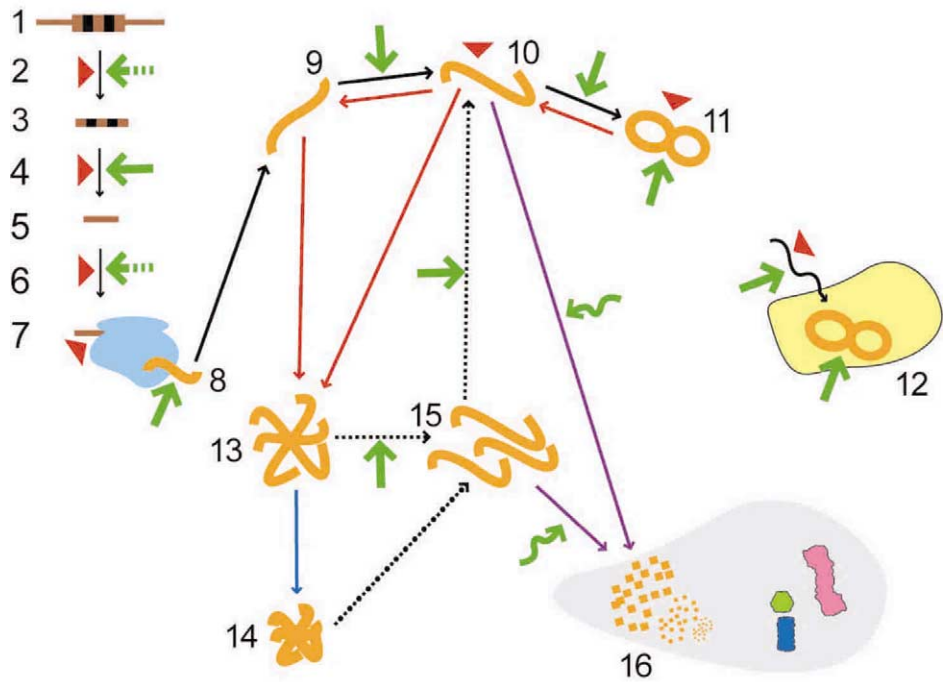

Fig. 1.

The functions of the chaperoning systems are varied and ubiquitous; probably few cellular functions involving proteins can be performed without assistance from chaperones (Fig. 1). Failure of a component of a single system may alter the relevant pathway, and by extension the chaperoning network, and cause pathologic effects. The extent and importance of these effects will depend on a number of factors, for example: the degree of failure (complete or partial, permanent or transitory) of a chaperone molecule; the possibility or lack thereof that the deficient chaperone pathway is replaced by another, or that the chaperoning deficiency is compensated by a different mechanism not involving protein chaperones; and the function most damaged by the chaperoning deficiency (critical or not for survival).

A great deal of attention has been directed towards defective proteins (proteinopathies) that tend to misfold, aggregate, and precipitate inside or around the cell, and cause disease, even in the presence of normal chaperoning systems—which seem unable to cope with so much demand from abnormal proteins in need of assistance. In parallel, extensive analysis of the anti-pathogenetic role of molecular chaperones has been carried out (references in Macario and Conway de Macario, 2000b, 2001a). Furthermore, it is possible that some chaperones contribute to the development of the pathologic protein deposits rather than to their prevention and dissolution: an area for investigation.

While proteinopathies have as primary manifestation a deficient (e.g. mutant) protein that ultimately forms precipitates, stress with its inherent power to induce protein denaturation is likely to enhance pathogenesis. Although stress sets in motion anti-stress mechanisms directly involved in the maintenance of a functionally sound set of cellular proteins, it does do so by causing some degree of protein denaturation. If the latter predominates over re-naturation (re-folding), a pathologic process will develop, which will be greatly facilitated if a cellular protein is aggregation-precipitation prone due to an inherent conformational deficiency (proteinopathy).

In addition to the above listed pathogenetic possibilities, we must now consider the role that might be played by sick chaperones (chaperonepathies) in the generation of disease, by themselves or in association with other proteinopathies.

4. Pathologic conditions with sick chaperones

Examples of pathologic conditions in which mutant chaperones seem to play a critical pathogenetic role have been reported in the last few years. These pathologic conditions provide a basis for examining the potential role of chaperonepathies in ageing, particularly in the perpetuation and acceleration of the ageing process.

4.1. AlphaB-crystallin

This chaperone belongs to the family of stress proteins called small heat-shock proteins (sHsp), which includes all those with a molecular mass of 35 kDa or less (references in Macario et al., 1999; Macario and Conway de Macario, 2000b). A missense mutation in the alphaB-crystallin chaperone gene has been reported to be associated with a myopathy characterized by the accumulation of desmin aggregates in muscle cells, both skeletal and myocardial (Vicart et al., 1998). Desmin-related myopathies (DRM) are inherited neuromuscular disorders that become clinically apparent in adulthood. Desmin is a protein that belongs to the type II intermediate filaments and, apparently, requires the chaperone alphaB-crystallin for proper folding and stabilization. In the publication mentioned above, it is shown that a mutation in the alphaB-crystallin gene maps in the DRM locus, strongly suggesting that this mutation is the cause of desmin aggregation-accumulation. Furthermore, the authors demonstrated that muscle cells transfected with the mutant gene showed aggregates containing alphaB-crystallin and desmin reproducing the picture seen in muscle fibers from DRM patients.

More recently, a new heterozygous desmin mutation (Q389P) was found (Goudeau et al., 2001). This novel mutation is located in the C-terminal region of the rod domain. Transfection of cell lines in culture with cDNA containing the mutation showed not only that Q389P cannot build up a functional intermediate filament network but that it also has a dominant negative effect on filament construction.

4.2. Group II chaperonins

Chaperonins belong to the 55–64 kDa family of Hsp or stress proteins (Macario et al., 1999; Archibald et al., 2001; Dunn et al., 2001). A mutation in a putative chaperonin gene has been implicated in the pathogenesis of a human developmental anomaly, the McKusick–Kaufman syndrome (MKS) (Stone et al., 2000). This syndrome is characterized by post-axial polydactyly, hydrometrocolpos, and congenital heart disease. Two patients were studied. One was found to be homozygous for an allele that had two missense substitutions in a gene that the authors named *MKKS*, whereas the other patient was a compound heterozygote for a frameshipt mutation—that would result in a truncated protein—and a missense mutation in the *MKKS* gene. The deduced protein encoded in *MKKS* had an amino-acid sequence similar to that of the chaperonin subunit alpha-thermosome from the archaeon *Thermoplasma acidophilum*. This protein belongs to the group II chaperonins, which occur in the cytoplasm of archaeal organisms and in the cytosol of eukaryotic cells. The participation of group II chaperonins in protein folding is under investigation and the data so far indicates that their role is critical, at least for a few important cellular proteins.

The same *MKKS* gene has been found mutated in cases of Bardet–Biedl syndrome (BBS) with polydactyly, pigmentary retinopathy, obesity, learning disability, hypogonadism, and renal abnormalities as major manifestations (Slavotinek et al., 2000). It was proposed that mutations in genes encoding chaperonins and their substrates are involved in the pathogenesis of BBS, retinitis pigmentosa, diabetes, obesity, and mental retardation—a suggestion that deserves testing.

The BBS situation seems complicated: the syndrome is still difficult to assess because of its phenotypic variability (Katsanis et al., 2001). There is considerable intra- and inter-familial variation and no evidence for race-specific bias. All this variability, which is evident as developmental (e.g. polydactyly) and progressive (e.g. retinal distrophy) defects, challenges the notion that BBS is caused by the anomaly of a single protein species. Part of the variation may be explained by recent findings showing genotypic heterogeneity—genetic and mutational data indicate that at least some BBS cases are due to mutations in more than one locus and/or some combinatorial mechanism involving different loci and mutations. However, part of the phenotypic heterogeneity along with the widespread nature of the defect throughout the body could be explained, at least in some BBS cases, if the affected protein is a chaperone. Chaperones are ubiquitous, and have multiple functions in every tissue and interact with a large variety of molecules (Fig. 1). Therefore, it will not be surprising to find that the occurrence of even a single type of chaperone functionally impaired due to a mutation, for example, is accompanied by heterogenous defects and pleiotropic phenotypes.

The implications of the above observations in understanding the role that chaperonepathies might have in the process of ageing are far reaching. Progressive failure of the chaperoning systems due to accumulation of mutations and wear and tear of their molecular components might very well be a feature of 'normal' senescence, but the impact of this expected decline of the chaperoning power with

age on the rest of cellular functions will be more devastating than a similar failure in other sets of molecules. The cell cannot afford the weakening of its chaperoning systems, much less so in a world full of stressors, because of the need for such systems throughout the body. Protein quality cannot be affected lest many functions are impaired. One may envisage a scenario in which sick chaperones due to inherited genetic defects, manifested early in life, will have an even bigger impact on the senescence process than that produced by chaperone defects simply arising as a consequence of 'normal' ageing. The impact of sick chaperones is expected to be serious and complex, as illustrated by the disorders discussed above.

4.3. Chaperones with the J domain

The archetypal chaperones with the J domain belong to the 35–54 kDa family of Hsp, but the domain is present in a variety of other molecules whose function as chaperones or co-chaperones has not yet been fully determined (references in Kelly, 1998; Macario et al., 1999). A gene causing the Charlevoix–Saguenay syndrome, an autosomal recessive spastic ataxia (in short ARSACS or SACS), has been identified (Engert et al., 2000). The *SACS* gene, a single very large exon of nearly 11 500 bp, encodes the protein sacsin. The gene is conserved in humans and mice and its deduced protein product has a sequence with three long segments similar to each other and to comparable segments (21–28% identities) of an ORF present in the genome of *Arabibdosis thaliana*. Sacsin bears a J domain near its C-terminus and two Hsp90 domains similar to the N-terminal region of Hsp90. The *SACS* gene is mutated in patients with the Charlevoix–Saguenay disorder, which is characterized by abnormal sensory and motor nerve conduction, hypermyelination of retinal-nerve fibers, and atrophy of the cerebellar vermis. As mentioned above, the J-domain occurs in many molecules from different organisms, but of specific interest is its presence in chaperones belonging to the 35–54 kDa family, and in molecules that may function as co-chaperones (see also Section 6.4). Thus, integrity of the J domain in several different families of molecules in any given cell is probably a requirement for normality of numerous cellular functions. Hence, mutations affecting the J domain, or other portions of molecules with it, are likely to affect a variety of tissues, including the nervous system, and enhance senescence.

Hsp90 belongs to the 81–90 kDa family of Hsp and, as discussed later in Section 6.3, it acts as a molecular chaperone in a variety of tissues (Buchner, 1999; Kanelakis et al., 1999; Mayer and Bukau, 1999; Galigniana et al., 2001). Therefore, its deficient functioning, or that of similar molecules with equivalent roles (an example of these would be sacsin), may very well lead to multiple, widespread effects such as those typical of senescence.

5. Abnormal expression of molecular chaperones

The abnormal proteins with a tendency to misfold and to form pathologic deposits that are characteristic of the better-studied proteinopathies do not nor-

mally function as chaperones (Macario and Conway de Macario, 2000b, 2001a). Furthermore, patients with these diseases are known or assumed to have normal chaperoning systems, although the role played by chaperones in pathogenesis is not completely understood. It is often assumed that the chaperoning systems are simply overwhelmed by the extraordinary demand posed by the abnormal abundance of pathologic peptides in need of assistance for folding and stability. Another aspect of proteinopathies, which was discussed in the preceding section, is failure of the chaperoning systems due to a structural abnormality caused by gene mutation in a molecular chaperone with partial or complete loss of its chaperoning ability.

There still is another possible cause of a deficient chaperoning system that could result in protein deposition and disease. Expression of a molecular chaperone gene may be abnormal (under- or overexpressed) and thus cause failure of a chaperoning pathway and protein misfolding and deposition, even if the gene's product, the molecular chaperone itself, is structurally normal. The central pathogenetic mechanism would then involve a deficient chaperone-gene regulation.

5.1. Neurodegenerative disorders

Alzheimer's is one of the so-called conformational diseases characterized by protein deposits in the central nervous system associated with neurodegeneration (references in Hutton et al., 2001; Selkoe, 2001). The deposits, extracellular plaques and intracellular fibrillary tangles, contain several proteins. Typically, the plaques contain amyloid-beta peptide, whereas the tangles contain tau, but these deposits and those found in other proteinopathies also contain other proteins, including components of the ubiquitin-proteasome system and molecular chaperones (Macario and Conway de Macario, 2000b, 2001a).

Abnormal expression of Hsp has recently been found to occur in the brains of patients with Alzheimer's disease (Yoo et al., 2001). Members of the 81–99, 65–80, 55–64 and 35 or less (sHsp of the alpha-crystallin group) kDa families were investigated in seven brains of adults with the disease; the methods used were two-dimensional polyacrylamide gel electrophoresis and matrix-associated laser desorption ionization mass spectroscopy (MALDI-MS). Hsp60, Hsp70RY, Hsc71, Grp75, Grp94, and alpha-crystallin B chain showed abnormal expression patterns, which varied depending on the brain region examined. In contrast, Hsp70.1, Grp78, and the epsilon subunit of the TCP-1 (CCT) chaperonin exhibited normal expression patterns. This rather comprehensive study suggests that abnormal gene-regulation mechanisms affecting chaperone genes ought to be scrutinized in proteinopathies to determine their role in pathogenesis and the possibility of findings new therapeutic targets.

5.2. Chaperones and the cardiovascular system

Senescence is accompanied by a poor response to stressors and diminished production of stress proteins in a variety of tissues (references in Snoeckx et al., 2001). For example, acute hypertension-induced synthesis of Hsp70 is considerably

reduced in the blood vessels of older animals as compared with younger counterparts (Udelsman et al., 1993). The mechanism responsible for the impaired stress response in aged tissues is not yet fully understood; it probably involves alterations in Hsp-gene regulation via one or more of the heat-shock factors (HSF) known to control heat-shock gene transcription in humans and other eukaryotes. The anatomic-functional decline of the heart and blood vessels associated with ageing could, at least in part, be explained by the 'normal' progressive failure of the chaperoning systems due to wear and tear of the chaperones themselves and of the molecules, like HSF, involved in the regulation of the genes that produce them under basal and stressful conditions.

6. Cellular functions that might be affected by sick chaperones and enhance senescence

Molecular chaperones participate in a wide variety of cellular processes, and many of these processes are known to depend heavily on molecular chaperones (Fig. 1). In addition, it is safe to say that new roles for molecular chaperones in cell physiology and in disease will be discovered. Thus, a wide array of manifestations is likely to occur if even a single chaperone species, say Hsp70, is abnormal and malfunctions due, for example, to mutation. Some examples were provided in the preceding section.

Which other chaperones are likely candidates to become major contributors to pathogenesis if defective? What cellular structures and processes might, if affected by a chaperonepathy, enhance senescence? A list of candidates is displayed in Table 1.

6.1. Hsp47 and collagen

Hsp47 interacts with procollagen during its folding, assembly, and transport from the endoplasmic reticulum (ER) to its final destination in mammalian cells (Tasab et al., 2000). Hsp47 interacts with triple-helical pro-collagen. Moreover, association of Hsp47 with pro-collagen is accompanied by collagen triple helix formation. The chaperone stabilizes correctly-folded collagen and protects it from the unfolding (denaturing) action of heat stress. Given the collagen deterioration characteristic of senescence, it is tempting to speculate that a sick Hsp47 would enhance considerably this manifestation of ageing.

6.2. The 100 and 65–80 kDa chaperone families and mRNA splicing

In eukaryotes, with the requirement for mRNA splicing, the role that might be played by molecular chaperones in this process is of particular interest. An alteration in the chaperoning system involved in the splicing machinery that impacted on splicing efficiency will have a widespread effect. In complex eukaryotes with many tissues and organs like humans, splicing deficiency will be felt through-

out the body. It will probably mean accumulation of abnormal molecules that might form aggregates and accelerate ageing of several tissues.

It has been demonstrated in the yeast *Saccharomyces cerevisiae* that chaperones of the 100 and 65–80 kDa families are directly involved in the maintenance of functional snRNPs, which are instrumental for mRNA splicing (Bracken and Bond, 1999). It was found that Hsp70 (Ssa) plays a critical role in snRNP assembly under physiological conditions and that this chaperone and Hsp104 are required for reassembly of snRNPs after heat shock, a stressor that seriously perturbs snRNP structure. Since stress caused by a variety of stressors of clinical import—including fever, sun exposure, and other types of hyperthermia—impacting on different tissues is unavoidable during life, it is probable that an individual with defective Hsp70 and/or Hsp104 will probably age faster than individuals with an efficient mRNA splicing mechanism that is not perturbed by the presence of abnormal chaperones.

Table 1
Predicted chaperones that if sick due to mutation, biosynthetic error, or other structural abnormality, would accelerate senescence of various tissues owing to the critical molecular functions and cellular processes that would be impaired

Chaperone	Target molecule, structure, or function most affected	Prediction based on data from
Hsp47	Collagen	Tasab et al., 2000
Hsp100 and Hsp70	mRNA splicing	Bracken and Bond, 1999
Hsp90, Hip, Hop, BAG	Steroid hormone receptor	Kanelakis et al., 1999
Hsp90, immunophilin	Steroid hormone receptor; dynein	Galigniana et al., 2001
Hsc70, Hsp40(Dnaj), J-domain proteins, CHIP and CHIP-like proteins	Neuronal synapse	Tobaben et al., 2001
Hsc70, J-domain proteins	Presynaptic terminals; synaptic vesicles	Morgan et al., 2001
Hsp70	Translation apparatus-protein synthesis machinery	Van Nieuwenhoven et al., 2001
Hsp90, p23	Telomerase	Holt et al., 1999
Prefoldin	Actin; tubulin	Rommelaere et al., 2001
Cytosolic chaperonin group II (CCT/TriC/TCP-1 complex)	Actin; tubulin; G-alpha-transducin; cyclin E; Von-Hippel-Lindau (VHL) tumor suppressor protein	Dunn et al., 2001

6.3. Hsp90 and steroid receptors

Hsp90 is necessary for ligand-dependent transcriptional activity of steroid receptors (Buchner, 1999; Kanelakis et al., 1999; Mayer and Bukau, 1999; Galigniana et al., 2001). Steroid receptor folding requires Hsp90, along with Hop and p23. Hsp90 binds to the hormone-binding domain of the glucocorticoid receptor, which is thus kept in a partially unfolded state endowed with high affinity for the glucocorticoid hormone. Dissociation of the Hsp90–receptor complex prevents hormone binding. A similar situation occurs for the progesterone and androgen receptors, although the mechanistic details are not identical. Here again is a situation that because of the extent of tissues affected, chaperone failure will have generalized consequences typical of ageing. The same scenario may be envisaged for any situation in which chaperones and co-chaperones (e.g. Hsp90, Hsp70, Hip, Hop, BAG; for references see Macario and Conway de Macario, 2001b) interact with signaling molecules and protect them from stressors, stabilize them and assist them in the performance of their functions. This is a promising area for investigation to discover new pathogenetic mechanisms that can be specifically targeted for treatment, and also because these mechanisms are likely to be involved in the ageing process throughout the body.

6.4. Hsc70, J-domain, and tetratricopeptide repeat containing molecules, and neuronal synapse

A chaperone complex that appears to play a key role in the maintenance of synapses in the nervous system has recently been described (Tobaben et al., 2001). The complex is formed by the chaperone Hsc70, the synaptic vesicle cysteine string protein (CSP), and the SGT protein. CSP is an interesting molecule: it participates in the exocytotic release of neurotransmitter, hormones, and enzyme precursors, and contains a J domain—this domain consists of about 70 amino acids, is well conserved in all organisms across the phylogenetic tree, and its main representative comes from the chaperone Hsp40(DnaJ)—see Section 4.3. Like this chaperone, CSP interacts with members of the 65–80 kDa family of chaperones; specifically, it was found that CSP strongly activates the Hsc70 ATPase.

The other component of the synaptic chaperone machine, SGT, has an amino-acid sequence with three copies of the tetratricopeptide repeat (TPR) motif, in tandem. TPR is a stretch of about 34 amino acids tandemly repeated (3 to nearly 20 times) in many different proteins distributed across the three phylogenetic domains. Among these proteins are chaperone co-factors like CHIP, the acronym for carboxyl terminus of Hsc70-interacting protein (Ballinger et al., 1999), and SGP might be considered one of them.

The results reported showed that: (i) the CSP/SGT/Hsc70 complex is located on the surface of the synapse vesicle and does not occur in CSP knockout mice; (ii) the complex reactivates partially denatured substrates in the presence of ATP; and (iii) over-expression of SGT in cultured neurons inhibits neurotransmitter release. These observations support the notion that the complex is a molecular chaperone machine

that must be assembled in a specific way and that is important for synapse function. Therefore, failure of any of the components of this trimeric machine that might occur with increasing age will cause perturbations throughout the nervous system, and thus contribute to senescence.

6.5. Hsc70, auxilin, and pre-synaptic terminals

Neurotransmitter release occurs very many times during life and this means a huge consumption of synaptic vesicles. As a consequence a local, efficient mechanism has evolved for vesicle recycling that provides them in an utilizable form, and fast. Clathrin-mediated endocytosis is one way of vesicle recycling that involves formation of clathrin-coated vesicles (CCV) and occurs in pre-synaptic terminals with the participation of numerous proteins. In addition, CCV must be uncoated before re-utilization, a process that requires the molecular chaperone Hsc70 and auxilin (Morgan et al., 2001). Auxillin is a clathrin-binding protein that belongs to the Hsp40(DnaJ) group of chaperones because it has a J-domain (see Sections 4.3 and 6.4), binds to Hsc70 and stimulates its ATPase activity. Via this interaction with Hsc70, auxilin facilitates the release of clathrin from CCVs. Auxilin homologs have been found in organisms of various phylogenetic branches and they all have the J-domain and a clathrin-binding domain.

It would appear that CCV uncoating starts by the formation of a clathrin-auxilin complex mediated by the clathrin-binding domain of auxilin. The J-domain in the latter would then recruit Hsc70 to the complex followed by activation of the Hsc70 ATPase activity, which will result in the release of clathrin monomers from the CCV. This model for CCV uncoating is supported by experimental data.

The above findings constitute still another promising line of research in the quest for mechanisms of ageing in which participation of sick chaperones might be a major force. Senescence is characterized among other things by a decline of neural functions. This decline could be due, at least to some extent, to the progressive ageing of the chaperoning system involved in the recycling of CVVs.

6.6. p23 and Hsp90, and telomerase

Telomere shortening is said to be the ageing clock as well as an ageing mechanism. The normal replication of the DNA-lagging strand does not copy the ends of linear templates, thus a gap occurs between RNA priming and terminus. This leads to telomere shortening at each round of DNA replication (cell division), which after a certain number of rounds will stop cell multiplication. The older a cell, the shorter its telomeres, and when the telomeres have been reduced down to a minimum, cell division stops with ensuing death as the inevitable consequence. However, there is a mechanism that counteracts telomere shortening. It involves telomerase, a ribonucleoprotein with enzymatic activity. The catalytic core of human telomerase, for example, acts as a reverse transcriptase that uses as template a sequence in its associated RNA to synthesize TTAGGG repeats—these repeats form the telomere, which contains thousands of them and are those progressively lost during cell senescence.

It has been reported that the chaperones p23 and Hsp90 are required for telomerase assembly and function (Holt et al., 1999). Although most normal human diploid cells have no detectable telomerase, it has been shown that expression of its catalytic subunit will function enzymatically as telomerase, and even overcome normal cell senescence in some instances.

The finding that molecular chaperones are necessary for the assembly and function of telomerase is a significant step forward in our understanding of the role of these ubiquitous molecules in important cellular processes. The door is now open to investigate the consequences that sick chaperones might have on telomere shortening and ageing. The potential exists for using telomerase to counteract senescence, but the need for healthy chaperones must be taken into account in any attempt at designing strategies against the ageing process. Furthermore, it is clear that chaperone failure will almost certainly lead to an acceleration of telomere shortening and ageing even in those cells that do have autochthonous telomerase.

7. Conclusion

Molecular chaperones are ubiquitous and lack specificity. They are present in all cells, interact with many different proteins and compounds, and play a role in a wide variety of cellular processes, normally and in response to stress. Given this universality of chaperones, the frequency of stress during life (perhaps an inherent feature of nature as is gravity), and considering the key role of chaperones in anti-stress mechanisms, if follows that chaperone failure will have widespread consequences, many of which will be indistinguishable from the manifestations of senescence. Indeed, chaperone defects, even subtle ones, might be an intrinsic component of the ageing process.

It is known that many diseases are caused by the occurrence of defective proteins, which are not chaperones, with a tendency to misfold, aggregate, and form intra- and extra-cellular deposits. These defective proteins depend on chaperones, even more than their normal counterparts, for correct folding and stabilization of a functional configuration at the cell locales where they exercise their physiological function. In patients with these diseases, the chaperoning systems are assumed to be normal; they would play a role in preventing misfolding and aggregation and in dissolving pathologic deposits as anti-disease mechanisms.

Here, we propose that sick chaperones due to wear and tear, mutation, or post-transcriptional abnormalities, and defective chaperoning systems due to perturbation in the regulation of chaperone-gene expression, contribute to the process of ageing. This idea is supported by recent findings reviewed in this article. One set of reported data suggests a role for chaperonepathies affecting the chaperone molecule itself in the pathogenesis of certain disorders—some of which show abnormal protein deposits containing the abnormal chaperone, while another set of results indicates that a defective regulation of chaperone-encoding genes is associated with neurodegenerative and cardiovascular diseases.

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