13 Heat Shock Proteins in Brain Function

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Abstract: Heat shock proteins are ubiquitous, highly conserved proteins helping the formation and repair of the correct conformation of other protein molecules. Cellular stress leads to heat shock protein (stress protein, molecular chaperone) induction, reflecting their protective role in cell survival. Heat shock proteins have a key importance in neuronal repair after brain damage, like trauma or stroke and in neurodegenerative diseases, such as in Alzheimer's, Parkinson's, and Huntington's type diseases. Because of the increasing amount of damaged proteins, heat shock proteins become overloaded during the aging process. This may lead to the release of heat shock protein-buffered, silent mutations, leading to the phenotypic exposure of previously hidden features and contributing to the onset of polygenic diseases such as neurodegenerative diseases. Heat shock protein induction and inhibition are promising pharmacological tools to protect neurons or to fight against brain tumors, respectively.

List of Abbreviations: ALS, amyotrophic lateral sclerosis; DnaJ, a co-chaperone of the 70-kDa heat shock protein, Hsp70; ER, endoplasmic reticulum; G-protein, small GTP-binding protein; Grp, glucose regulated protein; Hsc70, the constitutively expressed form of the 70-kDa heat shock protein, Hsp70; Hsp, heat shock protein; PU3, a purine-based inhibitor of the 90-kDa heat shock protein, Hsp90

1 Introduction

Protein folding has numerous steps, which need assistance in vivo. Heat shock proteins are required for many proteins to fold, or refold into native structures, for their oligomeric assembly and transport to their final destination inside the cell. This function is called chaperone function and, therefore, most heat shock proteins are also molecular chaperones. Heat shock proteins and their counterparts in the endoplasmic reticulum (and in mitochondria), glucose-regulated proteins form an ancient, primary system for "intracellular self-defense." Heat shock proteins have a profound importance in medical practice (Latchman, 1991; Welch, 1992; Hartl, 1996; Thirumalai and Lorimer, 2001). Their function is necessary for the homeostasis of the living cell, and becomes especially important in disease when our cells have to cope with a stressful environment. In damaged cells (such as in cells after heat shock), heat shock proteins will be up-regulated, which is an adaptive response of the cell to repair the increased amount of damaged proteins.

This chapter will briefly summarize and explain the role of heat shock proteins in cell survival, list a few of their recently uncovered specific functions, describe their role in neuroprotection, in the aging brain, in neurodegenerative diseases, and highlight some novel advances of heat shock protein-related medical therapies.

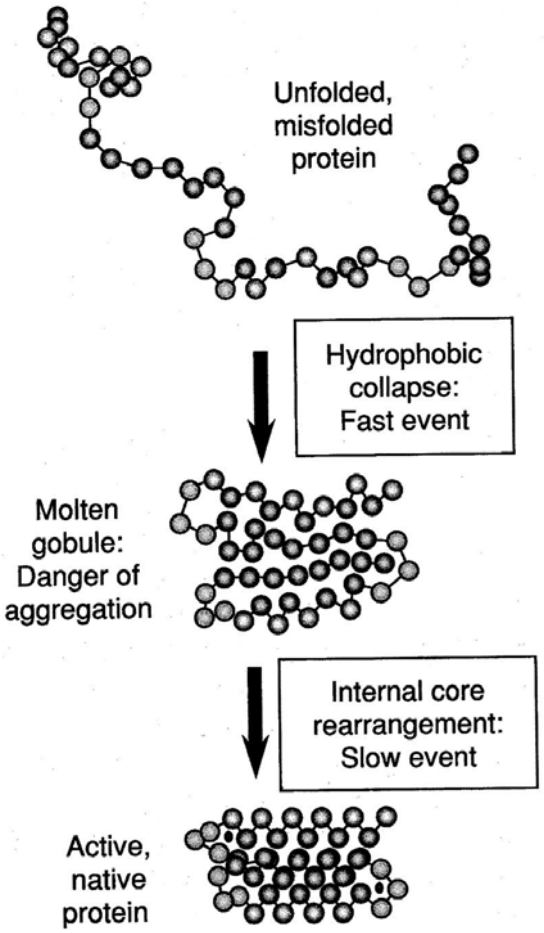
2 Heat Shock Proteins

2.1 Definition and General Functions

Heat shock proteins help protein folding. In ➲ *Figure 13-1* the two major steps of a usual folding process is shown. First, a fast collapse of the nascent or misfolded protein structure occurs, which leads to the development of the hydrophobic core. Here the hydrophobic amino acids become buried and thus their disturbing effect on the hydrogen-bonded water structure is prevented. However, this process is often incomplete and results in a molten globule, having hydrophobic amino acids on its surface, which make it prone to aggregation. When aggregation occurs, the hydrophobic amino acids on the surface of the partially folded protein are forced to bind to each other since this is the only way by which their energetically costly interaction with the water structure can be avoided. The second step of protein folding is usually slow. Here the hydrophobic core is rearranged, which results in the formation of the native protein structure.

However, protein folding is not a straightforward process. Dead-end pathways, reverse reactions, futile cycles are all characteristic of protein folding. A minor amount of fully folded, native protein always coexists with various forms of molten globules and with traces of remaining unfolded molecules. This unordered flow of events needs a lot of help. Aggregation of unfolded proteins and of molten globules is a great danger

Figure 13-1
Major steps of protein folding in vitro. First, a fast collapse of the protein structure occurs, which leads to the development of the hydrophobic core. However, this process is often incomplete and results in a molten globule, which is prone to aggregation. The second step is usually slow. Here the hydrophobic core is rearranged



that would drive the majority of folding intermediates to a nonproductive side-reaction, much before reaching their fully folded, competent state. Heat shock proteins serve to prevent this. They recognize and cover hydrophobic surfaces, successfully competing with the aggregation process. However, there is an important difference here. Unlike aggregating partners, heat shock proteins can leave their complex with misfolded proteins, utilizing the energy of ATP-hydrolysis-driven conformational changes. This function is called as chaperone function; therefore most of heat shock proteins are often called molecular chaperones.

Heat shock proteins (1) protect other proteins against 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 case of excessive damage, sequester damaged proteins to larger aggregates. Heat shock proteins are ubiquitous, highly conserved proteins, which utilize a cycle of ATP-driven conformational changes to re-fold their targets and which probably played a major role in the molecular evolution of modern enzymes (Hartl, 1996; Csermely, 1997, 1999; Thirumalai and Lorimer, 2001).

Cellular stress leads to the expression of heat shock proteins. Stress can be any sudden change in the cellular environment to which the cell is not prepared to respond, such as heat shock. However, almost all types of cellular stress induce heat shock proteins. Because of the generality of this phenomenon, heat shock proteins are often called stress proteins. The rationale behind this phenomenon is that after stress, there is an increased need for the chaperone function of heat shock proteins, which triggers their induction (Morimoto, 1998).

Heat shock proteins are best classified by their molecular weights, as there is significant overlap in their functions. Besides the major classes of heat shock proteins listed in ➤ *Table 13-1*, which generally target all

Table 13-1
Major classes of Hsp-s

Most important representatives[a]	Recent reviews
Hsp25[b], Hsp27, crystallins, small heat shock proteins	Arrigo, 1998; Haslbeck, 2002; Ganea, 2001
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; Picard, 2002; Pratt and Toft, 2003; Richter and Buchner, 2001; Sreedhar et al., 2004a; 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]The abbreviation "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

misfolded proteins with hydrophobic surfaces, there are also specialized heat shock proteins, like Hsp47, which is the procollagen-chaperone (Nagata, 1998). Heat shock proteins 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) (Hartl, 1996; Bukau and Horwich, 1998).

Heat shock proteins never work alone. They always form a complex with each other and recruit various smaller proteins, called co-chaperones, which regulate their ATP-ase cycle, therefore increase the rate of heat shock protein-assisted refolding. A central chaperone complex of the cytoplasm is assembled around the 90-kDa heat shock protein, Hsp90, and is called as the foldosome.

Nascent proteins have to fold when they are not even ready yet. The first protein segment, which leaves the ribosome, has a different energy minimum than the whole protein. In many cases, in vivo protein folding has to be delayed. Heat shock proteins are attached to the ribosomes "waiting" for the nascent protein chain. When it appears, the chaperones "sit on it," preventing premature protein folding before the rest of the protein is synthesized (Kim and Baldwin, 1990; Matthews, 1993).

Heat shock proteins also direct proteins inside the cell. Pores of the mitochondria or of the endoplasmic reticulum are too small to accommodate fully folded, globular proteins. Proteins have to unfold to get through and refold in the lumen of the organelle (Chirico et al., 1988).

Heat shock proteins help both the folding and degradation of damaged proteins. After a few futile refolding attempts—most probably due to the extension of the transit time of the unfolded target protein with the heat shock protein molecule—heat shock proteins (such as Hsp90 or Hsp70) recruit novel co-chaperones (like CHIP or the neuronal DnaJ proteins, HSJ1a and HSJ1b) and present their target to the proteasome (Chapple et al., 2004; Urushitani et al., 2004; Whittier et al., 2004). The proteasomal system, in fact, degrades a large amount of newly folded proteins in eukaryotic cells, accomplishing a very tight quality control during the translational process (Turner and Varshavsky, 2000). The proteasome itself is also behaving as a molecular chaperone, since its "cap" has to unfold damaged proteins to be able to insert them to the tight cavity of the protease domain (Braun et al., 1999). Heat shock proteins are also involved in lysosome-related protein degradation, such as autophagocytosis (Chiang et al., 1989). In case of massive protein damage, when the amount of degradable proteins exceeds the capacity of the intracellular proteolytic systems, chaperones help to form inclusion bodies to segregate damaged proteins (Mayer et al., 1991).

2.2 Nonconventional Roles: Dustmen of the Cells

Heat shock proteins are regarded as molecular chaperones and their major cellular function is thought to be associated with their role in protein folding. However, most protein folding experiments are conducted in an in vitro environment. When protein folding is studied in vitro, the experimenter has to use rather diluted conditions to prevent unwanted aggregation. Dilution also helps to make the kinetic analysis easier and spares precious research materials. On the contrary to these usual experimental conditions, the cellular environment is crowded (Zimmerman and Minton, 1993). Molecular crowding promotes protein aggregation and thus calls for an enhanced need for chaperone action. On the other hand, bona fide chaperones are not the only cellular solutions for aggregation-protection. Several "innocent bystanders," such as tubulin (Guha et al., 1998) or even small molecules (lipids, other amphiphyles, sugars, a class of compounds called as chemical chaperones; Welch and Brown, 1996) may assist folding and prevent aggregation albeit at much higher concentrations than the efficient concentration of heat shock proteins. Though we have several important lines of evidence, which undoubtedly show the necessity of chaperones in folding of numerous protein kinases, receptors, actin, tubulin, etc. (Hartl, 1996) we do not really know to what an extent heat shock proteins are really used for protein folding in the eukaryotic cell, which is mostly settled to degrade and not to repair its cellular proteins due to the energy surplus obtained from the acquired mitochondria (Frydman et al., 1994).

With the above statements I do not want to question the importance of heat shock proteins in assisting protein folding. Nevertheless, I would like to stress that there is enough room to think about other important functions of heat shock proteins related to, but not equal to their participation in protein folding. One of these possibilities lies in the peptide-binding properties of heat shock proteins. Heat shock proteins may behave as the "dustmen" of our cells. The proteasomal apparatus is most probably linked with oligo- and dipeptidases and therefore the peptide-endproducts of proteasomal degradation (Kisselev et al., 1998) are usually cleaved further into single amino acids. However, the coupled protein–peptide degradation can leak, which may especially happen under stressed conditions like in oxidative stress. Released peptide segments may often contain elements of important binding sites and thus may efficiently interfere with signaling and metabolic processes. If this happened at a massive scale, this would be a disaster for the cell. Peptides need to be eliminated, and safeguarding mechanisms must exist to correct the occasional "sloppiness" of degradative processes. Heat shock proteins are excellent candidates for this purpose and their role in the collection of "peptide-rubbish" must be considered besides their well-established function in peptide presentation for the immune system (Srivastava et al., 1998). Heat shock protein-mediated sequestration of bioactive molecules can be especially important in the brain where neuropeptides play a prominent role in interneuronal signaling.

2.3 Nonconventional Roles: Organization of the Cytoarchitechture

As another important and nonconventional aspect of heat shock proteins lies in their incredibly high affinity for complex formation. Chaperones often form dimers and tend to associate to tetramers, hexamers, octamers, and to even higher oligomers (Benaroudj et al., 1996; Trent et al., 1997; Csermely et al., 1998). Oligomerization usually affects only a few percent of the total protein; but by addition of divalent cations and certain nucleotides, heat treatment enhances oligomer formation. It is important to note that oligomerization studies were usually performed under "normal" in vitro experimental conditions using a few μg/ml of purified chaperone. The in vivo concentration of chaperones is estimated to be around a 100- or 1000-fold higher. This may significantly enhance the in vivo oligomerization tendencies of these proteins. Oligomer formation of chaperones might be further promoted by the large excluded volume effect of the "molecularly crowded" cytoplasm (Zimmerman and Minton, 1993).

Different chaperones also associate with each other. The Hsp90-organized foldosome may contain almost a dozen independent chaperones, or co-chaperones. The stoichiometry and affinity of these associations dynamically varies, and the variations are affected by the folding state of the actual target (or targets), which associate with the extensive folding machinery (Kamal et al., 2003).

Besides binding to themselves, to their sibling-chaperones, and to their targets, many chaperones bind to actin filaments, tubulin, and other cellular filamentous structures such as intermediate filaments. There is a chaperone complex associated with the centrosome (Wigley et al., 1999) and several chaperones, especially Hsp90, were considered to be involved in the direction of cytoplasmic traffic (Pratt and Toft, 2003).

The above model describing chaperones as a highly dynamic "appendix" of various, and often quite poorly identifiable, cytoplasmic filamentous structures is reminiscent of the early view (Wolosewick and Porter, 1979; Schliwa et al., 1981) about the microtrabecular lattice of the cytoplasm. Although later studies efficiently questioned the validity of the original electronmicroscopic evidence of the microtrabeculae, pointing out many possibilities for artefact formation during sample preparation, several indirect evidence, such as diffusion anomalies support the existence of a cytoplasmic mesh-like structure (Clegg, 1984; Jacobson and Wojcieszyn, 1984; Luby-Phelps et al., 1988). The major cytoplasmic chaperones (Hsp90, TCP1/Hsp60 and their associated proteins) may well form a part of this network in cells (Csermely, 2001a).

Our experiments showing the acceleration of the efflux of cytoplasmic constituents after the inhibition of the major cytoplasmic heat shock protein, Hsp90, both in case of numerous cell lines (Pato et al., 2001; Csermely et al., 2003; Sreedhar et al., 2003, 2004b) suggest the involvement of the 90-kDa molecular chaperone, Hsp90, in the maintenance of the cytoarchitecture. Interestingly, we did not see an acceleration of cytoplasmic release in *Escherichia coli*, which is in agreement with the lower level of cytoplasmic organization of prokaryotes compared with eukaryotes. We cannot ascertain at the moment that the faster release of cytoplasmic proteins after the disruption of Hsp90 complexes by Hsp90 inhibitors or anti-Hsp90 ribozyme treatment is a consequence of a disrupted cytoplasmic meshwork or shows the involvement of Hsp90 in the stabilization of the "traditional" cytoskeleton. However, future experiments analyzing the distribution of Hsp90 in the cytoplasm after these treatments as well as changes in the intracellular diffusion rates might answer this question.

The possible involvement of heat shock proteins in the organization of the cytoplasm were interesting in neural cells all the more since these cells utilize the cytoarchitecture in all important aspects of their signaling, contacts, and memory formation.

2.4 Nonconventional Roles: Buffering of Silent Mutations

In the last few years, several experiments were published, which suggested that chaperones behave as "buffers of evolutionary changes." Chaperones seem to correct the conformational changes caused by various mutations and make the genetic changes phenotypically silent in various organisms studied (Rutherford and Lindquist, 1998; Roberts and Feder, 1999; Fares et al., 2002; Queitsch et al., 2002). However, if a large stress occurs, the suddenly increased amount of damaged proteins may cause a "chaperone-overload," and may prevent the conformational repair of misfolded mutants. Therefore many previously hidden genotypical changes may appear in the phenotype, resulting in a "boom" of genetical variations in the whole population. This may help the selection of a beneficial change, which, in turn, may help the adaptation of the population to changed environmental conditions. Nevertheless, most of the exposed mutations are disadvantageous and tend to disappear from the population by natural selection.

Changes in living conditions and the significantly better medical care throughout life in the last 150 years have significantly reduced the occurrence of large physiological stresses that would normally result in significant intracellular proteotoxicity. There is little "chaperone overload" during reproductive years in the present times. Even major stressful events such as critical infections and extreme and unexpected changes in the environment that do cause a massive "chaperone overload" can be mitigated by improved medical care, thus saving lives that would otherwise have been lost. More people harboring deleterious mutations survive today and transmit their genes to later generations. Thus improved medical care may have led to a rise in phenotypically silent mutations in the human genome. As a consequence we may be carrying more and more chaperone-buffered, silent mutations from generation to generation (Csermely, 2001b).

The chance of the phenotypic manifestation of these mutations becomes especially large in aged subjects, where protein damage is abundant, and both chaperone induction and chaperone function are

impaired (Sőti and Csermely, 2000, 2002). Here the background of misfolded proteins increases and by competition prevents the chaperone-mediated buffering of silent mutations. Phenotypically exposed mutations may 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 disease, schizophrenia, etc.). Chaperone overload might be even more pronounced in neuronal cells, where selective apoptosis and clonal expansion cannot play a kind of natural selection as it is the case with other somatic cells. Aging neurons may begin to display more and more unexpected features. For quite a while the robust behavior of the neural system covers these deleterious changes; however system resistance is gradually lost and dysfunction occurs (Csermely, 2001b, 2004).

Recently, several other proteins, such as yeast prions, p53, and many others were shown to buffer genetic changes (Scharloo, 1991; True and Lindquist, 2000; Aranda-Anzaldo and Dent, 2003). On basis of theoretical studies it was proposed that the number of buffering proteins is even larger (Bergman and Siegal, 2003). However, these proteins are not all chaperones. What can be common property? Comparing the known examples with other information on complex systems, it was suggested that the formation of low-affinity, weak links is the most important common feature of these proteins. Indeed, weak links were shown to stabilize many systems from single macromolecules up to the human society (Csermely, 2004; 2006).

3 Heat Shock Proteins and Brain Function

Heat shock proteins have a complex role in most cellular functions. To have a comprehensive survey on their involvement in brain function needs further investigations. However, a few elements of their putative brain function have been already uncovered. Hsc70, together with the synaptic vesicle cysteine string protein, a DnaJ homologue, forms a chaperone complex of synaptic vesicles and is involved in neurotransmitter release. Targets for this chaperone machine include the vesicle protein VAMP/synaptobrevin and the plasma membrane protein syntaxin 1 (Chamberlain and Burgoyne, 2000; Tobaben et al., 2001). Another major chaperone, Hsp90, is necessary for the efficient neurotransmitter release at the presynaptic terminal. Moreover, Hsp90 is a critical component of the cellular machinery that constitutively delivers glutamate receptors into the postsynaptic membrane (Gerges et al., 2004).

4 Heat Shock Proteins in Neuroprotection

If heat shock proteins are generally cytoprotective, their beneficial effects should be observed in various cases of neuronal damage. Indeed, overexpression of the 70-kDa heat shock protein protected neuronal cells from ischemic damage in an experimental stroke model (Hoehn et al., 2001), which was also observed in epileptic models (Yenari et al., 1998). A heat shock protein coinducer molecule protected both motor and sensory neurons after damage, where the beneficial effect was most probably due to the enhanced expression of heat shock proteins (Kalmar et al., 2002, 2003).

5 Heat Shock Proteins and the Aging Brain

5.1 Protein Damage During Aging

During the lifespan 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. In several cases, age-related posttranslational modifications induce conformational changes and impair 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 nonnative, 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).

5.2 Protein Degradation in Aging

Accumulating misfolded proteins due to their vulnerability for aggregation pose a great danger to the aging cell. Since the reason for the folding anomaly is mostly a posttranslational modification, the change becomes irreversible and cannot 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 (Friguet et al., 1994; Bence et al., 2001; Bulteau et al., 2001). 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 post-translationally modified, misfolded proteins. In most tissues, cells die after a large proteotoxic damage and other cells start to proliferate to take over their functions (Sőti et al., 2003). However, in the brain, it is much more difficult than in other tissues and due to the increased cell loss, malfunction develops.

5.3 Heat Shock Proteins in Aging Brain

Accumulation of misfolded proteins in aged organisms requires an increased amount of heat shock proteins 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. On the other hand, a large number of reports demonstrate that the induction of various chaperones is impaired in aged organisms (Sőti and Csermely, 2000, 2002). 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 13-2*). 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 did not display a loss of capacity to accumulate Hsp70 in response to heat stress (Walters et al., 2001). This shows that calorie restriction, a well-known method to increase longevity

Table 13-2
Chaperone expression in aging brain

Chaperone	Change	References
Chaperone levels		
ubiquitin, Hsp27, αB-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 noninducible (cognate) form of Hsp70

(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 exhibited increased resistance of hippocampal neurons and striatal neurons to excitotoxic and metabolic stress (Bruce-Keller et al., 1999). Calorie restriction also attenuated the degeneration of dopaminergic neurons in mouse Parkinson models (Duan and Mattson, 1999).

6 Heat Shock Proteins and 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 (Goodman and Mattson, 1994; Gibson et al., 2000; Hemmer et al., 2001). Several molecular chaperones are involved in the maintenance of cellular redox status (Arrigo, 1998) and protect neurons 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.

6.1 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. The accumulation of Hsp90 and (to a smaller extent) Hsp60 was shown in the choroids plexus of brains affected with Alzheimer's disease. Neuronal chaperones were localized in neuritic plaques and neurofibrillary tangles (Hamos et al., 1991; Perez et al., 1991; Cisse et al., 1993; Renkawek et al., 1993; Shinohara et al., 1993; Anthony et al., 2003; Lukiw, 2004).

Accumulated chaperones participate 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 β-amyloid 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). Nonaffected nerve cells of Alzheimer victims, such as olfactory neurons (Getchell et al., 1995), also showed a decreased expression of Hsp70.

The pathologically hyperphosphorylated tau protein is often associated with β-amyloid fibers. Hsp27 has been shown to bind the hyperphosphorylated tau protein preferentially. The formation of this complex altered the conformation of pathological, hyperphosphorylated tau and reduced its concentration by facilitating its degradation and dephosphorylation. Hsp27 also prevented pathological hyperphosphorylated tau-mediated cell death (Shimura et al., 2004).

Since the amyloid precursor is an integral protein of the plasma membrane, which is usually processed in the endoplasmic reticulum (ER), the ER might be an especially important site 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).

6.2 Parkinson's Disease

Parkinson's disease is an age-related disorder characterized by a progressive degeneration of dopaminergic neurons in the substantia nigra and shows 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. Glial and astroglial cells of Parkinson's disease victims showed the expression of αB-crystallin, as seen in Alzheimer's disease, and aggregated proteins in Lewy bodies had a large content of various heat shock proteins, as observed in neurofibrillary tangles (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.

6.3 Huntington's Disease

Polyglutamine repeats make proteins vulnerable for aggregation. Diseases such as Huntington's disease, Kennedy spinal bulbar muscular atrophy, spinocerebral ataxia, Machado-Joseph disease all develop due to an expansion of polyglutamine segments in the respective proteins. Chaperones colocalize 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 (Cummings et al., 1998; Krobitsch and Lindquist, 2000; Carmichael et al., 2000; Hughes and Olson, 2001).

7 Heat Shock Protein-Related Therapeutic Approaches

The beneficial role of heat shock proteins in neuronal survival and their protection against various insults including ischemia, excitatory damage as well as as various forms of neurodegeneration make them prominent therapeutic targets. However, heat shock proteins are not only protecting damaged neurons, but they also protect malignantly transformed neural cells. Here the inhibition of neural heat shock proteins might be a good approach to fight against brain tumors.

7.1 Heat Shock Protein Inhibition: Brain Tumors

When heat shock proteins protect our malignant cells, they are not really beneficial. Still the inhibition of proteins, which have a profound role in the survival of all cells, seems to be a wild idea. However, if we consider that heat shock proteins are necessary for the folding of cyclin-dependent kinases and numerous other proteins which are upregulated in cancer (Neckers, 2003; Workman, 2004) and some of the heat shock protein inhibitors are selectively enriched in tumor cells (Chiosis et al., 2003) as well as selectively interact with tumor-specific forms of heat shock proteins (Kamal et al., 2003), we begin to believe that heat shock protein inhibition might be a valid pharmacological intervention against tumors. Indeed, heat shock protein inhibitors are currently in clinical trials against various forms of cancer (Neckers, 2003; Workman, 2004).

Since the 90-kDa molecular chaperone (Hsp90) has the most specific and most cell-permeable inhibitors and since this chaperone is the center of the kinase-related chaperone machinery, in most cases chaperone-based inhibition is achieved by using Hsp90 inhibitors. The first Hsp90 inhibitor drug was geldanamycin, a natural product isolated from *Streptomyces hygroscopicus*. Though the antitumor effects of geldanamycin were initially thought to be due to specific tyrosine kinase inhibition, later studies revealed

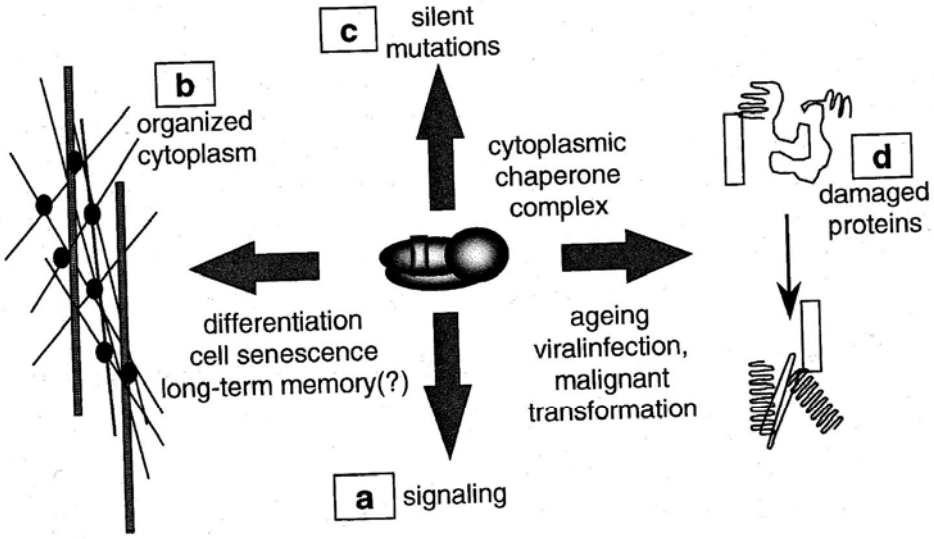
that the antitumor potential relies on depletion of oncogenic protein kinases via the proteasome (Whitesell et al., 1994). The major regulatory signaling proteins, which are affected by geldanamycin, include the protooncogene kinases ErbB2, EGF, v-Src, Raf-1, and Cdk4 (Neckers, 2003; Workman, 2004). Radicicol, another Hsp90 inhibitor (Soga et al., 1998), is a macrocyclic antibiotic isolated from *Monosporium bonorden*. As a recent development, PU3, a purine-based Hsp90 inhibitor was designed using X-ray crystallographic data. PU3 behaves like geldanamycin in inhibiting Hsp90 client protein degradation, and possesses a robust antitumor potential (Chiosis et al., 2002). Recently it was shown that Hsp90 contains a second nucleotide-binding site at the C-terminal domain (Marcu et al., 2000; Garnier et al., 2002; Sőti et al., 2002), which opens up new possibilities to develop Hsp90 inhibitors.

In agreement with the above considerations, the overexpression of Hsp27 and other small heat shock proteins has been found in gliomas and in other types of brain tumors (Hitotsumatsu et al., 1996; Zhang et al., 2003). Geldanamycin was shown to be effective in the treatment of medulloblastomas (Calabrese et al., 2003), gliomas, and glioblastomas (Yang et al., 2001; Zagzag et al., 2003).

7.2 Heat Shock Protein Induction: Brain Damage and Neurodegenerative Diseases

Induction of heat shock proteins is a part of cellular self-defense, which is mobilized in most disease states, e.g. by fever. However, heat shock protein induction may not be enough or the chronic disease may attenuate the level of induction. Heat shock protein induction becomes especially aggravated in aged organisms as described before. Because of these reasons, it was highly beneficial to help the expression of heat shock proteins. Several common drugs, such as aspirin (Jurivich et al., 1992), promote the induction of heat shock proteins; however, recently a specific heat shock protein coinducer drug family (Vígh et al.,

Figure 13-2
Competition for chaperone occupancy and its changes in the ageing process. Clockwise from bottom: *a*, 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 signaling cascades (Csermely et al., 1998; Pratt et al., 1999). *b*, Chaperones form low-affinity and highly dynamic extensions of the cytoskeleton participating in cellular traffic and in the organization of the cytoarchitecture (Csermely, 2001a; Pratt et al., 1999). *c*, phenotypically buffered, silent mutations require the assistance of chaperones to rescue them from folding traps (Rutherford and Lindquist, 1998; Csermely, 2001b). *d*, During the aging process, chaperones become more and more occupied by damaged proteins. As a consequence of this (a) signaling is impaired silent, (b) cell architecture becomes disorganized, and (c) mutations escape and contribute to the onset of polygenic diseases. The verification of these – presently largely hypothetical – changes requires further experimentation



1997; Török et al., 2003) extending the duration of DNA binding by the specific transcription factor inducing heat shock proteins (Hargitai et al., 2003) has also been described. These drug candidates work only in stressed cells, which already started the induction of heat shock proteins themselves. They protected both motor and sensory neurons after damage where the beneficial effect was most probably due to the enhanced expression of heat shock proteins (Kalmar et al., 2002, 2003). Moreover, heat shock protein coinducer drugs were shown to improve the conditions of superoxide dismutase mutant mice, which develop the symptoms of amyotrophic lateral sclerosis (ALS) or Lou Gehrig's disease (Kieran et al., 2004).

8 Closing Remarks

Numerous key elements of cellular life are competing with each other for the maintenance and repair function of heat shock proteins (damaged proteins, signaling proteins, silent mutations, and cytoarchitecture, see ❯ *Figure 13-2*). Therefore, heat shock proteins emerge as a central switchboard of the integration of cellular homeostasis. Their induction is highly beneficial to protect neurons against oxidative or neuroexcitatory damage. On the contrary, heat shock protein inhibition may be a promising tool to fight against brain tumors. I hope that with this short review I may increase the courage of some fellow scientists to enter this difficult, but very promising path of multidisciplinary research.

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