Structure, Function, and Regulation of the Hsp90 Machinery

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Heat shock protein 90 (Hsp90) is a molecular chaperone involved in the maturation of a plethora of substrates ("clients"), including protein kinases, transcription factors, and E3 ubiquitin ligases, positioning Hsp90 as a central regulator of cellular proteostasis. Hsp90 undergoes large conformational changes during its ATPase cycle. The processing of clients by cytosolic Hsp90 is assisted by a cohort of cochaperones that affect client recruitment, Hsp90 ATPase function or conformational rearrangements in Hsp90. Because of the importance of Hsp90 in regulating central cellular pathways, strategies for the pharmacological inhibition of the Hsp90 machinery in diseases such as cancer and neurodegeneration are being developed. In this review, we summarize recent structural and mechanistic progress in defining the function of organelle-specific and cytosolic Hsp90, including the impact of individual cochaperones on the maturation of specific clients and complexes with clients as well as ways of exploiting Hsp90 as a drug target.

One of the prerequisites for life is to adapt to constantly changing environmental conditions. Besides exposure to toxins, harmful radiation, or other stressors, buffering thermal stress is of pivotal importance. Proteins are particularly prone to the detrimental effects of stress, owing to their inherent instability, which is a premise for structural and functional dynamics. In the crowded cellular environment, protein unfolding may lead to the formation of aggregates in addition to the obvious loss of protein function.

To counter stresses on the cellular level, the increased expression of a set of stress proteins among them molecular chaperones is induced. Interestingly, molecular chaperones also fulfill essential functions under physiological conditions (Hartl et al. 2011). Molecular chaperones are defined as proteins that aid in the folding of their substrates (also called clients), while not being part of the correctly folded, active structure. In eukaryotes, Hsp70 (heat shock protein 70) and Hsp90 are among the most prominent members of this family. Although Hsp70 has a broad substrate range, Hsp90 seems to perform more specific functions and has a narrower set of clients. Hsp90 acts rather late in the maturation process of a client protein, which, in some cases, is believed to add an additional layer of regulation.

Historically, Hsp90 was first identified in complexes with steroid hormone receptors

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(SHRs) and the oncoprotein viral Src kinase (v-Src) (Brugge et al. 1981; Dougherty et al. 1984). Since then, hundreds of Hsp90 clients have been found, many of which are central parts of essential cellular processes often involved in cell growth and proliferation. Hence, the potential of targeting Hsp90 pharmaceutically in the context of cancer therapy has gained much interest.

Hsp90—EVOLUTION AND ISOFORMS

Hsp90 is one of the most abundant proteins in the cell and is conserved from bacteria to man with a sequence similarity of 50% between *Escherichia coli* Hsp90 (high temperature protein G [HtpG]) and human Hsp90 (hHsp90). Archaea lack Hsp90 (Chen et al. 2006), although most bacteria harbor one copy of Hsp90. Gene duplication gave rise to a constitutively expressed isoform, named 82 kDa heat shock cognate protein (Hsc82) in *Saccharomyces cerevisiae* and Hsp90β in *Homo sapiens* and a heat-induced isoform (Hsp82 in yeast and Hsp90α in humans) (Gupta 1995; Johnson 2012). The closely related constitutive and heat-induced Hsp90 isoforms display substantial functional overlap, but isoform-specific functions have also emerged (Morano et al. 1999; Millson et al. 2007). The increased importance of the Hsp90 system in eukaryotes is also reflected in the essentiality of at least one Hsp90 copy in yeast, even under normal growth conditions (Borkovich et al. 1989), whereas the loss of HtpG is compatible with bacterial life even in the presence of heat stress (Bardwell and Craig 1988; Borkovich et al. 1989). *S. cerevisiae* Hsp82 and Hsc82 share 97% sequence identity and either isoform can rescue the deletion of the other (Borkovich et al. 1989; Morano et al. 1999). Whereas the constitutively expressed Hsc82 is only induced 1.5–fold to twofold upon heat shock, Hsp82 levels increase 20-fold in yeast (Borkovich et al. 1989). Interestingly, despite the high sequence similarity, Hsp82 seems to be more efficient in suppressing heat sensitivity of a yeast strain expressing either Hsp82 and Hsc82 (Morano et al. 1999). In humans, the constitutively expressed Hsp90β and the heat-induced Hsp90α share ~85% sequence identity. There is evidence that some clients are preferably chaperoned by one of the isoforms, and that the isoforms convey different levels of stress protection or inhibitor sensitivity (Millson et al. 2007).

Additionally, organelle-specific members of the Hsp90 family have evolved in multicellular eukaryotes: TRAP1 (tumor necrosis factor receptor associated protein 1) in mitochondria, Grp94/Gp96 (glucose-regulated protein 94) in the endoplasmic reticulum (ER) and chloroplast Hsp90 (cHsp90). Of note, although less studied, cytosolic Hsp90 is also found in the extracellular matrix and on cell surfaces, where it might modulate cell migration and has therefore sparked interest in the field of cancer research (Sidera et al. 2004; Wong and Jay 2016). In particular, Hsp90α, which is secreted especially during stress (Clayton et al. 2005; Li et al. 2007), was found to interact with matrix metalloproteinase 2 and, thus, promotes cancer cell invasiveness (Eustace et al. 2004). Yet, how Hsp90 is secreted through the plasma membrane is still an open question because Hsp90 lacks known signal peptide sequences (Picard 2004; McCready et al. 2010). A mechanism involving the cleavage of the carboxy-terminal MEEVD motif as a prerequisite for secretion has been proposed (Wang et al. 2009). Additionally, a potential involvement in Hsp90α secretion has been attributed to a hydrophobic patch adjacent to the Hsp90 linker region between the amino-terminal domain (NTD) and middle domain (MD) (Tsutsumi et al. 2009).

ER-localized Grp94 arose most likely via gene duplication in metazoa (Gupta 1995; Marzec et al. 2012). Compared with its cytosolic paralogs, Grp94 has a more specialized role in the maturation process of particular secretory and membrane-bound proteins, such as Toll-like receptors (TLRs) and integrins (Randow and Seed 2001; Yang et al. 2007; Liu et al. 2010; Staron et al. 2010). Whether Grp94 is also involved in immunoglobulin folding and assembly together with the ER-located Hsp70-binding immunoglobulin protein (BiP) is not entirely clear (Melnick et al. 1994; Yang and Li 2005; Liu and Li 2008). Loss of Grp94 during organismal development has detrimental effects (Wanderling et al. 2007; Maynard et al. 2010). In contrast, isolated mam-

malian cells do not rely on the presence of Grp94 for viability and in *Leishmania*—one of the few unicellular organisms expressing Grp94—it is not essential but modulates virulence (Randow and Seed 2001; Descoteaux et al. 2002). This suggests that Grp94 fulfills rather specific functions and is not required for the maturation of extracellular proteins per se because cells lacking Grp94 do not show global defects of cell-surface receptor presentation (Randow and Seed 2001). Grp94 harbors a carboxy-terminal KDEL motif for ER-retention and lacks the carboxy-terminal MEEVD sequence found in cytosolic Hsp90 (see below). Only a single cochaperone, named CNPY3 (canopy fibroblast growth factor signaling regulator 3) has been identified for Grp94, which is ER-specific and seems to collaborate with Grp94 in a client-specific way (Wakabayashi et al. 2006; Liu et al. 2010).

The mitochondrial Hsp90 paralog TRAP1 has been considerably less well studied than Grp94, but both have been linked to diseases like Parkinson's and cancer (Song et al. 1995; Im 2016; Masgras et al. 2017). TRAP1 shares 50% amino acid similarity with Hsp90β. It contains an amino-terminal leader sequence for mitochondrial import (Felts et al. 2000; Kang 2012), lacks the carboxy-terminal MEEVD motif, and cochaperones do not seem to exist in mitochondria (Felts et al. 2000). TRAP1 confers antioxidant properties to the cell (Hua et al. 2007; Im et al. 2007; Montesano Gesualdi et al. 2007), regulates mitochondrial permeability (Kang et al. 2007), and may have antiapoptotic properties (Masuda et al. 2004; Hua et al. 2007; Im 2016). Additionally, TRAP1 functions as a metabolic switch regulating the balance between oxidative phosphorylation and aerobic glycolysis (Yoshida et al. 2013).

cHsp90 has attracted less attention in the past, although it has an essential function in plant development (Inoue et al. 2013). One of the major roles of cHsp90 is associated with protein import into the organelle (Li and Chiu 2010; Flores-Pérez and Jarvis 2013; Inoue et al. 2013) and the maturation of photosynthesis-related proteins (Lin and Cheng 1997; Cao et al. 2000). Importantly, in *Chlamydomonas reinhardtii*, a "foldosome" has been identified composed of Hsp90C, Hsp70B, the chloroplast DnaJ homolog CDJ1, and the chloroplast GrpE homolog CGE1, representing orthologs of cytosolic Hsp90, Hsp70, Hsp40, and the Hsp70 nucleotide exchange factor GrpE (Willmund and Schroda 2005; Willmund et al. 2008).

Hsp90—STRUCTURE AND CONFORMATIONAL CYCLE

Cytosolic Hsp90

All members of the Hsp90 family comprise a common domain structure consisting of the nucleotide-binding NTD, the MD and the carboxy-terminal domain (CTD) (Fig. 1). Carboxy-terminal dimerization of two Hsp90 protomers gives rise to a V-shaped dimer with substantial conformational dynamics, allowing transient amino-terminal dimerization, which is essential for chaperone function (Prodromou et al. 2000). Whereas this general architecture is conserved from bacteria to man, slight but functionally important differences between Hsp90 paralogs and orthologs are evident (Chen et al. 2006).

The NTD is rich in β-strands and forms a nucleotide-binding pocket sharing a Bergerat fold with members of the GHKL superfamily (gyrase subunit B [GyrB], histidine kinase, and DNA mismatch repair protein MutL) (Dutta and Inouye 2000). Binding of adenosine triphosphate (ATP) occurs with low affinity (K_D ~400 μM), whereas the affinity for adenosine diphosphate (ADP) is considerably higher (K_D ~10 μM), implying Hsp90 is only active if the cellular ATP:ADP ratio favors ATP binding (Prodromou et al. 1997; Scheibel et al. 1997; Young and Hartl 2000; McLaughlin et al. 2002). The "split ATPase" nature of GHKL ATPases requires conformational rearrangements in Hsp90, which reposition the NTD and MD so that the γ-phosphate of ATP bound to the NTD contacts Arg380 (in yeast Hsp82) from the MD to hydrolyze ATP (Meyer et al. 2003; Cunningham et al. 2012). The Hsp90 nucleotide-binding pocket forces ATP to be bound in a unique conformation (Prodromou et al. 1997), raising the opportunity for specific inhibition of Hsp90 with chemical compounds like radicicol (RD) and geldanamycin (GA) (Gre-

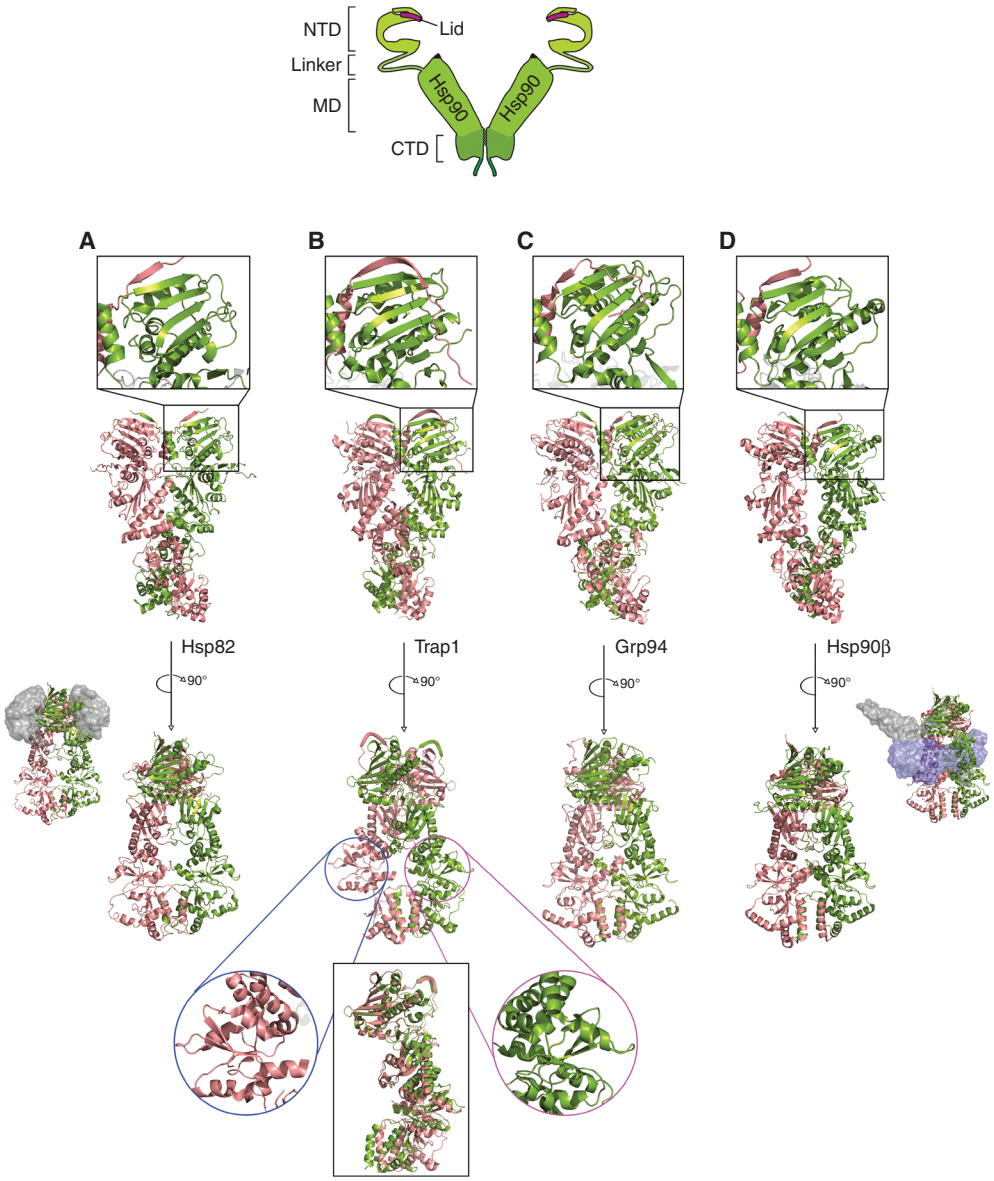

Figure 1. Structure of heat shock protein 90 (Hsp90) homologs. Structural models of (*A*) yeast Hsp90 (Protein Data Bank [PDB]: 2CG9), (*B*) mitochondrial tumor necrosis factor receptor associated protein 1 (TRAP1; PDB: 4IPE), (*C*) endoplasmic reticulum glucose-regulated protein 94 (Grp94; PDB: 5ULS), and (*D*) human cytosolic Hsp90β (PDB: 5FWL). For clarity, the Hsp90 protomers are distinctly colored. A schematic model of Hsp90 depicting the amino-terminal domain (NTD), middle domain (MD), and carboxy-terminal domain (CTD) as well as the linker and the adenosine triphosphate (ATP) lid is shown. In the *top* panel, the *insets* provide a zoomed view of the NTD of one protomer and the amino-terminal straps of the second protomer. In the *bottom* panel, the asymmetric conformation of the two protomers in TRAP1 is depicted (circles) and an alignment of the two protomers is shown (rectangle), highlighting the buckled conformation of the green protomer. Because the yeast Hsp82 and human Hsp90β structures were solved in complex with p23 (gray) or cell division control protein 37 (Cdc37; gray) and cyclin-dependent kinase 4 (Cdk4; blue), the full structures are shown on the *left* and *right*, respectively.

nert et al. 1997; Stebbins et al. 1997; Schulte et al. 1998; Roe et al. 1999; Chiosis et al. 2002). A charged, flexible linker (60 amino acids in yeast Hsp82) connects the NTD and MD, thus modulating domain contacts and Hsp90 chaperone function (Tsutsumi et al. 2012; Zuehlke and Johnson 2012; Jahn et al. 2014). Complete deletion of the linker is lethal in yeast and truncation of the linker interferes with client activation (Hainzl et al. 2009; Tsutsumi et al. 2012). Surprisingly, *E. coli* HtpG and mitochondrial TRAP1 lack a linker, although Grp94 harbors a linker that seems to be important for ATP binding and hydrolysis (Song et al. 1995; Schulte et al. 1999; Johnson 2012). The MD carries the binding site for Hsp90 clients and cochaperones. The CTD allows constitutive dimerization of Hsp90 through two carboxy-terminal helices forming a four-helix bundle (Ali et al. 2006; Pearl and Prodromou 2006). As mentioned, the carboxy-terminal MEEVD motif is present in cytosolic Hsp90 paralogs only. It mediates binding to tetratricopeptide repeat (TPR)-containing cochaperones.

After ATP binding to the NTD, the ATP lid closes over the bound nucleotide to yield a first intermediate state (Fig. 2). Further structural rearrangements lead to an amino-terminally

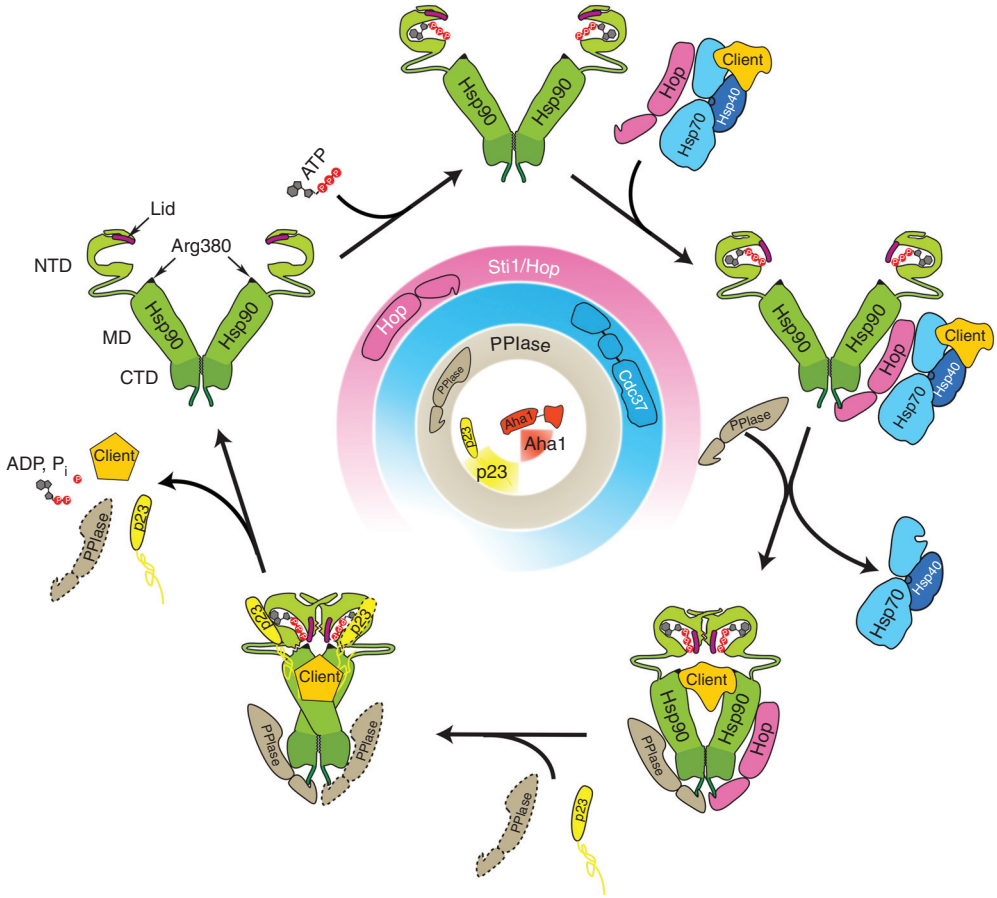

Figure 2. Hsp90 chaperone cycle. Hsp90 transitions through different conformational states during its ATPase cycle. Shown are intermediates of the cycle and how we envision client transfer and maturation. Some Hsp90 cochaperones preferentially bind specific Hsp90 conformations as indicated by the coloring of the circles in the middle and the association and dissociation of stress-inducible protein 1/Hsp70/Hsp90-organizing protein (Sti1/Hop) and large peptidylprolyl isomerases (PPIases) are depicted.

dimerized state (closed state 1) with swapped segments in the NTDs (Ali et al. 2006) and a compaction of the distance between the MD and NTD (closed state 2), resulting in the ATPase-competent conformation (Prodromou et al. 2000; Cunningham et al. 2008, 2012; Hessling et al. 2009). Subsequent release of ADP and phosphate as well as amino-terminal dissociation completes the Hsp90 chaperone cycle. Whereas the Hsp90 ATPase activity is essential in vivo (Obermann et al. 1998; Panaretou et al. 1998; Mishra and Bolon 2014), recent studies with the ATPase-deficient Hsp82 E33A mutant, which still binds ATP and undergoes conformational transitions, suggest that in principle the ability to sample different conformational states in defined dwell times is sufficient to support the essential function of Hsp90 in yeast (Zierer et al. 2016).

The general model of the Hsp90 ATPase cycle is applicable to all Hsp90 paralogs and isoforms. However, isoform-specific differences exist, which will be described below. Of note, eukaryotic Hsp90 works in a nondeterministic way and all conformations are accessible even in the absence of nucleotide, although bacterial HtpG seems to function in a more deterministic and nucleotide-dependent manner (Shiau et al. 2006; Southworth and Agard 2008; Graf et al. 2009; Mickler et al. 2009; Ratzke et al. 2012). In the light of an expansion of the "cochaperome" from none in bacteria via yeast (12 cochaperones) to humans (>20 cochaperones), it is tempting to speculate that the directionality of the conformational Hsp90 cycle in eukaryotes is increasingly influenced by cochaperones (Table 1) (Ratzke et al. 2014).

Hsp90 displays extremely slow ATPase kinetics that are limited by the large structural rearrangements during the cycle with conversion rates of 1 ATP min^{-1} for yeast Hsp82 and 0.1 ATP min^{-1} for hHsp90 (Prodromou et al. 1997; Scheibel et al. 1997; Stebbins et al. 1997; McLaughlin et al. 2002). Bacterial HtpG hydrolyzes ATP at a rate between that of Hsp82 and Hsp90. Interestingly, ATPase hydrolysis is pH-dependent (Cunningham et al. 2012; Jin et al. 2017). The ATPase rate of Grp94 is less clear and rates close to yeast Hsp90 and cytosolic hHsp90 have been reported (Dollins et al. 2007; Frey et al. 2007; Marzec et al. 2012). Different ATPase rates have also been suggested for human mitochondrial TRAP1, and importantly, ATPase activity seems to be strongly temperature-dependent with a 200-fold increase between 25°C and 55°C (Leskovar et al. 2008; Partridge et al. 2014; Jin et al. 2017).

Grp94 and TRAP1

The nucleotide-binding pocket of the NTD is considered the most conserved structural feature of Hsp90 (Fig. 1). The extended conformation of Grp94 is similar to Hsp82 and hHsp90α in the absence of nucleotides (Krukenberg et al. 2009). Similar to cytosolic, mammalian Hsp90, the equilibrium is only slightly shifted to the closed population when nucleotides are present (Krukenberg et al. 2009). Nucleotide binding induces large conformational changes in Grp94 that lead to a twisted conformation that is different from cytosolic Hsp90s (Dollins et al. 2007). This conformation is apparently not catalytically active, yet Grp94 possesses ATPase activity and this activity is required for chaperone function (Immormino et al. 2004; Dollins et al. 2007; Frey et al. 2007; Ostrovsky et al. 2009). In this structure of Grp94, the ATP lid, which contains a five-residue extension compared with cytosolic Hsp90, remains in an extended conformation, probably inhibiting ATPase function (Immormino et al. 2004; Dollins et al. 2007). Of note, a recent structure of fully closed Grp94 resolved a closed lid providing evidence for the generality of the Hsp90 ATPase cycle (Huck et al. 2017). Grp94, like cytosolic hHsp90α/β and TRAP1, carries an amino-terminal extension of 10–50 residues, which is absent in yeast and bacterial Hsp90 (Fig. 1C) (Chen et al. 2006). The Grp94 pre-N-domain "strap" is the longest in the Hsp90 family and was shown to suppress the Grp94 ATPase activity and regulate dimer closure (Dollins et al. 2007; Huck et al. 2017). In a crystal structure, the pre-N-domains swap across the two protomers to form loose contacts with the opposing monomer (Huck et al. 2017).

In TRAP1, the amino-terminal straps have been shown to form tighter contacts with the opposing protomer to stabilize the closed

Table 1. Diversity of heat shock protein 90 (Hsp90) cochaperones[a]

Cochaperone	Function	Expression levels in yeast (molecules per cell)[b]	Response to heat shock in yeast[c]	Affinity for Hsp90[d]	References
Yeast (Mammalia)					
Hsp82	Stress-induced isoform of Hsp90	445,000 T1/2 = 65 h	↑↑↑		
Hsc82	Constitutively expressed isoform of Hsp90	132,000–237,929 T1/2 = 16.6 h	-		
Sti1 (Hop)	Inhibits Hsp90 ATPase; Client handover from Hsp70 to Hsp90	52,260–67,600 T1/2 = 16.5 h	↑↑↑	Sti1: 0.04–0.24 μM (Hop: 0.7 μM)	Prodromou et al. 1999; Mayr et al. 2000; Richter et al. 2003; Siligardi et al. 2004; Onuoha et al. 2008
Cdc37	Inhibits ATPase by preventing dimer closing; kinase-specific	10,034–10,200 T1/2 = 11.7 h	-	yCdc37: 113 μM (hCdc37: 4 μM) (hCdc37 + yHsp82: 1.5–2.5 μM)	Siligardi et al. 2002; Roe et al. 2004; Zhang et al. 2004
Cns1 (Ttc4)	Genetic interaction with Cpr7 in yeast	672–3705 T1/2 = 9.4 h	-/↓	5 μM	Hainzl et al. 2004
Tah1	Part for the Rvb1-Rvb2-Tah1-Pih1 complex	1660	-/↓	0.8–1.6 μM	Millson et al. 2008; Eckert et al. 2010
Cpr6 (Cyp40)	PPIase	18,600–33,801 T1/2 = 18.8 h	↑↑↑	Cpr6: 0.014–0.3 μM (Cyp40: 0.23–3.8 μM)	Mayr et al. 2000; Pirkl and Buchner 2001; Onuoha et al. 2008
Cpr7 (Cyp40)	PPIase	1610–3230 T1/2 = 10.3 h	-	Cpr7: 0.06 μM	Mayr et al. 2000
Sba1 (p23)	Stabilizes closed state; inhibits ATPase; several Hsp90-independent functions	28,577–33,700 T1/2 = 9.9 h	-	Open Hsp82: 120 μM Closed Hsp82: 0.5–1.75 μM (Open Hsp90: 17 μM) (Closed Hsp90: 1.5 μM)	Richter et al. 2004; Siligardi et al. 2004; McLaughlin et al. 2006
Aha1	Activator of Hsp90 ATPase	13,900–17,266 T1/2 = 17.7 h	↑↑↑	0.16–3.8 μM	Panaretou et al. 2002; Meyer et al. 2004b; Koulov et al. 2010; Retzlaff et al. 2010; Li et al. 2013
Hch1	Homology with Aha1; yeast-specific	8530–28,484 T1/2 = 16.9 h	↑↑	1.9 μM	Panaretou et al. 2002

Continued

Table 1. *Continued*

Cochaperone	Function	Expression levels in yeast (molecules per cell)[b]	Response to heat shock in yeast[c]	Affinity for Hsp90[d]	References
Ppt1 (PP5)	Phosphatase; Regulates Cdc37	4688–6990 T1/2 = 8.7 h	↓↓↓	0.67 μM	Wandinger et al. 2006
Sgt1	Kinetochore assembly in yeast; TLR maturation in plants	1340–1666 T1/2 = 10.4 h	-	n.d.	
CHIP	E3 ubiquitin-ligase; involved in client degradation			(4.2–5 μM)	Millson et al. 2008; Kundrat and Regan 2010
FKPB51	PPIase			(0.2 μM)	Pirkl et al. 2001
FKBP52	PPIase			(0.06 μM)	Pirkl and Buchner 2001
NudC	Dynein-associated			n.d.	
UNC45	Assembly of myosin fibers			n.d.	

Hsc82, 82 kDa heat shock cognate protein; Sti1, stress-inducible protein 1; Hop, Hsp70/Hsp90-organizing protein; Cdc37, cell division control protein 37; Cns1, cyclophilin 7 suppressor 1; Ttc4, tetratricopeptide repeat (TPR) protein 4; Tah1, TPR-containing protein associated with Hsp90 1; Cpr 6, cyclosporin-sensitive proline rotamase 6; Cyp40, cyclophilin 40; Sba1, increased sensitivity to benzoquinone ansamycins 1; p23, p23/Sba1; Aha1, activator of Hsp90 adenosine triphosphatase (ATPase) protein 1; Hch1, high-copy Hsp90 suppressor 1; Ppt1, protein phosphatase T 1; PP5, protein phosphatase 5; Sgt1, suppressor of G2 allele of S-phase kinase-associated protein 1; CHIP, carboxyl terminus of Hsc70-interacting protein; FKBP51, tacrolimus (FK506)-binding protein 51; NudC, nuclear distribution protein C; UNC45, uncharacterized protein 45; Rvb 1, RuvB-like protein 1; Pih1, protein interacting with Hsp90 1; PPIase, peptidylprolyl isomerase; TLR, Toll-like receptor; hCdc37, human Cdc37; yCdc37, yeast Cdc37; yHsp82, yeast Hsp82; n.d., no data.

[a]This list is not conclusive but contains the best-studied Hsp90 cochaperones.

[b]Based on data in Ghaemmaghami et al. (2003), Christiano et al. (2014), and Kulak et al. (2014).

[c]Based on data in Gasch et al. (2000).

[d]Values for the yeast system are shown. When values for the human homologs are available, they are depicted in parentheses. Note that in some cases cochaperone binding is strongly dependent on salt concentrations as well as the nucleotide state of Hsp90.

conformation (Fig. 1D) (Lavery et al. 2014). Additionally, the pre-N-domain in TRAP1 is believed to convey temperature sensitivity to TRAP1 and regulate its ATPase rate (Partridge et al. 2014). Besides the missing charged linker between the NTD and MD, the most striking structural feature of TRAP1 is the marked asymmetry between the two monomers in the MD: CTD interface imparted by a helix swap, leaving one of the monomers in a buckled conformation (Fig. 1D) (Lavery et al. 2014). Different studies established a model for the TRAP1 ATPase cycle including an open apo-conformation, an intermediate state in which the NTDs come into proximity in a coiled coil dimer and a closed conformation (Leskovar et al. 2008; Lavery et al. 2014; Partridge et al. 2014; Sung et al. 2016; Elnatan et al. 2017). Of note, a sequential and deterministic mechanism for ATP hydrolysis has been proposed, in which the buckled monomer first hydrolyses ATP. Symmetry may then flip forcing the other monomer into a buckled conformation and after the second ATP hydrolysis the monomers can revert to an open state (Elnatan et al. 2017).

Hsp90—REGULATION

As Hsp90 is a key regulator of a plethora of cellular pathways that—if misregulated—may cause detrimental defects, its activity must be precisely controlled. The regulatory mechanisms that control Hsp90 have been reviewed in-depth elsewhere (Mollapour and Neckers 2012; Mayer and Le Breton 2015; Prodromou 2016; Sima and Richter 2018) and only an overview will be presented here. On the transcriptional level, Hsp90 expression is mainly induced by heat shock factor 1 (HSF1), which acts as a master regulator of the heat shock response (HSR) in eukaryotes (McMillan et al. 1998; Åkerfelt et al. 2010; Richter et al. 2010). HSF1 itself is regulated by chaperones, providing a direct link between proteome stress and expression of heat shock proteins (Voellmy and Boellmann 2007; Kijima et al. 2018; Zheng et al. 2018). Despite HSF1 function being conserved from yeast to man, yeast HSF1 is constitutively active and essential even under physiological conditions, whereas mammalian HSF1 is dispensable and remains in a repressed state in the absence of stress (Jakobsen and Pelham 1988; Sarge et al. 1993; Sistonen et al. 1994; Solís et al. 2016). Notably, a recent study showed that Hsp90 may play a role in modulating HSF1 dynamics by terminating HSF1 activity in mammalian cells (Kijima et al. 2018). Overexpression of Hsp70 and Hsp90 relieves the essential nature of HSF1 in yeast, stressing the central function of these chaperones in proteostasis (Solís et al. 2016). Importantly, other transcriptional regulators of Hsp90 like multicopy suppressor of SNF1 mutation 2/4 (Msn2/4) in yeast and signal transducer and activator of transcription 3 (STAT-3), nuclear factor for interleukin 6 (NF-IL6) and interferon γ (IFN-γ) in mammals have been identified (Gasch et al. 2000; Prodromou 2016).

Besides transcriptional regulation, Hsp90 is regulated by posttranslational modifications (PTMs), interaction with Hsp90 cochaperones (see below), and surprisingly, even by binding to clients. Numerous PTMs including small ubiquitin-like modifier addition (SUMOylation), acetylation, phosphorylation, and *S*-nitrosylation have been described and reviewed before (Mayer and Le Breton 2015; Prodromou 2016; Sima and Richter 2018). Notably, PTMs may not only have local effects that alter client and cochaperone binding, but modifications have been implicated in interdomain communication acting as conformational switches (Morra et al. 2009; Retzlaff et al. 2009; Mollapour and Neckers 2012; Soroka et al. 2012). We are far from understanding the effect of individual PTMs. Hyperphosphorylation of Hsp90 is negatively correlated with Hsp90 chaperone activity in vivo (Wandinger et al. 2006; Mollapour et al. 2011), yet, phosphorylation has also been reported to positively affect the maturation of some clients highlighting that dynamic PTMs are required for optimal Hsp90 function. Generally, acetylation and nitrosylation are considered to weaken the interaction of Hsp90 with clients, favoring their destabilization and degradation (Scroggins et al. 2007; Ai et al. 2009; Retzlaff et al. 2009; Zhang et al. 2010).

The up-regulation of Grp94 expression is one of the hallmarks of the unfolded protein response (UPR) and hence its regulation is distinct from

cytosolic Hsp90. Like the ER-Hsp70 BiP, Grp94 is regulated by the protein kinase R-like ER kinase/eukaryotic elongation factor 2 (PERK/eEF2), inositol-requiring enzyme 1/X-box-binding protein 1 (IRE1/XBP-1) and activating transcription factor 6 (ATF6) pathways and in homology with cytosolic Hsp90 and Hsp70, down-regulation of Grp94 induces the expression of BiP (Marzec et al. 2012). How TRAP1 activity is controlled is not very well understood (Altieri et al. 2012). It is up-regulated by the Myc oncogene, yet several posttranslational mechanisms are assumed to play a role in regulating mitochondrial TRAP1 levels because silencing of the transcription factor nuclear factor erythroid 2-related factor 2 (NRF2) resulted in reduced protein levels, despite transcription remaining unaltered (Coller et al. 2000; Kowalik et al. 2016). Both Grp94 and TRAP1 are also subject to PTMs, yet the biological function has remained elusive so far for many modifications (Cloutier and Coulombe 2013; Masgras et al. 2017).

Hsp90—COCHAPERONES

A plethora of cochaperones that interact with cytosolic Hsp90 have been identified, adding another layer of regulation to Hsp90 chaperone function (Table 1) (Schopf et al. 2017). Cochaperones are generally defined as proteins that interact with Hsp90 and assist its function, although they are not dependent on Hsp90 for their own folding and stability. All three Hsp90 domains harbor interaction sites for cochaperones (Li et al. 2012). These may bind in concert or antagonize each other. Moreover, they may play a role at different stages of the Hsp90 cycle, have different effects on the Hsp90 ATPase and show client specificity (Figs. 2 and 3; Table 1). Whereas they are structurally diverse, some structural commonalities have helped to categorize Hsp90 cochaperones.

Tetratricopeptide Repeat (TPR) Domain-Containing Cochaperones

Several cochaperones harboring the α-helical TPR domain interact with the carboxy-terminal Hsp90 MEEVD motif (Chen et al. 1998; Scheufler et al. 2000; Brinker et al. 2002). One of the best-studied TPR cochaperones is the adaptor Hop (Hsp70/90-organizing protein, stress-inducible protein 1 [Sti1] in yeast) acting as a linker between the Hsp70 and Hsp90 systems (Fig. 3). Containing three TPR domains (TPR1, TPR2A, and TPR2B), Sti1/Hop can simultaneously bind Hsp70 and Hsp90 and support client transfer from Hsp70 to Hsp90 (Chen and Smith 1998; Johnson et al. 1998; Wegele et al. 2006; Rohl et al. 2015). Additionally, Sti1/Hop contains two aspartate and proline-rich domains (DP domains) that are important for client activation in vivo (Flom et al. 2007; Schmid et al. 2012). Importantly, the binding of Hsp90 to Sti1/Hop significantly impacts the association of Hsp70 (Rohl et al. 2015). Recent studies reveal a bipartite architecture of Sti1/Hop, in which an amino-terminal module consisting of TPR1/DP1 and a carboxy-terminal module consisting of TPR2A/TPR2B and DP2 are connected via a flexible linker (Rohl et al. 2015). Sti1/Hop seems to keep Hsp90 in a client-acceptor state by preventing Hsp90 closing and is thus a strong non-competitive ATPase inhibitor (Prodromou et al. 1999; Richter et al. 2003; Li et al. 2011). A largely open issue is why Sti1/Hop harbors three Hsp90/Hsp70-binding sites and how the different TPR and DP modules modulate the interaction with Hsp70 and Hsp90. The inhibition of amino-terminal dimerization is mediated by additional contacts of Sti1/Hop with the Hsp90 MD (Schmid et al. 2012). Interestingly, direct contacts between Hsp90 and Hsp70 have been reported in bacteria, which lack cochaperones, and also in yeast, suggesting that a direct transfer is also possible (Genest et al. 2015; Kravats et al. 2018). Consistent with this idea, the deletion of Sti1 in yeast is not lethal (Chang et al. 1997) and some Hsp90 clients do not show Sti1 dependence for their activity (Sahasrabudhe et al. 2017).

Protein phosphatase 5 (PP5; protein phosphatase T 1 [Ppt1] in yeast) is a TPR-containing phosphatase and cochaperone that dephosphorylates Hsp90 and the kinase-specific cochaperone cell division control protein 37 (Cdc37) (Wandinger et al. 2006; Vaughan et al. 2008). Besides, PP5 was shown to also dephosphorylate

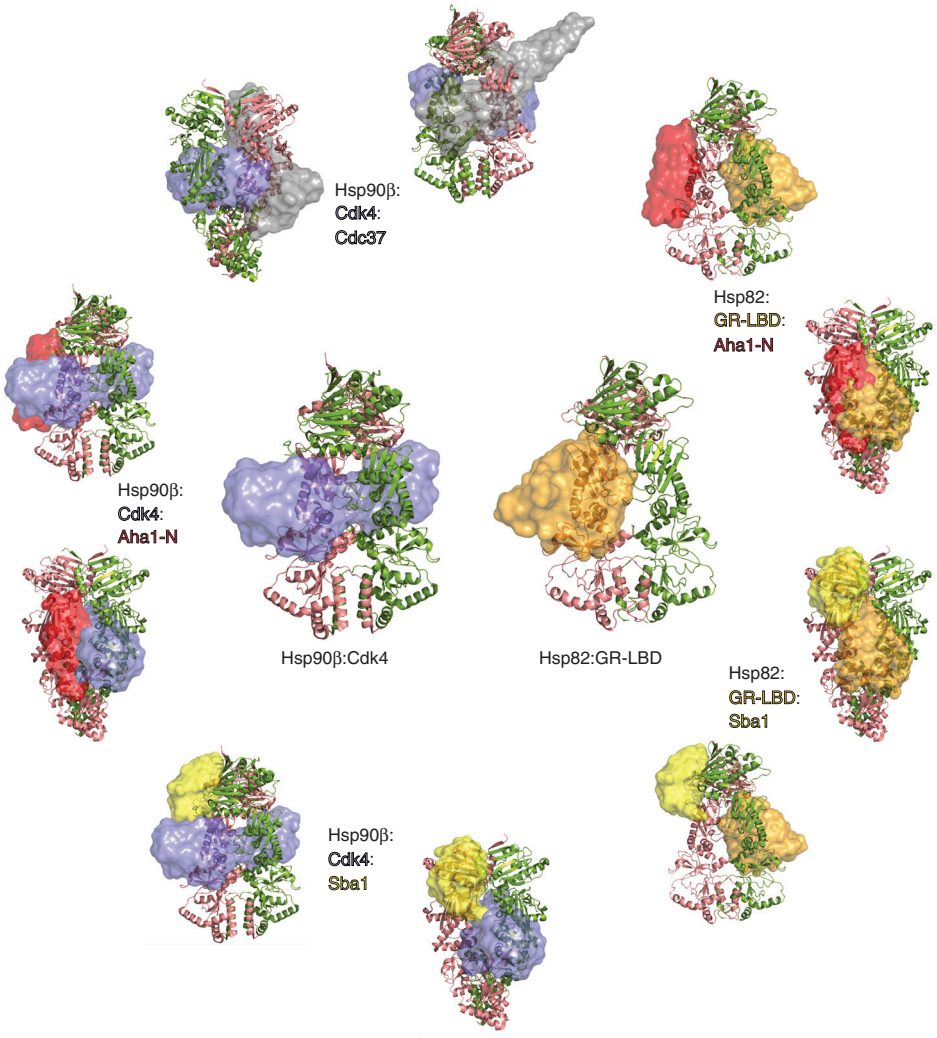

Figure 3. Hsp90 client comparison. Structural models showing the binding of Cdk4 (blue) to human Hsp90β and the glucocorticoid receptor (GR) ligand-binding domain (GR–LBD) (orange) to yeast Hsp82. The Hsp90β:Cdk4:Cdc37 complex is based on a cryogenic electron microscopy (cryo-EM) structure (PDB: 5FWL), whereas the Hsp90:GR–LBD:p23 structure is a pseudoatomic model combining data from cryo-EM, nuclear magnetic resonance (NMR) analysis, and small-angle X-ray scattering (SAXS) (Lorenz et al. 2014). Yeast Hsp82 (PDB: 2CG9) and human Hsp90β (PDB: 5FWL) were aligned in PyMOL to visualize the overlapping binding sites of the activator of Hsp90 ATPase protein 1 (Aha1) N-domain (red) and p23 (yellow) with the client-binding site. The binding site of the Aha1 N-domain was derived from an Aha1-N:Hsp90-MD crystal structure (PDB: 1USU) and docking experiments of Aha1-N on yeast Hsp82 (Retzlaff et al. 2010).

the tau protein, which interacts with microtubules and is involved in Alzheimer's disease (Shelton et al. 2017b). In the inactive state, PP5 is autoinhibited by the amino-terminal TPR domain blocking access to the carboxy-terminal phosphatase domain (Ramsey and Chinkers 2002; Yang et al. 2005). Hyperphosphorylation of Hsp90, attributable to the loss of Ppt1/PP5, has been shown to negatively affect client maturation and phosphorylation/dephosphorylation cycles of Cdc37, which are required for kinase maturation (Wandinger et al. 2006;

Vaughan et al. 2008; Soroka et al. 2012). Thus, Ppt1/PP5 is an important regulator of Hsp90 function.

cis/trans PPIases constitute another important group of TPR-containing cochaperones that include the structurally unrelated cyclophilin (Cyp) and tacrolimus (FK506)-binding protein (FKBP) families of prolyl isomerases. FKBP51, FKBP52, and Cyp40 represent the most important members of this cochaperone family in vertebrates. They all harbor a TPR domain that mediates Hsp90 binding and an amino-terminal PPIase domain (Ratajczak and Carrello 1996; Riggs et al. 2004). FKBP51, FKBP52, and Cyp40 show Hsp90-independent chaperone activity in vitro (Bose et al. 1996; Freeman et al. 1996; Pirkl and Buchner 2001). Strikingly, although the PPIase domain seems to be important for the function of PPIases, the enzymatic function seems to be dispensable for Hsp90-mediated client maturation (Riggs et al. 2003, 2007). The PPIase cochaperones present in yeast, cyclosporin-sensitive proline rotamase 6 (Cpr6) and Cpr7, are related to vertebrate Cyp40. Despite 47% sequence homology, Cpr6 and Cpr7 seem to have very different functions, which also holds true for FKBP51 and FKBP52 for which even opposing effects on glucocorticoid receptor (GR) activity have been shown (Hutchison et al. 1993; Smith and Toft 1993; Smith et al. 1993; Duina et al. 1996, 1998; Mayr et al. 2000; Riggs et al. 2003; Zuehlke and Johnson 2012; Zuehlke et al. 2013). Interestingly, although the PPIase activity of FKBPs seems irrelevant for GR activation, the differences between FKBP51 and FKBP52 seem to be largely defined by the structure of the PPIase domain (Riggs et al. 2007; Storer et al. 2011).

Little is known about the TPR-containing cochaperone cyclophilin 7 suppressor ([Cns1]; tetratricopeptide repeat protein 4 [Ttc4] in humans), one of the three essential cochaperones in yeast. An association of Cns1 and Cpr7 with the ribosome and a genetic interaction between Cns1 and Cpr7 have been shown, thus suggesting a link of Hsp90 to protein biosynthesis (Tesic et al. 2003; Tenge et al. 2015). Yet, the function of these cochaperones on translation has remained elusive so far.

Suppressor of G2 allele of S-phase kinase-associated protein 1 (Sgt1) is another essential cochaperone in yeast that also comprises a TPR domain in addition to a cysteine and histidine-rich domain (CHORD)-Sgt1 (CS) and a Sgt1-specific (SGS) domain (Azevedo et al. 2002). Surprisingly, it binds to the Hsp90 NTD in a TPR-independent manner (Zhang et al. 2008a). In yeast, Sgt1 was shown to be involved in kinetochore assembly and in higher eukaryotes in the activation of leucine-rich repeat (LRR) receptors that are pivotal in the innate immune defense (Kitagawa et al. 1999; Catlett and Kaplan 2006; Mayor et al. 2007).

Non-TPR-Containing Cochaperones

The preference of cochaperones for certain structural motifs in clients and the partaking in the maturation of specific clients is not limited to Sgt1 (Taipale et al. 2014). The cochaperone TPR-containing protein associated with Hsp90 1 (Tah1) and protein interacting with Hsp90 1 (Pih1) are suggested to specifically promote the assembly of the RuvB-like protein 1 (Rvb1)-Rvb2-Tah1-Pih1 (R2TP) complex that is required for small nucleolar ribonucleoprotein (snoRNP) biogenesis (Zhao et al. 2005, 2008). For the formation of this complex, the stabilizing effect of Tah1 on Pih1 is required, which in turn binds Rvb1/2 (Kakihara and Houry 2012).

Cdc37 represents the third essential cochaperone in yeast and is considered a kinase-specific cochaperone involved in the recruitment and maturation of up to 60% of human kinases that depend on Hsp90 (Grammatikakis et al. 1999; Citri et al. 2006; Taipale et al. 2012). Cdc37 forms contacts with the NTD of Hsp90 and partially inhibits its ATPase activity (Siligardi et al. 2002; Roe et al. 2004). Yet, contacts to the MD have also been proposed (Eckl et al. 2013, 2015). A recent cryogenic electron microscopy (cryo-EM) structure finally resolved the structure of Cdc37 and the kinase cyclin-dependent kinase 4 (Cdk4) bound to hHsp90 (Verba et al. 2016). Cdc37 is wrapped around one of the Hsp90 monomers with the kinase clamped between the Hsp90 monomers (Verba et al. 2016). This structure has important implications on

the mechanism of the Hsp90:kinase interaction (see below).

p23/Sba1 binds to a different binding site as shown in the crystal structure with yeast Hsp82 (Fig. 3) (Ali et al. 2006). The binding site is conserved in hHsp90 as determined by nuclear magnetic resonance (NMR) spectroscopy (Karagoz et al. 2011) and two p23 molecules seem to be able to bind simultaneously to opposite faces of the Hsp90 dimer (Richter et al. 2004; Ali et al. 2006; McLaughlin et al. 2006). The cochaperone stabilizes the closed Hsp90 dimer and inhibits the Hsp90 ATPase (Richter et al. 2004; McLaughlin et al. 2006). Notably, ATP is bound to the Hsp90:p23 complex, suggesting a noncompetitive mechanism of inhibition (Sullivan et al. 1997; Richter et al. 2004; Ali et al. 2006). p23/Sba1 comprises a folded domain and an unstructured carboxy-terminal tail that is important for chaperone function (Weikl et al. 1999; Weaver et al. 2000). Of note, it was also shown that p23 shows Hsp90-independent chaperone activity (Bose et al. 1996; Freeman et al. 1996). In addition to the central role p23/Sba1 plays in the Hsp90 cycle, p23/Sba1 also exerts Hsp90-independent functions. These include chromatin remodeling, ribosome biogenesis (Echtenkamp et al. 2011, 2016), and Hsp90-independent interaction with tumor-suppressor protein 53 (p53), which is supposed to compete with its DNA binding (Wu et al. 2018).

So far, activator of Hsp90 ATPase protein 1 (Aha1) is one of the few known cochaperones that strongly increases the Hsp90 ATPase rate. In yeast, Cpr6 and high-copy Hsp90 suppressor 1 (Hch1) were found to also moderately increase ATP hydrolysis (Panaretou et al. 2002). Mechanistically, Aha1 facilitates amino-terminal dimerization of yeast Hsp90 in an asymmetric manner, in which one Aha1 molecule is sufficient to accelerate the cycle (Figs. 2 and 3) (Panaretou et al. 2002; Meyer et al. 2004b; Retzlaff et al. 2010). Binding of Aha1 has been mapped to the Hsp90 NTD and MD (Meyer et al. 2004a; Retzlaff et al. 2010). It has been suggested that Aha1 acts as a regulator of the dwell time Hsp90 spends bound to a client (Koulov et al. 2010). This is consistent with findings that revealed a rescue of misfolded cystic fibrosis transmembrane conductance regulator (CFTR) upon Aha1 down-regulation in mammals and, surprisingly, activation of the GR in yeast when Aha1 was knocked out (Wang et al. 2006; Dunn et al. 2015; Sahasrabudhe et al. 2017). Overlapping binding sites of Aha1 and GR on Hsp90 suggest competitive binding as a way of regulating GR activity (Lorenz et al. 2014; Sahasrabudhe et al. 2017). The identification of small molecules that functionally impair the Aha1–Hsp90 interaction may therefore prove therapeutically useful in the future (Stiegler et al. 2017). Of note, Aha1 has a homolog, Hch1, in yeast that was lost during evolution in higher eukaryotes. Interestingly, phosphorylation of Tyr627 in hHsp90 is suspected to functionally replace Hch1 because this PTM has comparable effects on client activity as Hch1 has in yeast (Zuehlke et al. 2017).

Recently, Tsc1 from the tuberous sclerosis complex (TSC) has been identified as a bona fide Hsp90 cochaperone that inhibits Hsp90 ATPase and modulates client maturation (Woodford et al. 2017). Importantly, Tsc1 forms a trimeric complex with Tsc2 and Hsp90, in which Tsc2 is stabilized. How Tsc1 binds and regulates Hsp90 is largely unknown, but contacts with the CTD and MD have been proposed (Woodford et al. 2017).

Owing to the notable molar excess of Hsp90 over cochaperones in vivo (Table 1), symmetric complexes of two cochaperones bound to an Hsp90 dimer are unlikely. However, specific mixed cochaperone:Hsp90 complexes such as the Hsp90:Sti1:Cpr6 and Hsp90:Aha1:Cpr6 complexes have been identified in yeast that are important for cycle progression (Fig. 2) (Li et al. 2011, 2013). Analyses of complexes with hHsp90, Hsp70, Hop, and FKBP52 also revealed the prevalence of mixed Hsp90:cochaperone complexes in the hHsp90 system (Ebong et al. 2011). Besides synergistic interactions, some cochaperones also compete for Hsp90 binding, further increasing the complexity of Hsp90 regulation (Li et al. 2011; Freilich et al. 2018). Importantly, a number of cochaperones strictly bind specific conformational states of Hsp90, which positions them at distinct points of the cycle. In the current model for the cochaperone-

assisted conformational cycle of yeast Hsp90, Sti1/Hop binds the open Hsp90 conformation and facilitates substrate transfer from the Hsp70 system (Fig. 2). Cdc37 specifically recruits kinases to Hsp90, although Sti1 is also important for kinase maturation (Lee et al. 2004; Taipale et al. 2014; Sahasrabudhe et al. 2017). How Cdc37 and Sti1 collaborate in client recruitment is poorly understood. Additionally, spontaneous binding of clients to Hsp90 and direct transfer from Hsp70 to Hsp90 are possible. PPIases like Cpr6 can bind to the second carboxy-terminal MEEVD motif simultaneously to Sti1. Binding of Aha1 competes with Sti1 binding and also accelerates the Hsp90 cycle by promoting amino-terminal dimerization. In the presence of ATP, Hsp90 transitions to closed state 1 and after further compaction to closed state 2 forming the "late complex," in which p23/Sba1 binds together with a PPIase. This interaction also weakens the Sti1/Hop binding in the absence of Aha1. Importantly, this model lacks information about the influence of clients on the association of cochaperones. Recently, it was shown that even closely related Hsp90 clients rely on an individual subset of cochaperones for their activation and even point mutations affecting client folding could change the cochaperone dependency (Sahasrabudhe et al. 2017). Some cochaperones like p23/Sba1 and Sgt1 were shown to have more general effects, whereas others impacted folding in a client-specific way. This suggests that the general Hsp90 cochaperone cycle is modulated to optimize folding for different clients. Importantly, however, client specificity of cochaperones does not exclude parallel, subtle effects of all cochaperones on a wider range of clients, for example, attributable to modulation of the Hsp90 ATPase and competition for binding sites. In conclusion, the general Hsp90 cycle involves the successive binding of cochaperones along the cycle. Additionally, the effect of cochaperones is tuned in a client-dependent manner to provide a suitable folding platform for each client. Another level of complexity is added to the system when we consider that some clients like p53 may change the chaperone and cochaperone landscape by inducing the expression of a subset of cochaperones (Mattison et al. 2017).

Hsp90—HETEROGENEOUS EFFECTS ON CLIENTS

Client Spectrum

Because many Hsp90 clients represent central hubs of complex biological pathways, Hsp90 is a key factor of cellular regulation. A comprehensive list of Hsp90 clients is maintained by the Picard laboratory (see www.picard.ch/downloads; Echeverría et al. 2011). Historically, SHRs, in particular the GR and the progesterone receptor (PR) as well as kinases like v-Src are the most intensively studied Hsp90 clients (Brugge et al. 1981; Pratt and Toft 1997; Pratt and Dittmar 1998). Notably, it is estimated that 60% of the human kinome are dependent on Hsp90 to some degree (Taipale et al. 2012). An emerging class of Hsp90 clients are E3 ubiquitin ligases, of which 30% interact with Hsp90, whereas, surprisingly, only 7% of transcription factors are Hsp90 interactors, although transcription factors have long been seen as bona fide Hsp90 clients (Taipale et al. 2012). In particular, p53 has found significant attention as an Hsp90 client, including cancer-associated mutants (Blagosklonny et al. 1996; Sepehrnia et al. 1996; Whitesell et al. 1998; Deb et al. 1999; Nagata et al. 1999; Rudiger et al. 2002; Wang and Chen 2003; Müller et al. 2004; Walerych et al. 2004; Alexandrova et al. 2015).

Of note, we usually refer to Hsp90 clients as proteins that constitutively require Hsp90 to maintain an active state. A recent study using multiplexed proteome dynamics profiling revealed a remarkably high number of clients that require Hsp90 only transiently during de novo synthesis (Savitski et al. 2018). Because of the short-lived nature of the Hsp90 dependence of these proteins, they have found little recognition in the past, but become relevant for better understanding the pleiotropic effects of Hsp90 inhibitors in therapy.

How Hsp90 Recognizes and Folds Clients

The determinants that define an Hsp90 client are still subject to debate and not very well understood. No common binding motifs, such as hydrophobic sequences that occur in Hsp70 substrates, are known in Hsp90 clients. In addition,

the dichotomous separation in client and nonclient has been challenged because a continuous spectrum of Hsp90-binding affinities has been reported in human cells (Taipale et al. 2012). Insight into client specificity has been obtained from studies with closely related client/nonclient pairs like v-Src and the nonclient kinase cellular Src kinase (c-Src), which share 98% sequence identity, suggesting that a combination of factors contribute to Hsp90 dependence, including folding cooperativity, overall stability, and reduced compactness (Falsone et al. 2004; Taipale et al. 2012; Boczek et al. 2015; Keramisanou et al. 2016; Savitski et al. 2018). In addition, the recent structure of Hsp90 in complex with Cdk4 and Cdc37 revealed the structural motif in kinases that is recognized by Hsp90 (Verba et al. 2016).

Clients can be grouped in a few different categories depending on the way Hsp90 aids in their maturation. Kinases seem to require Hsp90 to maintain a specific active state (Grammatikakis et al. 1999; Polier et al. 2013; Boczek et al. 2015). In the case of kinases, Hsp90 facilitates ATP binding by the kinase, which in turn stabilizes the kinase (Eckl et al. 2016). Another category relies on Hsp90 for the assembly of protein complexes such as the kinetochore, the R2TP complex involved in snoRNP biogenesis, and also the purinosome required for purine biosynthesis (Kitagawa et al. 1999; Zhao et al. 2008; Pedley et al. 2018). Yet, in a third category Hsp90 action favors ligand binding as it does in SHRs (Pratt and Dittmar 1998; Kirschke et al. 2014; Lorenz et al. 2014). Also, the heme insertion into β- and γ-globins is dependent on Hsp90 (Ghosh et al. 2018). Furthermore, Hsp90 also promotes heme insertion into soluble guanylyl cyclase and inducible nitric oxide (NO) synthase (Ghosh et al. 2011; Ghosh and Stuehr 2012). In analogy, the RNA-induced silencing complex component Argonaute 2 (Ago2) requires the action of Hsp90 to reach an open conformation capable of binding RNA as a ligand (Iki et al. 2010; Iwasaki et al. 2010; Tsuboyama et al. 2018).

Hsp90 Client-Binding Site

As with all chaperones, client maturation requires regulated binding to and release from Hsp90, which is accompanied by large conformational changes connected to the ATPase cycle. Consistently, clients bind with rather moderate affinity in the lower micromolar range (Müller et al. 2004; Karagoz et al. 2014; Lorenz et al. 2014). Interestingly, for some clients like the GR, affinity is affected by the Hsp90 conformation, whereas others bind to different conformations of Hsp90 (Karagoz et al. 2014; Lorenz et al. 2014). Additionally, binding of cochaperones and also PTMs of Hsp90 modulate client binding. This inherently dynamic system has rendered it difficult to capture defined Hsp90:client complexes and define a client-binding site. Initial mutational studies suggested the MD of Hsp90 as the client-binding site (Bohen and Yamamoto 1993; Nathan and Lindquist 1995; Genest et al. 2013). By now, several models and structures of Hsp90:client complexes are available that confirmed the Hsp90 MD as the primary client-binding site (Shiau et al. 2006; Lorenz et al. 2014; Verba et al. 2016; Radli and Rüdiger 2018). In addition, the NTD seems to contribute, depending on the client used. In particular, hydrophobic residues that become buried between the monomers in the closed state of Hsp90 seem to be important for client binding.

A combination of small-angle X-ray scattering (SAXS), NMR, EM, and biochemical studies revealed a binding site for GR in the Hsp90 MD, with some additional contacts in the NTD and CTD (Fig. 3) (Lorenz et al. 2014). In the model, simultaneous binding of two GR molecules on the opposing faces of the Hsp90 dimer is possible (Lorenz et al. 2014). The binding site is in agreement with previous mutational studies and overlaps with the Aha1-binding site suggesting competitive binding (Lorenz et al. 2014). Of note, binding of the GR–LBD was tightest when Hsp90 was not entirely closed, suggesting that access to the inner surface of the Hsp90 monomers is required for efficient client interaction (Lorenz et al. 2014).

The cryo-EM structure of the hHsp90:Cdk4:Cdc37 complex recently provided a detailed picture of the kinase-binding site on hHsp90, which significantly overlaps with the proposed binding site for GR (Fig. 3) (Verba et al. 2016). Surprisingly, in the structure, the β4–β5 strands of the

kinase are ripped apart and threaded through the orifice formed by the closed Hsp90 monomers, leaving the kinase N- and C-lobe on opposite sides of the Hsp90 dimer (Verba et al. 2016). This complex resembles the model of two GR molecules binding simultaneously to Hsp90 (Lorenz et al. 2014; Verba et al. 2016). This structure also revealed the significance of Cdc37 in the complex, which forms contacts with the kinase N-lobe that mimic interactions normally occurring between the N- and C-lobes (Verba et al. 2016). The open kinase conformation is in line with an NMR-based model, in which Cdc37 challenges kinases by exerting unfolding pressure on the N-lobe to select thermally instable kinases from the kinase pool (Keramisanou et al. 2016). How the open kinase conformation benefits activation is not entirely clear. One may speculate that the open conformation bound to Hsp90 represents an intermediate state that is required for ATP binding (Eckl et al. 2016; Verba et al. 2016). The Hsp90-dependent stabilization of this state could in turn be defined by the overall kinase stability and cooperativity of folding (Taipale et al. 2012; Boczek et al. 2015). Additionally, the conformation of Cdc37 in the complex opposes previous findings, which mapped the Cdc37-binding site also to the Hsp90 NTD (Roe et al. 2004; Eckl et al. 2013, 2015). Hence, one could hypothesize that Cdc37 can sample different binding modes on Hsp90, yet how the transition between these states could occur remains unknown.

Contacts of clients with Hsp90 are not limited to the MD, but also spread to the NTD and CTD. An ensemble of structural models obtained by NMR spectroscopy of the tau protein suggested contacts of the client to the NTD and MD (Karagoz et al. 2014).

Despite extensive studies with p53, the exact binding site on Hsp90 is still elusive. NMR, docking, and biochemical studies mapped the interaction mainly to the Hsp90 MD and CTD and suggested a dynamic interaction (Hagn et al. 2011; Park et al. 2011b). On the p53 side, the DNA-binding domain (DBD) mediates interaction with Hsp90 (Rudiger et al. 2002; Müller et al. 2004; Walerych et al. 2004). Although Hsp90 generally acts late in the folding process, it is, however, an open question whether p53 binds to Hsp90 in an unfolded state, a "molten globule" conformation or in a native state (Rudiger et al. 2002; Hagn et al. 2011; Park et al. 2011a).

In summary, the evidence for the Hsp90 client-binding site converges to the Hsp90 MD. Although the general binding site seems to be conserved between different clients, additional client-specific contacts with the Hsp90 NTD and CTD are possible. Also, cochaperones contribute to the overall binding as seen in the case of Cdc37. The effect Hsp90 has on a client depends on the nature of the client and accordingly at which point in the folding pathway a client binds Hsp90 may vary greatly.

Contrary to a unidirectional model in which Hsp90 regulates its clients, also regulation of Hsp90 by clients has been shown (Rutz et al. 2018). The solvent-exposed W300 residue in yeast Hsp82, which is located at the rim of the client-binding site, has previously been shown to affect the maturation of different Hsp90 clients (Hawle et al. 2006; Flom et al. 2012). This residue acts as a long-range molecular switch, which transduces information about the binding of a client to the Hsp90 ATPase and thus, promotes progression of the cycle (Rutz et al. 2018). Additionally, clients can affect the Hsp90 ATPase rate to optimize the dwell time in different states for their specific requirements (Kirschke et al. 2014; Lorenz et al. 2014).

Hsp90—DISEASES

As one of the most abundant proteins in the cell, Hsp90 is known to counter different types of stresses (Borkovich et al. 1989; Taipale et al. 2010; Schopf et al. 2017). Accordingly, a variety of diseases that cause or are the result of proteotoxic stress are associated with Hsp90. Among others, Hsp90 modulates viral and protozoan infections, neurodegenerative diseases, and is implicated in cancer.

Hsp90 and Cancer

Transformed cells are usually characterized by dramatic changes in cellular metabolism and increased growth rate. Besides elevated protein

biosynthesis, which is required for cell growth, cancer cells express mutated oncoproteins, which may rely on chaperones to remain active. Hsp90 regulates several oncoproteins such as v-Src, human epidermal growth factor receptor 2 (ErbB2), telomerase, and hypoxia-inducible factor 1 (HIF1) and thus controls several hubs that are misregulated in cancer (Miyata et al. 2013). Hence, Hsp90 is involved in every hallmark of cancer (Hanahan and Weinberg 2011). Accordingly, cancer cells become not only oncogene-addicted but also addicted to Hsp90. Indeed, elevated levels of chaperones including Hsp90 are found in many cancer cells (Calderwood et al. 2006; Calderwood and Gong 2016) and are generally associated with a negative prognosis (Pick et al. 2007; Dimas et al. 2018). The most prominent and particularly well-studied Hsp90 client that is associated with cancer is the tumor-suppressor gene p53, which is mutated in >50% of human cancer patients. Importantly, ~75% of all mutations occur within the DBD that is chaperoned by Hsp90 in the wild-type and mutated variant (Whitesell et al. 1998; Whitesell and Lindquist 2005; Schulz-Heddergott and Moll 2018). Owing to the central role of p53 in tumor progression, cancer cells quickly become addicted to mutated p53. It was recently shown that pharmacological inhibition of Hsp90 that led to p53 degradation significantly extended survival in mice harboring mutated p53 (Alexandrova et al. 2015).

By buffering the effect of destabilizing mutations, Hsp90 may promote genetic variation attributable to the accumulation of mutations, thus contributing to cancer progression and possibly counteracting the efficiency of compounds (Rutherford and Lindquist 1998). Together, these results suggest Hsp90 inhibition as a promising therapeutic option in cancer treatment. In addition, the rewiring of the chaperone network in 50% of human cancers leading to the formation of the so-called epichaperome was reported. In these cancers, a highly connected network comprised of Hsp90, Hsp70, and various cochaperones exists, which expands the functions of the chaperones (Rodina et al. 2016). Of note, Hsp90 in these complexes bound inhibitors more strongly (Rodina et al. 2016).

Hsp90 and Neurodegenerative Diseases

Toxic protein aggregation is a common hallmark of many neuropathies like Huntington's, Alzheimer's, amyotrophic lateral sclerosis, and Parkinson's. In each case, a specific protein prone to misfolding is causative of neuronal cell death and thus, development of symptoms. Many of these proteins are regulated by chaperones and Hsp90 has been associated with all mentioned neuropathies (Brehme et al. 2014; Lackie et al. 2017). However, the precise role Hsp90 plays in the maturation process of these clients and how Hsp90 inhibition can be exploited therapeutically is unclear. Although Hsp90 inhibition may be detrimental to folding and actually exacerbate aggregate formation, the pathogenic species could also be preferentially targeted for the carboxyl terminus of Hsc70-interacting protein (CHIP)-mediated degradation after Hsp90 inhibition (Connell et al. 2001; Luo et al. 2010).

Aggregation of aberrant amyloid β (Aβ) and tau protein, which causes Alzheimer's disease was found to be inhibited by Hsp90 in vitro (Evans et al. 2006). Inhibition of Hsp90 decreased the levels of phosphorylated tau protein, possibly attributable to inhibition of Hsp90-dependent kinases that phosphorylate tau (Dickey et al. 2006, 2007). In mice, treatment with Hsp90 inhibitors attenuated Aβ toxicity (Chen et al. 2014). Aggregation of α-synuclein in Parkinson's is also modulated by Hsp90 and both protective and aggregation-promoting roles have been reported (Falsone et al. 2009; Putcha et al. 2010; Daturpalli et al. 2013). In a current model, the protective mechanism of Hsp90 inhibition is attributed to increasing Hsp70 levels after Hsp90 inhibition, portraying the complexity of intervening with the chaperone system (Sittler et al. 2001; Daturpalli et al. 2013; Lackie et al. 2017).

Hsp90 and Psychiatric Diseases

Mood affective disorders have experienced increasing attention and prevalence in recent years. Intriguingly, because Hsp90 regulates the GR and the mineralocorticoid receptor, it regu-

lates the major effectors of the hypothalamic–pituitary–adrenal axis, our central psychological stress-response system. Of note, both hyper- and hyposensitivity to glucocorticoids have been associated with mood disorders. In this system, especially the action of Hsp90 and its cochaperone FKBP51 on GR seem to play a pivotal role. In agreement with this notion, the FKBP51-encoding gene has been associated with anxiety, depression, and schizophrenia (Menke et al. 2013; Fujii et al. 2014; Szczepankiewicz et al. 2014; Stamm et al. 2016). FKBP51 levels increase with age and are additionally affected by single-nucleotide polymorphisms (SNPs) (Fujii et al. 2014; Sabbagh et al. 2014). FKBP51 provides a negative feedback loop: It attenuates hormone affinity in the Hsp90:GR:FKBP51 complex and GR signaling induces FKBP51 expression (Scammell et al. 2001; Sanchez 2012; Criado-Marrero et al. 2018). In conjunction with an environmental trigger, these factors can contribute to the onset of affective mood diseases and have further pleiotropic effects because FKBP51 competes with other cochaperones for Hsp90 binding (Pirkl and Buchner 2001; Schulke et al. 2010).

Hsp90 Cochaperones and Disease

The pivotal function of FKBP51 in psychiatric diseases indicates that in addition to Hsp90 itself, cochaperones may also play an important role in this context. Indeed, several lines of evidence support this hypothesis. As mentioned, studies in yeast showed that different clients were modulated by remarkably different subsets of cochaperones. Importantly, this even held true for mutants of the same client protein (Sahasrabudhe et al. 2017).

Mutations in the CFTR channel are causative for cystic fibrosis. Our current understanding suggests that mutated CFTR is bound and trapped in the Hsp90 cycle, which eventually leads to its degradation (Loo et al. 1998; Koulov et al. 2010). Intriguingly, wild-type and ΔF508 CFTR bind distinct subsets of cochaperones and knockdown of Aha1 by small interfering RNA (siRNA) could partially rescue ΔF508 CFTR function (Wang et al. 2006).

Another study showed that Aha1 promotes fibril formation of recombinant tau in an Hsp90-dependent manner, whereas Cdc37, p23, FKBP51, and FKBP52 had no effect (Shelton et al. 2017a). Importantly, Aha1 overexpression also led to enhanced tau aggregation in a transgenic mouse model and in vitro, chemical disruption of the Aha1:Hsp90 complex could reverse the Aha1-mediated effect (Shelton et al. 2017a). In contrast, PP5 is known to dephosphorylate tau and restore the microtubule-binding ability and the PPIase Cyp40 prevented tau-induced toxicity in vivo (Gong et al. 2004; Baker et al. 2017). Additionally, depletion of Hop or CHIP entailed accumulation of tau proteins, indicating that these chaperones are necessary for tau clearance (Dickey et al. 2008; Jinwal et al. 2013).

In cancer, several cochaperones have been associated with cell proliferation, migration, and drug resistance. Sti1/Hop was found to be overexpressed in certain cancers (Chao et al. 2013; Carvalho da Fonseca et al. 2014) and to promote invasion when present in the extracellular space (Walsh et al. 2011). Interestingly, mutated p53 seems to induce the expression of Sti1/Hop (Mattison et al. 2017) and p23 was shown to directly bind and modulate p53 in a Hsp90-independent manner (Wu et al. 2018). Silencing of Cdc37 destabilized kinases and thus, sensitized cancer cells to Hsp90 inhibition (Smith et al. 2009). A similar effect was observed when Aha1 expression was reduced (Holmes et al. 2008).

In summary, these recent findings revealed that besides the obvious regulation of clients by Hsp70 and Hsp90, the complex network of cochaperones significantly affects client activation. This urges a shifting of attention from solely targeting the chaperones to also address cochaperone interactions for therapeutic intervention.

Hsp90—INHIBITORS

Amino-Terminal Inhibitors

Amino-terminal inhibitors bind selectively to the Hsp90 nucleotide-binding pocket mimicking the rare, kinked conformation of bound ATP

(Prodromou et al. 1997). The most prominent examples of amino-terminal inhibitors are RD and the ansamycin GA (Whitesell et al. 1994; Roe et al. 1999). Notably, derivatives of RD and GA have been explored in phase II and phase III clinical trials, but they were discontinued owing to lack of efficacy at the tolerated doses (Sidera and Patsavoudi 2014; Khandelwal et al. 2016). Despite the unsuccessful trials with RD and GA derivatives, synthetic amino-terminal inhibitors (Chiosis et al. 2002) are currently undergoing clinical trials (Zuehlke et al. 2018). Additionally, the homing of amino-terminal inhibitors to cancer cells is exploited by inhibitors conjugated to cytotoxins. This promising concept has shown favorable effects in preclinical studies and is currently being tested in clinical trials (Heske et al. 2016).

Carboxy-Terminal Inhibitors

Induction of the HSR after Hsp90 inhibition and the increasing resistance to inhibition is one of the major problems amino-terminal inhibitors face (Whitesell and Lindquist 2005; Neckers and Workman 2012). Novobiocin is the best-known carboxy-terminal Hsp90 inhibitor, which selectively binds to a site near the carboxy-terminal dimerization site (Marcu et al. 2000; Matts et al. 2011). Although novobiocin binds with low affinity, derivatives display more favorable properties (Yin et al. 2009; Eskew et al. 2011). Silibinin, another carboxy-terminal Hsp90 inhibitor has been found to alleviate symptoms in an allograft model of Cushing's disease (Riebold et al. 2015). Despite promising preclinical data until now, no carboxy-terminal inhibitor has reached clinical trials.

Disruption of Cochaperone Binding

As illustrated before, the influence of cochaperones on disease progression has lately found increased attention. Inhibition of Hsp90 is linked to severe side effects, owing to the central position of Hsp90 in the broad spectrum of biological functions. Thus, disrupting the effect of individual cochaperones may reduce the side effects of interfering with the Hsp90 system and allow more focused therapeutic use. Several compounds have been described that lead to the modulation of cochaperone binding (Brandt and Blagg 2009).

Different inhibitors that disturb the Hsp90:Cdc37 complex, such as withaferin A (Yu et al. 2010), celastrol (Zhang et al. 2008b), derrubone (Hadden et al. 2007), or kongensin A (Li et al. 2016) have been studied. Most of them rely on blocking key interactions between Hsp90 and Cdc37 (Li et al. 2018). On the other hand, the derrubone inhibitory mechanism seems to rely on the detrimental stabilization of a Hsp90:Cdc37:kinase complex (Hadden et al. 2007). It should be mentioned that in many cases these inhibitors also have additional effects on Hsp90 function and induce an HSR (Garg et al. 2016).

Besides several Cdc37 inhibitors, a smaller, but growing number of inhibitors for other cochaperones have been identified. Gedunin was found to induce apoptosis by binding p23 and inhibiting its association with Hsp90, although only moderately inducing Hsp70 expression (Patwardhan et al. 2013). Intriguingly, treatment of human cells led to a selective destabilization of SHRs, making it an interesting candidate for diseases caused by misregulated GR activity (Patwardhan et al. 2013). Most recently, a substance specifically disrupting the Hsp90:GR:FKBP51 complex was found, which did not impede the binding of FKPB52 to Hsp90 (Sabbagh et al. 2018). This is of significant interest, given the described association of FKBP51 with mood affective disorders.

Recently, a small molecule was identified that could inhibit the ATPase-activating function of Aha1 without disrupting the Hsp90:Aha1 complex (Stiegler et al. 2017). This opens the possibility to selectively affect Aha1 in the context of cancers, neuropathies, and cystic fibrosis.

Selective activators of PP5 have been developed that release the autoinhibition and might find an application in Alzheimer's disease because tau is dephosphorylated by PP5 (Liu et al. 2005; Haslbeck et al. 2015).

In summary, a variety of Hsp90 and cochaperone inhibitors have been identified that modulate their activities by different mechanisms.

The way of inhibitors into therapeutics has not been achieved so far because of lacking efficacy in clinical studies. The reason is not entirely clear, but may be rooted in the differential rewiring of the chaperome in cancers and insufficiently matching the use of Hsp90 inhibitors to the genetic profile of the patient (Hong et al. 2013; Rodina et al. 2016).

Simultaneously, research of the recent past brought forth improved candidates that are now tested in clinical trials and promising new concepts of how Hsp90 can be exploited for therapy. Combination approaches with Hsp90 and kinase inhibitors have been proven beneficial (Chiosis and Neckers 2006; Proia and Kaufmann 2015). Additionally, the aforementioned conjugation drugs that exploit the tumor selectivity of Hsp90 inhibitors to target a cytotoxin, represent a promising strategy to use Hsp90 inhibitors in therapy (Proia et al. 2015; Heske et al. 2016). Concluding, inhibitors that specifically target Hsp90:cochaperone complexes propose an exciting new field wherein we can interfere with the Hsp90 system while reducing unspecific side effects.

CONCLUSION AND OUTLOOK

In the past years, the combined use of cell biological, biochemical, and biophysical approaches shed light on key aspects of the Hsp90 machinery. We now have structural data for all known Hsp90 paralogs defining the conservation and variation in structural elements. Several studies have been able to map the elusive client-binding site on Hsp90, a central first step for understanding how the Hsp90 chaperone machinery promotes the folding of its clients. Additionally, we are beginning to understand the wealth of regulatory mechanisms governing and fine-tuning the Hsp90 machinery, including PTMs, transcriptional regulation, and cochaperones. Although we have information on the binding modes of most cochaperones, how complexes of Hsp90 with different cochaperones or Hsp90:cochaperone:client complexes are formed is largely unknown. The hHsp90:Cdk4:Cdc37 EM structure suggests a binding mode for Cdc37 and the kinase that could not be anticipated from previous studies. In the context of therapeutic approaches, different Hsp90 inhibitors have been tested in clinical trials. But the outcome concerning therapeutic effect did not meet the expectation. New concepts have been developed and are on their way to clinical trials. Here, the Achilles heel is that what makes Hsp90 attractive as a target in the first place causes problems in treatment: Hsp90 is deeply involved in many cellular pathways as a central hub of the homeostasis network (Taipale et al. 2012, 2014; Costanzo et al. 2016; Kuzmin et al. 2018; Savitski et al. 2018). Thus, the systems perspective of Hsp90 has to be considered as a central component allowing altering of the cellular protein homeostasis network.

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