Evolution and function of diverse Hsp90 homologs and cochaperone proteins

Jill L. Johnson*

Department of Biological Sciences and the Program in Microbiology, Molecular Biology and Biochemistry, University of Idaho, Moscow ID 83844-3051, USA

Abstract

Members of the Hsp90 molecular chaperone family are found in the cytosol, ER, mitochondria and chloroplasts of eukaryotic cells, as well as in bacteria. These diverse family members cooperate with other proteins, such as the molecular chaperone Hsp70, to mediate protein folding, activation and assembly into multiprotein complexes. All examined Hsp90 homologs exhibit similar ATPase rates and undergo similar conformational changes. One of the key differences is that cytosolic Hsp90 interacts with a large number of cochaperones that regulate the ATPase activity of Hsp90 or have other functions, such as targeting clients to Hsp90. Diverse Hsp90 homologs appear to chaperone different types of client proteins. This difference may reflect either the pool of clients requiring Hsp90 function or the requirement for cochaperones to target clients to Hsp90. This review discusses known functions, similarities and differences between Hsp90 family members and how cochaperones are known to affect these functions. This article is part of a Special Issue entitled: Heat Shock Protein 90 (HSP90).

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Molecular chaperones assist the folding of newly synthesized or misfolded proteins, preventing their aggregation [1]. The highly conserved molecular chaperone Hsp90 (heat shock protein, 90 kDa) is a global cellular regulator critical for the folding and regulation of a wide array of cellular proteins, termed clients. Hsp90 interacts with clients in a dynamic ATP-dependent cycle. Although the features of client proteins that make them dependent on Hsp90 remain unclear, Hsp90 has conserved functions in client folding and/or stability, transport and/or assembly into multiprotein complexes. Cytosolic Hsp90 is a very abundant protein comprising up to 1–2% of soluble protein [2]. Genome-wide studies in Saccharomyces cerevisiae suggest that up to 10% of all proteins are directly or indirectly dependent on it for function [3,4].

There are up to four different homologs of Hsp90 in higher eukaryotes: cytosolic Hsp90, mitochondrial TRAP1 (tumor necrosis factor receptor-associated protein 1), GRP94 (94 kDa glucose-regulated protein) of the endoplasmic reticulum (ER) and chloroplast Hsp90C. The genes encoding Hsp90 family members have undergone multiple duplication events, and some homologs appear to have been lost from some species [5,6]. Lower eukaryotes, such as S. cerevisiae, contain only cytosolic Hsp90, where it is essential for viability [2]. Most eukaryotic species contain at least two genes encoding highly homologous isoforms of cytoplasmic Hsp90, which likely arose from separate gene duplication events [6]. Thus the two isoforms of H. sapiens or S. cerevisiae Hsp90 are more closely related to each other than to other homologs. Higher plants such as rice (O. sativa) have more than two genes encoding cytosolic Hsp90 (inset in Fig. 1). In contrast, Drosophila melanogaster, and Caenorhabditis elegans contain only one gene encoding cytosolic Hsp90.

Most bacteria have one homolog of Hsp90, known as HtpG (High temperature protein G). However, some bacterial species such as Streptomyces coelicolor, contain two homologs, although they share only 30% identity. HtpG is largely absent from Archaea [5]. Comparative analysis of available Hsp90 sequences has reached consensus about the evolution of some homologs and opposing views of the evolution of others [5–7]. Studies agree that GRP94 likely arose via gene duplication early during eukaryote evolution, and was subsequently lost from Fungi. Chloroplast Hsp90 likely arose after a duplicated form of GRP94 obtained a chloroplast leader sequence through mutation. The origin of TRAP1 is less clear. Chen et al., [5] suggested that the four eukaryotic lineages of cytoplasmic, ER, mitochondrial and chloroplast Hsp90s arose from duplications of the same version of HtpG. An alternative view is that the four lineages arose from an ancient ancestor of eukaryotes that harbored two distinct HtpG genes, one of which evolved into mitochondrial TRAP1, and the other which evolved into cytosolic and chloroplast forms [7]. Fig. 1 shows a phylogenetic tree based on alignment of Hsp90 family members present in representative prokaryotes (Escherichia coli and S. coelicolor) and eukaryotes (S. cerevisiae, Homo sapiens and Ozyra sativa). At the amino acid level, mammalian cytosolic Hsp90 shares
60% identity with *S. cerevisiae* Hsp90 and about 34% identity with *E. coli* HtpG.

2. In vivo functions of Hsp90 family members

Most of what is known about Hsp90 interaction with client proteins comes from analysis of cytosolic Hsp90, although specific clients of other homologs of Hsp90 have been identified in recent years. Table 1 lists representative clients of all Hsp90 family members [8–21]. Additional cytosolic Hsp90 clients are listed elsewhere [22]. The requirement for Hsp90 function is restricted to a subset of proteins that are inherently unstable or require help to fold properly. There is no discernable sequence or structural features shared between clients. Hsp90 appears to bind proteins that are partially folded or in a nearly native folded state [23,24]. Clients have been shown to interact with either the amino-terminal domain, or middle domain, or all three domains [23,25,26].

Hsp90 is required for activity of all clients, but there are differences in when and how Hsp90 is required. Hsp90 is continually required to maintain steroid hormone receptors in a nearly-completely folded conformation capable of ligand binding [27,28]. Some, but not all, protein kinases become unstable soon after Hsp90 function is reduced [29]. However, Hsp90 is continuously required for the activity, but not stability of the v-src kinase, and the p56-lck kinase requires Hsp90 only during synthesis [28,30]. A conserved function of Hsp90 is to promote assembly of multiprotein complexes; cytosolic Hsp90 stabilizes the Pih1 protein during assembly of the R2TP complex required for RNA processing [21] and HtpG stabilizes a rod linker polypeptide required for assembly of a light harvesting structure known as phycobilisomes in cyanobacteria [8]. Cytosolic Hsp90 also plays a role in transport of preproteins to the mitochondria and chloroplast [31,32].

The importance of the Hsp90 clients present in different cellular compartments is reflected in the requirement for the gene encoding the Hsp90 homolog for viability. Expression of at least one isoform of cytosolic Hsp90 is essential for viability of yeast, and cells expressing 5–10% of normal levels of Hsp90 exhibit temperature sensitive growth [33]. GRP94 is required for the function of insulin-like growth factor (IGF)-II and is essential for viability in mice [12]. The gene encoding Grp94 is also essential in *Drosophila*, and mutant larvae have defects in gut epithelium and exhibit a starvation-like phenotype [34]. Inhibition of TRAP1 function results in a severe reduction in mitochondrial function [35]. *Arabidopsis thaliana* containing an amino acid alteration in chloroplast Hsp90 exhibits pleitropic defects, including defects in chloroplast biogenesis [36]. Somewhat surprisingly, HtpG is not essential for viability, and HtpG disruption causes only mild growth defects in *E. coli*. However, HtpG was recently found to be required for thermotolerance in cyanobacteria and other functions have been proposed [8,9,37].

3. Functional differences between isoforms of Hsp90 expressed in the same cells

The mammalian isoforms of cytosolic Hsp90, Hsp90α and Hsp90β, are 85% identical, while the two isoforms of *S. cerevisiae*, Hsc82 and Hsp82 share 96% identity at the amino acid level. Although it is tempting to assume that highly homologous versions of Hsp90 that are found in the same cellular compartment have identical functions, isoform specific differences have been observed. Distinct functions of Hsp90α and Hsp90β have been identified. Hsp90α is secreted...
4. Hsp90 structure and function

Hsp90 is a flexible dimer that has weak ATPase activity (about 1 molecule of ATP per 1–2 min) [43–45]. All examined homologs of Hsp90 bind and hydrolyze ATP and contain three conserved domains: an N-terminal ATP-binding domain, a middle domain, and a carboxy-terminal dimerization domain. Hsp90 proteins range in size from 588 to 854 amino acids. The most highly conserved domain is the ATP binding domain (Fig. 2). Of the 17 amino acids conserved in all analyzed sequences, most of those amino acids are required for ATP binding and/or hydrolysis [5], TRAP1, GRP94 and Hsp90C contain amino terminal leader sequences, and GRP94 also contains an ER retention sequence. Hsp90C, a long hydrophilic leader peptide, is targeted to the mitochondria and/or hydrolysis domain [47]. A charged linker region of varying length that separates the ATP binding domain from the middle domain contributes to conformational flexibility. A charged linker that separates the ATP binding domain from the middle domain can form an additional dimerization interface [47]. Although the isolated amino-terminal domain is able to bind ATP, ATP hydrolysis requires interaction of flexible loop from the middle domain. A charged linker region of varying length that separates the ATP binding domain from the middle domain contributes to conformational flexibility in yeast [48]. Evolutionarily, Hsp90 is related to other ‘split ATPases’ such as DNA gyrase, the MutL family of DNA mismatch repair proteins and type II and type IV DNA topoisomerases [49].

All examined homologs of Hsp90 undergo conformational changes as they bind and hydrolyze ATP. In a simplified model, in the absence of ATP, Hsp90 is dimerized only at the carboxy-terminus, in what is known as the open conformation. Nucleotide binding promotes, but does not strictly require for, a series of conformational changes that result in the formation of an additional dimerization interface between the two amino-terminal domains. ATP hydrolysis results in formation of a distinct ADP-bound conformation. Differing rates of ATP hydrolysis and/or hydrolysis between the three conformational states have been observed between HtpG, yeast and human cytosolic Hsp90, GRP94 and TRAP1 [43,44,50–54]. It is unclear how these differences affect client folding, although they likely contribute to the ability of diverse homologs of Hsp90 to chaperone a wide range of client proteins in distinct cellular environments.

One of the conserved features of the different Hsp90 homologs is that nearly all of them are subject to inhibition by radicicol, geldanamycin, or derivatives of GA such as 17-AAG (various inhibitors and their effects are reviewed in [55]). Geldanamycin is a naturally occurring ansamycin compound produced by Streptomyces hygroscopicus that binds the ATP binding pocket, preventing ATP hydrolysis. The ability of these compounds to selectively inhibit purified Hsp90 or Hsp90 in live cells or lysates has been instrumental in identifying a wide range of clients and to understand how Hsp90 affects protein function. More selective Hsp90 inhibitors that target organellar TRAP1 or secreted Hsp90α have been developed [35,38]. Interestingly, Hsp90 isolated from C. elegans will not bind GA, and GA does not cause harmful growth defects in C. elegans. Since both C. elegans and S. hygroscopicus both live in soil, this is believed to be a case of adaptive evolution [56].

5. Cytosolic Hsp90 has a longer charged linker and interacts with multiple cochaperone proteins

Cytosolic Hsp90 has two structural features that are not present or as predominant in other homologs. One of these is the flexible charged linker that separates the ATP binding domain from the middle domain. Short deletions of the linker do not significantly disrupt Hsp90 function in S. cerevisiae. However, longer deletions disrupt the essential functions of Hsp90 and the ability of Hsp90 to undergo ATP-dependent conformational changes that result in formation of the closed complex characterized by dimerization of the amino-terminal domains [48]. Although the charged linker is recognizable in most other homologs of Hsp90, the linker is longest in cytosolic Hsp90. The other unique feature of cytosolic Hsp90 is the conserved motif (MEEVD) that is present at the extreme carboxy-terminus. This sequence, along with the similarly conserved terminal EEDV motif of cytosolic Hsp70, serves as the binding site for multiple cochaperones containing tetratricopeptide repeat (TPR) motifs [46].

As discussed in more detail below, the molecular chaperone Hsp70 also assists protein folding in an ATP-dependent manner. Hsp70 has two types of cochaperones, J proteins (named for the Hsp40 DnaJ) and NEFs (nucleotide exchange factors), which stimulate ATP hydrolysis and promote nucleotide exchange, respectively (reviewed in [57,58]). Cytosolic Hsp90 has multiple cochaperones (twelve in S. cerevisiae, more in mammalian cells), including some that stimulate ATP hydrolysis and some that inhibit ATP hydrolysis. Other cochaperones do not affect ATPase activity, but instead appear to target clients to Hsp90 or have other, as yet unknown, functions.

A list of the better-known cochaperones and some of their characteristics, such as binding site on Hsp90 is shown in Table 2 [59–75]. Hsp90 is more abundant than any individual cochaperone, and many of these

### Table 1: Known functions and clients of diverse Hsp90 family members.

<table>
<thead>
<tr>
<th>Hsp90 family member</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>HtpG: client stabilization during assembly of large multiprotein complexes; regulation of client activity</td>
<td>[8]</td>
</tr>
<tr>
<td>Phycobilisome rod linker polypeptide L10R</td>
<td>[9]</td>
</tr>
<tr>
<td>Ureoporphyriogen decarboxylase (HemE)</td>
<td>[10]</td>
</tr>
<tr>
<td>TRAP1: client stabilization and/or activation</td>
<td>[11]</td>
</tr>
<tr>
<td>Calcium binding protein Sorcin</td>
<td>[12]</td>
</tr>
<tr>
<td>Tumor necrosis factor receptor</td>
<td>[13]</td>
</tr>
<tr>
<td>GRP94: client stabilization and/or activation</td>
<td>[14]</td>
</tr>
<tr>
<td>Insulin-like growth factor (IGF)-II</td>
<td>[15]</td>
</tr>
<tr>
<td>Toll-like receptors</td>
<td>[16]</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>[17]</td>
</tr>
<tr>
<td>Hsp90C: Vesicle-inducing protein in plastids (VIPP1)</td>
<td>[18]</td>
</tr>
<tr>
<td>Hsp90: client stabilization during assembly of large multiprotein complexes; regulation of client activity; targeting of proteins to mitochondria or chloroplasts</td>
<td>[19]</td>
</tr>
<tr>
<td>Progesterone receptor, glucorticoid receptor</td>
<td>[20]</td>
</tr>
<tr>
<td>v-src, Raf-1, Akt Kinasers, Telomerase</td>
<td>[21]</td>
</tr>
<tr>
<td>Rvb1 and Rvb2 helicases</td>
<td>[22]</td>
</tr>
<tr>
<td>Many others</td>
<td>[23]</td>
</tr>
</tbody>
</table>

### Fig. 2: Schematic diagram of the domains of Hsp90.

Accession numbers used: E. coli HtpG: NP_415006.1, H. sapiens TRAP1: NP_057376, GRP94 NP_003290.1, Hsp90α P07900, O. sativa Hsp90C: XP_483065.1. Purple, leader sequence or ER retention sequence, blue ATP binding domain, gray, charged linker region, orange, middle domain, salmon dimerization domain, black co-chaperone binding site. The overall amino acid identity of these homologs ranges from 33 to 49% in pairwise comparison. The most conserved domain is the ATP binding domain, which is 41–55% identical in pairwise comparisons.

---

**Table 2:**

<table>
<thead>
<tr>
<th>Amino acid identity</th>
<th>41-55%</th>
<th>30-45%</th>
<th>22-25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP Middle D HtpG</td>
<td>624 aa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP Middle D Trap1</td>
<td>704 aa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP Middle D Grp94</td>
<td>803 aa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP Middle D Hsp90C</td>
<td>785 aa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP Middle D Hsp90</td>
<td>732 aa</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
cochaperones compete for the same binding site on Hsp90 or bind alternate conformations [76,77]. Thus, distinct Hsp90-cochaperone complexes may exist at any time. Some of the cochaperones have catalytic activities. PPS/Ppt is a protein phosphatase that is able to dephosphorylate either Hsp90 [78] or the cochaperone Cdc37 [79]. Cyp40 and FKBP51/52 are peptidyl-prolyl isomerases that are capable of binding immunosuppressants such as cyclosporine A or FK506, respectively [71]. As shown in Table 2, although most TPR-containing cochaperones bind the carboxy-terminal MEEVD acceptor site, two such cochaperones, GCUNC-45 and Sgt1 bind the amino-terminus of Hsp90. GCUNC-45 binds a similar sequence in the amino-terminal domain of Hsp90 [64]. Sgt1 binds Hsp90 through a p23-related domain, although p23/ Sba1 and Sgt1 have surprising differences in contact sites with Hsp90 [63].

Until recently, cytosolic Hsp90 was the only homolog known to be dependent on cochaperones for function. Homologs of the known cochaperones listed in Table 2 are not recognizable in bacteria or eukaryotic organelles. However, a recent report identified a cochaperone of Grp94 that is required to promote folding of a variety of Toll-like receptors. This protein, CNPY3, appears to bind the amino terminus of the nucleotide-free form of Grp94, and may play a role in targeting Toll-like receptors to Grp94 [78].

6. Conserved cooperation of Hsp70 and Hsp90 during client folding

Hsp70 and Hsp90 sequentially cooperate during client folding [80]. Hsp70 is a ubiquitous ATP-dependent molecular chaperone that is highly conserved and present in all species and all cellular compartments [81]. Hsp70s bind unfolded or partially unfolded polypeptides in an ATP-regulated cycle. Hsp70 functions with co-chaperone proteins that modulate its ATPase activity, including DnaJ/Hsp40, which stimulate Hsp70’s ATPase activity as well as a variety of NEFs (reviewed in [57,58]). For many proteins, interaction with the Hsp70/DnaJ/NEF system is sufficient for folding. However, some need additional folding by Hsp90, although the reason for this requirement remains vague. In S. cerevisiae, mutations in the Hsp40 Ydj1 or the NEF Sse1 are known to disrupt Hsp90 client activity [82,83], providing additional evidence of the important role of Hsp70 and Hsp90 cochaperones for Hsp90 client folding.

Purified cytosolic mammalian Hsp70 and Hsp90, in conjunction with DnaJ from E. coli are capable of refolding denatured luciferase, a model unfolded protein [84]. Other Hsp90 homologs have also been shown to cooperate with Hsp70 during client folding. HtpG was recently shown to cooperate with Hsp70, DnaJ and the NEF GrpE in luciferase refolding [85]. Organellar Hsp90 also cooperates with Hsp90, as evidenced by sequential interaction of BIP, an Hsp70 with DnaJ from GrpE in luciferase refolding [84,85]. Organellar Hsp90 also cooperates with Hsp70, DnaJ and the NEF [80]. Hsp90, although the reason for this requirement remains vague. In E. coli, mutations in the Hsp70 ATnPase domain, ATPase activity as well as a variety of NEFs (reviewed in [57,58]). For many proteins, interaction with the Hsp70/DnaJ/NEF system is sufficient for folding. However, some need additional folding by Hsp90, although the reason for this requirement remains vague. In S. cerevisiae, mutations in the Hsp40 Ydj1 or the NEF Sse1 are known to disrupt Hsp90 client activity [82,83], providing additional evidence of the important role of Hsp70 and Hsp90 cochaperones for Hsp90 client folding.

Purified cytosolic mammalian Hsp70 and Hsp90, in conjunction with DnaJ from E. coli are capable of refolding denatured luciferase, a model unfolded protein [84]. Other Hsp90 homologs have also been shown to cooperate with Hsp70 during client folding. HtpG was recently shown to cooperate with Hsp70, DnaJ and the NEF GrpE in luciferase refolding [85]. Organellar Hsp90 also cooperates with Hsp90, as evidenced by sequential interaction of BIP, an Hsp70 of the ER with Grp94 with immunoglobulin chains [14]. A complex of Hsp70 and Hsp90 homologs in the chloroplast has also been detected, suggesting similar functions [15].

What else is known about the ability of differing homologs of Hsp90 to chaperone the same client? The ability of purified cytosolic Hsp90/Hsp70/DnaJ or purified HtpG/DnaK/DnaJ/GrpE to refold denatured luciferase [84,85] suggests that at least some of the factors governing client specificity are shared among diverse family members. Cytosolic Hsp90 present in rabbit reticulocyte or wheat germ lysates was able to refold clients from a variety of sources, including steroid hormone receptors, the sigma1 protein of reovirus and the duck hepatitis virus reverse transcriptase [16,86-88]. Furthermore, heterologous steroid hormone receptors (such as glucocorticoid and estrogen receptors) or the v-src kinase expressed in yeast form complexes with Hsp90 and function in an Hsp90-dependent manner [89,90]. Few studies have examined the ability of non-cytosolic homologs of Hsp90 to rescue functions of cytosolic Hsp90, and vice versa. However, TRAP could not replace Hsp90 using a purified five-component system to reactivate the progesterone receptor [51]. Client specific functions may also exist even among the same type of Hsp90 isolated from different species. For example, purified E. coli HtpG could not prevent aggregation of phytochelatins rod linker polypeptide L30R protein, but purified HtpG from cyanobacteria was able to prevent aggregation [8].

7. Specific roles of cytosolic Hsp90 cochaperones during client folding

The cooperation of Hsp70, Hsp40, Hsp90 and Hsp90 cochaperones is exemplified by the model of folding of the progesterone and glucocorticoid receptors [91]. Newly synthesized receptor interacts first with Hsp40 and Hsp70. Transfer of the receptor to Hsp90 is mediated by the cochaperone Hop/Sti1, which directly binds both Hsp70 and Hsp90 through separate TPR domains. At this stage, receptor and Hop/Sti1 are bound to Hsp90 in the nucleotide free, open conformation in what is termed the intermediate complex. Progression to the mature complex of the receptor is dependent on ATP binding to Hsp90, which results in displacement of Hop/Sti1 by other TPR containing-cochaperones such as Cyp40/Cp6, formation of the closed Hsp90 conformation, and binding of p23/Sba1 to the dimerized amino-termini. A recent study suggests that a key intermediate in this process is formation of an Hsp90 complex containing one molecule of Hop/Sti1 and one molecule of Cyp40/Cp6 bound to the two separate MEEVD sites within an Hsp90 dimer [92]. Only the mature complex of the receptor characterized by p23 interaction is capable of high-affinity hormone binding. Hsp90 inhibitors such as GA block ATP binding, resulting in accumulation of the receptor in the intermediate conformation in vitro and reduced receptor ability to bind hormone in vivo and in vitro.

There is increasing evidence that some Hsp90 clients require the function of only a subset of cochaperones. For example, folding of

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>p23</td>
<td>Sba1</td>
<td>No</td>
<td>Weakly inhibits</td>
<td>N-terminus</td>
<td>Stabilizes closed conformation</td>
<td>[59-61]</td>
</tr>
<tr>
<td>Sgt1</td>
<td>Sgt1</td>
<td>Yes</td>
<td>No effect</td>
<td>N-terminus</td>
<td>TPR domain and domain with homology to p23</td>
<td>[62,63]</td>
</tr>
<tr>
<td>GCUNC-45</td>
<td>Cdc37</td>
<td>Yes</td>
<td>No effect</td>
<td>N-terminus</td>
<td>TPR domain</td>
<td>[64]</td>
</tr>
<tr>
<td>PS0/Cdc37</td>
<td>Cdc37</td>
<td>Yes</td>
<td>Inhibits</td>
<td>N-terminus</td>
<td>Binds kinase clients</td>
<td>[65]</td>
</tr>
<tr>
<td>Aha1</td>
<td>Aha1, Hch1</td>
<td>No</td>
<td>Stimulates</td>
<td>N-terminus and Middle domain</td>
<td>Potent activator of Hsp90 ATPase activity</td>
<td>[66,67]</td>
</tr>
<tr>
<td>Hop</td>
<td>Sti1</td>
<td>No</td>
<td>Sti1 inhibits</td>
<td>C-terminus</td>
<td>TPR domains, binds Hsp70 and Hsp90</td>
<td>[68,89]</td>
</tr>
<tr>
<td>PPS</td>
<td>Ppr1</td>
<td>No</td>
<td>None</td>
<td>C-terminus</td>
<td>TPR domain, phosphatase domain</td>
<td>[70]</td>
</tr>
<tr>
<td>FKBP51,52</td>
<td>Cpr6, Cpr7</td>
<td>No</td>
<td>None</td>
<td>C-terminus</td>
<td>TPR domain, peptidyl-prolyl isomerase</td>
<td>[71]</td>
</tr>
<tr>
<td>Cyp40</td>
<td>Cpr6, Cpr7</td>
<td>No</td>
<td>None</td>
<td>C-terminus</td>
<td>TPR domain</td>
<td>[72]</td>
</tr>
<tr>
<td>TTC4</td>
<td>Cpr6, Cpr7</td>
<td>No</td>
<td>None</td>
<td>C-terminus</td>
<td>TPR domain, activates ATPase activity of Hsp70</td>
<td>[73,74]</td>
</tr>
<tr>
<td>XAP2</td>
<td>Cpr6, Cpr7</td>
<td>No</td>
<td>None</td>
<td>C-terminus</td>
<td>TPR domain</td>
<td>[71]</td>
</tr>
<tr>
<td>AIP1</td>
<td>Cpr6, Cpr7</td>
<td>No</td>
<td>None</td>
<td>C-terminus</td>
<td>TPR domain</td>
<td>[75]</td>
</tr>
</tbody>
</table>
Hsp90-dependent protein kinases in vivo and in vitro is dependent on p50/Cdc37 [93,94]. Relatively few studies have used purified proteins to dissect the minimum components for client folding. Hsp70, the Hsp70 cofactor DnaJ/Hsp40 and Hsp90 are critical for client folding. Additional proteins are required in a client-specific manner. Refolding of the progesterone and glucocorticoid receptors required the presence of the Hsp90 cochaperones p23 and Hop [95,96]. In contrast, refolding of the Chk1 kinase required Hsp70, DnaK, Hsp90, the Hsp90 cochaperone Cdc37, and the protein kinase CK2, which is required for Cdc37 function [94].

8. Roles of cochaperones in targeting Hsp90 to specific targets

The presence of cochaperones in Hsp90-client complexes varies in a client-specific manner, with some cochaperones preferring certain types of clients. For example, Cdc37 binding is observed almost exclusively to protein kinases [93]. The type of bound cochaperone may dramatically alter client function. Cyp40/Cpr6, FKBP51 and FKBP52 have similar ATP-dependent interactions with Hsp90 and all are present in complexes with the progesterone and glucocorticoid receptors [71]. However, the relative ratio of the three cochaperones bound to differing steroid hormone receptors varies. In the case of the glucocorticoid receptor, FKBP52 is able to potentiate receptor activity, but this effect is blocked by expression of increasing amounts of FKBP51 [97].

9. Eukaryotic cells from different organisms contain differing collections of cochaperone proteins

Early comparisons of Hsp90 complexes with cochaperone proteins from different sources revealed the presence of similar complexes in yeast, rabbit reticulocyte lysate and wheat germ extracts [98,99]. However, as more cochaperones have been identified, it is clear that the types and numbers of cochaperones vary from species to species, and this may have dramatic effects on client folding. To get a better idea of just how much the composition of the Hsp90 complex varies, we looked for homologs of well-studied cochaperones in the genomes of 19 disparate eukaryotic organisms [100]. Quite surprisingly, no individual cochaperones were present in all 19 species, although five, Hop/Sti1, Pp5/Ppt1, Ahal, p23 and Sgt1 were present in 16/19 organisms examined. All co-chaperones were present in all examined branches of eukaryotes, suggesting that cochaperones have been selectively lost from various species. This suggests that each organism has its own complement of cochaperones, and thus the pattern of cochaperones bound to a particular client may vary in a species-specific manner.

The lack of conserved Hsp90 cochaperones is surprising given that Hsp70s are always assisted by a DnaJ/Hsp40 protein [reviewed in [57]]. However it is less surprising given that no cochaperones have yet been identified for HtpG, TRAP1 or Hsp90C, and a cochaperone for GRP94 was only recently discovered [78]. It is possible that a wider array of clients require cytosolic Hsp90, resulting in an increasingly complex Hsp90 cochaperone machine to accommodate a diverse clientele. For example, one of the key roles of Cdc37 is to prebind protein kinase clients and target them to Hsp90 [101]. Hop/Sti1 plays a similar role in presenting Hsp70-bound clients to Hsp90 [80]. Non-cytoplasmic Hsp90 homologs may not require clients to be presented in such a manner. Alternatively, chaperones may not be required for other homologs due to inherent differences in ATPase activities and/or ability to shift between the three conformational states [50].

One possible explanation for the absence of cochaperones in some organisms is that cochaperones have overlapping functions. The finding that each organism examined contained a gene encoding either Hop/Sti1 or Cdc37, the two cochaperones that are inhibitors of Hsp90 activity [69,102], suggests that the ability to fine tune the ATPase activity of Hsp90 in order to effect changes in client folding is essential. Overexpression of CDC37 was able to rescue defects in folding of the Ste11 kinase observed in a sti1 deletion strain [103]. However, overexpression of STI1 is not known to be able to rescue defects caused by loss or mutation of the essential gene CDC37. Thus it appears that the ability to chaperone a particular client is dependent on more than just the ability of these cochaperones to regulate Hsp90’s ATPase activity. Information about functional overlap between other cochaperones is sparse and comes mostly from studies in yeast. Loss of CPR7 causes a slow growth defect and Hsp90 client defects. Surprisingly, these defects cannot be rescued by overexpression of CPR6, which shares 38% amino acid identity with Cpr7, but they can be rescued by overexpression of CNST [73]. As more studies identify specific in vivo roles for individual cochaperones, it will be possible to determine the extent of overlapping functions.

An alternate explanation for the absence of cochaperones in some species is that clients that are dependent on a specific cochaperone in one species may not require Hsp90 for function in other species. Many mammalian kinases are dependent on both Cdc37 and Hsp90 for function (reviewed in [93]). In yeast, up to 65% of all kinases require Cdc37 for activity and stabilization [104]. However, a gene encoding Cdc37 was not evident in 10/19 species examined [100]. It is not known whether protein kinases from organisms lacking Cdc37 bind a different cochaperone or whether those kinases evolved in such a way as to be independent of Hsp90 for function. Since little is known about why a protein becomes dependent on Hsp90 for activity or stability, it is very hard to predict whether a protein requires Hsp90 for folding. The timing of client mutations that resulted in Hsp90 dependence could result in significant variation in the scope of Hsp90 clients in different organisms.

To date there have been no attempts at studying coevolution of Hsp90-cochaperone-client pairs. One approach to this question is to isolate the homologous client from different species and determine if it is dependent on Hsp90 and the same cochaperones across various species. Few such studies have been done. Hsp90 and Sgt1 have conserved roles in the folding and activation of NLR (nucleotide-binding domain and leucine-rich repeat containing) proteins in both plants and animals. Together, this demonstrates a conserved role for Hsp90 and Sgt1 in mediating innate immunity [105]. An alternate approach is a comparison of the spectrum of proteins found in complex with Hsp90 or cochaperones in different species. A recent study identified proteins bound to the p23 homolog in Toxoplasma gondii [106]. Many of these were protein kinases, but others were proteins that do not have established functional connections with Hsp90. Additional studies such as these may provide a valuable glimpse into how much the scope of Hsp90 clients varies among diverse organisms.

10. Concluding remarks

The varied composition of the Hsp90 molecular chaperone machine, both in different cellular compartments and in different organisms indicates that Hsp90 is very adaptable. The ability of Hsp90 to bind and stabilize proteins containing alterations that would otherwise result in reduced or altered function is thought to have played a role in evolution by allowing accumulation of polymorphic variants of critical signaling pathways [107]. As reviewed elsewhere in this volume, the adaptability of the Hsp90 system also lends itself to manipulation by cancer cells, viruses and other human pathogens. Hsp90 and many cochaperones are overexpressed in cancer cells, which are highly dependent on Hsp90 for growth [108-111]. Genes encoding Hsp90 are not present in any viruses, but many viral proteins are dependent on the host Hsp90 [112,113]. Other human pathogens are also dependent on Hsp90, either for function or during development of gene resistance [114]. A greater understanding of the mechanism of how Hsp90 and associated cochaperones recognize and mediate the functions of client proteins will shed light on all known functions of Hsp90, and undoubtedly uncover new functions as well.
References


J.C. Young, F.J. Hartl, Polypeptide release by Hsp90 involves ATP hydrolysis and is enhanced by the co-chaperone p23, EMBO J. 19 (2000) 5930–5940.


