Chapter 6

Heat shock proteins

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Contents

Table 6.1. Effects inducing the synthesis of stress proteins. (For details please consult the following references: Lindquist (1986), Welch (1992) and Feige *et al.* (1996).)

Heat shock	Too much Ca^{2+} inside the cell
Cold shock	Viral infection
UV radiation	Bacterial products
Electrosmog	Parasite toxins
Amino acid analogues	Acute phase reaction
Alcohol	Overload of the endoplasmic reticulum
Heavy metal ions	Phagocytosis
Arsenite	Hormonal effects
Too much oxidation	Increased cell proliferation
Too much reduction	Cell differentiation
Too little glucose	Increased blood pressure
Too little ATP	Too little exercise
"Feeding" of cell cultures	Too much exercise
Osmotic shock	Mental stress

6.1 Introduction

When a cell experiences environmental stress, it stops, or at least slows down most of its original functions, such as transport processes, DNA, RNA and protein synthesis. However, there is a peculiar set of proteins, called stress proteins, which are preferentially expressed under these, restrictive conditions. The archetypal of stress response is a sudden rise in the outside temperature, called heat shock. The heat shock response was first discovered by Feruccio Ritossa (1962), who observed an enlargement of special sections of *Drosophila melanogaster* chromosomes (heat shock puffs) after heat treatment of the flies. Later it became clear that these chromosome segments encoded a special class of proteins, heat shock proteins. Heat shock proteins (Hsp-s) are induced by a large variety of stimuli besides heat shock itself. Table 6.1 lists a few examples of environmental stresses leading to the expression of heat shock, or other stress proteins.

Heat shock proteins play an essential role in the etiology of numerous diseases, with a rapidly increasing role in clinical practice (Latchman, 1991; Welch, 1992; Jindal, 1996; van Eden and Young, 1996; Feige *et al.*, 1996). 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. Our increasing knowledge about the function and biochemistry of heat shock proteins gives us more and more tools to characterize them in patients, enabling us to monitor and improve the status of this intracellular defense system during infections, diseases and aging.

6.2 Heat shock proteins, molecular chaperones

Heat shock proteins form an ancient, primary system for "intracellular self-defense". Why are heat shock proteins

beneficial? How does this defense system save and, in fact, extend our life? Most of the heat shock proteins are molecular chaperones. Chaperones have been defined as "proteins that bind to and stabilize an otherwise unstable conformer of another protein – and, by controlled binding and release, facilitate its correct fate *in vivo*: be it folding, oligomeric assembly, transport to a particular subcellular compartment, or disposal by degradation" (Hartl, 1996). The majority of chaperones prevent the aggregation of "sticky" protein folding intermediates. By binding to their targets, chaperones are acting as "collectors" of damaged proteins. Two classes of chaperones which are especially effective in completing this job are the small heat shock proteins and the 90 kDa heat shock proteins (the Hsp90 family, Table 6.2) may thus be considered as "recycling-concerned dustmen" of cells, binding their targets and keeping them in a folding competent state until the whole cell recovers, and becomes able to provide the energy for the refolding process. Members of the 90 kDa chaperone family bind to various peptides both *in vitro* and *in vivo* (Menoret *et al.*, 1999), which may extend their "collector/dustman" role to free intracellular peptides (Baranyi, Juhasz, Csermely, unpublished observations). These residual peptides, which escape from the major cytoplasmic proteolytic apparatus, the proteasomal system, may seriously interfere with signaling processes (Blum *et al.*, 2000), and therefore pose a great threat to cellular function.

A class of chaperones (in yeast: the 100 kDa heat shock proteins) is able to deseggregate proteins from loose protein aggregates (Glover and Lindquist, 1998). Other classes of chaperones (mainly the 60 and 70 kDa heat shock proteins, Table 6.2) rescue misfolded proteins from folding traps, giving them renewed chance for spontaneous folding. Chaperones do not determine the tertiary structure of proteins, but help them find their structure more efficiently. This mechanism increases the yield, but not the rate, of protein folding. Only a few chaperones behave as true catalysts by increasing the speed of folding. These special chaperones, peptidyl prolyl cis/trans isomerases and protein disulfide isomerases, are therefore better named "folding catalysts" (Hartl, 1996). All these mechanisms have paramount importance following stress, when the cell has to refold damaged proteins and re-establish its original structure.

Chaperone protein monomers never work alone. Most chaperones form oligomers and/or are helped by co-chaperones. A special multimeric chaperone machine is the eukaryotic "foldosome", which governs the folding of various signaling kinases and nuclear hormone receptors (Pratt and Toft, 1997). Refolding of proteins requires energy. Therefore it is not surprising that most chaperones bind and hydrolyze ATP. ATP cleavage induces a conformational change in chaperone structure, which is an essential part of the folding assistance. However, chaperones are rather poor ATP-ases. The inefficient and slow hydrolysis of ATP enables them to provide enough time to the target protein to re-fold. The rate of ATP hydrolysis and exchange of the product ADP to another ATP is regulated by several co-chaperone molecules. Co-chaperones may also influence binding and dissociation of target molecules as well as direct the targets, or the whole chaperone complex to various destinations inside the cell.

Figure 6.1 shows the "working cycles" of the two major classes of molecular chaperones, Hsp60 and Hsp70. Hsp60 binds its target to the internal cavity of the oligomeric protein. In most cases the client protein is completely isolated from the outside world by the acquisition of an Hsp10-heptamer behaving as a "cap". Due to this insulation of the target, Hsp60-type chaperone machines are often called as Anfinsen-cages after Christian Anfinsen, a Nobel-laureate

Table 6.2 Major families of molecular chaperones

Chaperone families	*Prokaryotic family members*	*Major functions of chaperone families*
Small heat shock proteins (e.g. Hsp27*)		Prevent the aggregation of other proteins, by collecting protein "garbage", act as "dustmen" of cells
Hsp60 family	GroEL (co-chaperone: GroES)	Assistance in protein folding and re-folding
Hsp70 family	DnaK (co-chaperones: DnaJ, GrpE)	Assistance in protein folding and re-folding
Hsp90 family	HtpG	Stabilize substrate proteins and maintain their active, or inactive state, prevent the aggregation of other proteins, by collecting protein "garbage", act as "dustmen" of cells
Hsp100 family	ClpA	Deseggregation of proteins
Protein disulfide isomerases	DsbA	Assistance in gradual oxidation of secreted proteins, promotion of correct disulfide-bridge formation, reorganization of disulfide bridges
Peptidyl prolyl cis/trans isomerases (rotamases, FKBP-s, cyclophilins, parvulins)		Catalysis of the cis/trans isomerization of peptide-bonds besides proline residues to set the correct conformation of protein segments

* The "Hsp" abbreviation stands for "heat shock protein", numbers refer to the molecular weight in kDa.

pioneer in protein-folding studies. The protected environment of the Hsp60-cavity prevents the aggregation of the client protein completely (Hartl, 1996). Binding and hydrolysis of ATP induces large conformational changes in Hsp60 (see http://www.cryst.bbk.ac.uk/~ubcg16z/cpn/chaperone.html), which help to loosen the hydrophobic core of the target protein in a partial unfolding process. During this multi-directional pulling of the target, water may enter its hydrophobic core and facilitate reorganization (Csermely, 1999). Hydrolysis of ATP and binding of a new target on the other side of the chaperone-machine releases the Hsp10 cap, and liberates the target protein. For most unfolded or damaged proteins a single round of Hsp60-assisted folding is not enough, and they are recycled through multiple unfolding-refolding cycles. The above mechanism has been deciphered by studying the *E. coli* Hsp60, GroEL and its co-chaperone, GroES. Substrate specificity of eukaryotic Hsp60 chaperones tends to be restricted to actin and tubulin, and details of the folding mechanism may differ (Bukau and Horwich, 1998).

Hsp70 binds the target proteins utilizing the help of its co-chaperone, Hsp40 (in *E. coli*: DnaK and DnaJ, respectively Figure 6.1B). During Hsp70 ATP hydrolysis some proteins acquire their native conformation, while others require the successive action of the Hsp60-machine. Both Hsp70 and Hsp40 have many isoforms, which enables members of these families to perform highly specialized functions. In contrast to Hsp60, which surrounds its target, Hsp70 binds a small peptide-segment of the target protein. Among the Hsp70-bound 7 amino acids, 3–4 are usually hydrophobic. The Hsp70-bound peptide segment adopts a highly extended conformation, which requires considerable unfolding of local secondary structures (Bukau and Horwich, 1998).

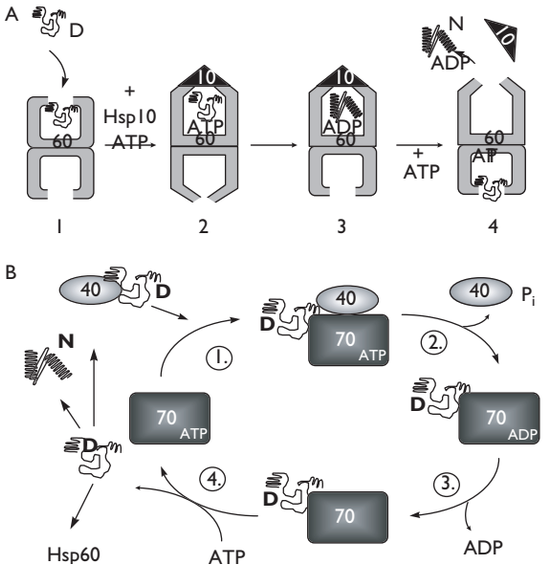

Figure 6.1 A, B Catalytic cycle of the 60 kDa (*panel A*) and 70 kDa (*panel B*) molecular chaperones. Abbreviations: 60, Hsp60; 10, Hsp10 – a co-chaperone of Hsp60; 70, Hsp70; 40, Hsp40 – a co-chaperone of Hsp70; D, denatured protein; N, native protein.

6.3 Synthesis of heat shock proteins

Chaperones (such as heat shock proteins) are ubiquitous, highly conserved proteins which probably played a major role in the pre-biotic evolution of modern enzymes (Csermely, 1997). Chaperones are vital throughout the whole lifetime of our cells. However, they are needed even more after environmental stress, which induces protein damage. In eukaryotic organisms the expression of heat shock protein messenger RNA-s is mediated by a family of transcription factors, called heat shock factors. Heat shock factor I (HSF-I) plays a major role in heat shock response, while other members of the family are activated after prolonged stress, or participate in processes such as embryonic development, or cell differentiation. In resting cells HSF-I is complexed with various heat shock proteins, such as with Hsp70, or with Hsp90. After stress, damaged proteins become abundant and liberate the heat shock factor from its Hsp70/Hsp90 complexes. This process sets the stage for the trimerization, nuclear translocation and phosphorylation of HSF-I, which are all pre requisites for its binding to the special nucleotide segments, called heat shock elements, in the promoter region of heat shock protein genes. All these steps are modulated by numerous co-chaperones of the major heat shock proteins, Hsp70 and Hsp90, and most probably by other proteins as well (Morimoto, 1999; Figure 6.2). The nucleosomal structure of the DNA-segment containing the heat shock element is reorganized by a special protein-machine, called the GAGA-factor (Tsukiyama *et al.*, 1994). Interestingly, many heat shock protein genes recruit an active DNA-dependent RNA polymerase II even in the absence of heat shock factor. This "pausing polymerase" transcribes a small segment of the gene, but becomes arrested by its binding to the initial complex of TATA-binding general transcription factors. Binding of the heat shock factor-trimer to the heat shock element sets the polymerase free, which can proceed to complete the transcription of the heat shock RNA. During stress, all the subsequent steps of protein synthesis (RNA splicing, nuclear export and translation itself) are blocked. Heat shock RNA-s developed various strategies to circumvent these problems. Primary transcripts (such as Hsp70 RNA) usually do not contain introns, or the open reading frame encoding the protein itself begins after the intron and the initialization may proceed from the intron as well (e.g. Hsp90, see Csermely *et al.*, 1998). Recognition of heat shock RNA-s also utilizes special routes avoiding those translational initialization factors, which became inactivated during stress.

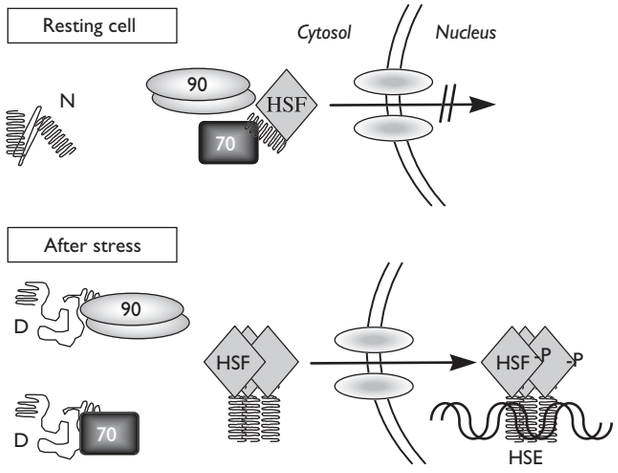

Figure 6.2 Induction of heat shock factors during stress. Abbreviations: 70, Hsp70; 90, Hsp90; D, denatured protein; HSE, heat shock element – a binding site for heat shock factor in the promoter region of heat shock protein genes; HSF, heat shock factor; N, native protein. "-P" represents the phosphorylation of heat shock factor during stress.

Another set of stress proteins, glucose regulated proteins, reside in the endoplasmic reticulum (ER) and are expressed when the ER experiences stress. ER stress can be provoked by any circumstances, which hinder folding and maturation of secreted proteins. Thus changes of redox conditions, calcium concentration, deprivation of the glycosylating apparatus from its building blocks, monosaccharides, as well as the "ER-overload", when more secretory proteins are synthesized than the amount which can be handled by the ER folding apparatus, all lead to an ER stress response. The endoplasmic reticulum uses a unique mechanism for stress-signaling. A transmembrane receptor protein, called IRE, binds unfolded proteins in the lumen of the ER. Occupation of yeast IRE leads to the activation of its endonuclease activity in the cytosol. The endonuclease is specific for the primary RNA of the transcription factor, Hac. With the coordinated action of an RNA-ligase Hac-RNA undergoes a nonconventional splicing event. Only the spliced Hac mRNA can be translated. The Hac protein binds to the promoter region of ER-chaperones, and activates their transcription. Unfortunately a human substitute for Hac has not been identified yet, but several pieces of evidence suggest that a similar mechanism may also operate in mammals (Kaufman, 1999).

6.4 Heat shock proteins and signal transduction

Almost all chaperone classes are involved in various signaling events. Small heat shock proteins become phosphorylated by stress-kinases, and increase the amount of reduced glutathione in the cytoplasm (Arrigo, 1998). Similarly, Hsp70 protects cells against oxidative stress, and inhibits stress kinases and apoptosis (Gabai *et al.*, 1998). Other chaperones, such as mitochondrial Hsp60 (which is liberated after the disruption of mitochondrial membrane), promote apoptosis by activating caspases (Samali *et al.*, 1999). Another mechanism, the peptidyl prolyl cis/trans isomerase, Pin1, is required for the activation of several key modulators of the cell cycle.

Hsp90 has a special role among "signaling-chaperones". As we mentioned before, Hsp90 is a key organizer of several cytoplasmic complexes. It binds to steroid receptors and to several serine and tyrosine kinases including the Src, Raf, focal adhesion kinases and protein kinase CK-II, or cyclin-dependent kinases-4, 6 and 9 (Buchner, 1999; Csermely *et al.*, 1998; Miyata and Yahara, 1992; Pratt and Toft, 1997). Hsp90 helps these proteins to reach their fully signaling-competent form, as well as regulating their association with other proteins and membranes. Figure 6.3 shows the maturation of the steroid receptor. Chaperones bind to the *de novo* synthesized receptor protein in a sequential manner. First Hsp70 is attached with its co-chaperones, Hsp40 and Hip. In the next step, Hsp90 associates with the "bridging" chaperone, Hop. Finally, peptidyl prolyl cis/trans isomerases from the FK506-binding class join the complex helping to direct the receptor to its final destination in the cell. The small co-chaperone, p23 stabilizes the complex preventing its premature dissociation. When the steroid hormone binds to its receptor, the receptor dissociates from Hsp90 and becomes translocated to the nucleus resulting in the dimerization-coupled binding of the receptor-hormone complex to the steroid response element DNA-region. Chaperones like

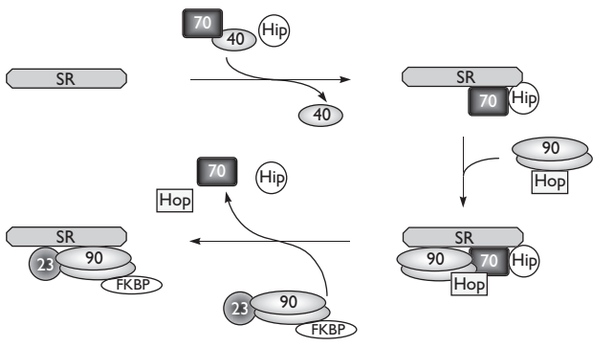

Figure 6.3 Involvement of the Hsp90-organized foldosome in the folding of steroid receptors. Abbreviations: 23, a co-chaperone of Hsp90; 40, Hsp40 – a co-chaperone of Hsp70; 70, Hsp70; 90, Hsp90; FKBP, FK506-binding protein, a peptidyl prolyl cis/trans isomerase binding to Hsp90; Hip, a co-chaperone of Hsp70; Hop, a chaperone, which connects Hsp70 with Hsp90; SR, steroid receptor.

Hsp90 may also play a role in the termination of the steroid response promoting the recycling of the receptor to the cytoplasm. The above mechanism gives an overview of activation of glucocorticoid receptors. Activation of other steroid receptors differ in the extent of chaperone involvement in the maturation/translocation of the receptor, and in the termination of the response.

A similar Hsp90-organized foldosome helps to acquire the activation-competent state of several protein kinases. The major difference from the steroid-parenting foldosome is that, in case of kinase-folding, the foldosome contains Cdc37, which is a kinase-specific "destination-tag" on the folding complex. Recent data also indicated the involvement of Hsp90 in the maturation of NO-synthase, telomerase and viral reverse transcriptase (Buchner, 1999; Csermely *et al.*, 1998; Pratt and Toft, 1997).

6.5 Heat shock proteins and disease

6.5.1 Chaperones as general helper of cell survival

Molecular chaperones are responsible for the "conformational homeostasis" of cellular proteins. When the homeostasis of the host organism is perturbed, an increased capacity of the chaperones is highly advantageous. Many of the perturbations (such as alcohol, other poisons, sunburn, anxiety, etc.) may induce the synthesis of these chaperone proteins *per se*, but in case of bacterial and viral infections the developing fever also helps this process. Several common drugs, such as aspirin, also promote the induction of heat shock proteins (Jurivich *et al.*, 1992).

Ischemia and the consecutive oxidative damage of reperfusion are also common perturbations in higher organisms. Since Currie *et al.* (1988) have shown that the induction of molecular chaperones, most notably Hsp70, may prevent damage to cardiac muscle by both ischemia and reperfusion, molecular chaperones are actively being investigated as possible tools in the treatment of heart attack or stroke. Indeed, transgenic animals, where Hsp70 has been constitutively expressed either in heart or brain, are much less likely to develop heart attack or stroke. Induction of heat shock proteins is also beneficial in transplanted organs, where moderate heat treatment reduces transfer-damage and the risk of organ rejection (Perdrizet *et al.*, 1993).

6.5.2 Chaperones in aging and in neurodegenerative diseases

Aging is frequently described as a consequence of an impaired function of repair processes (immune system, DNA-repair, elimination of free radicals, etc.). Molecular chaperone-catalyzed refolding of damaged proteins may well be one of these crucial repair processes. In agreement with this hypothesis, aged organisms contain an increased amount of misfolded proteins, and the induction of Hsp70 is impaired in both aged rats and humans. On the contrary, better induction of heat shock proteins leads to an increased life expectancy in yeast, *Drosophila* or *C. elegans* (Tatar *et al.*, 1997).

Protein damage becomes especially dangerous when it affects neuronal cells, which, generally, cannot renew themselves by multiple mitotic events. In most of the neurodegenerative diseases, such as in Alzheimer's disease, in Parkinson's disease, in Huntington's disease, in Wilson's disease, in Alexander's disease and in prion-related human syndromes, nerve cells develop massive protein aggregates. These inclusion bodies usually contain various heat shock proteins, such as ubiquitin-tags, the small heat shock protein, Hsp27, Hsp70 and Hsp90 (Mayer *et al.*, 1991). Heat shock protein co-aggregation may reflect the fight of these chaperones against the aggregation process. In accordance with this view, over expression of Hsp70, or other heat shock proteins protects *Drosophila*-s from neurodegeneration in Huntington disease-like polyglutamine-induced aggregation.

6.5.3 Chaperones and the immune response

Molecular chaperones are one of the most conserved proteins in living organisms (Lindquist, 1986). Invading bacteria experience major changes in their environment when entering their host. These changes and the activation of defense mechanisms (depletion of nutrients, pH changes, osmotic changes, digestive enzymes, peroxides, superoxides and an increase in temperature) induce numerous heat shock proteins in bacteria, among which some are also expressed on the bacterial surface. Because of their conservative structure, these bacterial heat shock proteins, especially the bacterial homologue of Hsp70 become a common recognition signal, and therefore provoke a general, high-capacity immune response (van Eden and Young, 1996). There are at least two dozen infectious diseases in which immune responses to heat shock porteins have been reported, including tuberculosis, leprosy, legionnaire's disease, Chagas's disease, lyme disease, chlamidyal infections and Q fever.

In some unfortunate cases (such as in rheumatoid arthritis, in lupus erythematosus, in multiple sclerosis and in insulin dependent diabetes mellitus, IDDM) certain proteins of the host organism resemble some epitopes of these bacterial heat shock proteins. In these patients the common, antibacterial immune response attacks the cells bearing these host-proteins, and a severe autoimmune response develops. Vaccination with modified epitopes of a bacterial Hsp70 homologue diminish, and in some cases prevent the development of the disease (van Eden and Young, 1996). Some recent reports raise the possibility that expression of human Hsp60 on the surface of epithelial cells may be one of the initial events of arterial plaque development.

6.6 Therapeutic approaches

6.6.1 Chaperone induction

As we have mentioned earlier, if an organism experiences stress, the induction of heat shock proteins is beneficial for its survival. Fever and some common drugs, such as aspirin, help to induce heat shock protein expression. Other general methods, such as sauna, or hyperthermia can also be efficient. Recently a non-toxic drug candidate has been described, which behaves as a co-inducer of various heat shock proteins (Vígh *et al.*, 1997). However, due to the pleiotropic effects of chaperones, in most cases an organ-specific induction of special heat shock proteins is sought. Though recent data revealed the complexity of the regulation of the heat shock response at the molecular level (Morimoto, 1999), which may expose new targets for therapeutic interventions, we are still far from an efficient "stress-pill", which would reduce the risk of stroke, or heart attack. Moreover, the expression- and induction-pattern of various heat shock proteins seems to be rather complex. Each individual, but most probably each cell at each point of its history, has a unique pattern of heat shock protein level and transcription. This "stress-status" may be a complex readout of the individual "stress-history" of the given cell. This complexity makes a general therapeutic intervention even more difficult. As an additional note of caution we must emphasize that artificially high levels of heat shock proteins are extremely cytotoxic (Feder *et al.*, 1992). Therefore limitless chaperone-induction may not always be advantageous.

6.6.2 Chaperone inhibition

Chaperones help survival, regardless of whether the host cells are "normal" or malignant. Tumor cells experience a lot of stress, such as hypoxia or glucose-limitation. Various chemotherapy, phototherapy, radiation therapy and hyperthermia protocols increase heat shock protein induction and consequent tumor cell survival even further. Under these conditions the inhibition of chaperones would be highly beneficial. When thinking about chaperone-inhibition based, signaling-related therapeutic approaches we find two major difficulties:

1 chaperones have many general functions in cells
2 there are only a few compounds which bind to one or another chaperone specifically.

Fortunately in several chaperone functions, chaperone-isoforms, or even chaperones from different classes, may substitute each other, and the number of chaperone-specific pharmacological agents is increasing.

As we have mentioned earlier, the most important signaling-chaperone is Hsp90. It has numerous distinct, and overlapping binding sites on its surface. Hsp90 contains at least two chaperone-sites, one in its N-terminal domain, and another in the C-terminal domain. The highly charged connecting bridge after the N-terminal domain binds protein kinase CK-II, and there are additional binding sites for calmodulin, peptidyl prolyl cis/trans isomerases and other co-chaperones. Hsp90 forms dimers, binds ATP in the N-terminal domain, and changes its conformation after ATP addition (Csermely, 1998; Maruya *et al.*, 1999; Minami *et al.*, 1994).

This abundance of binding-sites and the conformational flexibility of Hsp90 suggests the presence of a large variety of active surfaces on Hsp90, which may all be potential binding sites for various pharmacological compounds. Moreover, different occupancy and conformation may expose different binding surfaces of this chaperone. Indeed, the number of Hsp90-binding drugs is steadily increasing. Figure 6.4 shows an overview of the Hsp90-binding pharmacological agents identified so far. Geldanamycin, and an unrelated antibiotic, radicicol, occupy the ATP-binding pocket of the N-terminal domain of Hsp90. This ATP-binding site is rather unique, does not resemble the nucleotide binding site of protein-, or other kinases. The only distant homologs are some DNA-binding bacterial topoisomerases. Therefore geldanamycin and radicicol seem to be specific agents for the modification of the function of the 90 kDa chaperones (Hsp90, and its homologs in the endoplasmic reticulum and mitochondria). Treatment of cells with these cell-permeable drugs induces the dissociation of Hsp90-complexes and the subsequent degradation of most Hsp90 client proteins by the proteasome.

Recently other compounds, such as the coumarine-derivative novobiocin and cisplatin, have been found which bind to the C-terminal domain of Hsp90. The exact binding site of

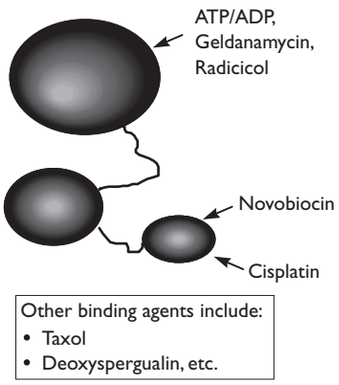

Figure 6.4 Various drugs and drug-candidates binding to the 90 kDa molecular chaperone, Hsp90. The Figure shows the domain structure of Hsp90 and lists those drugs which bind to the N-terminal (top) domain, or to the C-terminal (bottom) domain of Hsp90. Both domains contain a binding site for target proteins and peptides.

another widely applied anti-tumor agent, taxol, on Hsp90 has not been identified yet (Byrd *et al.*, 1999; Itoh *et al.*, 1999a; Marcu *et al.*, 2000; Neckers *et al.*, 1999). It will be an exciting task to explore whether some of these compounds specifically affect the efficiency of the N-terminal, or C-terminal chaperone site of Hsp90. Various Hsp90 conformations and/or complexes may be stabilized by one, or another of these drugs, and therefore different compounds (or their combination) may inhibit different functions of Hsp90.

Another possible target, where chaperone-inhibition may help, is immune-suppression. Indeed, widely used immune-suppressants, such as cyclosporin, or FK506 bind to different classes of peptidyl prolyl cis/trans isomerases, and block various signaling pathways in T lymphocytes. Other chaperones are also targets of immune-suppressive drugs: Hsp70 and Hsp90 bind deoxyspergualin (Nadeau *et al.*, 1994), while Hsp60 binds mizobirine (Itoh *et al.*, 1999b).

6.6.3 *Chaperones as anti-cancer or anti-viral vaccines*

As we have discussed before, tumor cells experience a lot of stress (hypoxia, nutrient deprivation, etc.). Stressful conditions induce heat shock protein synthesis, and several types of cancer cells expose heat shock proteins on their surface (Multhoff and Hightower, 1996). These cells are recognized and killed by a special class of T lymphocytes, the $\gamma\delta$-cells. There are ongoing clinical trials, where tumor cells are exposed to various types of stresses to prime them for a subsequent immune attack.

At the beginning of the eighties, several research groups isolated molecular chaperones (Grp94 and Hsp90) as tumor-specific transplantation antigens, i.e. surface proteins, which provoked a highly specific immune response against a certain type of tumor. When painstaking research efforts made it clear that neither the primary structure, nor major post-translational modifications were different in chaperones coming from normal versus malignant tissue, researchers in the field were puzzled. Pramod Srivastava first suggested that the differences may come from the peptides, which are carried by the chaperones. In a 1994 hypothesis paper he proposed the existence of a relay-type mechanism, where various cytoplasmic chaperones, such as Hsp70 and Hsp90 as well as their counterparts in the endoplasmic reticulum, such as Grp94, give each other the peptides and help their presentation to the MHC-I complex, specialized to present the "self" antigens to the immune system. MHC-I molecules induce a cytotoxic T-cell response, which is a fast and local immune attack, against a cell expressing "false" self-antigens (e.g. after a viral infection). This may significantly enhance the efficiency of the MHC-II immune response, which is based on helper T lymphocytes, and generates a slow and general immune attack. According to Srivastava's hypothesis, chaperones carrying tumor-peptides may enter the MHC-I pathway of macrophages and dendritic cells (Figure 6.5) and activate a cytotoxic immune response against a "foreign" antigen. Later studies proved that each major step of the original hypothesis was correct (Srivastava *et al.*, 1998). Chaperone-induced "cross-priming" (channeling of the MHC-II peptides to the MHC-I presenting pathway) is an interesting phenomenon by itself, but its real importance lies in the clinical application.

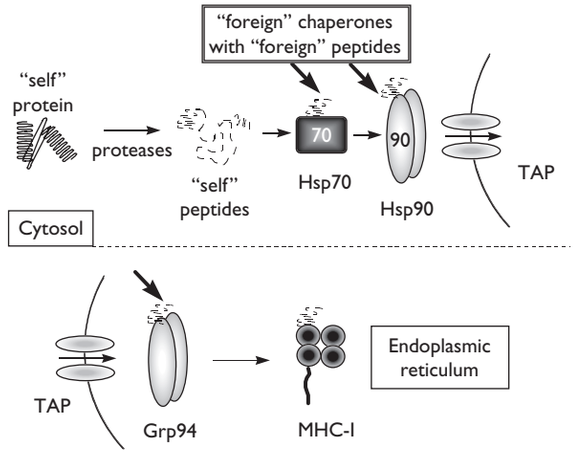

Figure 6.5 Heat shock proteins may enable the presentation of "foreign" peptides (antigens) to the MHC-I complex specialized to present "self" antigens. TAP represents the protein responsible for peptide transport through the endoplasmic reticulum membrane and Grp94 is a homologous protein to Hsp90 in the endoplasmic reticulum.

Srivastava's discovery made it possible to induce an anti-tumor immune response by vaccination. Isolation of peptide-chaperone complexes from the primary tumor and administration of the complex to the dendritic cells as a vaccine provokes an efficient anti-tumor immune response and is the subject of ongoing clinical trials (Srivastava *et al.*, 1998). In the future the establishment of common important peptide-antigens in certain type of tumors, such as prostate cancer, may alleviate the need for time consuming and costly "personalized vaccines". Similar approaches may be used to provoke an anti-viral immune response by the help of viral-specific peptide-chaperone complexes.

6.7 Final remarks

Molecular chaperones were necessary for the establishment of life on Earth, for the bursts of activity during evolution, and are crucial for the protection of our own life against proteotoxic stress. Chaperones are one of the most abundant proteins in our cells, and may help to maintain and remodel the structure of the cytoplasm in eukaryotes (Csermely *et al.*,

1998; Pratt and Toft, 1997). These pleiotropic effects make chaperones an attractive target for pharmacological interventions, but also create difficulty for the researcher looking for proper compounds and methods which specifically modify one or another chaperone function. Recent developments in Hsp90-related pharmacology and the establishment of several clinical methods which utilize the role of chaperones in peptide presentation make this field an exciting area for future clinical studies. Even in our own private life we may take a lesson from chaperones: small, but frequent challenges lead to a modest induction of heat shock proteins, which increases our fitness and (at least in animal studies) prolongs life expectancy.

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