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The 90-kDa Molecular Chaperone Family: Structure, Function, and Clinical Applications. A Comprehensive Review

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ABSTRACT. The 90-kDa molecular chaperone family (which comprises, among other proteins, the 90-kDa heat-shock protein, hsp90 and the 94-kDa glucose-regulated protein, grp94, major molecular chaperones of the cytosol and of the endoplasmic reticulum, respectively) has become an increasingly active subject of research in the past couple of years. These ubiquitous, well-conserved proteins account for 1–2% of all cellular proteins in most cells. However, their precise function is still far from being elucidated. Their involvement in the aetiology of several autoimmune diseases, in various infections, in recognition of malignant cells, and in antigen-presentation already demonstrates the essential role they likely will play in clinical practice of the next decade. The present review summarizes our current knowledge about the cellular functions, expression, and clinical implications of the 90-kDa molecular chaperone family and some approaches for future research. PHARMACOL. THER. 79(2):129–168, 1998. **© 1998 Elsevier Science Inc.**

KEY WORDS. Chaperone, hsp90, grp94, endoplasmin, gp96.

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ABBREVIATIONS. BiP, grp78, a 70-kDa glucose-regulated protein of the endoplasmic reticulum; CK-II, protein kinase CK-II (previously known as casein kinase II); Cyp, Cyclosporin A-binding immunophilin; eIF-2-α, initiation factor 2-α-subunit; ER, endoplasmic reticulum; FKBP, FK506-binding immunophilin; FKBP52, 52-kDa immunophilin (former names: hsp56, hsp59, HBI); gp96, 94-kDa glucose-regulated protein (other names: grp94, endoplasmin; formerly: hsp100, hsp110); grp94, 94-kDa glucose-regulated protein (other names: gp96, endoplasmin; formerly: hsp100, hsp110); Hip, co-chaperone of hsc70; Hop, 60-kDa protein linking hsp70 and hsp90 in the cytoplasmic chaperone complex (other names: p60, STI); hsc70, constitutively expressed 70-kDa heat-shock protein; HSE, heat-shock element; hsp70, 70 kDa heat-shock protein, member of the 70-kDa molecular chaperone family; hsp75/TRAP-1, a novel eukaryotic homologue belonging to the hsp90 molecular chaperone family; hsp90, 90-kDa heat-shock protein; HtpG protein, prokaryotic hsp90 (originally: high temperature protein G); IME, element of the early meiotic transcriptional cascade; MHC, major histocompatibility complex; NLS, nuclear localization signal; p23, a small, hsp90-associated chaperone; PP-5, phosphoprotein phosphatase-5, a tetratricopeptide repeat containing immunophilin; TPR, tetratricopeptide repeat; TRAP-1, Type 1 tumor necrosis factor receptor-interacting protein 1, a small cytosolic hsp90 homologue; also called hsp75; URS, upstream regulatory sequence.

1. INTRODUCTION: THE 90-kDa MOLECULAR CHAPERONE FAMILY

Molecular chaperones recently 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). Chaperones do not determine the tertiary structure of the folding proteins, but help them find their structure more efficiently. However, only a few chaperones behave as true catalysts by increasing the rate of protein folding. These special chaperones, peptidyl prolyl isomerases and protein disulfide isomerases, are, therefore, better called "folding catalysts." The majority of the chaperones prevents incorrect interactions of "sticky" protein-folding intermediates and frequently helps these intermediates to refold from folding traps, giving them a new chance for spontaneous folding. This mechanism increases the yield, but not the rate, of protein folding (Hartl, 1996).

Chaperones are ubiquitous, highly conserved proteins that probably played a major role in the evolution of modern enzymes (Csermely, 1997). Chaperones are vital for our cells during their entire lifetime. However, they are needed even more after environmental stress, which induces protein damage. Stress (heat shock, poisoning, almost any abrupt change in the cellular environment, and mental stress as well) induces the synthesis of many chaperones, which, therefore, are called heat-shock, or stress, proteins. Chaperones play an essential role in the aetiology of numerous diseases, with a rapidly increasing role in clinical practice (Latchman, 1991; Welch, 1992; Burdon, 1993; Snyder and Sabatini, 1995; Jindal, 1996; van Eden and Young, 1996; Welch and Brown, 1996; Brooks, 1997).

Lacking a settled view about their exact and specific cellular functions, chaperones are still best classified by their molecular weights. The major chaperone families are listed in Table 1. The characteristic chaperone functions of the different families show that the 90-kDa molecular chaperones are somewhat different from the others, being the most "passive," since in most cases, they only prevent the aggregation of unstable protein conformers, which is a rather general feature of almost all proteinaceous and chemical chaperones (Welch and Brown, 1996). The specificities of chaperone functions of the 90-kDa chaperones are further discussed in Section 3.1.

Members of the 90-kDa molecular chaperone family are introduced in Table 2. The prokaryotic HtpG protein (after its original name: high temperature protein G) is not as well characterized as its eukaryotic counterparts, the 90-kDa heat-shock protein hsp90 and the 94-kDa glucose-regulated protein grp94. hsp90 is largely a cytosolic protein, while the majority of grp94 resides in the endoplasmic reticulum (ER). The two proteins are 50% identical, and their existence is most probably a result of a gene duplication that occurred at a very early stage in the evolution of the eukaryotic cell (Gupta, 1995). Translocation of these proteins to other organelles has been observed; however, a *bona fide* nuclear, or mitochondrial, hsp90 homologue has not been discovered yet. Recently, two highly homologous proteins, hsp75 and TRAP-1, were reported. These proteins differ from each other only in their N- and C-termini. hsp75/TRAP-1 is a distant eukaryotic relative of hsp90, resembling both in size and in structural organization the HtpG protein (Song *et al.*, 1995; Chen, C. F. *et al.*, 1996). Recently, Cho *et al.* (1997) described yet another seemingly novel nuclear 90-kDa heat-shock protein; but, lacking sequence data, its exact relation to existing hsp90 struc-

TABLE 1. Major Molecular Chaperone Families

Some common names of eukaryotic chaperone family members	Characteristic chaperone function	Recent reviews
hsp27, crystallins, small heat-shock proteins	Prevent protein aggregation, release proteins from aggregates	Ciocca *et al.*, 1993; Groenen *et al.*, 1994; Buchner, 1996
hsp60, chaperonins	Prevent protein aggregation, help protein folding	Hartl, 1996; Fenton and Horwich, 1997
hsp70, grp78, BiP	Prevent protein aggregation, help protein folding	Cyr *et al.*, 1994; Haas, 1994; Hartl, 1996
hsp90, grp94	Prevent protein aggregation	Jakob and Buchner, 1994; Buchner, 1996; Pratt, 1997; Johnson and Craig, 1997
hsp110	Release proteins from aggregates	Schirmer *et al.*, 1996; Wawrzynow *et al.*, 1996

Neither the co-chaperones (chaperones that help the function of other chaperones listed, such as hsp10, dnaJ homologues, Hip, Hop, Hup, etc.), 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.

tures is presently unknown. Chadli *et al.* (1997) purified a 440-kDa cytosolic glycoprotein having 9 peptide sequences highly homologous to hsp90. The protein is heavily glycosylated, and its peptidic moiety has a molecular mass of 78 kDa.

hsp90 has two isoforms, hsp90-α and -β, which are 76% identical and are the consequences of a gene duplication about 500 million years ago (Moore *et al.*, 1989; Krone and Sass, 1994). hsp90-β is somewhat larger than hsp90-α, and until recently, was frequently denoted as hsp86 and hsp84. hsp90-β is a somewhat less inducible protein than hsp90-α, and sometimes is called hsc90, emphasising that it is the (more or less) constitutively expressed cognate protein of the 90-kDa chaperones (in this nomenclature hsp90-α retains the hsp90 abbreviation). Here we use the "α-β" nomenclature to better distinguish between the two proteins. Due to the high degree of structural and functional homology between animal and human hsp90, in most cases, we do not discriminate between these hsp90 species.

2. STRUCTURE AND CHARACTERIZATION OF 90-kDa MOLECULAR CHAPERONES

The prokaryotic 90-kDa molecular chaperone, the HtpG protein, is about 40% similar to its eukaryotic counterparts (Bardwell and Craig, 1987). It is a dimeric phosphoprotein (Spence and Georgopoulos, 1989) that displays chaperone characteristics similar to hsp90, forms oligomers, has a higher thermostability than the eukaryotic homologues (Jakob *et al.*, 1995b), and probably binds to many prokaryotic proteins, e.g., to the prokaryotic heat-shock factor σ^{32} (Nadeau *et al.*, 1993). However, in contrast to hsp90, deletion of HtpG is not lethal to eubacteria, and only makes them somewhat more heat-sensitive, resulting in a slight growth disadvantage (Bardwell and Craig, 1988). The molecular characteristics of hsp90 and grp94, much better established than those of the HtpG protein, are summarized in the following two sections.

2.1. *Molecular Characteristics and Structure of hsp90*

Like the prokaryotic HtpG protein, hsp90 is also a phosphorylated dimer (Rose *et al.*, 1987; Lees-Miller and Anderson, 1989a,b; Radanyi *et al.*, 1989; Minami *et al.*, 1991) containing 2–3 covalently bound phosphate molecules per monomer (Iannotti *et al.*, 1988). Dimerization is necessary for the vital functions of hsp90 (Minami *et al.*, 1994). In the presence of nonionic detergents, and after heat treatment, it preferentially forms oligomers (Lanks, 1989; Minami *et al.*, 1991). The tendency for oligomerization is characteristic of "native" hsp90 as well, especially in the presence of divalent cations, nucleotides, and higher hsp90 concentrations (Minami *et al.*, 1993; Jakob *et al.*, 1995b; Nemoto *et al.*, 1996; Freitag *et al.*, 1997).[1] hsp90 dimers have a rather elongated structure, as indicated by sedimentation studies (Welch and Feramisco, 1982; Rose *et al.*, 1987) and by electron microscopy (Koyasu *et al.*, 1986).

Like many other chaperones, hsp90 is a rather hydrophobic protein and its hydrophobicity further increases after heat shock (Iwasaki *et al.*, 1989; Yamamoto *et al.*, 1991). On the other hand, hsp90 also contains two highly charged domains: one is the hinge-domain between the N-terminal and C-terminal domains (this structure is present only in the eukaryotic hsp90 homologues), and the other lies in the C-terminal domain. These structures (together with the exposed hydrophobic surfaces) are probably also involved in determining the protein binding characteristics of hsp90 (Binart *et al.*, 1989). In agreement with this prediction, initial studies indicated that hsp90 shows a binding preference either for positively charged, or for hydrophobic, proteins (Csermely *et al.*, 1997). Surface charges of hsp90 are further increased by the heavy phosphorylation of the protein, which forms complexes with numerous protein kinases (see Section 3.2.2), and many of them, especially protein kinase CK-II (previously known as casein kinase II), preferentially phosphorylate the protein (Dougherty *et al.*, 1987; Lees-

[1] Cs. Sőti, and P. Csermely, unpublished observations.

TABLE 2. Members of the 90-kDa Molecular Chaperone Family

Name	Characteristic localization	First sequence information
HtpG	*Escherichia coli*	Bardwell and Craig, 1987
hsp75/TRAP-1	Cytoplasm	Song *et al.*, 1995; Chen, C. F. *et al.*, 1996
hsp90-α, hsp90-β	Cytoplasm	Farrelly and Finkelstein, 1984
grp94 (endoplasmin, gp96)	ER	Kulomaa *et al.*, 1986; Sorger and Pelham, 1987

Miller and Anderson, 1989a; Miyata and Yahara, 1992, 1995). Interestingly, in spite of the fact that hsp90 forms complexes with a large number of tyrosine kinases, tyrosine phosphorylation of the protein has not been observed. Besides its high affinity for protein kinases, hsp90 is co-isolated with the phosphatidylinositol-4-kinase (Flanagan and Thorner, 1992) and binds phosphoprotein phosphatases, such as the immunophilin-like phosphoprotein phosphatase-5 (PP-5) (Chen, M. S. *et al.*, 1996; Silverstein *et al.*, 1997).

hsp90 is probably one of the "stickiest" proteins of the cytosol, a kind of "molecular glue" in our cells. Besides kinases and phosphatases, hsp90 binds a wide range of other proteins, including various nuclear hormone receptors (see Pratt, 1997), actin (Koyasu *et al.*, 1986; Czar *et al.*, 1996), tubulin (Sanchez *et al.*, 1988; Redmond *et al.*, 1989; Fostinis *et al.*, 1992; Williams and Nelsen, 1997), the heat-shock factor-1 (Nadeau *et al.*, 1993), calmodulin (Minami *et al.*, 1993), calpain,[2] and the proteasome (Tsubuki *et al.*, 1994; Wagner and Margolis, 1995). hsp90 forms a large cytosolic complex (designated as the foldosome) with numerous other molecular chaperones, such as hsc70, immunophilins, CDC37, and p23 (Hutchison *et al.*, 1994; Pratt, 1993), the functional consequences of which are described in Section 3.9.

Earlier studies demonstrated that hsp90 possesses an ATP-binding site and an ability to phosphorylate itself (Csermely and Kahn, 1991). It also undergoes a large conformational change after ATP addition (Csermely *et al.*, 1993). Purified hsp90 displays ATPase activity (Nadeau *et al.*, 1992, 1993) and is even more active as a GTPase (Nardai *et al.*, 1996). This activity, however, either is due to an impurity in the hsp90 preparations or its manifestation requires a mandatory co-inducer protein (which may be either a nucleotide exchanger or an ATP/GTPase activator protein, or both) (Nadeau *et al.*, 1994; Shi *et al.*, 1994; Nardai *et al.*, 1996). Based on low autophosphorylating and ATPase activities of hsp90 preparations, on the rather low affinity of ATP-binding, and on the fact that the chaperone activity of hsp90 does not require the presence of ATP (see Jakob and Buchner, 1994; Buchner, 1996), ATP binding of hsp90 recently has been questioned (Jakob *et al.*, 1996). However, in the interim, ATP was shown to induce the dissociation of hsp90 from actin filaments (Kellermayer and Csermely, 1995), and to be necessary for the interaction of p23 and hsp90 (Johnson *et al.*, 1996; Sullivan *et al.*, 1997). Recently, the ATP- and ADP-complexes of the N-terminal domain of hsp90 were crystallized (Prodromou *et al.*, 1997a), and methods with higher resolution using spin-labeled conformational probes also confirmed the binding of ATP to hsp90, albeit with a rather low affinity (apparent K_d around 200–400 μM) (Csermely and Kahn, 1991; Csermely *et al.*, 1993; Kellermayer and Csermely, 1995; Scheibel *et al.*, 1997; Grenert *et al.*, 1997). Other nucleotides, such as ADP (Grenert *et al.*, 1997) or CTP (Freitag *et al.*, 1997), have a higher affinity for hsp90 than ATP.

Although the primary structure of hsp90 was described many years ago (Farrelly and Finkelstein, 1984), relatively little is known about the functional role of various segments of the protein. Biochemical and electron microscopic studies indicate that it contains two clearly distinguishable domains attached to each other by a relatively flexible, highly charged loop (Fig. 1A) (Koyasu *et al.*, 1986; Itoh and Tashima, 1993). The C-terminal domain itself may also have a bilobular structure (Joachimiak, 1997; Nemoto *et al.*, 1997).

2.1.1. Structure of hsp90: the N-terminal domain. The crystallization and three-dimensional structure analysis of the N-terminal domain (Stebbins *et al.*, 1997; Prodromou *et al.*, 1997a,b) is one of the most important recent developments in the characterization of hsp90. The tertiary structure of human (Stebbins *et al.*, 1997) and yeast (Prodromou *et al.*, 1997b) N-terminal domains are almost identical: a highly twisted, eight-stranded β-sheet covered on one side by α helices (Fig. 1B). At the center of the helical side, a deep pocket penetrates to the surface of the buried β-sheet and forms a binding site for ATP/ADP (Prodromou *et al.*, 1997a; Grenert *et al.*, 1997) and for the hsp90-specific antitumor drug geldanamycin (Stebbins *et al.*, 1997; Grenert *et al.*, 1997). The geldanamycin-binding site probably overlaps with the binding site of another hsp90-binding antibiotic, radicicol (Soga *et al.*, 1998). The N-terminal domain is involved in the binding of target proteins (Prodromou *et al.*, 1997b; Young *et al.*, 1997), and it contains a 60 amino acid stretch highly homologous with the intramolecular chaperone region of *Vibrio cholerae* cytolysin protein (Nagamune *et al.*, 1997).

2.1.2. Structure of hsp90: the highly charged connecting hinge region. The central, highly charged region of hsp90, specific to eukaryotic cells (Gupta, 1995), has been shown

[2]T. Schnaider, Cs. Sőti, and P. Csermely, unpublished observations.

to participate in association of the protein with steroid receptors (Tbarka *et al.*, 1993; Cadepond *et al.*, 1993; Dao-Phan *et al.*, 1997) and with protein kinase CK-II (Miyata and Yahara, 1995). Alternating lysine and glutamic acid residues (so-called "KEKE-motifs") may be generally involved in protein-protein interactions (Realini *et al.*, 1994a,b) and may serve as a binding site of hsp90 for the proteasome. Genetic studies, however, indicate that the region is not essential for the life-sustaining functions of hsp90 (Louvion *et al.*, 1996), and may be involved in some "back-up" or regulatory functions.

hsp90 is a calcium-binding protein (Kang and Welch, 1991; Minami *et al.*, 1993). As a part of its "KEKE-region," two α-helix pairs were predicted showing a high similarity to calcium binding EF-hand structures (Nardai *et al.*, 1996). The putative hsp90-EF-hands contain two major *in vivo* phosphorylation sites of hsp90, which account for approximately one-half of the *in vivo* phosphorylation of the protein and which can be phosphorylated by protein kinase CK-II, a kinase known to form a complex with hsp90 (Dougherty *et al.*, 1987; Lees-Miller and Anderson, 1989a; Miyata and Yahara, 1992, 1995; Shi *et al.*, 1994). An overlap of the phosphorylation sites and the putative calcium-binding sites suggests that phosphorylation of the major phosphorylation sites may be a requirement for calcium binding of hsp90. As a possible consequence of this, the Ca^{2+}-dependent autophosphorylation of hsp90 requires the occupancy of the major phosphorylation sites (Csermely and Kahn, 1991).

As further evidence for the regulatory role of the central, highly charged hinge region of hsp90, Szyszka *et al.* (1989) demonstrated that hsp90 is able to enhance the kinase activity of the initiation factor 2-α-subunit (eIF-2-α) kinase only after its phosphorylation with protein kinase CK-II. Control experiments indicated that although the cyclic AMP-dependent protein kinase is also able to phosphorylate hsp90, this phosphorylation does not result in a structure of hsp90 that would be able to activate the eIF-2-α kinase (Kudlicki *et al.*, 1985). Experiments with the phosphoprotein phosphatase inhibitor okadaic acid also point to involvement of hsp90 phosphorylation in the regulation of the stability of hsp90/v-Src complexes (Mimnaugh *et al.*, 1995). hsp90 also becomes methylated on 1–3 lysine residues soon after its translation has been completed (Wang *et al.*, 1981, 1982), but the importance of this post-translational modification remains to be clarified.

A small portion of hsp90 is known to reside in and/or translocate to the cell nucleus in resting cells and after heat shock (Collier and Schlessinger, 1986; Gasc *et al.*, 1990; Morcillo *et al.*, 1993; Biggiogera *et al.*, 1996). Nuclear transport of hsp90 may be mediated by a bipartite nuclear localization sequence located next to the EF-hand-like structures (Nardai *et al.*, 1996) (Fig. 1A). Under normal conditions, this signal seems to be hidden in the interior of the protein, but its exposure in deletion mutants shifts the truncated hsp90 to the nucleus (Meng *et al.*, 1996). The hsp90 nuclear localization signal (NLS) may participate in the nucleo-cytoplasmic shuttle of steroid receptors as well (Csermely *et al.*, 1995b).

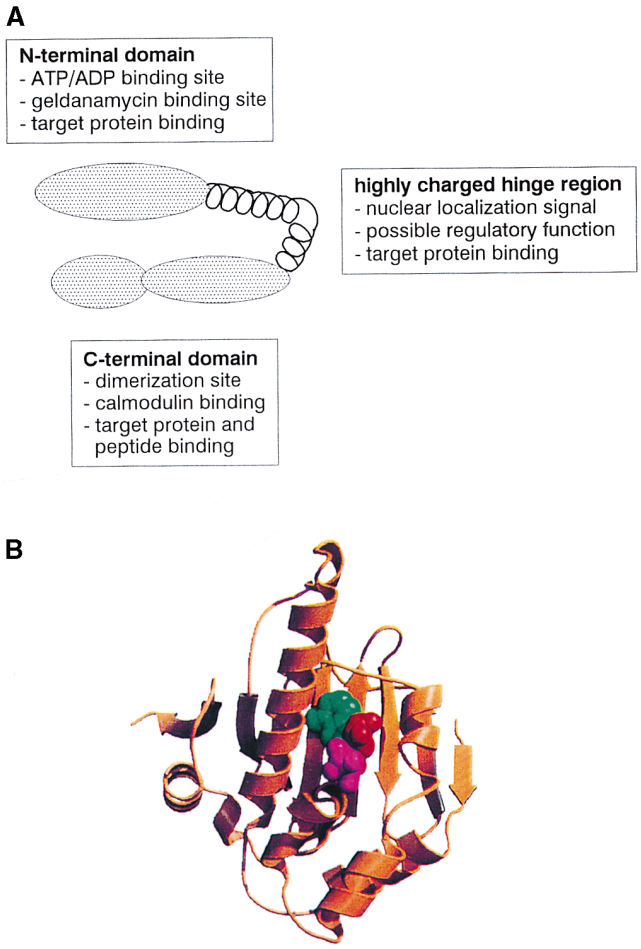

FIGURE 1. Structure of hsp90. A: Domain structure of hsp90. The highly conserved primary sequence and the available data suggest that grp94 possibly has a quite similar structural and functional organization of its domains to that of hsp90. For clarity, the formation of dimer structures is not shown. For further details see the text. B: Three-dimensional structure of the N-terminal domain of yeast hsp90, showing the position of bound ADP/ATP. The base, ribose, and phosphates of the bound nucleotide are colored green, red, and magenta, respectively. B is reproduced from Prodromou *et al.* (1997a), with permission of the authors and the copyright holder, Cell Press, Cambridge.

2.1.3. Structure of hsp90: the C-terminal domain. The C-terminal domain harbors the binding site for calmodulin (Minami *et al.*, 1993) and the hsp90 dimerization site (Minami *et al.*, 1994; Nemoto *et al.*, 1995; Meng *et al.*, 1996) (Fig. 1A). The dimerization site lies close to the epitope of the AC-88 monoclonal anti-hsp90 antibody (Schlatter *et al.*, 1992; Sullivan and Toft, 1993), which also recognizes some heterogeneous nuclear ribonucleoproteins around 40–50 kDa (Harry *et al.*, 1990). Binding of AC-88 to hsp90 interferes with the binding of several proteins, including steroid receptors and actin filaments, which may indicate

involvement of the C-terminal region of hsp90 in protein binding (Sullivan *et al.*, 1985; Schlatter *et al.*, 1992; Cadepond *et al.*, 1993; Sullivan and Toft, 1993; Kellermayer and Csermely, 1995). In agreement with this assumption, Shue and Kohtz (1994) localized the helix-loop-helix transcription factor folding activity of hsp90 to a 48-amino acid segment close to the C-terminus of the protein. As further evidence for the role of the C-terminal domain in the chaperon function of hsp90, Young *et al.* (1997) demonstrated that this domain binds both proteins and the antigenic octapeptide of the vesicular somatitis virus G-protein.

Our earlier findings (Csermely and Kahn, 1991) indicated the presence of an ATP-binding consensus sequence in the C-terminal half of hsp90. Later studies (Jakob *et al.*, 1996) pointed out that the degree of homology is not well preserved, and the discovery of a rather nonconventional ATP/ADP binding site (different from the "Walker-type" ATP-binding sites [Walker *et al.*, 1982] present in all ATP-binding chaperones) in the N-terminal domain (Prodromou *et al.*, 1997a), also made it unnecessary to presume the existence of a C-terminal ATP-binding site. However, photoaffinity labeling of hsp90 with ATP analogues shows rather scattered labeling of almost all tryptic fragments, and Hill plots of ATP-dependent hsp90 activities, such as autophosphorylation or the associated ATPase activity, also suggest cooperativity of two binding sites[3] (Sőti and Csermely, 1998). Part of these observations can be explained by interaction of the two N-terminal ATP-binding sites of the hsp90 dimer, but may also imply that hsp90 contains two nucleotide-binding sites, like members of the hsp110 chaperone family (Wawrzynow *et al.*, 1996).

Concluding our structural analysis of hsp90, we should point out that some experimental data can be reconciled by assuming that the N- and C-terminal domains of hsp90 closely interact with each other. Comparison of the rather large and different conformational changes of hsp90 after ATP (Csermely *et al.*, 1993) and/or geldanamycin addition[4] with the rather similar tertiary structure of the ATP- (Prodromou *et al.*, 1997a) and geldanamycin- (Stebbins *et al.*, 1997) complexes may indicate an N-terminal domain-triggered conformational change in the C-terminal domain after the binding of various ligands. Similarly, various proposals involving the participation of all three major domains of hsp90 in peptide binding, and the preference of hsp90 for both hydrophobic and basic residues (Csermely *et al.*, 1997), may reflect either several different peptide-binding sites or a concerted action of all three domains in the low-affinity trapping of various peptide segments.

2.2. *Molecular Characteristics and Structure of grp94*

grp94, the most abundant protein of the ER (Koch *et al.*, 1986), is approximately 50% homologous with its cytoplasmic counterpart hsp90 (Gupta, 1995). This high degree of homology already suggests that many of the characteristic features of hsp90 will be similarly expressed by grp94. However, our knowledge about the structure and characteristics of grp94 is rather limited compared with the rapidly expanding molecular data on hsp90.

Like hsp90, grp94 also forms dimers (Nemoto *et al.*, 1996; Wearsch and Nicchitta, 1996b) and is phosphorylated by numerous kinases, including CK-II (Cala and Jones, 1994; Csermely *et al.*, 1995a; Wearsch and Nicchitta, 1997). CK-II phosphorylates the protein in the middle highly charged region and at four C-terminal threonine residues (Cala and Jones, 1994). The degree of *in vivo* phosphorylation may vary from cell type to cell type (Welch *et al.*, 1983; Lee *et al.*, 1984). Various methods, including rotary-shadowing electron microscopy, indicated that grp94 dimers show a trinodular elongated rod-like shape (Koyasu *et al.*, 1986; Wearsch and Nicchitta, 1996b). Dimerization is promoted by hydrophobic interactions and results in a tail-to-tail organization of two grp94 molecules (Wearsch and Nicchitta, 1996b). Under oxidizing conditions, grp94 dimerization may be further stabilized by a disulfide-bridge between cysteines 117 of the two monomers (Poola and Lucas, 1988; Qu *et al.*, 1994). The *in vitro* oligomerization of grp94 is probably not so pronounced as that of hsp90 (Nemoto *et al.*, 1996), but given the extremely high protein concentration of the ER lumen, one may predict that *in vivo*, the high degree of "molecular crowding" (Zimmerman and Minton, 1993) leads to the appearance of grp94 oligomers.

grp94 is a hydrophobic protein and tends to associate with the membrane of the ER and Golgi apparatus. This avid binding to lipid structures led to the early assumption that grp94 was a transmembrane protein (Lewis *et al.*, 1985; Mazzarella and Green, 1987). However, later studies suggested that the majority of the protein resides in the ER lumen (Kang and Welch, 1991; Cala and Jones, 1994; Wearsch and Nicchitta, 1996a). Especially if the cell encounters stressful conditions, grp94 tends to redistribute to the Golgi apparatus (Booth and Koch, 1989), becomes somewhat enriched in the nucleus (Welch *et al.*, 1983), and is partially secreted to the extracellular space (McCormick *et al.*, 1982; Takemoto *et al.*, 1992), or to the outer surface of the plasma membrane (Altmeyer *et al.*, 1996; see also Section 3.8 for further references). Interestingly, surface-expressed grp94 has been reported to exist in an N- and/or C-terminally truncated form as well (Poola and Lucas, 1988; Poola and Kiang, 1994), which may help it "escape" from the ER by losing the C-terminal KDEL ER retention signal. Based on our present knowledge about the localization of grp94, it seems to be a somewhat puzzling, but certainly an extremely versatile, marker of the "stress-status" of the ER and of the host cell and organism. Clearly, further studies are needed to establish the exact causes and mechanisms of grp94 redistribution between the various cell compartments. Easy mobility seems to be a general phenomenon for many proteins of the ER lumen; therefore, studies on grp94 redistribution will also significantly advance our understanding about the general function of the ER under stress.

[3] P. Csermely, unpublished observations.
[4] Cs. Sőti and P. Csermely, unpublished observations.

Like hsp90, grp94 associates with numerous other proteins, such as protein kinases (Cala and Jones, 1994; Csermely *et al.*, 1995a; Ramakrishnan *et al.*, 1997; Trujillo *et al.*, 1997), actin filaments, calmodulin (Koyasu *et al.*, 1986, 1989), and other molecular chaperones of the ER, such as grp78 (BiP) (Pouyssegur and Yamada, 1978; Melnick *et al.*, 1992), calreticulin, calnexin (Tatu and Helenius, 1997), the ERp72 protein disulfide isomerase, grp170 (Kuznetsov *et al.*, 1997), and the collagen-specific chaperone hsp47 (Ferreira *et al.*, 1994, 1996). The ratio of the various chaperones might change in the chaperone complex of different ER subcompartments, since calreticulin is confined mainly to the rough ER, while grp94 resides in the smooth ER (Peter *et al.*, 1992). The ER chaperone complex is not as well characterized as the cytoplasmic foldosome, but if one takes into account the extremely high protein concentration of the ER lumen (estimated to be around 100 mg/mL), it is reasonable to assume that grp94 might be part of an even more complex supramolecular organization than hsp90.

grp94 is a calcium-binding protein (Koch *et al.*, 1986; Kang and Welch, 1991; Cala and Jones, 1994) harboring 4 high-affinity (K_d, 2 μM) and approximately 10 low-affinity (K_d, 600 μM) calcium-binding sites (Van *et al.*, 1989; Hubbard and McHugh, 1996), and contains several EF-hand structures (Csermely *et al.*, 1995a), which may serve as some of the calcium-binding sites of the protein. Since the lumenal calcium concentration of the ER may reach 400 M (Miyawaki *et al.*, 1997), calcium may play an important role in the regulation of grp94 functions. In accordance with this assumption, calcium binding causes a conformational change of grp94 reflected by a decrease of its α-helix content from 40 to 34% (Van *et al.*, 1989).

Unlike hsp90 and many of the other ER chaperones, grp94 is a glycoprotein. Under normal conditions, it is N-glycosylated at Asn-196 (Qu *et al.*, 1994), where a core oligosaccharide, containing 8 mannose and 2 *N*-acetyl-glucosamine residues, is attached to the protein (Lewis *et al.*, 1985; Van *et al.*, 1989). Oligosaccharide side chains may also contain minor amounts of galactose and *N*-acetyl-galactosamine (Poola and Lucas, 1988). Interestingly, the O-glycosylation of grp94 has also been reported. The O-linked moiety most probably contains a neutral disaccharide and sialo tri- and tetrasaccharides (Poola and Lucas, 1988; Hayes *et al.*, 1994; Poola and Kiang, 1994). O-glycosylation is an important regulatory modification, which, in many cases, has a reciprocal relationship with phosphorylation (Hart, 1997) and thus, may play an important role in the regulation of grp94 function. O-linked *N*-acetyl-glucosamine transferase is a tetratricopeptide repeat (TPR)-containing protein in the cytoplasm and in the nucleus (Kreppel *et al.*, 1997; Lubas *et al.*, 1997). Taking into account the intimate association of the highly homologous hsp90 with numerous TPR-containing proteins (see Section 3.3), the O-glycosylation of grp94 may be related to its direct association with the respective transferase enzyme.

The glycosylation pattern of grp94 tends to change after cellular stress, reflected by an increased resistance to endoglycosidase H digestion (Booth and Koch, 1989), indicating processing of the glycosyl side-chains by *N*-acetylglucosaminyltransferase I, a typical Golgi enzyme. The appearance of endoglycosidase H resistance seems to depend strongly on the cell type and on the type of stress experienced (Kang and Welch, 1991). However, this change also occurs in several diseases, such as in cancer (Feldweg and Srivastava, 1995) or in diabetes (Csermely, 1994), making it likely that cells experience a general ER stress under these conditions, resulting in partial translocation of ER chaperones to the Golgi apparatus. Depending on the rate of grp94 synthesis, hyperglycosylation may also occur at secondary, C-terminal glycosylation site(s) of the protein (Qu *et al.*, 1994; Wearsch and Nicchitta, 1996b). The existence and structure of the attached oligosaccharide also depend on the availability of the respective sugars (Pouyssegur and Yamada, 1978; Lewis *et al.*, 1985; Wearsch and Nicchitta, 1996a), making the glycosyl side chains of grp94 a sensitive marker of aberrant cellular metabolism occurring, for example, in diabetes (see Section 5.3). This raises the possibility that the status of grp94 glycosylation may play an important role in the regulation of ER chaperone activity after stress.

Similarly to hsp90, grp94 is also an ATP-binding protein (Clairmont *et al.*, 1992; Li and Srivastava, 1993; Nigam *et al.*, 1994; Csermely *et al.*, 1995a) with a relatively low affinity for ATP or GTP. Binding of the nucleotides leads to autophosphorylation of grp94 (Dechert *et al.*, 1989; Csermely *et al.*, 1995a) or an ATPase activity (Li and Srivastava, 1993). Interestingly, Anderson *et al.* (1994) reported a stimulation of grp94-related ATPase and ADPase activity after interferon-α treatment of Daudi cells. The manifestation of ATPase activity, however (similarly to that of hsp90), may require additional proteins and is not observed in highly purified grp94 preparations (Csermely *et al.*, 1995a). This, together with the low affinity of ATP-binding, may explain why the detection of these features of grp94 is not always straightforward (Van *et al.*, 1989; Nigam *et al.*, 1994; Wearsch and Nicchitta, 1997; Ramakrishnan *et al.*, 1997; Trujillo *et al.*, 1997). As another similarity to hsp90, peptide binding to grp94 is also not dependent on the presence of nucleotides (Wearsch and Nicchitta, 1997). However, some observations suggest that the recognition of larger protein substrates may be influenced by ATP (Li and Srivastava, 1993; Melnick *et al.*, 1994). Nigam *et al.* (1994) observed the ATP-dependent release of grp94 from denatured protein affinity columns, but their experiments did not directly address the question as to whether individual grp94 molecules were released or whether grp94 was eluted as part of a larger chaperone complex containing grp78 (BiP), which is known to dissociate from its targets upon addition of ATP.

Predictive studies indicate that the N-terminal domain of grp94 may have a tertiary structure similar to that of hsp90 (see Fig. 1B) (Gerloff *et al.*, 1997). If so, it may also contain a nucleotide-binding site and a binding site for geldanamycin, which also affects the function of grp94 (Chavany *et al.*, 1996). However, the C-terminus of the protein seems to be

required for its autophosphorylation to occur (Csermely *et al.*, 1995a). This may indicate that the C-terminal domain also contributes to the binding of nucleotides or harbors its "own," independent, second nucleotide binding site.

The C-terminal domain of grp94 contains the segment responsible for dimer formation (Nemoto *et al.*, 1996; Wearsch and Nicchitta, 1996b) and a C-terminal KDEL sequence, which is the common retention signal for ER proteins (Sorger and Pelham, 1987). grp94 is able to form a heterodimer with hsp90 (Nemoto *et al.*, 1996), which shows that the dimerization properties are important, and evolutionarily conserved, features of the 90-kDa chaperone family.

3. POSSIBLE CELLULAR FUNCTIONS OF hsp90 AND grp94

Addressing the cellular functions of hsp90 and grp94 in eukaryotes, we first summarize the most important aspects of their key contributions to major cellular functions, and in Section 3.9, we present our own view about the importance of the various functions described.

3.1. *hsp90 as a Part of Chaperone Machines, Foldosomes in the Cytosol*

Our understanding of the chaperone properties of hsp90 followed the usual path, starting from relatively simple systems (purified hsp90 itself) to the more and more complex assemblies of chaperone complexes. Purified hsp90 suppresses the aggregation of unstable proteins, such as guanidinium.HCl-unfolded and partially renatured citrate synthase and rhodanese (Wiech *et al.*, 1992),[5] heat-denatured citrate synthase (Jakob *et al.*, 1995a), or protein kinase CK-II at low ionic strength (Miyata and Yahara, 1992, 1995). hsp90 is also able to disaggregate the loose aggregates of CK-II occurring after low-salt treatment. However, it does not promote disaggregation of severely denatured protein kinase CK-II aggregates (Miyata and Yahara, 1992, 1995). hsp90 also somewhat enhances the yield of refolding of denatured citrate synthase or antibody Fab fragments (Wiech *et al.*, 1992). Studies with heat-denatured luciferase (Yonehara *et al.*, 1996) or with guanidinium.HCl-denatured β-galactosidase (Freeman and Morimoto, 1996) indicated that hsp90 alone is unable to aid the refolding of these proteins. However, by binding to the partially renatured forms of these targets, hsp90 maintains the non-native substrate in a "folding-competent" state, which can be rescued and successfully refolded by the addition of other chaperones, such as the hsc70/hdj1-complex (Freeman and Morimoto, 1996) or reticulocyte lysate (Yonehara *et al.*, 1996). The above effects do not require the presence of nucleotides, which makes a clear distinction between the chaperone actions of hsp90 and those of hsp60 and hsc70/hsp70 (Jakob and Buchner, 1994; Buchner, 1996). The recent study of Young *et al.* (1997) demonstrated that hsp90 has two independent chaperone sites and, therefore, its function in the help of protein folding might be more complex than previously thought. The important feature that the N-terminal chaperone site can be inhibited by the hsp90-specific drug geldanamycin gives an excellent tool to elucidate the role of the two sites in the complex function of hsp90.

In agreement with its conformational changes, higher hydrophobicity and enhanced oligomerization at elevated temperatures (see Section 2.1), hsp90 displays a heat-induced chaperone activity above 46°C (Yonehara *et al.*, 1996). Divalent cations, such as Mg^{2+}, greatly suppress the chaperone activity of the protein (Jakob *et al.*, 1995b). Based on kinetic studies, Jakob *et al.* (1995a) proposed that hsp90 recognizes early unfolding intermediates, which have a defined secondary structure, but whose tertiary structure has not been completed yet. This assumption is supported by the fact that in contrast to the unstructured reduced carboxymethyl α-lactalbumin, the "stable molten globule" casein is able to compete with hsp90-bound dihydrofolate-reductase (Yonehara *et al.*, 1996) and binds to hsp90 with relatively high affinity (Csermely *et al.*, 1997). This binding preference places hsp90 "behind" hsc70 in a folding cascade, since hsc70 recognizes unfolded proteins with a less developed structure than hsp90 (Buchner, 1996; Johnson and Craig, 1997).

As noted above, in most cases, hsp90 alone is insufficient to help refolding of partially denatured proteins, and requires other chaperones to complete this task. Most of our initial understanding about the hsp90-associated chaperone system came from the analysis of the inactive steroid receptor and oncogenic protein kinase complexes (Smith and Toft, 1993; Pratt, 1997; Pratt and Toft, 1997; Johnson and Craig, 1997). It turned out that besides hsp90, at least nine other proteins participate in the complete folding process. hsc70 may initiate the process by binding of the unstructured target protein together with its co-chaperone Hip, helped by a homologue of the prokaryotic dnaJ protein (Fig. 2). The next step is most probably the binding of Hop (formerly called p60), which links hsc70 with hsp90. Together with hsp90, either of the three immunophilins, the rapamycin-binding FKBP52 (formerly called hsp56), FKBP51, or the Cyclosporin A-binding immunophilin (Cyp)40, and p23 is added to the complex. Parallel with this, hsc70, Hip, and Hop dissociate from the mature complex. The smallest component, p23, plays an important role in retarding the dissociation of the foldosome from its target, thus allowing the completion of folding (Dittmar *et al.*, 1997; Pratt and Toft, 1997). Finally, the target is released, which leads to activation of the respective protein (for more details, see Sections 3.2.1 and 3.2.2). In the case of oncogene protein kinases, the details of the process are not as clear as the maturation steps of the steroid receptors outlined above. Kinase targets are recognized (and perhaps targeted to the plasma membrane) by a specific component of the "kinase-foldosome" CDC37 (formerly called p50) (Stepanova *et al.*, 1996). The situation is made even more complex by the fact that besides the "classical" chaperones hsc70 and

[5]T. Schnaider, Cs. Sőti, and P. Csermely, unpublished observations.

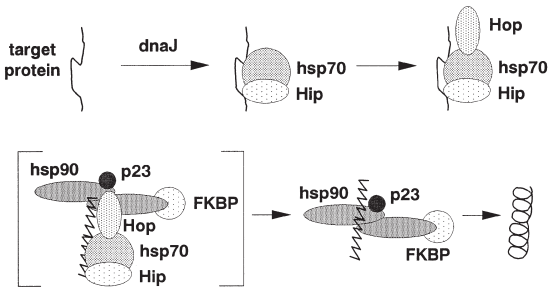

FIGURE 2. The hsp90-related folding pathway. The folding complexes have been best elucidated in the folding process of the steroid receptors. With protein kinases, the details of the folding steps are not as clear, but they are most probably very similar to the ones detailed here. Partially unfolded kinases are recognized by a specific component of the "kinase-foldosome" CDC37 (formerly called p50), which, for better clarity, is not indicated in this figure (for more details see text). dnaJ, a eukaryotic homologue of the prokaryotic dnaJ protein.

hsp90, almost all the other components have chaperone activities in *in vitro* assays when added alone (Duina *et al.*, 1996; Bose *et al.*, 1996; Freeman *et al.*, 1996; Kimura *et al.*, 1997).

Renaturation studies of heat-denatured firefly luciferase in reticulocyte lysate also indicated the cooperation of hsc70 and hsp90 chaperone complexes in the process (Nimmesgern and Hartl, 1993; Schumacher *et al.*, 1996; Thulasiraman and Matts, 1996). In this complex system, the dissection of the role of hsp90 is greatly aided by the specific hsp90-binding drug geldanamycin (Whitesell *et al.*, 1994; Stebbins *et al.*, 1997) or by the structurally related herbimycin A. Addition of these ansamycin antibiotics inhibited the release of luciferase from hsp90 complexes, both in reticulocyte lysates (Thulasiraman and Matts, 1996; Schneider *et al.*, 1996) and in *in vivo* whole cell studies, and resulted in an increased degradation of the incompletely folded target protein (Schneider *et al.*, 1996). Thus, the hsp90-mediated folding cascade seems to be connected with the proteolytic apparatus (most probably with the proteasome; see Section 3.7).

3.2. *Role of hsp90 in Signalling*

The *in vivo* chaperone activities of hsp90 hitherto reported (see previous section) are almost exclusively related to the folding of various nuclear hormone receptors and a number of protein kinases, all of which are involved in signalling. In the following section, we summarize our present knowledge of the involvement of hsp90 in these signal transduction processes.

3.2.1. hsp90 in the steroid response. hsp90 is necessary for proper steroid action *in vivo* (Picard *et al.*, 1990; Bohen and Yamamoto, 1993; Nathan and Lindquist, 1995). As described in the preceding section, folding of steroid receptors occurs via a sequential process, where hsp90 plays a crucial role as a central organizer of the "early" (hsc70- and Hop-containing) and "late" (p23-containing) chaperone complexes, which aid the maturation of the receptors (see Fig. 2 and Section 3.1 for further details). hsp90 binds to the hormone-binding domain of steroid receptors (Pratt, 1997; Pratt and Toft, 1997). Such binding is conceived as a trap for the hormone-binding domain, keeping it in a partially unfolded state, which is for the glucocorticoid receptor the only state where the steroid can bind with high affinity. Presence of the "early" (hsp90.Hop.hsc70) chaperone complex is enough to achieve this hormone-binding state (Dittmar and Pratt, 1997). If hsp90 dissociates in the absence of the hormone, the glucocorticoid receptor hormone binding domain collapses and loses its steroid binding ability (Bresnick *et al.*, 1989; Picard *et al.*, 1990). The progesterone receptor behaves similarly, while the androgen receptor requires hsp90 only for the development of high-affinity ligand binding (Fang *et al.*, 1996). The estrogen receptor does not seem to depend on hsp90 to assume a steroid-binding conformation.

Binding of the steroid destabilizes the steroid receptor-hsp90 complex and leads to dissociation (or only low affinity, transient binding) of hsp90. Upon dissociation of hsp90, the receptor is able to bind to DNA and (in case of the glucocorticoid and mineralocorticoid receptors) its nuclear translocation is also facilitated (Smith and Toft, 1993; Pratt, 1997). Dissociation of hsp90 most probably enhances nuclear translocation via an increased accessibility of the NLS of the receptor. Experiments by Kang *et al.* (1994), where co-expression of an hsp90-NLS fusion product with an NLS-deleted glucocorticoid or progesterone receptor targeted the cytoplasmic receptors to the cell nucleus with a "piggyback" mechanism, indicated that hsp90 may be at least transiently bound to the steroid receptor until it reaches the nucleus. Steroid receptors constantly shuttle back and forth between the cytoplasm and the cell nucleus (DeFranco *et al.*, 1995; Csermely *et al.*, 1995b). This shuttle can be disrupted by both geldanamycin and molybdate, agents more or less specific to hsp90 action. Geldanamycin prevents the receptors from entering the nucleus (Czar *et al.*, 1997), while molybdate facilitates the export of glucocorticoid receptors from the nucleus and may trap the receptors in the cytoplasm (Yang and DeFranco, 1996; Yang *et al.*, 1997). The immunophilin FKBP52 most probably also participates in directing steroid receptors to the nucleus (Gasc *et al.*, 1990; Czar *et al.*, 1994, 1995).

Besides the steroid receptors, hsp90 is also necessary for the maturation of the aryl-hydrocarbon (dioxin) receptor (Carver *et al.*, 1994; Whitelaw *et al.*, 1995), which behaves like the glucocorticoid receptors in that its contact with hsp90 is necessary for development of its high-affinity ligand binding, and that the dissociation of hsp90 is a prerequisite for the DNA binding of the receptor (Wilhelmsson *et al.*, 1990; Pongratz *et al.*, 1992; Coumailleau *et al.*, 1995b). The steps of aryl-hydrocarbon receptor maturation may be similar to those of steroid receptors described for the general folding mechanism of the hsp90-chaperone system in the preceding section (Antonsson *et al.*, 1995; Nair *et al.*, 1996). In contrast to the zinc-finger DNA-binding domains of steroid receptors, which do not bind to hsp90 directly,

the helix-loop-helix DNA-binding domain of the aryl-hydrocarbon receptor can form a stable complex with hsp90 (Antonsson *et al.*, 1995). Binding of the helix-loop-helix domain to hsp90 occurs in addition to complex formation of the ligand-binding domain of the receptor with hsp90, which is a common feature of all steroid receptors (Whitelaw *et al.*, 1993; Coumailleau *et al.*, 1995b). A recent study of Blankenship and Matsumura (1997) described the association of the c-Src kinase with the aryl-hydrocarbon receptor/hsp90-complex.

Holley and Yamamoto (1995) reported that a 20-fold reduction of hsp90 level severely compromises the activation of retinoid receptors and impairs the development of high-affinity retinoic acid binding, suggesting that involvement of hsp90 in maturation/signalling may be a general phenomenon for all nuclear hormone receptors, involving at least a transient interaction between the receptor and hsp90.

3.2.2. hsp90 and protein kinases. The first hsp90-kinase complex (with the v-Src tyrosine kinase) was identified more than 15 years ago (Brugge *et al.*, 1981; Oppermann *et al.*, 1981). Since then, numerous other tyrosine and serine/threonine protein kinases have been reported to form stable complexes with hsp90 (summarized in Table 3). Genetic evidence extends the list of kinases in Table 3 even further by demonstrating that hsp90 is necessary for the activity of the Sevenless and Torso kinases in *Drosophila* (Cutforth and Rubin, 1994; Doyle and Bishop, 1993).

hsp90 is necessary for the correct folding, and thus, for the activity of many of these kinases, such as the v-Src kinase (Xu and Lindquist, 1993; Nathan and Lindquist, 1995), the Raf kinase (van der Straten *et al.*, 1997), and the eIF-2-α kinase (Uma *et al.*, 1997). There is good reason to suppose that the hsp90-related chaperone pathway (see Section 3.1 for details) mediates the folding of many (if not all) of the kinases forming a stable complex with hsp90.

Kinases such as v-Src or Raf bind to hsp90 via their catalytic domain (Jove *et al.*, 1986; Stancato *et al.*, 1993). When bound to hsp90, v-Src is hypophosphorylated and lacks protein kinase activity. Concomitant with their dissociation, both hsp90 and v-Src become multiply phosphorylated, v-Src gains kinase activity and associates with membrane fractions (Mimnaugh *et al.*, 1995; Hunter and Poon, 1997). Raf kinase also requires hsp90 for its membrane association (Schulte *et al.*, 1995), and seems to retain hsp90 in its membrane-bound active complex (Wartmann and Davis, 1994). hsp90 protects the kinase from phosphatase-mediated inactivation (Dent *et al.*, 1995). Both Src- and Raf-hsp90 complexes can also be prematurely dissociated by the hsp90-specific drugs geldanamycin and radicicol. This type of dissociation often leads to increased degradation of the respective kinase, most probably via the proteasome (Whitesell *et al.*, 1994; Schulte *et al.*, 1995, 1996, 1997; Stancato *et al.*, 1997; Pratt, 1997; Soga *et al.*, 1998).

Besides hsp90, a "kinase-specific" 50-kDa protein is almost always found in these complexes (Hunter and Poon, 1997). Its binding is completed by the same Hop (p60) protein involved in the formation of steroid receptor-folding chaperone complexes (Owens-Grillo *et al.*, 1996). Recent studies demonstrated that the 50-kDa protein p50 (at least in the cases examined so far) is identical with CDC37 (Hunter and Poon, 1997). CDC37/p50 is a chaperone (Kimura *et al.*, 1997) that probably is involved in directing the immature kinase complexes to their final destination, in most cases, to the plasma membrane (Owens-Grillo *et al.*, 1996; Pratt, 1997).

Interestingly, not only hsp90, but its homologue in the ER grp94, also seems to form complexes with kinases. A recent report showed that p185-erbB2 (also known as her-2/neu, a receptor-like tyrosine kinase overexpressed in many breast, ovarian, and prostate carcinomas and associated with poor prognosis) could be depleted from SKBr3 human breast carcinoma cells by geldanamycin. Geldanamycin binds to a 100-kDa protein, shown to be grp94, forming a stable complex with p185-erbB2 (Chavany *et al.*, 1996). After geldanamycin treatment, the grp94/p185-erbB2 complex dissociates and the kinase is degraded by the proteasome (Mimnaugh *et al.*, 1996). grp94 is also known to be

TABLE 3. Protein Kinases That Form a Complex with hsp90 and with Its "Kinase-Targeting Co-Chaperone" CDC37/p50[1]

Protein kinase	Reference
Tyrosine kinases	
v-Src, c-Src[2]	Brugge *et al.*, 1981; Oppermann *et al.*, 1981; Hutchison *et al.*, 1992; Blankenship and Matsumura, 1997
v-Fes, c-Fes, v-Fgr, v-Fps, v-Ros, v-Yes	Adkins *et al.*, 1982; Lipsich *et al.*, 1982; Ziemiecki, 1986; Ziemiecki *et al.*, 1986; Nair *et al.*, 1996
Lck, c-Fgr	Hartson and Matts, 1994; Hartson *et al.*, 1996
p75-v-erbA	Privalsky, 1991
p185erbB2[3]	Chavany *et al.*, 1996
Wee1	Aligue *et al.*, 1994
Insulin receptor	Takata *et al.*, 1997
Serine-threonine kinases	
v-Raf, c-Raf, B-Raf	Stancato *et al.*, 1993; Wartmann and Davis, 1994; Jaiswal *et al.*, 1996
Gag-Mil	Lovric *et al.*, 1994
MEK	Stancato *et al.*, 1997
CDK4	Stepanova *et al.*, 1996; Dai *et al.*, 1996
eIF-2-α kinase	Rose *et al.*, 1987; Matts and Hurst, 1989
eEF-2-α kinase	Nygard *et al.*, 1991; Palmquist *et al.*, 1994
Protein kinase CK-II	Dougherty *et al.*, 1987; Miyata and Yahara, 1992, 1995; Shi *et al.*, 1994

[1] The identity of CDC37 with the 50-kDa protein (p50) of the hsp90-kinase complexes has been directly established only in a few cases, and the participation of p50 in the complexes itself has to be demonstrated in the case of Lck, c-Fgr, Wee1, Gag-Mil, eIF, eEF, and CK-II kinases.

[2] Association of c-Src and hsp90 has not been demonstrated yet, only as part of the hsp90/aryl hydrocarbon receptor complex (Blankenship and Matsumura, 1997), probably because of the extremely low levels of the kinase. The reconstruction of the c-Src/hsp90 complex in reticulocyte lysate was also successful (Hutchison et al., 1992; Hartson and Matts, 1994).

[3] Forms a complex with grp94, the hsp90 homologue in the ER.

associated with protein kinase CK-II (Cala and Jones, 1994; Csermely *et al.*, 1995a; Ramakrishnan *et al.*, 1997; Trujillo *et al.*, 1997).

3.2.3. Other links to signalling components. Besides protein kinases, phosphoprotein phosphatases, such as the tetratricopeptide domain-containing immunophilin PP-5, are also part of hsp90 complexes. Similarly to the kinases, PP-5 seems to be rather inactivated when bound to hsp90 (Chen, M. S. *et al.*, 1996; Silverstein *et al.*, 1997).

A recent review presented an interesting hypothesis about the possible involvement of hsp90 in the folding, and subsequent association, of the β- and γ-subunits of signal-transducing G-proteins (Pratt, 1997), based on the observations of Inanobe *et al.* (1994), who found that hsp90 binds to the β/γ-subunits of G-proteins. As another possible link to receptor signalling, a novel eukaryotic homologue of hsp90, hsp75/TRAP-1, has been identified as a binding protein of the cytoplasmic domain of the Type 1 tumor necrosis factor receptor at a site that may link it to the activation of nuclear factor-κB (Song *et al.*, 1995).

Besides nuclear hormone receptors, hsp90 associates with and modulates the effects of a number of other transcription factors (see Section 3.4).

3.3. *hsp90 Oligomers, Cytoskeleton, and the Microtrabecular Lattice*

We have already briefly referred to both the oligomerization tendency of hsp90 and its binding to microfilamentous and microtubular structures (Section 2.1). However, to stress the importance of these properties of hsp90, a chaperone constituting 1–2% of the total cytoplasmic proteins, we now extend our earlier description.

hsp90 dimers tend to associate into tetra-, hexa-, octamers, and into even higher oligomers. Oligomerization usually affects only a few percent of the total protein, but addition of divalent cations, certain nucleotides, heat treatment, or the presence of nonionic detergents enhances oligomer formation (Lanks, 1989; Minami *et al.*, 1991, 1993; Jakob *et al.*, 1995b; Nemoto *et al.*, 1996; Freitag *et al.*, 1997).[6] It is important to note that oligomerization studies were usually performed under "normal," *in vitro* experimental conditions, using a few micrograms/milliliter of purified hsp90. The *in vivo* concentration of hsp90 is estimated to be around 1–5 mg/mL (Scheibel *et al.*, 1997). This may significantly enhance the *in vivo* oligomerization tendencies of the protein. Oligomer formation of hsp90 might be further promoted by the large excluded volume effect of the "molecularly crowded" cytoplasm (Zimmerman and Minton, 1993).

hsp90 crosslinks filamentous actin *in vitro* (Koyasu *et al.*, 1986; Nishida *et al.*, 1986; Kellermayer and Csermely, 1995). Analyzing the *in vivo* co-localization of actin filaments and hsp90, Akner *et al.* (1992) and Fostinis *et al.* (1992) could not demonstrate the existence of stable hsp90-actin complexes in human fibroblasts and in human endometrial adenocarcinoma cells, respectively. The lack of *in vivo* stable hsp90-actin association in these cells might be explained by the findings of Kellermayer and Csermely (1995), who observed that millimolar ATP concentrations induce the dissociation of hsp90 from actin filaments. Since under normal conditions the intracellular ATP concentration is in this range (Scheibel *et al.* [1997] calculated that 70% of hsp90 is saturated with ATP under similar circumstances), it is likely that *in vivo* hsp90 forms a stable complex with actin filaments only after severe stress, when cellular ATP levels drop significantly.

hsp90 also binds to tubulin (Sanchez *et al.*, 1988; Redmond *et al.*, 1989; Fostinis *et al.*, 1992; Czar *et al.*, 1996) and seems to be involved in the protection of microtubules after heat shock (Williams and Nelsen, 1997). Several laboratories (Fostinis *et al.*, 1992; Czar *et al.*, 1996) have also described co-localization of hsp90 with non-microtubular and non-microfilamental structures of the cytoplasm, sometimes resembling intermediate filaments.

Data about the involvement of microtubules and microfilaments in the trafficking of the steroid receptor-hsp90 complexes from the cytoplasm to the nucleus are rather contradictory. Miyata and Yahara (1991) reported that *in vitro*, the glucocorticoid receptor binds to actin filaments via hsp90. Akner *et al.* (1990) found that the steroid receptor-hsp90 complex co-localizes with the microtubular, but not with the microfilamental, network. However, other authors found that nuclear translocation cannot be inhibited by disruption of the cytoskeleton using nocodazole or the combination of colcemid and cytochalasin (Perrot-Applanat *et al.*, 1992).

The above contradictory findings may be rationalized by assuming that hsp90 binds to many cytoplasmic filamentous structures simultaneously (this would explain, if one of these is disrupted, how the respective transport processes can utilize the remaining elements) and that hsp90 binds to all these structures with a relatively low affinity. This low-affinity binding, and the presumably highly dynamic equilibrium between the bound and free forms of hsp90 complexes, may explain the difficulties in finding a stable co-localization between hsp90 and the filamentous structures, and may also be a prerequisite for the translocation of the hsp90 complexes along these structures.

The above model describes hsp90, and the (thousand-and-one) hsp90-associated proteins, as a highly dynamic "appendix" of various, and often quite poorly identifiable, cytoplasmic filamentous structures reminiscent of the early view (Wolosewick and Porter, 1979; Schliwa *et al.*, 1981) about the microtrabecular network of the cytoplasm. Although a rather energetic debate has developed about the validity of the electron microscopic evidence of the microtrabeculae, several independent findings support the existence of a cytoplasmic mesh-like structure (Clegg, 1984; Jacobson and Wojcieszyn, 1984; Luby-Phelps *et al.*, 1988; Penman and Penman, 1997). The major cytoplasmic chap-

[6] Cs. Sőti and P. Csermely, unpublished observations.

erones (TCP1/hsp60 and hsp90 and their associated proteins) may well form a part of this network in cells. This hypothesis was recently further supported by the discovery of Trent and co-workers (1997) that TCP1/hsp60 forms extensive filaments in the archaebacterium *Sulfolobus shibatae*, and may constitute a kind of cytoskeleton in this organism. While there is very little chance for a similarity between this "archaic" structure and the organization of eukaryotic cells, recent observations demonstrated that archaebacteria, in fact, are closer relatives of eukaryotes than the whole prokaryotic kingdom (Olsen and Woese, 1997), which somewhat increases the likelihood that the highly conserved chaperones may have a similar role in the organization of the two organisms.

What can be the functional importance of the above dynamic interactions between hsp90 and the cytoskeleton? Besides its putative role in the organization of the cytoplasm, hsp90 most probably protects the filamentous structures after stress. Environmental stress often leads to ATP-depletion of the stressed cells, which is highly detrimental to these structures (Kabakov and Gabai, 1997). By stress-induced association to existing filaments and/or by formation of partially novel filamentous structures, hsp90 may significantly contribute to preservation of the structural integrity of the cell after stress.

Besides the putative role of hsp90 in building and maintaining the cytoarchitecture, several observations suggest that hsp90 and the hsp90-related chaperone complex is not a static, purely structural, participant/attachment of various cytoplasmic filaments, but might also play a role in the cytoplasmic traffic along these trajectories. This hsp90-mediated transport hypothesis has been best developed by Pratt (Pratt, 1992, 1997; Pratt *et al.*, 1993; Owens-Grillo *et al.*, 1996). Interestingly, hsp90 displays a significant homology with the movement proteins of several plant viruses (Koonin *et al.*, 1991), which may indicate a shared mechanism in the promotion of particle migration.

What is the mechanism that helps the putative "hsp90-based translocator" to decide where to go? hsp90 binds to various proteins containing a TPR domain. The (most probably incomplete) list of these proteins includes the hsp90-hsp70 connecting protein Hop (p60) and the hsp90-binding immunophilins (FKBP52, Cyp-40, PP-5). FKBP52 has been suggested to participate in directing steroid receptor holo-complexes to the cell nucleus (Gasc *et al.*, 1990; Czar *et al.*, 1994, 1995); its dissociation from hsp90 is promoted by its phosphorylation of protein kinase CK-II, a predominantly nuclear protein kinase (Miyata *et al.*, 1997). The CDC37 protein, which has a binding site on hsp90 adjacent to the TPR-binding portion of the protein, is probably involved in directing many hsp90-associated protein kinases to the plasma membrane. Finally, association of the TPR-containing mitochondrial import receptor with hsp90 has also been demonstrated. Binding of these "directing" components is mutually exclusive, meaning that hsp90 can form a complex with only one of them (Ratajczak and Carrello, 1996; Owens-Grillo *et al.*, 1996; Pratt, 1997). These findings raise the possibility that the above proteins play a decisive role in directing hsp90 and its specific targets along intracellular trajectories.

3.4. *A Possible Role for hsp90 in the Cell Nucleus*

3.4.1. Nuclear transport. The end of the previous section summarized our present knowledge about the possible directing of hsp90-related protein complexes along the cytoplasmic filamentous structures by various proteins binding to hsp90 via their TPR domain. However, almost all the initially identified members of the TPR-containing protein family participate in mitosis, transcription, splicing, and protein import, each a predominant function of the cell nucleus (Goebl and Yanagida, 1991). Although a direct interaction of these "original" TPR-proteins with hsp90 has not been demonstrated yet, they are likely to participate in the various nuclear functions of hsp90 (Csermely *et al.*, 1998) summarized in the present section.

About 5–10% of cellular hsp90 is known to be localized to the cell nucleus. An additional fraction of hsp90 translocates to the nucleus after a single or repeated heat shock (Arrigo *et al.*, 1980; Collier and Schlessinger, 1986; van Bergen en Henegouwen *et al.*, 1987; Berbers *et al.*, 1988; Wilhelmsson *et al.*, 1990; Gasc *et al.*, 1990; Akner *et al.*, 1992; Morcillo *et al.*, 1993; Biggiogera *et al.*, 1996). At first sight, a few percent of a protein may seem negligible; however, hsp90 is one of the most abundant proteins in most cells, so that even a small proportion may be significant. Although the intranuclear localization of hsp90 may vary under different conditions, its association with the nucleoli (van Bergen en Henegouwen *et al.*, 1987; Pekki, 1991) and with the perichromatin ribonucleoprotein fibrils (Carbajal *et al.*, 1990; Vazquez-Nin *et al.*, 1992) has also been reported.

Nuclear transport of hsp90 may be mediated by other components of the hsp90 complex, such as FKBP52, steroid receptors, or certain protein kinases. However, a bipartite nuclear localization sequence is located in the middle, highly charged region of hsp90 (Nardai *et al.*, 1996; Fig. 1A; see Section 2.1). The nuclear localization sequence is preceded by a poly-Glu tract shown to facilitate the nuclear translocation of nucleoplasmin (Vancurova *et al.*, 1997). These signals are most probably hidden in the interior of the hsp90 dimer, but their exposure in some deletion mutants shifts these truncated hsp90s to the nucleus (Meng *et al.*, 1996). Furthermore, hsp90 harbors numerous sequences similar to other known "traditional" or "alternative" nuclear import and export signals (Table 4). This may explain and further substantiates the assumption that like the steroid receptors and hsp70, hsp90 is also constantly shuttling back and forth between the cell nucleus and the cytoplasm (Yang *et al.*, 1997).

hsp90 and the hsp90-related chaperone complex most likely participate in the transport of a subset of proteins, characterized by certain nuclear hormone receptors and protein kinases, to the cell nucleus. In accordance with this, hsp90 has been suggested, and shown, to bind NLS sequences (Chambraud *et al.*, 1990; Schlatter *et al.*, 1992;

TABLE 4. Similarities to Nuclear Import and Export Signals in the Primary Structure of hsp90

Nuclear import/export signals[1]	Sequence position[2]	Reference
Nuclear import signals		
"Traditional" NLS		
KKxxxxxKKKxK	Consensus sequence	Dingwall and Laskey, 1991
KKdgd-kKKKKK	hsp90 268–278	Nardai *et al.*, 1996
"Alternative" NLS-candidate		
ENKR--LxRR	hsp70 NLS sequence	Lamian *et al.* 1996
ENRKkknniK	hsp90 352–361	Present review
Nuclear export signal		
OOxxxOOxxxLxLx[3]	An emerging consensus sequence	Fischer *et al.*, 1996; Kim *et al.*, 1996; Fritz and Green, 1996; Iovine and Wente, 1997; Nigg, 1997
nTf--YSnkeIfLr	hsp90 34–45	Present review
FYe-qFSk-nIkLg	hsp90 436–445	Present review
LVi-lLYetaL-Ls	hsp90 661–672	Present review

[1]In the consensus sequences "x" denotes any amino acid; underlined amino acids show identical or highly similar sequences; hyphens correspond to gaps introduced for better alignment.

[2]The position of the homologous sequences is given using the sequence of human hsp90-α.

[3]In the two "OO"-diads, the first "O" denotes any hydrophobic amino acid of L,I,F,W; the second "O" corresponds to any of the more hydrophilic amino acids of S,T,V,P,Q,G,Y,N. The number of bridging amino acids ("x") may be less than indicated.

Miyata and Yahara, 1995; Csermely *et al.*, 1995b).[7] The hsp90-related protein complex may also play a role in the calcium-, calmodulin-, and ATP-dependent nuclear protein import system described by Sweitzer and Hanover (1996), which probably becomes quite significant during calcium-dependent signalling events and under stressful conditions. On its return to the cytoplasm, hsp90 may also accelerate protein and/or RNA export processes from the cell nucleus. As a proposed nuclear chaperone (Csermely *et al.*, 1995b, 1998), hsp90 may also modulate the structure of DNA, RNA, and DNA/RNA-protein complexes. We now summarize our current knowledge about these putative activities of hsp90.

3.4.2. DNA binding and its possible consequences. hsp90 is able to bind both DNA and RNA with relatively low affinity (Szántó *et al.*, 1996).[8] Interestingly, a 60 amino acid stretch around the LKVIRK epitope of hsp90 displays significant homology with the single-stranded DNA/RNA binding region of several plant viruses (Koonin *et al.*, 1991). The ability of hsp90 to bind to RNA sequences makes it possible that it participates in the assembly of various viral reverse transcriptase/RNA complexes (Hu and Seeger, 1996; Hu *et al.*, 1997; see Section 5.2), both as a chaperone of the protein and of the respective RNA species. As described in Section 3.4.1, hsp90 has been reported to associate with nucleoli and with perichromatin ribonucleoprotein fibrils. After heat shock, hsp90 is localized in chromatoid bodies of mouse male germ cells (Biggiogera *et al.*, 1996). Thus, hsp90 can be found in nuclear structures that are actively involved in RNA synthesis and processing.

The ATP/ADP-binding N-terminal domain of hsp90 shows a significant homology with DNA topoisomerases and DNA gyrases (Gerloff *et al.*, 1997; Bergerat *et al.*, 1997). As a possible consequence, highly purified hsp90 preparations show a topoisomerase/nuclease-like activity (Szántó *et al.*, 1996). hsp90 associates with specific heat-shock puffs (hsr omega) in polytene chromosomes of *Drosophila melanogaster*, *D. hydei*, *Chironomus thummi*, and *Chironomus tentans* (Morcillo *et al.*, 1993), pointing to its participation in DNA rearrangements after heat shock, and also in embryonic development. The functional interaction of hsp90 and hsr omega is also supported by genetic studies (Lakhotia and Ray, 1996).

3.4.3. Modulation of DNA-protein interactions. hsp90 avidly binds histone molecules (Csermely *et al.*, 1994, 1997). In the presence of hsp90, both histones H1 and nucleosomal core histones display a tighter, salt-resistant, binding to DNA (Csermely *et al.*, 1994). Comparison of hsp90 primary structure with the polyglutamic acid sequence of nucleoplasmin, which plays an important role in the assembly of nucleosomal structure (Dingwall *et al.*, 1987), reveals its similarity to a highly charged region in the hinge region of hsp90 (Fig. 1A) (Nardai *et al.*, 1996). In agreement with this homologous sequence, circular dichroism measurements of DNA and added histones indicated that hsp90 may have a nucleoplasmin-like activity by promoting the assembly of histones and DNA at physiological salt concentrations (Csermely *et al.*, 1994). Some of these effects may also be caused by the relatively minor amount of hsp90 present or translocated to the cell nucleus. However, a much better chance for hsp90-histone or -DNA interactions occurs in the mitotic process where the nuclear barrier for the bulk of hsp90 is abrogated. In agreement with this, hsp90-α of *Saccharomyces cerevisiae* has been identified as an early meiotic gene induced by the IME1-IME2 transcriptional cascade (Szent-Gyorgyi, 1995). The cell cycle-related changes of hsp90 are summarized in Section 3.5. Another specific oc-

[7]Cs. Sőti and P. Csermely, unpublished observations.

[8]E. Nagy, T. Schnaider, and P. Csermely, unpublished observations.

casion when a major rearrangement of the nuclear structure occurs is in oogenesis, embryogenesis, and the differentiation of various cells. In accordance with an increased demand for nuclear chaperone action, nuclear translocation of hsp90-β has been observed in amphibian embryogenesis (Coumailleau *et al.*, 1997). The involvement of hsp90 in cell differentiation and development is further detailed in Sections 3.5 and 4.5.

Segments of the middle, highly charged, domain of hsp90 strongly resemble DNA (Binart *et al.*, 1989). Thus, it is not surprising that besides histones, hsp90 interacts with other DNA-binding proteins, such as transcription factors. A summary of presently known hsp90-binding transcription factors is listed in Table 5. Although in most cases the formation of the hsp90-transcription factor complex may reflect an hsp90-mediated maturation step of the respective transcription factor, and thus, may occur mostly in the cytoplasm, there are some observations that suggest a role for hsp90 in the modulation of the nuclear functions of the transcription factors as well. If hsp90 forms only a low-affinity transient complex with the respective transcription factor, it usually enhances DNA binding. hsp90 promotes DNA binding of several helix-loop-helix transcription factors, such as MyoD1 or E12 (Shaknovich *et al.*, 1992; Shue and Kohtz, 1994) via this mechanism. The "helix-loop-helix folding site" resides in a 48-residue region close to the hsp90 C-terminus (Shue and Kohtz, 1994). By contrast, if hsp90 forms a stable complex with the transcription factor, it decreases or prevents DNA binding. Thus, a stable complex with hsp90 in the absence of the respective hormone prevents DNA binding of most nuclear hormone receptors (Pratt, 1997). An altered dominance of the concomitant effects of hsp90-induced folding and modification of DNA binding may lead to seemingly opposite results, such as the hsp90-mediated enhancement (Inano *et al.*, 1994) and inhibition (Sabbah *et al.*, 1996) of estrogen receptor binding to the estrogen-response DNA element. As yet another mode of hsp90 action on DNA-protein complexes, hsp90 competes with DNA in binding to protein kinase CK-II in *in vitro* experiments (Miyata and Yahara, 1995). This is promoted by the highly charged middle region of hsp90 resembling the DNA structure (Binart *et al.*, 1989).

Similarly to hsp90, a small fragment of grp94 is known to be translocated to the cell nucleus after heat shock (Welch *et al.*, 1983). The recently described immunologically different hsp90 homologue is a predominantly nuclear protein (Cho *et al.*, 1997), and a significant portion of the novel member of the hsp90 family, hsp76, also becomes nuclear after heat shock (Chen, C. F. *et al.*, 1996). However, at present, there is practically no information about the possible role of these hsp90 homologues in the above nuclear functions.

TABLE 5. Transcription Factors Forming a Complex with hsp90

Transcription factor	Reference
Zinc finger proteins	
Steroid receptors	Pratt, 1997
v-erbA	Privalsky, 1991
Helix-loop-helix proteins	
Dioxin receptor	Perdew, 1988; Denis et al., 1988
Single-minded homologues	McGuire et al., 1995; Probst et al., 1997
MyoD1	Shaknovich et al., 1992
E12	Shue and Kohtz, 1994
Hypoxia-inducible factor 1 α	Gradin et al., 1996
Heat-shock factor 1	Nadeau et al., 1993; Nair et al., 1996
Specific DNA-binding sequences	
Mutant p53 tumor suppressor	Selkirk et al., 1994; Sepehrnia et al., 1996; Blagosklonny et al., 1996

3.5. *hsp90 and grp94 in the Cell Cycle, in Cell Differentiation, and in Apoptosis*

As already described in Section 3.2.2, hsp90 (together with its kinase-specific co-chaperone CDC37/p50) is necessary for the folding of several cell cycle-related protein kinases, such as the cyclin-dependent kinase CDK4 and the cyclin-dependent kinase regulator Wee1 (Aligue *et al.*, 1994; Stepanova *et al.*, 1996; Hunter and Poon, 1997). The expression pattern of several hsp90 isoforms is cell cycle-dependent, which further substantiates their role in regulation. hsp90-α mRNA is induced at the G1/S transition of chicken hepatoma cells (Jerome *et al.*, 1993), and hsp90-α has also been identified as an early meiotic gene induced by the IME1-IME2 transcriptional cascade in yeast (Szent-Gyorgyi, 1995). The novel hsp90 homologue hsp75/TRAP-1 associates with the retinoblastoma protein during meiosis and after stress, most probably aiding the refolding of the retinoblastoma product after dephosphorylation and stress-induced denaturation, respectively (Chen, C. F. *et al.*, 1996). Interestingly, the ATP concentration is reported to rise from 2 to 4 mM during mitosis of fibroblasts (Marcussen and Larsen, 1996). Cell cycle-dependent fluctuations in ATP concentration may affect some functions of hsp90, a low-affinity ATP-binding protein (see Section 2.1).

Several observations describe changes in hsp90 or grp94 expression during cell differentiation. Differentiation of embryonal carcinoma cells is paralleled by the induction of hsp90-β (Kohda *et al.*, 1991) and a nonidentified isoform of hsp90 (Maruyama *et al.*, 1996). Osteoblast and HL-60 cell differentiation results in a reduced level of hsp90-α and (with a delay) also in a reduced hsp90-β level (Shakoori *et al.*, 1992). The expression of hsp90-α increases during the proliferative phase of the myometrium (Komatsu *et al.*, 1997). Finally, grp94 expression is down-regulated in quiescent keratinocytes (Honore *et al.*, 1994). In brief, the regulation of 90-kDa chaperone expression varies from cell to cell, but two major trends may be fairly general: (1) expression of 90-kDa chaperones is usually lowered when the cells leave vigorous proliferation; (2) this may be particularly true for hsp90-α, which also undergoes the most profound changes among the 90-kDa chaperones during the cell cycle.

The involvement of hsp90 in the diversion of the normal cell cycle towards apoptosis most probably depends on the type of apoptotic signal. Reduced hsp90 levels correlate with a protection against tumor necrosis factor-α-induced apoptosis of U937 cells (Galea-Lauri *et al.*, 1996). This may be related to the possible involvement of the hsp90 homologue hsp75/TRAP-1 in Type 1 tumor necrosis factor receptor signalling reported by Song *et al.* (1995). Induction of grp94 helps to prevent thapsigargin-induced apoptosis. This effect of grp94 might be a consequence of grp94-mediated repair functions in the ER lumen after the calcium depletion induced by the calcium-pump inhibitor thapsigargin (McCormick *et al.*, 1997). The protective effects of hsp90 during oxidative damage may also protect the host cell from several types of apoptosis mediated by reactive oxygen species (Punyiczki and Fésüs, 1998). As is obvious from the above, our present knowledge about the involvement of the 90-kDa chaperones in the cell cycle and apoptosis is rather fragmentary. However, these areas may well provide significant major advances in the understanding of hsp90 function in the near future.

3.6. *grp94 in the Quality Control of the Endoplasmic Reticulum*

The ER harbors a refined network of molecular chaperones acting as a quality control mechanism for proteins secreted from the cell or transported to the plasma membrane (Hammond and Helenius, 1995; Wei and Hendershot, 1996; Brooks, 1997). ER also behaves as a fine-tuned sensor of irregularities, stressful conditions in the calcium metabolism, redox status, and level of malfolded proteins in the ER lumen and membrane (Pahl and Baeuerle, 1997). At present the role of grp94 in these processes is even less understood than the role of its cytoplasmic counterpart hsp90 in the maintenance of the structural integrity of the cytoplasm and of its constituent proteins.

grp94 associates with numerous other molecular chaperones of the ER, such as grp78 (BiP), calreticulin, calnexin, the protein disulfide isomerase ERp72, the hsp70-homologue grp170, and the collagen-specific chaperone hsp47 (Melnick *et al.*, 1992; Ferreira *et al.*, 1994, 1996; Tatu and Helenius, 1997; Kuznetsov *et al.*, 1997). Overexpression of grp94 prolongs the folding of thyroglobulin (Muresan and Arvan, 1997). The exact order and mechanism of chaperone cooperation in the ER, as well as the role of grp94 in this process, is not clear yet. However, data of Melnick *et al.* (1992, 1994) suggest that grp94, the most abundant chaperone of the ER lumen, might act similarly to hsp90 by binding proteins after a preceding "pre-folding" step by the ER hsp70 homologue grp78/BiP. As further support of this view, treatment of cells with the grp94-binding drug geldanamycin resulted in an increase in the association of unfolded proteins with grp78 (Lawson *et al.*, 1998). grp94 also has been reported to bind an elongated mutant of protein C (Katsumi *et al.*, 1996). Thus, grp94 may also recognize protein segments with significant secondary structure, but with a fluctuating tertiary structure, and may act as a "buffer" or "sink," keeping the excess of folding proteins in a folding competent state to prevent the overload of other chaperones of the folding machinery during ER stress. The preference for structured folding intermediates may also explain why neither grp94 (Dierks *et al.*, 1996) nor hsp90 (Wiech *et al.*, 1993) seem to play a major role in protein transport to the ER.

On the other hand, grp94 is also able to bind a great variety of smaller peptides with sizes ranging from tetramers to 18-mers (Li and Srivastava, 1993; Nieland *et al.*, 1996; Udono and Srivastava, 1997). grp94 serves as one of the receptors of the peptides arriving at the ER lumen via the transporter associated with antigen processing (Lammert *et al.*, 1997), and it is involved in peptide presentation to the major histocompatibility complex (MHC) Class I molecules at a 10-fold higher efficiency than hsp90 (Udono and Srivastava, 1994). Peptide binding of grp94 seems to be nucleotide-independent (Wearsch and Nicchitta, 1997). At present, it is not known whether structured proteins and peptides bind to the same site on grp94 and/or if they bind to the same subpopulation of grp94 proteins. A report of Li and Srivastava (1993) that only casein, but not peptides, is able to stimulate the grp94-associated ATPase activity, is similar to the finding of Melnick *et al.* (1994), who showed that after ATP depletion, grp94 was absent from grp78/immunoglobulin complexes. This points to some possible differences in the mode of handling of the two types of substrates by grp94.

3.7. *Role of hsp90 and grp94 in Protein Presentation to the Proteolytic Machinery*

The quality control mechanism of the ER also involves the presentation of excess malfolded proteins to the proteasome, which is most probably attached to the outer membrane of the ER (Kopito, 1997). Recent data using the 90-kDa chaperone-selective drug geldanamycin indicate that at least in case of some selected targets, such as the p185erbB2 protein, grp94 might be involved in the presentation of these substrates to the proteasome. This substrate presentation occurs most probably via a retrograde transport of the folding-arrested p185erbB2 protein through the ER membrane (Chavany *et al.*, 1996; Mimnaugh *et al.*, 1996). The coupling of grp94 to the proteasome is further substantiated by the fact that only the proteasome inhibitor lactacystine is able to induce grp94 expression, in contrast to inhibitors of cysteine, serine, and metalloproteases, which have no effect on grp94 levels (Bush *et al.*, 1997). Several groups have reported the degradation of highly purified preparations of grp94 (Srivastava *et al.*, 1986; Anderson *et al.*, 1994; Lammert *et al.*, 1997),[9] which may also indicate an intimate association of grp94 with proteases.

Addition of geldanamycin also induced the degradation of several hsp90-bound proteins, such as luciferase (Schneider *et al.*, 1996), mutant p53 (Whitesell *et al.*, 1997), or the Src and Raf kinases (Whitesell *et al.*, 1994; Schulte *et al.*, 1995,

[9]T. Schnaider, Cs. Sőti and P. Csermely, unpublished observations.

1996; Stancato *et al.*, 1997; Pratt, 1997). Interestingly, the enhanced proteolysis was paralleled by an increase in the amount of luciferase-hsp90 complexes, in contrast to the Src and Raf kinases, where a dissociation of the hsp90-kinase complex occurred. This different behavior probably reflects differences in the specificity of hsp90 interaction with these targets. The specific, stable kinase-hsp90 complexes must dissociate to route the kinase for degradation. By contrast, the nonspecific association of luciferase with hsp90 might occur at a different site of the chaperone, which responds to geldanamycin by an increase in binding affinity. As an alternative explanation, the presence of different co-chaperones (such as CDC37/p50) in the two complexes might induce opposite changes in the chaperone-target stability after geldanamycin addition.

In agreement with the above-mentioned involvement of hsp90 in proteasome action, hsp90 associates with the proteasome (Tsubuki *et al.*, 1994; Wagner and Margolis, 1995; Conconi *et al.*, 1996).[10] Association may occur via the highly charged "KEKE" motif (Realini *et al.*, 1994a,b) of the middle, linker region of hsp90. hsp90 inhibits the proteasome-mediated proteolysis of exogenous substrates (Tsubuki *et al.*, 1994; Wagner and Margolis, 1995). This may indicate an hsp90-mediated shift in the preference of the proteasome from substrates arriving by random diffusion to substrates channeled by the heat-shock protein. The hsp90-proteasome association seems to be age-dependent, being more prevalent in younger than in aged animals (Wagner and Margolis, 1995). Interestingly, the "small hsp90 homologue" hsp75/TRAP-1 has also been reported to co-associate with the Type 1 tumor necrosis factor receptor and the proteasome (Song *et al.*, 1995; Tsurumi *et al.*, 1996; Hampton *et al.*, 1996; Dunbar *et al.*, 1997). The proteasome is known to be attached to microfilaments and microtubules (Arcangeletti *et al.*, 1997). Association of hsp90, an actin- and tubulin-binding protein, with the proteasome may also mediate this structural organization of the major proteolytic apparatus of the cytoplasm. hsp90 also associates with calpain, suggesting a functional interaction of the two proteolytic systems (Pariat *et al.*, 1997).[10] A recent genetic study, showing a functional interaction between the p60 protein and the proteasome (Yamashita *et al.*, 1996), raises the interesting possibility that the hsp90-related chaperone complex (the foldosome) participates not only in the regulation, but also in the assembly and/or repair, of the proteasome complex.

3.8. *Surface Expression of grp94 and hsp90 and Their Role in Antigen Presentation*

In 1986, both human grp94 (termed gp96) and mouse hsp90 were identified as tumor-specific antigens expressed on the surface of various tumor cells (Srivastava *et al.*, 1986; Ullrich *et al.*, 1986). Expression of grp94 and hsp90 on the surface of resting or stressed cells has also been reported by numerous other laboratories (Pouyssegur *et al.*, 1977; Shiu *et al.*, 1977; Pouyssegur and Yamada, 1978; McCormick *et al.*, 1982; Hughes *et al.*, 1983; Carbajal *et al.*, 1986; La Thangue and Latchman, 1988; Maki *et al.*, 1990; Erkeller Yuksel *et al.*, 1992; Altmeyer *et al.*, 1996). Surface expressed grp94 is able to bind transferrin with relatively high affinity (Poola and Lucas, 1988; Hayes *et al.*, 1994; Poola and Kiang, 1994). grp94 can be shed by human fibroblasts (McCormick *et al.*, 1979, 1982), and is secreted by exocrine pancreatic cells (Takemoto *et al.*, 1992) and by certain calcium ionophore-treated lines of cultured fibroblasts (Booth and Koch, 1989). Similarly, the secretion of hsp90-α by human-human hybridoma SH-76 cells has also been reported. Extracellular hsp90-α had a stimulatory effect on the growth of some lymphoid cell lines (Kuroita *et al.*, 1992). Presently, neither the molecular details of the surface attachment of grp94 and hsp90 nor the exact mechanism of their secretion are known. Some evidence suggests that the observed phenomena cannot be explained by a nonspecific lysis of certain cells (Multhoff and Hightower, 1996).

The most likely major function of both the surface-expressed grp94 and hsp90 is their role in antigen presentation, which is helped by their binding capacity for a great variety of peptides. Surface-expressed grp94 has been identified as the major tumor rejection antigen of several tumors (Srivastava *et al.*, 1986). In some tumors, the glycosylation pattern of grp94 shows some differences, but these minor variations in grp94 glycosylation cannot account for the major differences in the immunogenicity of surface-expressed grp94 species. This apparent discrepancy led Srivastava to suggest that the grp94-related (and possibly the hsp90-related) immunogenicity resides in a great variety of peptides, which are noncovalently associated with grp94 and thus, "presented" by this chaperone (Srivastava and Heike, 1991; Srivastava and Maki, 1991). In accordance with this, later studies identified grp94 as a peptide-binding protein (Li and Srivastava, 1993; Nieland *et al.*, 1996; Udono and Srivastava, 1997). Later experiments also showed that grp94 preparations from normal tissues did not elicit antitumor immunity (Udono and Srivastava, 1994) and that grp94 acts as one of the receptors of the peptides transported to the ER (Lammert *et al.*, 1997). The functional and/or physical association of both grp94 and hsp90 with the proteasome (see Section 3.7) also supports their role in peptide presentation.

Endogenously synthesized antigenic determinants are generally presented on MHC Class I molecules, whereas exogenous antigens are presented by MHC Class II molecules. Heat-shock and glucose-regulated proteins (hsp70, hsp90, and grp94) may present their bound peptides to MHC Class I molecules. Under normal (nonstressed) conditions, this may be a helper mechanism for loading of the MHC Class I molecules in the ER. However, stress proteins may carry their immunogen peptides to MHC Class I molecules other than those of their original cells by lysis of the original cell and subsequent phagocytosis by macrophages, or by direct macrophage engulfment of the whole cell. Since heat-shock proteins are highly conserved, this phenomenon may also occur after the lysis or phagocytosis of foreign cells

[10] T. Schnaider, Cs. Sőti, and P. Csermely, unpublished observations.

with different haplotypes. Hence, foreign chaperones may "disguise" their bound foreign peptides as self. Thus, insertion of the nondiscriminating stress proteins to the peptide/antigen-presenting "relay" may explain the phenomenon of cross-priming, i.e., that not all the processing of the antigens occurs via the haplotype-restricted MHC Class I molecules of the immunized mouse, but at least some of peptide/antigens are salvaged by the macrophages of the immunized mouse directly from the chaperones of the immunizing cells (having a different haplotype) (Srivastava *et al.*, 1994).

The above hypothesis of Srivastava *et al.* (1994) is supported by the direct demonstration of the role of cytotoxic T lymphocytes and macrophages in the grp94-elicited tumor-specific immune response of BALB/cJ mice (Udono *et al.*, 1994). Later studies provided further experimental evidence for the chaperone-mediated cross-priming, i.e., for the channeling of exogenous antigens by exogenously added grp94 to the endogenous pathway presented by C57BL/6 mouse macrophage MHC Class I molecules and activating cytotoxic T lymphocytes (Suto and Srivastava, 1995; Arnold *et al.*, 1995). Reduction of grp94 levels to 10% of their original amount in P136 mastocytoma cells did not disturb MHC Class I-mediated antigen presentation (Lammert *et al.*, 1997). However, this finding may be explained by the redundance of various ER chaperones or by assuming other channeling mechanisms in MHC Class I-restricted endogenous peptide presentation. Interestingly, while hsp70 was equally potent in immunogenic peptide presentation in BALB/cJ mice, like grp94, hsp90 had only about 10% the efficiency of grp94 (Udono and Srivastava, 1994). This may reflect a difference in substrate recognition by the two proteins, pointing to a lower affinity of hsp90 for smaller peptides than grp94.

The involvement of grp94 and hsp70 in antigen presentation (Udono and Srivastava, 1997) also means that in an organism experiencing the stress of infection, the MHC nonrestricted presentation of non-self antigens becomes more dominant: a response that increases the efficiency of immune surveillance. Peptide-loaded chaperones (via the peptide-presenting macrophage-MHC Class I molecules) may prime cytotoxic lymphocytes even after the lysis of the originally infected or malignant cells, which extends the cytotoxic response and also makes it more efficient (Srivastava *et al.*, 1994).

The 90-kDa (and 70-kDa) chaperone-mediated "escape route" of cytotoxic lymphocyte priming from the restrictive self-MHC molecules described above has profound consequences in vaccination. The vaccination procedure does not necessarily have to use autologous or HLA-matched cells, which may extend its use to shared tumor antigens or viral antigens (Blachere *et al.*, 1993; Srivastava *et al.*, 1994; Heike *et al.*, 1996).

3.9. *Speculations on the Major Cellular Functions of hsp90 and grp94*

To supplement our description of the possible cellular functions of hsp90 and grp94 (Sections 3.1–3.8), we now summarize our present view about the importance of the surprisingly many possibilities as to how the 90-kDa chaperones might help the everyday life of cells and enable them to retain their viability after environmental stress. We first recall that deletion of the 90-kDa chaperones is lethal for eukaryotic cells, and that these chaperones are one of the most abundant cellular proteins. The most important questions that arise from these facts are:

(A) What makes these chaperones so important?
(B) Why do we need constitutively so much of them?

There are several possible answers to these questions. A recent review (Johnson and Craig, 1997) described hsp90 as a general chaperone of the eukaryotic cytosol, orchestrating the folding of many eukaryotic proteins with the help of hsp70 and the "thousand-and-one" co-chaperones they bind. In a summary of an elegant study investigating the *in vitro* folding of four different hsp90-substrates, Nair *et al.* (1996) give a somewhat more elaborate list of functions for the hsp90-related chaperone complex involving (1) repression of the target's activity, (2) protection of the target from proteolysis, (3) dynamic docking of the target to regulate its oligomerization, and (4) providing phenotypic diversity for the target by stabilizing its alternative conformational states.

Although each of the above "definitions" for the cellular function of hsp90 is correct, we would like to raise some arguments suggesting that the major cellular function of hsp90 is probably not its chaperone behavior, but its dynamic participation in the organization and maintenance of the cytoarchitecture.

3.9.1. hsp90-mediated folding of nascent proteins does not seem to be a general phenomenon. The "classical" chaperone function, as an aid in protein folding, is a very good candidate to answer both questions A and B above. Assisted folding is a vital function that requires a large amount of the chaperone. However, recent findings indicate that hsp90 is probably not necessary as a general chaperone for *de novo* synthesized proteins. In contrast to eukaryotic hsp90, the eubacterial homologue, the HtpG protein, is not necessary for cell survival (Bardwell and Craig, 1988). Interestingly, the *in vitro* chaperone properties of the two proteins are rather similar (Jakob *et al.*, 1995b). The recent discovery that the folding of nascent proteins occurs mostly cotranslationally in eukaryotes, whereas in eubacteria it is mainly a post-translational event (Netzer and Hartl, 1997), shows that in eukaryotes, where hsp90 has vital functions, the need for general post-translational chaperoning is limited. In contrast, in eubacteria, where the need for chaperones is much more expressed, hsp90 deletion is not lethal. Moreover, hsp90 (in contrast to hsp70) has not been observed as part of the ribosome-attached chaperone machinery (Beckmann *et al.*, 1990; Nelson *et al.*, 1992), and *in vivo* examples of hsp90-mediated protein folding are quite limited (Johnson and Craig, 1997). The elegant data of Lindquist and co-workers (Nathan *et al.*, 1997) gave further evidence

for the hsp90 independence of the *de novo* folding of most proteins. Thus, the involvement of hsp90 in folding of nascent proteins may be restricted to a subset of eukaryotic proteins, which harbor large hydrophobic surfaces for their ligands, or for protein/membrane-binding (in the case of steroid receptors and signalling kinases, respectively), and, therefore, need a temporary stabilization of their otherwise collapsing or aggregating structure.

Since hsp90-mediated folding of some kinases (e.g., CDK4) may be a good enough reason for the lethal consequences of hsp90 deletion, the importance of hsp90 (question A) most probably has been elucidated. However, these specific folding events should not require a 1000-fold excess (Buchner, 1996) of the respective chaperone. Question B still remains open.

3.9.2. hsp90-mediated folding after stress. The other possibility for *in vivo* utilization of the *in vitro* chaperone activity of hsp90 is to refold damaged proteins after cellular stress, such as heat shock. Some *in vitro* observations show that, indeed, the chaperone activity of hsp90 becomes activated at higher temperatures, corresponding to the usual range of cellular heat shock (Yonehara *et al.*, 1996; Jakob *et al.*, 1995a). *In vitro* hsp90 was also shown to retain partially denatured proteins in a folding-competent state (Freeman and Morimoto, 1996), which may be an important mechanism of its *in vivo* rescue function after cellular stress. As further *in vivo* proof for the importance of hsp90-mediated protein folding after stress, higher levels of hsp90 increase the heat-resistance of the respective cells (Yahara *et al.*, 1986; Heads *et al.*, 1995).

Thus, hsp90 may become a fairly general chaperone after stress that requires a large amount of the protein. However, recent data of Nathan *et al.* (1997) suggest that hsp90 generally does not protect proteins from thermal inactivation, but enhances the rate at which a heat-damaged protein is reactivated. From this perspective, 1–2% of the total cellular protein seems to behave like a "fireman" of the cell, sitting quietly and doing nothing most of the time. Such a luxury is seldom tolerated by evolution. Thus, the explanation of the constitutively large amounts of the 90-kDa chaperones (question B) most probably involves another function that requires a large amount of the protein and that is specific for eukaryotes.

3.9.3. hsp90 and the organization and maintenance of the cytoarchitecture. The organizational role of hsp90 in the foldosome (Section 3.1), in signalling events (Section 3.2), and in proteolytic degradation (Section 3.7), together with its participation in various forms of the cytoskeletal structure (Section 3.3), raises the possibility that hsp90 may participate in the maintenance and remodeling of the cytoarchitecture by guiding some selected *de novo* synthesized or damaged targets to their proper destination within the cytoplasm. hsp90 may be similar in this respect to the other major cytoplasmic chaperone the hsp60-TCP1 protein (Trent *et al.*, 1997). In stressed cells, hsp90 may also function by helping to preserve the structural integrity of both the cytoplasm and the nucleus. These functions require constitutively high levels of hsp90, are fairly specific to eukaryotes, are vital for the everyday life of cells, and, therefore, represent an adequate answer to question B.

The above hypothesis suggests that the current uniform chaperone definition may likely be reformulated in the near future, applying the participation in protein folding as a major principle for eubacteria (where folding of nascent proteins is post-translational) (Netzer and Hartl, 1997) and defining chaperones of the eukaryotic cytoplasm (where folding of nascent proteins occurs co-translationally) (Netzer and Hartl, 1997) as parts of the cellular structure involved in directed transfer of proteins. Although our knowledge about the function of grp94 is rather fragmentary, it may also play a similar structural-organizational role in the lumen of the ER.

4. EXPRESSION OF hsp90 AND grp94

As also shown by its abbreviated name, hsp90 is a ***h***eat-***s***hock ***p***rotein, while grp94 is a ***g***lucose-***r***egulated ***p***rotein; they are induced by elevated temperatures and by glucose starvation, respectively. In subsequent sections, we summarize our knowledge about the molecular mechanism of their induction and the various conditions known to induce these proteins.

4.1. *Gene Structure and Mechanism of Gene Expression*

The human gene encoding the inducible hsp90-α has been mapped to chromosome band 14q32.3. The chromosome segments 1q21.2–q22, 4q35, and 11p14.1–p14.2 most probably contain pseudogenes of hsp90-α (Ozawa *et al.*, 1992; Vamvakopoulos *et al.*, 1993). The constitutively expressed hsp90-β gene family consists of a gene at chromosome band 6p21 and two pseudogenes at chromosome bands 4q21–q25 and 15pter–q21 (Durkin *et al.*, 1993; Takahashi *et al.*, 1994). As a rather unique feature of hsp90, compared with the generally intronless heat-shock proteins, both the hsp90-α and -β genes contain intron sequences. As a consequence of this, gene structure splicing of hsp90 mRNA is inhibited after severe heat shock in *Drosophila* cells (Yost and Lindquist, 1986). Interestingly, splicing of hsp90 mRNA in whole *Drosophila* larvae seems to be much more resistant to heat shock than that of the individual cells (Shen *et al.*, 1993).

Heat-shock protein expression is regulated by a family of specific transcription factors, the heat-shock factors. Binding of the properly activated heat-shock factor to its specific site (to the heat-shock element [HSE]) in the promoter region of the heat-shock genes enhances binding and/or allows the start of the prebound (so-called "pausing") RNA polymerase along the coding region of the gene (for recent reviews, see Lis and Wu, 1993; Wu, 1995; Morimoto *et al.*, 1992, 1996). To the good fortune of those working in the 90-kDa chaperone field, the promoter region of the yeast hsp90 genes is a favorite object of studies on the regulation of heat-shock gene transcription. A map of the upstream regulatory sites of yeast hsp90 is shown in

Fig. 3. Interestingly, out of the two heat-shock factor-binding sites, only one, HSE1, is occupied. However, upon heat shock, a weak binding of heat-shock factor also occurs at the HSE2 site of hsp90-α. The promoter of hsp90-β contains an upstream regulatory sequence (URS1), which is a site for the early meiotic cascade-induced activation of hsp90-β expression (Erkine *et al.*, 1995; Giardina and Lis, 1995; Szent-Gyorgyi, 1995). Critical segments of the promoter region of the hsp90-α gene contain two sequence-positioned nucleosomes. This nucleosomal structure is disrupted by the yeast heat-shock factor, alleviating the nucleosome repression of the core promoter (Lee and Garrard, 1991; Gross *et al.*, 1993).

The human hsp90-α promoter region contains a putative SP1 binding site and a serum-response element, besides a "perfect" and several "imperfect" HSEs (Hickey *et al.*, 1989). This suggests that the transcription of human hsp90 may be multiply regulated by cross-talk of various transcription factors. A distal HSE of hsp90-α plays a synergistic role with a proximal HSE in reporter-gene assays (Zhang and Shen, 1995; Y. F. Shen, personal communication). Interestingly, there are two typical HSEs in the first intron of the human hsp90-β gene. Eighty percent of the constitutively expressed hsp90-β is initiated from the intron promoters, while upon heat shock, almost all the inducible transcription is driven by the intron promoters (Liu *et al.*, 1995; Shen *et al.*, 1997).

The human gene family of grp94 is similar to that of hsp90, with one coding gene and two pseudogenes. The coding gene has been localized to the chromosome band 12q24.2–12q24.3, while the pseudogenes are found on chromosomes 1p22 and 15q25–15q26 (Maki *et al.*, 1993).

Regulation of grp94 expression is highly similar and linked to the regulation of the expression of grp78 (BiP). Deficiency in grp94 induction also impairs induction of grp78 (Little and Lee, 1995), while both overexpression of grp78 and an antisense grp78 fragment reduce the induction of grp94 (Dorner *et al.*, 1992; Liu *et al.*, 1997). Both human grp78 and grp94 promoter regions contain a CG/CAAT and a GC-rich sequence motif, which are important for basal and induced expression of the genes (Liu and Lee, 1991). The promoter regions also contain Sp1, Ap2-binding sites, and interferon-stimulated response elements (Chang *et al.*, 1989; Anderson *et al.*, 1994). A minimum of 6 proteins bind to grp94 promoter sequences, ranging from 55 kDa to 210 kDa. One of the binding proteins is the Ku auto-antigen, a DNA helicase subunit of the double-stranded DNA-dependent protein kinase (Liu and Lee, 1991). Promoter-binding of the heteromeric CCAAT-binding protein (CBF) has also been identified (Ramakrishnan *et al.*, 1995). An increase in the amount of malfolded proteins in the ER (an "ER-overload") induces the expression of glucose-regulated proteins, including grp94 (see, e.g., Lenny and Green, 1991). The exact mechanism of signal transduction from the ER to the transcriptional complexes is not known. However, several pieces of evidence suggest that serine/threonine and tyrosine phosphorylation both play an important role in this process (Cox *et al.*, 1993; Mori *et al.*, 1993; Cao *et al.*, 1995).

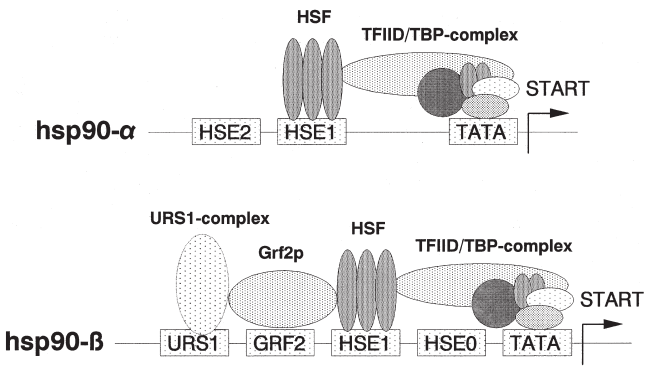

FIGURE 3. Structure of the promoter regions of the yeast hsp90 genes. GRF2, binding site for the ancillary yeast protein Grf2p; HSE, binding site for the heat-shock factor 1; HSF, heat-shock factor 1; hsp90-α, the inducible hsp90; hsp90-β, the constitutively expressed hsp90; TATA box, thymidine- and adenine-rich sequence forming the binding site for the general transcription factors and for RNA polymerase-II; URS1, a site for the early meiotic cascade-induced activation of hsp90-β expression. Data from Erkine *et al.* (1995, 1996) and Szent-Gyorgyi (1995).

4.2. *hsp90 Isoforms*

As mentioned in the preceding section, hsp90 has two isoforms: the somewhat more inducible hsp90-α (other names: hsp90, hsp84) and the somewhat less inducible, and more constitutively expressed, hsp90-β (other names: hsc90, hsp86). Besides heat shock, hsp90-α can be induced by a variety of other agents (see Section 4.3). We now summarize the available data on the differences in function of these two isoforms.

Both hsp90-α and -β form mostly homodimers. Slight differences in the C-terminal dimerization domain render the hsp90-β dimers less stable than the α-homodimers. This difference in stability also explains why the majority of hsp90 monomers comes from the β isoform (Minami *et al.*, 1991; Nemoto *et al.*, 1995). Besides the differences in dimer stability, the low abundance of hsp90 heterodimers may also be explained by the observation of Sullivan and Toft (1993) that the turnover of dimers is slow; therefore, newly synthesized hsp90 does not form a dimer with the pre-existing pool of the protein.

hsp90-β was found to be unevenly distributed in the cytoplasm, with a larger portion of the protein localized in the vicinity of the nuclear envelope (Perdew *et al.*, 1993). In agreement with this localization, hsp90-β (but not α) can be phosphorylated by the double-stranded DNA-dependent protein kinase at its N-terminal threonine residues (Lees-Miller and Anderson, 1989b). The complex formations of the two isoforms are rather similar: both α and β can be found in nuclear hormone receptors and filamentous actin complexes (Mendel and Orti, 1988; Minami *et al.*, 1991; Rexin *et al.*, 1991; Perdew *et al.*, 1993). hsp90-α predominates in the brain and in testis, while hsp90-β is enriched in other peripheral organs (Vamvakopoulos, 1993).

Data from the foregoing show no major differences between the molecular characteristics and functions of hsp90-α and -β analyzed so far. On the other hand, if one takes into account that hsp90-α is usually more inducible than hsp90-β (see Section 4.3), it is quite likely that there must be a substantial difference in the function of the two isoforms. hsp90-α may be a better candidate for the cytoplasmic organizational role of the chaperone described in Section 3.9. Changes in the oligomerization/complex formation properties of hsp90-α may require a concentration threshold reached by the slight elevation of the protein levels during the cell cycle (Section 3.5), in embryonic development (Section 4.5), or in cancer (Section 5.4). The elucidation of the different functions of hsp90-α and -β at the cellular level is an important task for the near future.

4.3. *hsp90 after Heat Shock, Its Expression by Other Inducers*

Heat shock induces the expression mainly of the α isoform of hsp90 (Meng *et al.*, 1993). The term "heat shock" is highly relative, depending on the previous acclimatization of the organism, e.g., for winter-acclimatized eurythermal goby fish, a 28°C habitat is a marked inducer of hsp90, while for the summer-acclimatized fish of the same species, even a 30°C bath does not induce any significant synthesis of hsp90 (Dietz and Somero, 1992). Interestingly, in spinach and in *Brassica napus*, hsp90 mRNA is also induced by cold shock (Krishna *et al.*, 1995).

Heat shock increases the oligomerization and the *in vitro* chaperone activity of hsp90 (Yonehara *et al.*, 1996). The improved chaperone activity is most probably derived from the α isoform, which has a higher potential for oligomerization (Minami *et al.*, 1991; Nemoto *et al.*, 1995). Heat shock also increases the turnover of phosphate residues on hsp90 (Legagneux *et al.*, 1991), which may reflect a greater flexibility in regulation. After heat shock, the "sticky" hsp90 most probably displays an even higher binding efficiency than it does in resting cells, for a variety of reasons. Heat shock increases the hydrophobicity/unfolding of hsp90 (Yamamoto *et al.*, 1991; Lanks *et al.*, 1992; Csermely *et al.*, 1993), which, by itself, may enhance its binding to various partially unfolded target proteins, having exposed hydrophobic surfaces. A stress-induced drop in the cellular ATP level (Kabakov and Gabai, 1997) may also lead to the association of hsp90 with actin filaments (Kellermayer and Csermely, 1995).

In contrast to hsp70 and to other heat-shock and glucose-regulated proteins, the ribosomal recognition of hsp90 mRNA does not seem to use a special mechanism (Zapata *et al.*, 1991). hsp90 plays an important role in inhibition of the general translation process during heat shock by helping the activation of eIF-2α kinase and the subsequent phosphorylation of eIF-2α. The molecular details of this process have not been fully elucidated (Pal *et al.*, 1996).

Besides heat shock, hsp90 can be induced by a variety of stimuli. Some of the physiologically important inducers, as well as some pathological states that also lead to an increased expression of hsp90, are listed in Table 6. Expression and role of hsp90 in some diseases of exceptional importance, such as in ischaemia, in various infections, in autoimmune diseases, and in cancer, are summarized separately in Sections 5.1.–5.4. Since hsp90 is a stress protein, it can be induced by almost any substances used and abused by mankind and studied until now, exemplified by ethanol (Miles *et al.*, 1994), cocaine (Salminen *et al.*, 1997), etc. The number of various (mostly justified) animal-poisoning experiments investigating the expression of hsp90 is increasing exponentially, and the limits of the present review do not allow us to list them. However, we refer to some toxicological applications of hsp90 induction in Section 5.5.

4.4. *grp94 in the "Stressed" Endoplasmic Reticulum*

grp94 is classically induced by glucose starvation and by calcium ionophores such as A23187 (Pouyssegur *et al.*, 1977; Shiu *et al.*, 1977; McCormick *et al.*, 1979; Wu *et al.*, 1981; Welch *et al.*, 1983; Lee, 1987). In contrast to our knowledge about the stress-induced functional changes of hsp90, which may be regarded as "fragmentary," we have practically no information about the changes in the function of grp94 following the effects of various stressors to the cells and to the ER.

In Table 6, we have summarized a number of physiological and pathophysiological conditions that induce grp94. Many of these changes actually lead to an accumulation of malfolded proteins in the ER, resulting in overexpression of grp94 by the mechanism outlined in Section 4.1. The glycosylation pattern tends to change after some types of cellular stress and also seem to occur in several diseases, e.g., in certain types of cancer or in diabetes (Section 2.2). This raises the possibility that the status of grp94 glycosylation may play an important role in the regulation of ER chaperone activity after stress.

grp94 is involved in the recognition and folding of proteins (so-called "quality control") in the ER (Hammond and Helenius, 1995). In several genetic disorders, such as various forms of cystic fibrosis, or α_1-antitrypsin deficiency, this quality control mechanism is "overreacting" and retains the partially malfolded, but otherwise functional, molecule in the ER (Brooks, 1997; Welch and Brown, 1996). The changes of grp94 function in this type of stress (often called "ER-overload") await further investigation.

4.5. *Role of hsp90 and grp94 in Development and in Aging*

As described in Section 3.2.2, hsp90 is required for the expression and functioning of several development-related protein kinases in *Drosophila*, such as Torso (Doyle and Bishop, 1993) and Sevenless (Cutforth and Rubin, 1994). *Drosophila* accumulates hsp90 in the ovaries during oogenesis and in early stages of embryonal development (Zimmerman *et al.*, 1983; Ding *et al.*, 1993). The same is true for the amphibian *Pleurodeles waltl*, where a characteristic nuclear transfer of hsp90 occurs in Stage VI oocytes and up to the

TABLE 6. Induction of hsp90 and grp94 by Various Physiological Agents and by Pathological Conditions

Protein/inducer	Reference
hsp90[1]	
Heat shock	Welch and Feramisco, 1982
Cold shock	Krishna *et al.*, 1995
Transforming growth factor-β	Takenaka and Hightower, 1992, 1993
Glucocorticoids[2]	Kasambalides and Lanks, 1983; Patchev *et al.*, 1994
Estradiol	Olazábal *et al.*, 1992; Shyamala, 1993
Prostaglandin A1	Pica *et al.*, 1996
Erythrophagocytosis	Clerget and Polla, 1990
T lymphocyte activation	Ferris *et al.*, 1988
hsp90-α	
Heat shock	Meng *et al.*, 1993
Phorbol ester	Jacquier-Sarlin *et al.*, 1995
Serum, insulin, insulin-like growth factor-1, epidermal growth factor, platelet-derived growth factor	Kasambalides and Lanks, 1985; Jerome *et al.*, 1991
Estradiol	Wu *et al.*, 1996
interleukin-4	Metz *et al.*, 1996
Glutathione depletion[2]	Rokutan *et al.*, 1996
hsp90-β	
Interleukin-6	Stephanou *et al.*, 1997
Lymphocyte activation	Hansen *et al.*, 1991
Familial glucocorticoid resistance	Brönnegard *et al.*, 1995
grp94	
Glucose starvation, calcium ionophores	Pouyssegur *et al.*, 1977; Shiu *et al.*, 1977; McCormick *et al.*, 1979; Wu *et al.*, 1981; Welch *et al.*, 1983; Lee, 1987
Geldanamycin	Lawson *et al.*, 1998
Estrogen	Baez *et al.*, 1987; Shyamala, 1993; Hayes *et al.*, 1994; Poola and Kiang, 1994
Interferon-α and -γ	Anderson *et al.*, 1994
Interleukin-6	Haverty *et al.*, 1997
Lactacystine (proteasome inhibitor)	Bush *et al.*, 1997
Epileptic seizures	Little *et al.*, 1996
Congenital hypothyroid goiter	Medeiros-Neto *et al.*, 1997
Osteoarthritis	Takahashi *et al.*, 1997
CNS injury	Lowenstein *et al.*, 1994

[1] The up-regulated hsp90-isoform has not been specified.
[2] Represses the induction of the respective 90-kDa chaperone.

blastula stage (Coumailleau *et al.*, 1995a, 1997). In *Saccharomyces cerevisiae*, hsp90 accumulates prior to sporulation (Kurtz and Lindquist, 1984; Kurtz *et al.*, 1986). The dormant dauer larva of *Caenorhabditis elegans* develops a 15-fold enriched mRNA message that decreases after recovery (Dalley and Golomb, 1992). As a general "rule of thumb," it may be concluded that major changes in cellular structure and organization during embryonal development usually bring about an increase in the hsp90 mRNA message. Further studies will certainly make the details of this clearer by dissecting the role of various hsp90 isoforms in this process.

hsp90 is similarly upregulated during oogenesis or early embryogenesis of higher organisms (Morange *et al.*, 1984; Barnier *et al.*, 1987; Harry *et al.*, 1990; Curci *et al.*, 1991). hsp90-β seems to participate in neural, retinal, and in bone development (Kojima *et al.*, 1996; Loones *et al.*, 1997; Walsh *et al.*, 1997), while the other hsp90 isoform, hsp90-α, is closely related to muscle development by activating the helix-loop-helix transcription factor myoD (see also Section 3.4), both in zebrafish and in humans (Bornman *et al.*, 1996; Sass *et al.*, 1996; Sass and Krone, 1997). hsp90 interacts with centrin in *Xenopus* oocytes, and this complex dissociates upon calcium-dependent activation of the oocyte (Uzawa *et al.*, 1995). Thus, hsp90 may modulate the assembly of centrosomes in early embryonic development. grp94 is constitutively expressed in mouse embryos during early stages of oogenesis and is localized particularly within the developing heart, neuroepithelium, and surface ectoderm tissues (Barnes and Smoak, 1997).

Cellular aging of fibroblasts is known to impair the induction of both hsp70 and hsp90 (Liu *et al.*, 1989a,b). grp94 mRNA levels seem to remain unchanged in aging mice (Spindler *et al.*, 1990). As is clear from the above examples, despite a fairly general defect of heat-shock protein expression in aged organisms (Heydari *et al.*, 1994; Liu *et al.*, 1996), the impairment of hsp90 synthesis during aging or age-related diseases, like various neurodegenerative syndromes, has not been investigated adequately.

5. THE 90-kDa MOLECULAR CHAPERONES IN DISEASE: CLINICAL APPLICATIONS

The induction and function of hsp90 and grp94 in several pathological states have already been referred to in Sections 4.3 and 4.4. We now discuss their role in some diseases of exceptional importance, such as cerebro- and cardiovascular diseases (ischaemia and reperfusion), in various infections, in autoimmune diseases, and in cancer.

5.1. *Ischaemia and Reperfusion*

Ischaemia and reperfusion are stress phenomena accompanying most cerebrovascular disease states. The study of the cellular protective mechanisms against hypoxia- or oxidative stress-induced damages is of paramount importance in therapy of heart attacks and strokes.

As a general rule, glucose-regulated proteins (such as grp94) are mostly induced during the ischaemic period, while heat-shock proteins (such as hsp90) are overexpressed during the oxidative stress of reperfusion (Sciandra *et al.*, 1984). hsp90 mRNA levels do not increase *during* a cerebral ischaemic period (Higashi *et al.*, 1994), and contrary to the protective role of hsp70, overexpression of hsp90 is not protective against ischaemic damage (Heads *et al.*, 1995; Amin *et al.*, 1996). However, *existing* levels of hsp90 may play a role in ischaemic signalling by binding to the hypoxia-inducible factor 1-α (Gradin *et al.*, 1996), and in contrast to hsp90, grp94 is strongly induced after acute kidney ischaemia (Kuznetsov *et al.*, 1996).

Reperfusion induces hsp90-α in heart (Nishizawa *et al.*, 1996), brain (Katsumi *et al.*, 1996; Kawagoe *et al.*, 1993; Wagstaff *et al.*, 1996), kidney (Morita *et al.*, 1995; Turman *et al.*, 1997), and EL-4 thymoma cells (Gabai and Kabakov, 1994). Similarly to the effect of reperfusion, direct oxidative damage is a strong inducer of hsp90 in kidneys (Fukuda *et al.*, 1996) and in lymphocytes (Marini *et al.*, 1996). In a recent study, a markedly decreased hsp90 level was found in the interventricular septum of so-called "sudden-death pigs" with inherited hypertrophic cardiomyopathy, which may indicate the importance of hsp90 in protecting the heart muscle from oxygen fluctuation-induced damage (Lee *et al.*, 1996). However, in contrast to the detailed evidence for the conditioning effect of hsp70 in ischaemia, our knowledge of hsp90-induced protection during reperfusion is rather limited.

5.2. *Infections*

When a parasite or bacterium enters the host organism, it usually finds the environment highly stressful. Temperature, pH, ionic strength and milieu, and nutritional composition are all abruptly changed, not to mention the highly hostile reception by the immune system. Thus, it is not surprising that the infectious invader usually overexpresses a large panel of various heat-shock proteins to protect itself. Many of these proteins are also expressed on the surface of the parasites or bacteria, providing an easy target for immune recognition. Since the structure of the heat-shock proteins has been highly conserved during evolution, the "stress-epitope repertoire" found on the surface of a wide variety of infecting agents is rather similar. Therefore, a very strong and generalized immune response develops against these proteins at an early stage of postnatal immune maturation and acts as a "first line of defence" during later infections (Kaufmann, 1990; Cohen and Young, 1991).

In agreement with the foregoing general picture, hsp90 overexpression protects many infectious organisms, e.g., *Leishmania* (Salotra *et al.*, 1995; Streit *et al.*, 1996), yeast (Hodgetts *et al.*, 1996), etc. Surface-expressed parasitic hsp90 also serves as an antigen in many infections, such as Chaga's disease (Dragon *et al.*, 1987), ascariasis (Kumari *et al.*, 1994), *Leishmania* (Skeiky *et al.*, 1995), and *Schistosoma mansoni* (Johnson *et al.*, 1989). Therefore, a proper antibody against the dominant hsp90 epitope, or vaccination by the respective hsp90 protein, or by its fragment, can provide significant protection against the infection. Protection by hsp90 antibodies, or by vaccination, has been demonstrated in infections of *Streptococcus oralis* (Burnie *et al.*, 1996), *Plasmodium falciparum* (Bonnefoy *et al.*, 1994), and *Candida albicans* (Matthews and Burnie, 1992). There is an increasing number of patents and applications, such as US patent 5288639, which describes the isolation of an hsp90 homologue from *Candida albicans* and the use of an antibody against this protein as an immune therapy against the pathogen. A similar immune therapy might be useful in the treatment of AIDS patients (Voellmy, 1996). The primary *Candida* antigen is a 47-kDa proteolytic fragment of the *Candida* hsp90, having a major epitope at the conserved hsp90 sequence LKVIRK (Matthews and Burnie, 1992). Interestingly, the segment of hsp90 around the LKVIRK sequence is highly similar to the RNA-binding region of several plant virus proteins (Koonin *et al.*, 1991; Section 3.4.2). Antibodies against this sequence were found to be useful in controlling other infections with hsp90-related immunodominance (Burnie *et al.*, 1996). However, as expected, cross-reactivity is only limited, since recombinant *Leishmania* hsp90 is recognized by sera of patients with leishmaniasis, but not by sera of patients with Chaga's disease (de Andrade *et al.*, 1992).

Early stages of viral infections and intracellularly growing bacteria are stressful not only for the infecting organism, but also for the infected cells. This is reflected by an increased expression of heat-shock proteins, including hsp90 (Garry *et al.*, 1983; Khandjian and Turler, 1983; Cheung and Dosch, 1993; Schwan and Goebel, 1994; Cho *et al.*, 1997). hsp90 may also be expressed on the surface of infected cells, as in the case of herpes simplex virus infection (La Thangue and Latchman, 1988), where it may serve as a signal for elimination of the infected cell. Relatively little is known about the role of heat-shock proteins in the development of infection. The situation is especially interesting in the case of viral infections, where the virus has to "steal" the chaperones of its host to facilitate its own assembly. hsp90 has been reported to associate with the capsid protein of Sindbis virus and with the nucleocapsid protein of

vesicular stomatitis virus (Garry *et al.*, 1983). As another example of the viral use of host hsp90, hsp90 is necessary for the assembly of the reverse transcriptase/RNA complex of hepatitis B virus (Hu and Seeger, 1996; Hu *et al.*, 1997). In some interesting cases, chaperones of microorganisms themselves are structurally related to hsp90, as in the case of the intramolecular chaperone of *Vibrio cholerae* cytolysin (Nagamune *et al.*, 1997) or in the case of movement proteins of several plant viruses (Koonin *et al.*, 1991).

5.3. *Autoimmune Diseases, Diabetes*

The general immune response against the conserved and, in many cases, surface-expressed, heat-shock proteins of the invading organisms described in the preceding section sometimes recognizes a similar sequence of proteins of the host organism that leads to development of an autoimmune disease (Cohen and Young, 1991). Auto-antibodies against both hsp90 and grp94 have been detected in systemic lupus erythematosus (Minota *et al.*, 1988; Dhillon *et al.*, 1993; Boehm *et al.*, 1994; Latchman and Isenberg, 1994), where the usually constitutive hsp90-β becomes overexpressed (Twomey *et al.*, 1993). Antibodies against surface-expressed hsp90 of infectious organisms (such as systemic candidiasis, invasive aspergillosis, etc.) frequently cross-react with the highly homologous human hsp90 and behave as an auto-antibody. The epitopes of these (auto)antibodies are usually different from those of systemic lupus erythematosus (al-Dughaym *et al.*, 1994).

Several aspects of the aetiology of diabetes are related to autoimmune processes. Although recurrent findings invoke various heat-shock proteins as target auto-antigens, as well as heat-shock protein-related immune responses as autoimmune attacks leading to diabetes, so far neither hsp90 nor grp94 have been demonstrated as diabetes-related auto-antigens. Since diabetes is a chronic disease, changes in the chaperone-related repair mechanisms may be crucial for the onset of chronic effects of diabetes, such as angiopathy and neuropathy (Vígh *et al.*, 1997; Bíró *et al.*, 1997). In spite of the intimate link between changes in the extracellular glucose level and the regulation of the synthesis of glucose-regulated proteins, our knowledge of their function in diabetes is rather limited. Our initial studies show a diabetes-related decrease in grp94 mRNA. There is a similar decrease in the immunorecognizable grp94 by the 9G10 monoclonal antibody, which is not reflecting the decrease of the total grp94 protein and may be related to changes in the glycosylation pattern of diabetic grp94 (Csermely, 1994; Szántó *et al.*, 1995).

5.4. *Cancer*

Both hsp90 and grp94 are frequently up-regulated in tumor cells experiencing various types of stress, such as acidic pH, a scarcity of nutrients, and fluctuations of oxygen supply (Gabai and Kabakov, 1994). Thus, constitutively elevated levels of hsp90 (including most of the time the otherwise not constitutively expressed α isoform) were found in ras-transformed cells (Lebeau *et al.*, 1991), in other malignant cell lines (Legagneux *et al.*, 1989; Ferrarini *et al.*, 1992; Gabai *et al.*, 1995), in acute leukemias (Yufu *et al.*, 1992; Chant *et al.*, 1995), in melanomas (Pia Protti *et al.*, 1994), in gastrointestinal cancers (Ehrenfried *et al.*, 1995), in ovarian cancers (Mileo *et al.*, 1990), and in pancreatic and endometrial carcinomas (Gress *et al.*, 1994; Nanbu *et al.*, 1996). grp94 was found to be up-regulated in colon adenocarcinoma (Menoret *et al.*, 1994) and in large, radiation-induced mouse fibrosarcomas (Cai *et al.*, 1993). Both hsp90 and grp94 are overexpressed in human breast cancer (Jameel *et al.*, 1992; Franzen *et al.*, 1996, 1997; Haverty *et al.*, 1997), where overexpression of hsp90-α is usually associated with poor prognosis (Yano *et al.*, 1996). Complementing these changes, the down-regulation of hsp90-β has been observed in the invasive and tumorigenic BC-61 subline of 8701-BC breast carcinoma cells (Luparello *et al.*, 1997). The "take-home thumb-rule" of Section 3.5 on cell cycle, differentiation, and apoptosis seems to be valid for malignant transformation as well: a higher level of heat-shock proteins, particularly hsp90-α, seems to be closely correlated with the overall proliferative potential of malignant cells. Elevated levels of heat-shock proteins may participate in the reorganization of chromatin structure, help in the maintenance of steroid- (especially estrogen-) dependent growth, and confer a significant advantage on tumor cells to survive in a hostile environment. Increased amounts of hsp90 may also lead to an increased drug resistance of certain tumors (Bertram *et al.*, 1996).

Interestingly, Kojika *et al.* (1996) reported some low-molecular mass (80 and 43 kDa), "aberrant" forms of hsp90 in human leukemic cells. Some tumor types show a variation in grp94 glycosylation detected by a change in the endoglycosidase H-sensitivity and by a different recognition by the 9G10 anti-grp94 antibody (Feldweg and Srivastava, 1995). These changes most probably reflect the versatile behaviour of grp94 in cells experiencing various degree of stress described in Section 2.2. The putative heparanase and protease (aminopeptidase) activities of grp94, together with its frequent expression on the surface of tumor cells (Srivastava *et al.*, 1986; De Vouge *et al.*, 1994; Graham, 1994; Srivastava, 1994; Lammert *et al.*, 1996),[11] may enable grp94 to act as a mediator of metastasis generation. However, the testing of the putative role of grp94 in promotion of metastasis formation is a task for future research.

More than 10 years ago, both grp94 and hsp90 were identified as tumor-specific antigens expressed on the surface of various tumor cells (Srivastava *et al.*, 1986; Ullrich *et al.*, 1986). Tumor immunogenicity resides not in the chaperones themselves, but in the great variety of associated tumor-specific peptides they carry. Tumor-specific, grp94-presented peptides are taken up by macrophages and presented by the macrophage MHC Class I molecules. These macrophages are able to prime cytotoxic T lymphocytes for an antitumor attack (Srivastava *et al.*, 1994; Udono *et al.*, 1994; Suto and Srivastava, 1995; Arnold *et al.*, 1995; see

[11] T. Schnaider, Cs. Sőti, and P. Csermely, unpublished observations.

Section 3.8 for details). The involvement of grp94 (and of the other peptide-presenting chaperones hsp70 and hsp90) in peptide presentation offers new and more flexible routes for antitumor vaccination by circumventing the strict requirements for autologous or HLA-matched cells (Blachere *et al.*, 1993; Srivastava *et al.*, 1994; Heike *et al.*, 1996; Udono and Srivastava, 1997; Tamura *et al.*, 1997).

5.5. *Stress Monitoring in Toxicology and in Public Health*

Heat-shock (stress) proteins are often used as biomarkers in environmental toxicology and in public health (Ryan and Hightower, 1996). hsp90 was found to be overexpressed after a treatment with pesticides (Bagchi *et al.*, 1996), antibiotics (Ohtani *et al.*, 1995), anticancer drugs (Satoh *et al.*, 1994), etc. grp94 was induced after cadmium exposure (Goering *et al.*, 1993). Due to their relatively minor inducibility, monitoring induction of hsp90 (or grp94) alone is not enough to judge the extent of stress in most cases. However, hsp90 expression in *Xenopus laevis* has been proposed as a potential additional biomarker besides the expression of hsp70 mRNA (Ali *et al.*, 1996). Following the expression of the 90-kDa chaperones in blood cells of workers in high-risk environments may also provide useful additional information to judge their exposure to the harmful effects.

6. CONCLUSIONS AND PERSPECTIVES

Bearing in mind the more than 500 references cited, it may seem rather provocative to state that we do not know too much about the cellular functions of the 90-kDa molecular chaperones. This recalls the well-known Indian story about the elephant and the blind men. We touch it, we smell it, but we still do not see it. We do have many of the important specific elements of the action of both hsp90 and grp94, but the frame is missing. Novel approaches are required to explore the "secret life of hsp90," the highly dynamic and rather low-affinity protein complexes of the protein. These approaches may shed light on the details of its association with the cytoskeleton and its possible involvement in protein targeting, in nuclear and in mitotic events.

The discovery of the "small brother," hsp75/TRAP-1, as a member of the 90-kDa chaperone family, further increases the number of open questions about the possible similarities and dissimilarities in the action of hsp90-α and -β isoforms. The elucidation of their role in various signalling events, in cell proliferation, in cell differentiation, and in development certainly will be a fruitful area of intensive research in the near future.

The crystallization of the N-terminal domain of hsp90 has significantly improved our understanding of the structure/function relationships of the protein. Further exploration of the three-dimensional structure of 90-kDa chaperones may elucidate the nature of their protein-binding sites and provide some clues to the versatile nature of the localization of grp94. Regulation of the 90-kDa chaperone function is also a highly unexplored area of hsp90- and grp94-related basic research.

Among clinical applications, hsp90-based vaccination or antibody treatment certainly will be a powerful tool in our fight against many infectious diseases. The grp94-mediated peptide presentation, which circumvents the self/nonself restrictions imposed by the MHC (see Section 3.8), offers new areas for antiviral and antitumor vaccination.

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Note Added in Proof

During the printing process of this review, an excellent overview has been published from Lindquist's laboratory on the possible *in vivo* functions of hsp90. Their data are in agreement with our assumption detailed in Section 3.9, suggesting that "hsp90 is not required for the *de novo* folding of most proteins, but it is required for a specific subset of proteins that have greater difficulty reaching their native conformations. Under conditions of stress, hsp90 does not generally protect proteins from thermal inactivation, but does enhance the rate at which a heat-damaged protein is reactivated (Nathan *et al.*, 1997). An elegant study of Nemoto and Sato (1998) lists suggestive evidence that hsp90 forms higher oligomers *in vivo*, an assumption in agreement with our proposal that hsp90 is involved in the organization of the cytoplasm as a possible constituent of a microtrabecular lattice-type meshwork.

hsp90 was shown to be esssential for the activation of the endothelial nitric oxide synthase by vascular endothelial growth factor, histamine, and fluid shear stress (Garcia-Cardena *et al.*, 1998). A recent report from Toft's laboratory provided experimental evidence for ATP binding to the N-terminal domain of hsp90 and showed that binding of its co-chaperone, p23, probably requires an interaction of both the N- and C-terminal domains (Grenert *et al.*, 1997). Hop, the co-chaperone of hsp90 and hsp70, binds to the ADP form of both proteins, and its binding to hsp90 is mutually exclusive with the binding of p23 (Johnson *et al.*, 1998). Recently, the existence of two target-binding sites located in its N- and C-terminal domains of hsp90 (Young *et al.*, 1997) were confirmed (Scheibel *et al.*, 1998). The protein and peptide binding specificity of the two sites are different, and binding to the N-terminal site can be completed with geldanamycin or ATP.

Recent studies provided a better identification of the binding elements of the grp94 promoter. The element contains a consensus repeat of CCAAT-N_9-CCACG called

"ERSE" after the name "ER stress response element," which binds various transcription factors, including the CCAAT binding protein, nuclear factor Y, Yin-Yang factor 1, and possibly the homologues of the transcription factor for the yeast "unfolded protein response" Hacl (A. Lee, H. Yoshida, and T. Yura, personal communication).

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