View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Biochimica et Biophysica Acta 1823 (2012) 624–635

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

The Hsp90 chaperone machinery: Conformational dynamics and regulation by co-chaperones ☆

Jing Li [1], Joanna Soroka [1], Johannes Buchner *

Center for Integrated Protein Science at the Department Chemie, Technische Universität München, Germany

ARTICLE INFO

Article history:
Received 16 July 2011
Received in revised form 7 September 2011
Accepted 8 September 2011
Available online 16 September 2011

Keywords:
Co-chaperones
Hsp90 clients
Conformational regulation
ATPase
Posttranslational modifications

ABSTRACT

Hsp90 is a dimeric molecular chaperone required for the activation and stabilization of numerous client proteins many of which are involved in essential cellular processes like signal transduction pathways. This activation process is regulated by ATP-induced large conformational changes, co-chaperones and posttranslational modifications. For some co-chaperones, a detailed picture on their structures and functions exists, for others their contributions to the Hsp90 system is still unclear. Recent progress on the conformational dynamics of Hsp90 and how co-chaperones affect the Hsp90 chaperone cycle significantly increased our understanding of the gearings of this complex molecular machinery. This article is part of a Special Issue entitled: Heat Shock Protein 90 (Hsp90).

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Hsp90 is an evolutionarily conserved and highly abundant molecular chaperone that mediates many fundamental cellular processes including cell cycle control, cell survival, hormone signaling and response to cellular stress [1–5]. Thus, Hsp90 function is a key component providing maintenance of cellular homeostasis [6,7]. In eukaryotes, Hsp90 promotes the formation of the correct conformation and activation of more than 200 proteins referred as Hsp90 'clients' [2,8–10]. Since many of these proteins are deregulated in cancers, Hsp90 inhibition appears to be a promising therapeutic strategy for cancer treatment [11–13]. By buffering oncogenic mutations and protecting oncoproteins, Hsp90 assists the stabilization of tumorogenic cells that are often considered to be 'addicted' to Hsp90 [14,15]. However, there are numerous indications linking Hsp90 with cancer development and progression. Hsp90 is involved in cellular defense against cancer by directly interacting and stabilizing the tumor suppressor protein p53 [16]. Hsp90 chaperone activity is of high importance as mutations in this transcription factor were identified in more than half of all human tumors studies. In this context, Hsf1, whose activity is attenuated by association with Hsp90, has been shown to be a potent modifier of carcinogenesis [17]. Apparently, also normal cells use Hsp90 to increase their inherent genetic heterogeneity: compelling evidence indicates that Hsp90 plays a pivotal role in evolutionary processes by buffering mutations that occur during normal morphological evolution [18–20].

The recruitment and assembly with client proteins requires collaboration of eukaryotic Hsp90 with Hsp70 and a multitude of the accessory proteins called 'co-chaperones' to form large dynamic multi-chaperone complexes [21–23]. To accomplish its function, the Hsp70/Hsp90 machinery acts in concert with the ubiquitin-proteosome system directing misfolded proteins for degradation and thus plays an active role in protein quality control [24–26]. Unlike Hsp70 which binds to the nascent polypeptide chain, the association with Hsp90 occurs at a later stage of the client folding process.

In eukaryotes, Hsp90 is found in the cytosol, the nucleus and in organelles. The nuclear localized Hsp90 represents a small fraction of cytosolic Hsp90 under physiological conditions [27]. Two cytosolic Hsp90 isoforms exist: an inducible and a constitutive form, Hsp90α and Hsp90β in man and Hsp82 and Hsc82 in yeast [28]. Plants express several additional cytosolic isoforms and their high expression levels protect cells from deleterious effects of diverse environmental fluctuations and pathogens [29–31]. Organelle-specific Hsp90 forms exist in mitochondria (TRAP1), chloroplasts and endoplasmic reticulum (Grp94) [30,32,33]. Recent studies show that Hsp90 is not only present inside the cell, but also on the cell surface of various cell types and secreted into the extracellular space suggesting distinct extracellular chaperoning activity [12,34,35]. It seems that Hsp90 evolved to exert diverse functions in a variety of different organisms from bacteria to man. However, while being an essential protein in eukaryotes, its bacterial homologue HtpG appears to be dispensable [3,36]. Additional differences in the modes of action between eukaryotic and

☆ This article is part of a Special Issue entitled: Heat Shock Protein 90 (Hsp90).

* Corresponding author at: Center for Integrated Protein Science at the Department Chemie, Technische Universität München, Lichtenbergstrasse 4, 85747 Garching, Germany. Tel.: +49 89 289 13341; fax: +49 89 289 13345.
E-mail address: johannes.buchner@ch.tum.de (J. Buchner).
[1] These authors contribute equally to this article.

0167-4889/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2011.09.003

prokaryotic/organellar Hsp90s exist. While in eukaryotes cytosolic Hsp90 requires the assistance of large cohort of co-chaperones, its procaryotic homologue seems to act on its own. However, the structural organization and the mechanism of the ATPase cycle necessary for the Hsp90 chaperone activity are conserved among cytosolic and organellar species [37–41]. The clientele of prokaryotic Hsp90 is to be identified [42,43].

2. Structure and conformational dynamics of Hsp90

Hsp90 forms flexible homodimers, where each protomer contains an N-terminal ATP-binding domain (N-domain), followed by charged region of a variable length, a middle domain (M-domain) with binding sites for client proteins and co-chaperones [44–50] and a C-terminal dimerization domain (C-domain) with its C-terminal MEEVD motif anchoring various tetratricopeptide repeat (TPR) domain-containing co-chaperones [51–53]. Structural studies [54,55] revealed that Hsp90 adopts a number of structurally distinct conformations (Fig. 1). To establish a kinetic model of conformational rearrangements in Hsp90, sophisticated biophysical techiques including ensemble or single molecule fluorescence resonance energy transfer (FRET) and analytical ultracentrifugation (aUC) have been applied (Fig. 2a–c). This allowed dissecting the steps of the ATPase cycle in detail [56,57].

In the apo state, Hsp90 adopts predominantly an open V-shaped conformation. Binding of ATP to the N-terminal domain triggers repositioning of a lid segment and leads to the formation of the first intermediate state (I1). Concomitant structural changes induce accommodation of a closed state, in which the N-domains are dimerized and associated with the M-domains. This structural compaction represents the second intermediate state (I2), in which ATP is hydrolyzed. After ATP hydrolysis, the N-domains dissociate, release ADP, Pi and Hsp90 returns to its original conformation. The speed of the ATPase cycle which is dominated by these conformational changes, is slow, compared to other known ATP-dependent chaperones. Hsp90 from yeast hydrolyzes one ATP molecule per 1–2 min [58,59], and the ATP hydrolysis by human Hsp90 is tenfold slower than that of its yeast homologue [37,60,61]. Hsp90 belongs to the family of GHKL ATPases, which all share a similar architecture of the ATP binding pocket [38].

Though the role of nucleotide in structural movements has been well established, the conformations found in the absence of nucleotide are less well understood. Importantly, open and closed forms of Hsp90 have been detected even in the absence of nucleotide [57,62] suggesting that these movements can occur spontaneously and that a dynamic conformational equilibrium between different conformations exists. Further analysis of the C-terminal dimerization revealed anti-correlated motions of the C- and N-domains showing important communication pathways between remote regions of the protein [63]. The activity of Hsp90 is additionally regulated by posttranslational modifications of important switch point regions (Len Neckers and co-workers, this BBA issue).

3. Hsp90 co-chaperones

Various co-chaperones associate dynamically with Hsp90 during the chaperone cycle (Table 1). In eukaryotic cells, more than 20 co-chaperones have been identified to regulate the function of Hsp90 in different ways, such as the inhibition and activation of its ATPase activity as well as recruitment of specific client proteins [52,64–67]. Among them, the TPR co-chaperones, which recognize the C-terminal MEEVD motif in Hsp90 through a highly conserved clamp domain, are a prominent example. Structurally, TPR motifs consist of degenerated 34-amino acid repeats forming two anti-parallel α helices separated by a turn [68]. The helix–turn–helix motifs stack upon each to form a superhelical groove, which interacts with TPR acceptor modules [51]. TPR domain-containing co-chaperones include Hop [69] (yeast homologue Sti1 [70]), protein phosphatase PP5 [71] (yeast homologue Ppt1 [72]), J domain containing protein TPR2 [73], the myosin folding factor Unc45 [74,75] and members of peptidylprolylisomerase (PPIase) family, like Fkbp52 [76–79], Fkbp51 [80] and Cyp40 [81] (yeast homologues Cpr6/Cpr7 [82]).

The protein Hop/Sti1 binds and stabilizes the open conformation of Hsp90 and thus inhibits its ATPase activity [52,69,83]. The presence of three TPR domains allows for its simultaneous binding and modulation of Hsp70 and Hsp90, which leads to the facilitation of client

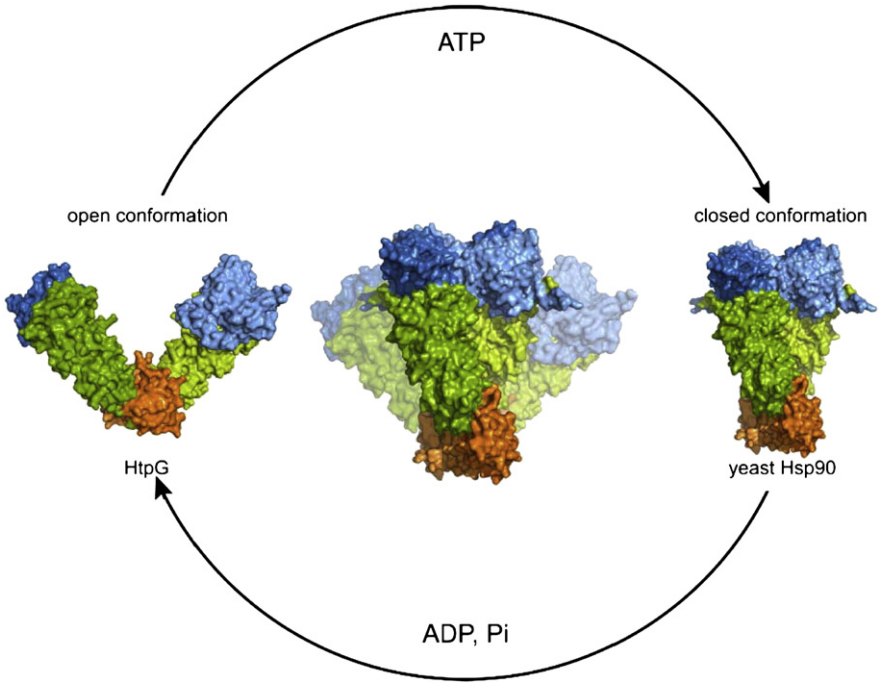

Fig. 1. Hsp90 structures crystal structures of full length Hsp90 from *E. coli* (HtpG) in the open conformation (left, PDB 2IOQ) and nucleotide-bound yeast Hsp90 in the closed conformation (right, PDB 2CG9). The N-domain is depicted in blue, the M-domain in green and the C-domain in orange.

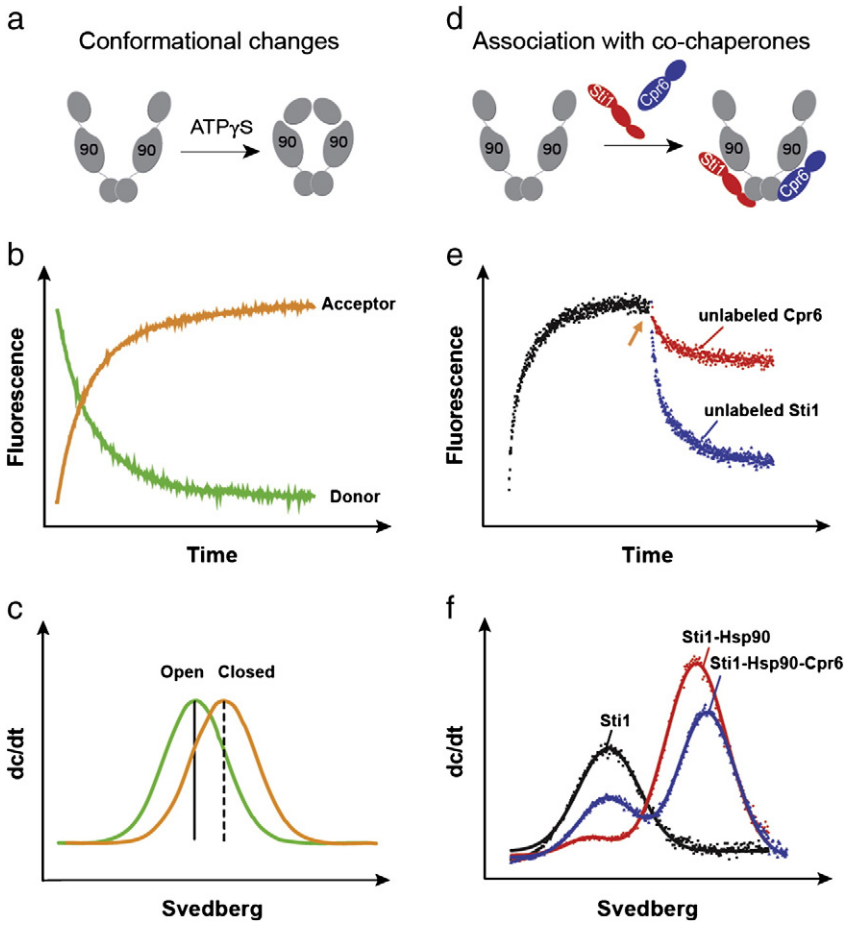

Fig. 2. Biophysical methods to investigate the Hsp90 chaperone machinery (a) Schematic representation of the conformational changes induced by binding of nucleotide to Hsp90. (b) Nucleotide-induced conformational rearrangements can be monitored by FRET or (c) analytical ultracentrifugation. (d) Schematic representation of complex formation with Hsp90 and the co-chaperones Sti1 and Cpr6. Association between Hsp90 and the co-chaperones can be detected using (e) FRET or (f) analytical ultracentrifugation.

protein transfer [67,84,85]. Biochemical studies showed that the TPR1 and TPR2a domain bind to the EEVD-containing C-terminus of Hsp70 and Hsp90, respectively (Fig. 3a) [51,86,87]. Recent results showed that Hop/Sti1 is a monomeric protein [88,89] and current models suggest that binding of one Hop/Sti1 monomer to one Hsp90 dimer is sufficient to inhibit the ATPase activity of Hsp90 dimer [89]. In this context it also needs to be considered that the concentration of Hsp90 co-chaperone is significantly lower than that of Hsp90 [90]. Functional analysis in yeast shows that Hop/Sti1 is not an essential protein, but lethality can be induced when Hop/Sti1 is knocked out with other genes such as the Hsp40 homologue Ydj1 or the Hsp90 co-chaperone p23/Sba1 [91–93]. Reconstitution studies showed that Hop/Sti1 is indispensable for maintaining the hormone binding activity of the progesterone receptor [94]. Recent results indicate that Hop/Sti1 has an influence on many different Hsp90 clients. For example, Lin and co-workers suggest that in *Drosophila* Hop/Sti1 is important for phenotypic stability and this involves a complex of Hop/Sti1 with Hsp90 and the protein Piwi [95]. S-nitrosylation or knockdown of Hop contributes to the maturation of mutant form cystic fibrosis transmembrane conductance regulator (CFTR) [96], similar to the co-chaperone Aha1 [97] (see also below), qualifying it as a new target for the treatment of cystic fibrosis.

In contrast to Hop/Sti1, p23/Sba1 binds specifically to the closed conformation of Hsp90 [98,99]. It was identified as a component in steroid receptors complex together with Hsp90 and a PPIase [100]. p23/Sba1 is a small acidic protein containing an unstructured C-terminal tail, which plays an important role in its intrinsic chaperone activity [101]. Structural studies revealed that the contact sites are prominently located in the N-domain of Hsp90 (Fig. 3b), but also with minor M-domain interaction [54]. In yeast, deletion of p23/Sba1 results in mild growth defects at both low and high temperature [91]. In mice, p23/Sba1 is not essential for prenatal development but necessary for perinatal survival, as the development of lungs functions is substantially impaired in p23/Sba1 knockout embryos [102,103].

p23/Sba1 facilitates the maturation of client proteins by stabilizing the closed conformation of Hsp90 [104]. As a result, the ATP hydrolysis, which is indispensable for the release of the client protein [58,105], is partially inhibited in the presence of p23/Sba1 [65,106]. From previous studies we know that p23/Sba1 is the limiting component for the stability of Hsp90-client protein heterocomplex [107]. Since p23/Sba1 possesses chaperone activity [108,109], it may interact directly with the client protein and may thus serve as the control of its conformation. For this function, the C-terminal tail of p23/Sba1 seems important [101,110].

Cdc37 is another co-chaperone which inhibits the ATPase activity of Hsp90 [111,112]. Originally, Cdc37 was identified in *S. cerevisiae* as a gene essential for cell cycle progression [113,114]. During the investigation of the oncoprotein v-Src, Cdc37 was found as a part of the Hsp90-kinase complex [115,116]. Further work in different organisms showed that Cdc37 is specific for chaperoning kinases [117]. It interacts with kinases through its N-terminal domain and binds to the N-domain of Hsp90 via its C-terminal parts (Fig. 3c). The ATPase arrest is mediated by the insertion of the Cdc37 R167 side chain into the nucleotide binding pocket of Hsp90. This directly inhibits the binding of ATP [54]. Furthermore, the binding of the Hsp90 lid segment prevents its closing of the ATP binding sites and blocks the access of catalytic residue of the

Table 1
Summary of Hsp90 co-chaperones.

Co-chaperones				Function
TPR co-chaperones				
Mammals		Yeast	Plant	
protein	Gene name (human)			
Hop	STIP1	Sti1	Hop	Scaffold for Hsp90/Hsp70 interaction; involved in client protein maturation; inhibition of Hsp90 ATPase
Fkbp52	FKBP4	None	AT5G48570[a]	Peptidy-prolyl-isomerase; chaperone; involved in client protein maturation
Fkbp51	FKBP5	None	ROF1	Peptidy-prolyl-isomerase; chaperone; involved in client protein maturation
Cyp40	PPID	Cpr6/Cpr7	SQN	Peptidy-prolyl-isomerase; chaperone; involved in client protein maturation
AIP	AIP	None	None	Complex with AhR (aryl hydrocarbon receptor), PPARα (peroxisome proliferator-activated receptor α), Hbx (Hepatitis B virus X protein)
CHIP	STUB1	None	CHIP	Ubiquitin ligase, tagging protein for degradation
PP5	PPP5C	Ppt1	PP5.2	Phosphatase
Tpr2	DNAJC7	None	ATP58IPK[a]	Tpr2 recognizes both Hsp70 and Hsp90 through its TPR domains. It may mediates the retrograde transfer of substrates from Hsp90 onto Hsp70
Sgt1	SUGT1	Sgt1	SGT1B	Forms complex with Hsp90 and CHORD proteins; involved in the function of NLR receptors in plant and animal innate immunity
Unc45	UNC45B	She4	None	Assembly of myosin fibers
Ttc4	TTC4	Cns1	AT1G04130[a]	Nuclear transport protein; putative tumor suppressor involved in the transformation of melanocytes
Tom70	TOMM70A	Tom70p	None	Mitochondrial protein import
None		None	Toc64	Chloroplast protein import
Tah1	RPAP3/FLJ21908	Tah1	AT1G56440[a]	Forms complex with Pih1 and Hsp90
Non-TPR co-chaperones				
Aha1	AHSA1	Aha1	AT3G12050[a]	Stimulates ATPase activity; induces conformation changes in Hsp90
p23	PTGES3	Sba1	AT3G03773[a]	Involved in client protein maturation; inhibition of Hsp90 ATPase; chaperone
Cdc37	CDC37	Cdc37	None	Kinase-specific co-chaperone; inhibition of Hsp90 ATPase, chaperone
Chp1/Melusin	CHORDC1	None	Rar1	Forms complex with Hsp90 and Sgt1; involved in the function of NLR receptors in plant and animal innate immunity
NudC	NUDC	NudC	AT4G27890[a]	CHORD domain-containing chaperone; dynein-associated nuclear migration protein; plays multiple roles in mitosis and cytokinesis

[a] Several homologues are uncharacterized in plants. The listed gene names are for *Arabidopsis thaliana*.

Hsp90 M-domain to the ATP binding pocket. Finally, Cdc37 holds the N-domain in an open state and precludes its dimerization [66].

Unlike the co-chaperones discussed above, Aha1 is so far the most prominent activator of the ATPase activity of Hsp90. In yeast, both Aha1 and its homologue Hch1 are not essential [118]. Nevertheless, the activation of specific clients such as v-Src and hormone receptors is severely affected in the double knockout cells [119]. Interestingly, Aha1 seems to play an important role in the quality control pathway of the CFTR. Down-regulation of Aha1 could rescue the phenotype caused by misfolded CFTR [120]. Based on biochemical and co-crystallization studies, Aha1 binds the M-domain of Hsp90 [48,119]. A recent NMR analysis further suggests that there are also interactions involving the N-domain of Hsp90 (Fig. 3d) [45,97]. In the suggested asymmetric activation mechanism, one Aha1 molecule is sufficient to stimulate the ATPase activity of one Hsp90 dimer [45]. Binding of Aha1 induces an Hsp90 domain orientation, where the N-domains are in a closed state, which accelerates the progression of the ATPase cycle [45,56]. FRET measurements show that the presence of Aha1 enables Hsp90 to bypass the I1 state and to directly reach the I2 state in the ATPase cycle [56].

Studies of steroid hormone receptor (SHR) complexes led to the identification of another subset of Hsp90 co-chaperones, the TPR-containing peptidyl-prolyl cis-trans isomerases, such as Fkbp52, Fkbp51 and Cyp40 in mammals [22,77,121–123] and Cpr6, Cpr7 in yeast [124]. These proteins contain a PPIase domain, which catalyzes the interconversion of the cis-trans isomerization of peptide bonds prior to proline residues [125], and a TPR domain(s) for the interaction with C-terminal end of Hsp90 (Fig. 3e). Most of these large PPIases show independent chaperone activity [108,109,121]. However, the function of PPIases in SHR complexes is not well understood. They may be selected by specific client proteins. For example, Cyp40 is most abundant in ER complexes [126] and Fkbp52 mediates potentiation of GR but not ER [78]. Notably, TPR containing PPIases are not only restricted to chaperoning SHR but also influence the function of other proteins. For example, AIP was shown to be a negative regulator of PPAR α (Peroxisome proliferator-activated receptor family member, regulation of enzymes involved in fatty acid metabolism) [127]. It also facilitates the AhR [Aryl-hydrocarbon Receptor, a transcription factor that belongs to bHLH/PAS (basic helix–loop–helix/-Per–Arnt–Sim) family] signaling pathway by preventing nucleocytoplasmic shuttling of the unliganded receptor [128–131]. Fkbp38 affects neuronal apoptosis by inhibiting the anti-apoptotic function of Bcl-2 [132], and its isoform Fkbp8 plays a positive role in the RNA replication of Hepatitis C virus [133].

Pp5/Ppt1 is special among the co-chaperones as it is a protein phosphatase which associates with Hsp90 through its N-terminal TPR domain (Fig. 3f). Binding to Hsp90 results in the abrogation of the intrinsic inhibition of Pp5/Ppt1 [134]. In yeast, Ppt1 specifically dephosphorylates Hsp90 and Cdc37 [72,135]. This influences the maturation of client proteins. In Ppt1 knockout strains, activity of Hsp90-specific clients is significantly reduced, which implies that the tight regulation of Hsp90 phosphorylation state is necessary for the efficient processing of client proteins [72].

Sgt1 is a co-chaperone required for innate immunity in plants and animals [136]. It interacts with the N-domain of Hsp90 through its CS domain, which is structurally similar to p23/Sba1 (Fig. 3g) [137,138]. However, the binding surfaces are different from each other and Sgt1 has no inherent Hsp90 ATPase regulatory activity. Interestingly,

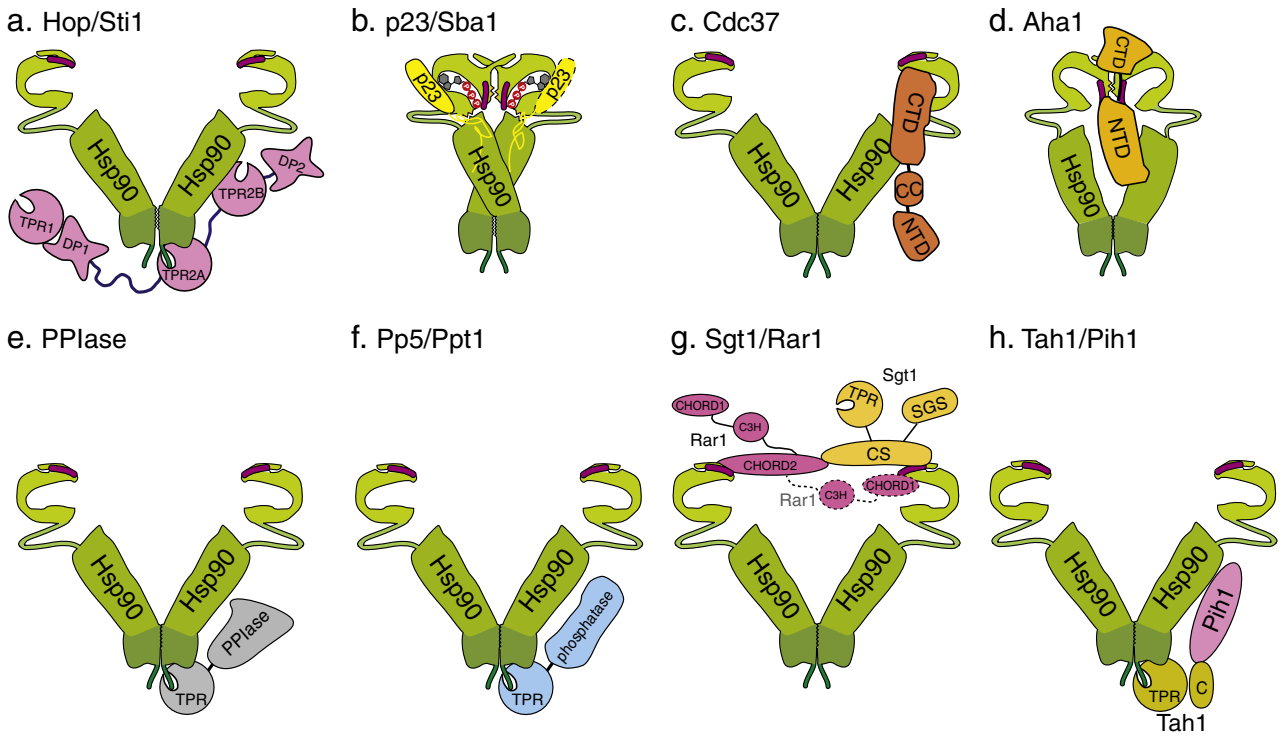

Fig. 3. Co-chaperones interact with distinct surfaces on Hsp90 a. Hop/Sti1 binds to the C-terminal MEEVD motif of Hsp90 through its TPR2A domain, additional binding sites in the M-domain may exist. b. p23/Sba1 associates with the N-domain of Hsp90, with minor contact in the M-domain. c. Cdc37 interacts through its C-domain with the N-domain of Hsp90. d. Binding sites for Aha1 are located in the N- and the M-domain of Hsp90. Association with Aha1 induces a partially closed conformation of Hsp90. e. PPIases bind to the C-terminal MEEVD motif of Hsp90 through their TPR domains. f. Pp5/Ppt1 is known to interact with the C-terminal MEEVD motif of Hsp90 through its TPR domain. g. Sgt1, Rar1 and Hsp90 form a ternary complex. Sgt1 binds to the N-domain of Hsp90 via its CS domain. The binding surface is different from that of p23/Sba1. The CHORD2 domain mediates the interaction with the N-domain of Hsp90 (the exact position of CHORD1 domain in the ternary complex is unknown [141], as indicated by the two positions in the cartoon). h. Tah1 anchors to the C-terminal MEEVD motif of Hsp90 through its TPR domain. Pih1 interacts with the M-domain of Hsp90 and the C-domain of Tah1.

although Sgt1 also contains a TPR domain, it is not involved in the interaction with Hsp90 [139]. Functionally, Hsp90 and Sgt1 form a ternary complex with another co-chaperone, Rar1 (Fig. 3g), which acts as a core modulator in plant immunity [140]. Recent co-crystallization studies provide a structural basis for the assembly of the Hsp90–Sgt1–Rar1 protein complex. Rar1 interacts with Hsp90 through the C-terminal lobe of its CHORD domain (cysteine and histidine-rich domain), opposite to the Sgt1-interacting region [141]. This complex may be involved in the recruitment and activation of NLRs (nucleotide-binding leucine-rich repeat receptors) [140].

Another ternary assembly, the Hsp90–Tah1–Pih1 complex, was recently discovered in chromatin remodeling and small nuclear RNP maturation. Tah1 interacts with Hsp90 through its TPR domain (Fig. 3h) and its C-terminal region binds Pih1, an unstable non-TPR co-chaperone of Hsp90. The Hsp90–Tah1 complex stabilizes Pih1 *in vivo* and prevents its aggregation *in vitro* [142]. Recent biochemical work points out that the Tah1–Pih1 heterodimer binds to Hsp90 with similar affinity as Tah1 alone and inhibits the ATPase activity of Hsp90 suggesting that the Pih1–Tah1 complex may act as a 'client adaptor' recruiting specific clients to the Hsp90 machinery [143].

The above examples provide a glimpse on the gearings of the Hsp90 co-chaperone system. For some co-chaperones we have obtained a quite detailed picture on their structures and functions, for others we are beginning to understand their contributions to the Hsp90 system. Co-chaperones are also involved in other physiological processes not discussed here (Table 1), such as mitochondrial/chloroplast protein import (Tom70/Toc64), nuclear migration (NudC) and melanoma progression (TTC4), Hsp90/Hsp70-dependent protein degradation (CHIP). Thus, the picture will be expanding in the coming years.

4. The chaperone cycle of Hsp90

During the maturation of the client protein, such as SHRs and kinases, Hsp90 functions in concert with a large set of co-chaperones (Table 1), which are crucial to drive the chaperone cycle of Hsp90-client protein interactions [76,77,79,82,121,144]. Some co-chaperones, such as Hop/Sti1 and PPIases, have strong influences on the activation of the SHRs, most of which strictly depend on the interaction with the Hsp90 machinery [78,145]. Research on the assembly of Hsp90 with SHRs has shown that several distinct Hsp90-co-chaperone complexes are formed during the maturation processes [8,22,100,146]. According to the models based on reconstitution experiments, the assembly of SHRs involves the chronological progression through three complexes with different co-chaperone compositions [22]. In the first, 'early complex' Hsp70 and Hsp40 bind the receptor [146–148]. After association with Hsp90, the 'intermediate complex' is formed [22]. Hop/Sti1 is an important component in this process. It serves as an adaptor protein between Hsp70 and Hsp90 [67,69,84]. In addition to the Hop/Sti1–Hsp90 complex, a third complex, which contains a PPIase and the co-chaperone p23/Sba1, has also been found to be a part of the chaperone cycle at a later stage [22,100,104,106,149,150], termed the 'late complex'. Notably, similar heterocomplexes can be found from yeast to mammals and seemingly independent of the presence of client protein [22]. However, the regulation of the progression from one complex to another remained unclear.

Recent biochemical studies using fluorescence resonance energy transfer (FRET), analytical ultracentrifugation (aUC) (Fig. 2d–f), NMR spectroscopy and electron microscopy, provided insight into how the exchange of co-chaperones is regulated. Based on these results, a new model of the chaperone cycle emerges, in which

first one Hop/Sti1 binds to the open conformation of Hsp90 and inhibits its ATPase activity (Fig. 4). The other TPR-acceptor site is then preferentially occupied by a PPIase, leading to an asymmetric Hsp90 intermediate complex. After the binding of ATP and p23/Sba1, Hsp90 adopts the 'closed' conformation which weakens the binding of Hop/Sti1 and therefore promotes its exit. Another PPIase or TPR co-chaperone can potentially bind to form the late complex together with Hsp90 and p23/Sba1 [89]. After ATP hydrolysis, p23/Sba1, PPIase and the folded client are released from Hsp90 (Fig. 4).

5. Hsp90 function is regulated by posttranslational modifications

In recent years, an additional layer of regulation, the covalent modification of Hsp90, gained increasing significance. Hsp90 is tightly controlled by several posttranslational modifications including phosphorylation, acetylation, nitrosylation. These modifications influence the chaperone activity of Hsp90 and thus the maturation of selected clients. Transient posttranslational modifications ensure fast and efficient responses to extra- and intra-cellular stimuli. Thus, spatially distant residues allow precisely adjusting Hsp90 chaperone activity to cellular requirements. Up to date, several of the modified positions have been mapped and the mechanism of Hsp90 modification has been elucidated.

5.1. Phosphorylation

Phosphorylation is the most frequently occurring posttranslational modification of Hsp90. Generally, Hsp90 is phosphorylated at multiple sites located in distinct regions of the dimeric protein and its hyperphosphorylation in the absence of the phosphatase Ppt1 leads to a decreased activation of client proteins in *S. cerevisiae* [72]. Hsp90 phosphorylation has also been linked to the posttranslational assembly of the C-terminal globular head of the reovirus attachment protein σ1 [151] supporting the notion that dynamic phosphorylation/dephosphorylation events represent a key regulatory mechanism for chaperone function.

Hsp90 appears to be predominantly phosphorylated on serine residues, though threonine and tyrosine phosphorylations were also reported [152]. The role played by individual phosphorylation sites in modulating the cellular functions of Hsp90 is currently under investigation. Gratton and colleagues found that c-Src-mediated Hsp90β phosphorylation leads to increased association of Hsp90 and endothelial nitric oxide synthase (eNOS), and thus NO release from endothelial cells [153]. Hsp90 phosphorylation is also linked to apoptosis. In leukemic cells, suppression of Hsp90β phosphorylation increases its association with apoptotic peptidase activating factor 1 (APAF1), abrogates cytochrome *c*-induced apoptosome assembly and promotes apoptosome inhibition [154]. In a recent study, Neckers and co-workers [155] reported that the Wee1/Swe1-mediated phosphorylation of Hsp90 on a tyrosine moiety in the N-domain is cell-cycle associated, affects geldanamycin binding and reduces cancer cell sensitivity to Hsp90 inhibition. The CKII-mediated phosphorylation of Hsp90 at a threonine moiety in the N-domain affected association with specific clients and co-chaperones [156]. Interestingly, most of these effects were compensated by overexpression of the co-chaperone Aha1. Since both phospho-sites are important determinants of Hsp90 drug sensitivity, these observations might provide a new strategy to increase the cellular potency of Hsp90 inhibitors [157].

Hsp90 can be phosphorylated in an isoform-specific manner. DNA-dependent protein kinase (DNA-PK) has been found to phosphorylate Hsp90α, but not the β isoform, at two unique threonine residues in the N-terminal domain [158]. Recent studies demonstrated that the non-ubiquitous calmodulin kinase Pnck perturbs Hsp90 chaperone activity by phosphorylating both human Hsp90 isoforms [159] leading to the degradation of selected clients. Since in the cellular context, the two Hsp90 isoforms are not functionally redundant, unique phosphorylation events may allow further isoform-specific variations [160].

Though many individual phosphorylation sites have been shown to specifically affect Hsp90 function, the question that still remained was how the global control of Hsp90 toward its diverse clientele is achieved by phosphorylation. The recent quantitative analysis of yeast Hsp90 phosphorylation performed by Buchner's lab determined multiple phosphorylation sites in different regions of Hsp90 and allowed addressing the specific role of the phosphatase Ppt1 (Soroka J. and Buchner J., unpublished data). These unpublished data show that phosphorylation of key residues specifically and by different molecular mechanisms modulate conformational rearrangements during the ATPase cycle of Hsp90 with profound effects on client activation. The sites of phosphorylation seem to work as functional switches, allowing adapting chaperone activity to the cellular environment.

Interestingly, many kinases that regulate the Hsp90 phosphorylation status, including CKII, Wee1, Src, Raf1 or Cdk4 [155,161] are at the same time Hsp90 clients. This suggests that changes in phosphorylation are coupled to the ability of Hsp90 to fold and activate this selected group of clients.

5.2. Acetylation

Acetylation is a second prominent Hsp90 modification and its influence on the Hsp90 chaperone machinery was extensively examined in the last years. p300 has been reported to be an acetyltransferase responsible for acetylating Hsp90 [162]and several groups recently discovered HDAC6 as an Hsp90 deacetylase. Kovacs et al. demonstrated that HDAC6-mediated reversible deacetylation is important for regulating the chaperone function of Hsp90 [163]. Their study shows a direct physical interaction between HDAC6 and Hsp90. Several reports confirmed that blocking HDAC6 activity, either with specific inhibitors or after silencing with siRNA, compromised complex formation with client proteins including key oncogenic proteins and led to their destabilization [163–165]. Several lysine moieties in Hsp90 are modified. Neckers and colleagues demonstrated that changes in the acetylation state serves as a key regulator of Hsp90 function both in yeast and man influencing client protein maturation and co-chaperone binding [166]. In summary, these studies highlight a link between Hsp90 acetylation and cell signaling events, nuclear transport or processing of a selected group of clients controlling gene expression.

5.3. Nitrosylation

Hsp90 is also a target of S-nitrosylation and NO modification of a cysteine residue in the C-terminal domain of human Hsp90α was shown to affect its chaperone function [167,168]. S-nitrosylation negatively influenced the Hsp90 ATPase activity *in vitro* and reduced association with eNOS in endothelial cells. The authors proposed a model in which S-nitrosylation down-regulates Hsp90 chaperone properties and provides a feedback mechanism to inhibit further eNOS activation. To undercover how the modification of the C-terminal nitrosylation site inhibits the ATPase activity of Hsp90, Retzlaff and colleagues tested a set of point mutants [169]. The authors found that this cysteine residue in the C-domain functions as a sensitive switch point regulating the inter-domain communication in the Hsp90 dimer and consequently affecting client activity.

5.4. Methylation

Increasing evidence suggests that Hsp90 plays an important role in chromatin remodeling. It has been proposed that specific DNA methylation and chromatin modifications, which might be functionally linked to Hsp90, are interdependent processes involved in

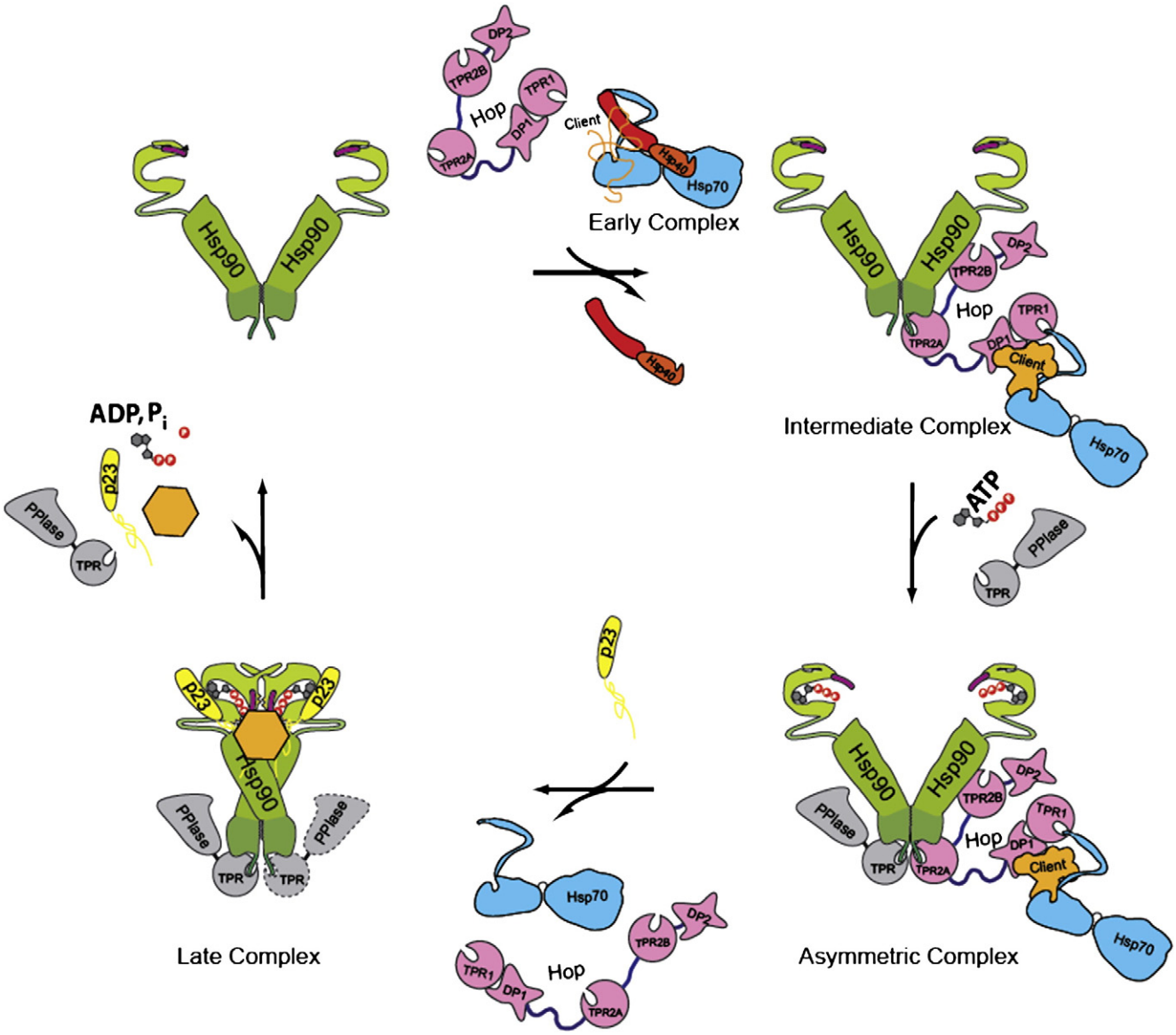

Fig. 4. Co-chaperone cycle of the Hsp90 machinery Hsp70, Hsp40 and a client protein form an 'early complex'. The client protein is transferred from Hsp70 to Hsp90 through the adaptor protein Hop/Sti1. One Hop/Sti1 bound is sufficient to stabilize the open conformation of Hsp90. The other TPR-acceptor site is preferentially occupied by a PPIase, leading to an asymmetric intermediate complex. Hsp90 adopts the ATPase-active (closed) conformation after binding of ATP. p23/Sba1 stabilizes the closed state of Hsp90, which weakens the binding of Hop/Sti1 and promotes its exit from the complex. Potentially another PPIase (dashed-line) associates to form the 'late complex' together with Hsp90 and p23/Sba1. After hydrolysis of ATP, p23/Sba1 and the folded client are released from Hsp90.

chromatin silencing [170,171]. Hsp90 residues subjected to methylation still await mapping. Hamamoto et al. identified a novel histone lysine methyltransferase, SMYD3, up-regulated in several cancers and discovered that its catalytic activity was dramatically enhanced by interaction with Hsp90 [172,173]. An outstanding issue is whether SMYD3 can also methylate Hsp90 and how this process affects its chaperone cycle.

6. Hsp90 client proteins

In the past decades, more than 200 client proteins have been identified which show Hsp90 dependence (see http://www.picard.ch/downloads/Hsp90interactors.pdf). Furthermore, proteome-wide studies suggest that the number of clients will further increase [10,174]. Early work on Hsp90 clients mainly focused on two classes: protein kinases and nuclear receptors [146,175–177]. Besides those well-studied clients, many others related to e.g. viral infection, innate immunity and RNA modification have been discovered in recent years [142,178–180]. To date, Hsp90 clients involve almost all physiological events such as signal transduction, cell cycle progression and transcriptional regulation. The interaction with the Hsp90 machinery enables their correct folding, activation, transport and even degradation [25,181–184] (Fig. 4).

Hsf1 is the central player controlling the heat stress response. Under heat shock conditions it upregulates several hundred genes including Hsp90. Under normal condition, as a client protein, Hsf1 is kept in an inactive monomeric form through the transient interaction with Hsp90 [185,186]. This complex is highly dynamic and Hsf1 constantly associates and dissociates from Hsp90. During stress, Hsp90 binds to unfolding proteins which compete for binding to Hsf1. Upon dissociation from Hsp90, Hsf1 homotrimerizes, undergoes phosphorylation and translocates to the nucleus. Thus, Hsp90 functions as an Hsf1 regulator monitoring the cellular stress response [185,187].

Interestingly, also viral proteins are Hsp90-dependent. Viral proteins, such as Picornavirus capsid proteins, hepatitis B virus (HBV) core proteins and hepatitis C virus (HCV) nonstructural protein NS3

have been identified as clients of Hsp90 as their folding and assembly requires Hsp90 machinery [188–190]. Pharmacological inhibition of Hsp90 resulted in the failure of virus replication in cell cultures and infected animals [133,188,191,192]. As well, some toxins, such as diphtheria toxin and binary actin-ADP-ribosylating toxin have been identified as the client proteins of Hsp90 [193,194]. Hsp90 facilitates the translocation of these toxins. In consequence, inhibition of Hsp90 prevents cellular uptake and thus protects cells from intoxication [193].

Recent studies in plants and mammals revealed that Hsp90 is vital to stabilize NLR proteins, which are conserved immune sensors to recognize pathogens [179,195]. In humans, 21 NLR proteins are involved in innate immune responses [196]. Accumulating evidence indicates that Hsp90 and its co-chaperones Sgt1, Rar1 are involved in the maturation of these proteins [140,197].

Hsp90 is also known to chaperone nuclear proteins and therefore involved in DNA replication, DNA repair, DNA metabolism, RNA transcription and RNA processing [180,198]. The telomere protein system is a well-studied example. Freeman and co-workers found that Hsp90 facilitates telomere DNA maintenance by mediating the switch between its capping and extending structure [199]. Impairment of Hsp90 functions resulted in the decreased activity of telomerases [198]. Latest studies show that the assembly of small nucleolar ribonucleoproteins and RNA polymerase as well requires Hsp90. The R2TP complex (consisting of Tah1, Pih1 and the AAA+ ATPase Rvb1 and Rvb2) is the client-specific co-chaperone system involved in RNA processing [142,180].

A long standing open question is the molecular basis of client recognition by Hsp90. To date, no common sequence or motif has been identified among the numerous client proteins. The αC-β4 loop in the kinase domain was found to be an important region for the association with Hsp90 [200,201]. However, it is not the only determinant for the interaction, since other regions adjacent to the kinase domain have also an influence on the binding to Hsp90 [202,203]. Probably the association with Hsp90 is determined by the conformation or stability of the client protein instead of the primary structure. Prominent examples here are the Src kinases. Hsp90 is able to stably associate with viral Src kinase (v-Src), but it only transiently interacts with its normal cellular counterpart (c-Src) [204], although they are almost identical (95% sequence identity). Despite this high level of sequence identity, c-Src is more resistant to chemical and heat denaturation and v-Src is prone to aggregation [204]. Moreover, also co-chaperones can contribute to the process of client selection and recognition. For example, Cdc37 seems to be a co-chaperone specific for kinases, while Sgt1 plays an important role in the processing of NLR proteins as discussed above.

The structural analysis of the interaction of Hsp90 with client proteins is a challenging task, as most of them are highly unstable and aggregation-prone. The EM reconstruction of the Hsp90–Cdc37–Cdk4 complex provided a first view of the client-loaded Hsp90 complex. The model suggests that clients bind in an asymmetric manner to one N- and M-domain of Hsp90 [48]. Recent structural studies using a model client protein showed that the Hsp90 M-domain preferentially binds a locally structured region in the intrinsically unfolded model protein [49]. Binding induces a partially closed conformation of Hsp90 and enhances the ATPase activity [49,60,205].

However, the conformations of Hsp90-bound clients are yet to be answered, as the present results on this issue are controversial. Studies using the model client citrate synthase indicated that Hsp90 interacts with structured intermediates [206]. This is consistent with the notion derived from the experiments with SHRs [8] and also the structure of a kinase in the cryo EM kinase complex [48].

A well characterized client is p53 [207]. Biochemical experiments suggest that p53 interacts with Hsp90 in a rather folded state [16,207]. However, recent results imply that p53 may be destabilized by Hsp90 [208]. There are also several studies in which NMR-based approaches were used to determine the conformation of Hsp90-bound p53 and the sites of interaction (Stefan Rüdiger and co-workers, this BBA issue). For heat-treated p53, Hsp90 was suggested to bind the largely unfolded protein [209]. For the interaction of Hsp90 with native p53, the binding sites on Hsp90 could be mapped to the M-domain [210,211] and to the C-domain [211]. Some NMR studies suggest that Hsp90 binds to heat-unfolded p53 [209]. However, there is no consensus on the structure of the bound p53 in this case. Park et al. [210] propose that Hsp90 domains induce a molten globule state in p53. In contrast, Hagn et al. report a native-like structure of bound p53 [211]. Further analysis will be required to resolve this conundrum and to determine the folding states of different Hsp90-bound client proteins.

7. Perspectives

The extensive research over the past decades witnessed a rapid expansion of our knowledge on the mechanism of the Hsp90 machinery. A combination of experimental approaches enabled dissecting the ATP-induced conformational changes and progression of the chaperone cycle in detail. For some of the Hsp90 co-chaperones we have now an idea of their influence on Hsp90 and their integration in the conformational cycle. Also the analysis of posttranslational modifications provides further understanding of the regulatory mechanisms governing the Hsp90 chaperone machinery. Nevertheless, many characteristics remain to be explored due to the dynamic nature and inherent complexity of the Hsp90 system. For example, how does Hsp90 recognize different client proteins? What is the location of the client binding site or are there several sites? What is the specific contribution of each co-chaperone to the maturation of the client proteins? How do multiple, different posttranslational modifications influence the function of Hsp90 and its co-chaperones? In the future, it will be important to further analyze the Hsp90 chaperone machinery in the presence of client proteins to see how they influence co-chaperone interaction and other aspects of the conformational cycle.

In recent years, Hsp90 has emerged as an important anti-cancer drug target and commenced a new era in the field of cancer therapeutics [12,13]. The initial success suggests also great promise for other diseases in which Hsp90 is involved, such as inflammatory or neurodegenerative disorders. The discovery that Hsp90 secretion enhances wound healing [212] and is correlated with cancer metastasis [35] indicates that novel cell-impermeable Hsp90 modulator may allow selectively targeting these species. Thus, the comprehensive study of the Hsp90 machinery will not only contribute to the understanding of cellular protein folding mechanisms but also to the treatment of human diseases.

References

[1] S.K. Wandinger, K. Richter, J. Buchner, The Hsp90 chaperone machinery, J. Biol. Chem. 283 (2008) 18473–18477.
[2] R. Zhao, M. Davey, Y.C. Hsu, P. Kaplanek, A. Tong, A.B. Parsons, N. Krogan, G. Cagney, D. Mai, J. Greenblatt, C. Boone, A. Emili, W.A. Houry, Navigating the chaperone network: an integrative map of physical and genetic interactions mediated by the hsp90 chaperone, Cell 120 (2005) 715–727.
[3] K.A. Borkovich, F.W. Farrelly, D.B. Finkelstein, J. Taulien, S. Lindquist, hsp82 is an essential protein that is required in higher concentrations for growth of cells at higher temperatures, Mol. Cell. Biol. 9 (1989) 3919–3930.
[4] J.C. Young, I. Moarefi, F.U. Hartl, Hsp90: a specialized but essential protein-folding tool, J. Cell Biol. 154 (2001) 267–273.
[5] K. Richter, M. Haslbeck, J. Buchner, The heat shock response: life on the verge of death, Mol. Cell 40 (2010) 253–266.
[6] D.C. Dezwaan, B.C. Freeman, HSP90: the Rosetta stone for cellular protein dynamics? Cell Cycle 7 (2008) 1006–1012.
[7] M. Taipale, D.F. Jarosz, S. Lindquist, HSP90 at the hub of protein homeostasis: emerging mechanistic insights, Nat. Rev. Mol. Cell Biol. 11 (2010) 515–528.
[8] W.B. Pratt, D.O. Toft, Steroid receptor interactions with heat shock protein and immunophilin chaperones, Endocr. Rev. 18 (1997) 306–360.

[9] D. Picard, Heat-shock protein 90, a chaperone for folding and regulation, Cell. Mol. Life Sci. 59 (2002) 1640–1648.
[10] A.J. McClellan, Y. Xia, A.M. Deutschbauer, R.W. Davis, M. Gerstein, J. Frydman, Diverse cellular functions of the Hsp90 molecular chaperone uncovered using systems approaches, Cell 131 (2007) 121–135.
[11] L. Neckers, A. Kern, S. Tsutsumi, Hsp90 inhibitors disrupt mitochondrial homeostasis in cancer cells, Chem. Biol. 14 (2007) 1204–1206.
[12] J. Trepel, M. Mollapour, G. Giaccone, L. Neckers, Targeting the dynamic HSP90 complex in cancer, Nat. Rev. Cancer 10 (2010) 537–549.
[13] Y.S. Kim, S.V. Alarcon, S. Lee, M.J. Lee, G. Giaccone, L. Neckers, J.B. Trepel, Update on Hsp90 inhibitors in clinical trial, Curr. Top. Med. Chem. 9 (2009) 1479–1492.
[14] T.A. Sangster, S. Lindquist, C. Queitsch, Under cover: causes, effects and implications of Hsp90-mediated genetic capacitance, Bioessays 26 (2004) 348–362.
[15] R. Bagatell, L. Whitesell, Altered Hsp90 function in cancer: a unique therapeutic opportunity, Mol. Cancer Ther. 3 (2004) 1021–1030.
[16] P. Muller, P. Ceskova, B. Vojtesek, Hsp90 is essential for restoring cellular functions of temperature-sensitive p53 mutant protein but not for stabilization and activation of wild-type p53: implications for cancer therapy, J. Biol. Chem. 280 (2005) 6682–6691.
[17] C. Dai, L. Whitesell, A.B. Rogers, S. Lindquist, Heat shock factor 1 is a powerful multifaceted modifier of carcinogenesis, Cell 130 (2007) 1005–1018.
[18] C. Queitsch, T.A. Sangster, S. Lindquist, Hsp90 as a capacitor of phenotypic variation, Nature 417 (2002) 618–624.
[19] S.L. Rutherford, S. Lindquist, Hsp90 as a capacitor for morphological evolution, Nature 396 (1998) 336–342.
[20] D.F. Jarosz, S. Lindquist, Hsp90 and environmental stress transform the adaptive value of natural genetic variation, Science 330 (2010) 1820–1824.
[21] W.B. Pratt, D.O. Toft, Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery, Exp. Biol. Med. (Maywood) 228 (2003) 111–133.
[22] D.F. Smith, Dynamics of heat shock protein 90-progesterone receptor binding and the disactivation loop model for steroid receptor complexes, Mol. Endocrinol. 7 (1993) 1418–1429.
[23] H. Wiech, J. Buchner, M. Zimmermann, R. Zimmermann, U. Jakob, Hsc70, immunoglobulin heavy chain binding protein, and Hsp90 differ in their ability to stimulate transport of precursor proteins into mammalian microsomes, J. Biol. Chem. 268 (1993) 7414–7421.
[24] W.B. Pratt, Y. Morishima, M. Murphy, M. Harrell, Chaperoning of glucocorticoid receptors, Handb. Exp. Pharmacol. (2006) 111–138.
[25] W.B. Pratt, Y. Morishima, H.M. Peng, Y. Osawa, Proposal for a role of the Hsp90/Hsp70-based chaperone machinery in making triage decisions when proteins undergo oxidative and toxic damage, Exp. Biol. Med. (Maywood) 235 (2010) 278–289.
[26] M. Gamerdinger, P. Hajieva, A.M. Kaya, U. Wolfrum, F.U. Hartl, C. Behl, Protein quality control during aging involves recruitment of the macroautophagy pathway by BAG3, EMBO J. 28 (2009) 889–901.
[27] T. Langer, S. Rosmus, H. Fasold, Intracellular localization of the 90 kDA heat shock protein (HSP90alpha) determined by expression of a EGFP-HSP90alpha-fusion protein in unstressed and heat stressed 3T3 cells, Cell Biol. Int. 27 (2003) 47–52.
[28] P. Csermely, T. Schnaider, C. Soti, Z. Prohaszka, G. Nardai, The 90-kDa molecular chaperone family: structure, function, and clinical applications. A comprehensive review, Pharmacol. Ther. 79 (1998) 129–168.
[29] T.A. Sangster, C. Queitsch, The HSP90 chaperone complex, an emerging force in plant development and phenotypic plasticity, Curr. Opin. Plant Biol. 8 (2005) 86–92.
[30] P. Krishna, G. Gloor, The Hsp90 family of proteins in *Arabidopsis thaliana*, Cell Stress Chaperones 6 (2001) 238–246.
[31] D. Meiri, A. Breiman, *Arabidopsis* ROF1 (FKBP62) modulates thermotolerance by interacting with HSP90.1 and affecting the accumulation of HsfA2-regulated sHSPs, Plant J. 59 (2009) 387–399.
[32] S.J. Felts, B.A. Owen, P. Nguyen, J. Trepel, D.B. Donner, D.O. Toft, The hsp90-related protein TRAP1 is a mitochondrial protein with distinct functional properties, J. Biol. Chem. 275 (2000) 3305–3312.
[33] R.P. Shiu, J. Pouyssegur, I. Pastan, Glucose depletion accounts for the induction of two transformation-sensitive membrane proteinsin Rous sarcoma virus-transformed chick embryo fibroblasts, Proc. Natl. Acad. Sci. U. S. A. 74 (1977) 3840–3844.
[34] K. Sidera, E. Patsavoudi, Extracellular HSP90: conquering the cell surface, Cell Cycle 7 (2008) 1564–1568.
[35] B.K. Eustace, T. Sakurai, J.K. Stewart, D. Yimlamai, C. Unger, C. Zehetmeier, B. Lain, C. Torella, S.W. Henning, G. Beste, B.T. Scroggins, L. Neckers, L.L. Ilag, D.G. Jay, Functional proteomic screens reveal an essential extracellular role for hsp90 alpha in cancer cell invasiveness, Nat. Cell Biol. 6 (2004) 507–514.
[36] J.C. Bardwell, E.A. Craig, Ancient heat shock gene is dispensable, J. Bacteriol. 170 (1988) 2977–2983.
[37] K. Richter, J. Soroka, L. Skalniak, A. Leskovar, M. Hessling, J. Reinstein, J. Buchner, Conserved conformational changes in the ATPase cycle of human Hsp90, J. Biol. Chem. 283 (2008) 17757–17765.
[38] R. Dutta, M. Inouye, GHKL, an emergent ATPase/kinase superfamily, Trends Biochem. Sci. 25 (2000) 24–28.
[39] U. Jakob, I. Meyer, H. Bugl, S. Andre, J.C. Bardwell, J. Buchner, Structural organization of procaryotic and eucaryotic Hsp90. Influence of divalent cations on structure and function, J. Biol. Chem. 270 (1995) 14412–14419.
[40] S. Frey, A. Leskovar, J. Reinstein, J. Buchner, The ATPase cycle of the endoplasmic chaperone Grp94, J. Biol. Chem. 282 (2007) 35612–35620.
[41] A. Leskovar, H. Wegele, N.D. Werbeck, J. Buchner, J. Reinstein, The ATPase cycle of the mitochondrial Hsp90 analog Trap1, J. Biol. Chem. 283 (2008) 11677–11688.
[42] J. Buchner, Bacterial Hsp90 — desperately seeking clients, Mol. Microbiol. 76 (2010) 540–544.
[43] T. Sato, S. Minagawa, E. Kojima, N. Okamoto, H. Nakamoto, HtpG, the prokaryotic homologue of Hsp90, stabilizes a phycobilisome protein in the cyanobacterium *Synechococcus elongatus* PCC 7942, Mol. Microbiol. 76 (2010) 576–589.
[44] P. Meyer, C. Prodromou, C. Liao, B. Hu, S. Mark Roe, C.K. Vaughan, I. Vlasic, B. Panaretou, P.W. Piper, L.H. Pearl, Structural basis for recruitment of the ATPase activator Aha1 to the Hsp90 chaperone machinery, EMBO J. 23 (2004) 511–519.
[45] M. Retzlaff, F. Hagn, L. Mitschke, M. Hessling, F. Gugel, H. Kessler, K. Richter, J. Buchner, Asymmetric activation of the hsp90 dimer by its cochaperone aha1, Mol. Cell 37 (2010) 344–354.
[46] S. Sato, N. Fujita, T. Tsuruo, Modulation of Akt kinase activity by binding to Hsp90, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 10832–10837.
[47] J. Fontana, D. Fulton, Y. Chen, T.A. Fairchild, T.J. McCabe, N. Fujita, T. Tsuruo, W.C. Sessa, Domain mapping studies reveal that the M domain of hsp90 serves as a molecular scaffold to regulate Akt-dependent phosphorylation of endothelial nitric oxide synthase and NO release, Circ. Res. 90 (2002) 866–873.
[48] C.K. Vaughan, U. Gohlke, F. Sobott, V.M. Good, M.M. Ali, C. Prodromou, C.V. Robinson, H.R. Saibil, L.H. Pearl, Structure of an Hsp90–Cdc37–Cdk4 complex, Mol. Cell 23 (2006) 697–707.
[49] T.O. Street, L.A. Lavery, D.A. Agard, Substrate binding drives large-scale conformational changes in the hsp90 molecular chaperone, Mol. Cell 42 (2011) 96–105.
[50] L. Pullen, D.N. Bolon, Enforced N-domain proximity stimulates Hsp90 ATPase activity and is compatible with function in vivo, J. Biol. Chem. 286 (2011) 11091–11098.
[51] C. Scheufler, A. Brinker, G. Bourenkov, S. Pegoraro, L. Moroder, H. Bartunik, F.U. Hartl, I. Moarefi, Structure of TPR domain–peptide complexes: critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine, Cell 101 (2000) 199–210.
[52] C. Prodromou, G. Siligardi, R. O'Brien, D.N. Woolfson, L. Regan, B. Panaretou, J.E. Ladbury, P.W. Piper, L.H. Pearl, Regulation of Hsp90 ATPase activity by tetratricopeptide repeat (TPR)-domain co-chaperones, EMBO J. 18 (1999) 754–762.
[53] S. Chen, W.P. Sullivan, D.O. Toft, D.F. Smith, Differential interactions of p23 and the TPR-containing proteins Hop, Cyp40, FKBP52 and FKBP51 with Hsp90 mutants, Cell Stress Chaperones 3 (1998) 118–129.
[54] M.M. Ali, S.M. Roe, C.K. Vaughan, P. Meyer, B. Panaretou, P.W. Piper, C. Prodromou, L.H. Pearl, Crystal structure of an Hsp90-nucleotide-p23/Sba1 closed chaperone complex, Nature 440 (2006) 1013–1017.
[55] A.K. Shiau, S.F. Harris, D.R. Southworth, D.A. Agard, Structural Analysis of E. coli hsp90 reveals dramatic nucleotide-dependent conformational rearrangements, Cell 127 (2006) 329–340.
[56] M. Hessling, K. Richter, J. Buchner, Dissection of the ATP-induced conformational cycle of the molecular chaperone Hsp90, Nat. Struct. Mol. Biol. 16 (2009) 287–293.
[57] M. Mickler, M. Hessling, C. Ratzke, J. Buchner, T. Hugel, The large conformational changes of Hsp90 are only weakly coupled to ATP hydrolysis, Nat. Struct. Mol. Biol. 16 (2009) 281–286.
[58] B. Panaretou, C. Prodromou, S.M. Roe, R. O'Brien, J.E. Ladbury, P.W. Piper, L.H. Pearl, ATP binding and hydrolysis are essential to the function of the Hsp90 molecular chaperone in vivo, EMBO J. 17 (1998) 4829–4836.
[59] T. Scheibel, T. Weikl, J. Buchner, Two chaperone sites in Hsp90 differing in substrate specificity and ATP dependence, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 1495–1499.
[60] S.H. McLaughlin, H.W. Smith, S.E. Jackson, Stimulation of the weak ATPase activity of human hsp90 by a client protein, J. Mol. Biol. 315 (2002) 787–798.
[61] N. Wayne, D.N. Bolon, Dimerization of Hsp90 is required for in vivo function. Design and analysis of monomers and dimers, J. Biol. Chem. 282 (2007) 35386–35395.
[62] D.R. Southworth, D.A. Agard, Species-dependent ensembles of conserved conformational states define the Hsp90 chaperone ATPase cycle, Mol. Cell 32 (2008) 631–640.
[63] C. Ratzke, M. Mickler, B. Hellenkamp, J. Buchner, T. Hugel, Dynamics of heat shock protein 90 C-terminal dimerization is an important part of its conformational cycle, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 16101–16106.
[64] B. Panaretou, G. Siligardi, P. Meyer, A. Maloney, J.K. Sullivan, S. Singh, S.H. Millson, P.A. Clarke, S. Naaby-Hansen, R. Stein, R. Cramer, M. Mollapour, P. Workman, P.W. Piper, L.H. Pearl, C. Prodromou, Activation of the ATPase activity of hsp90 by the stress-regulated cochaperone aha1, Mol. Cell 10 (2002) 1307–1318.
[65] K. Richter, S. Walter, J. Buchner, The co-chaperone Sba1 connects the ATPase reaction of Hsp90 to the progression of the chaperone cycle, J. Mol. Biol. 342 (2004) 1403–1413.
[66] S.M. Roe, M.M. Ali, P. Meyer, C.K. Vaughan, B. Panaretou, P.W. Piper, C. Prodromou, L.H. Pearl, The mechanism of Hsp90 regulation by the protein kinase-specific cochaperone p50(cdc37), Cell 116 (2004) 87–98.
[67] S. Chen, D.F. Smith, Hop as an adaptor in the heat shock protein 70 (Hsp70) and hsp90 chaperone machinery, J. Biol. Chem. 273 (1998) 35194–35200.
[68] A.K. Das, P.W. Cohen, D. Barford, The structure of the tetratricopeptide repeats of protein phosphatase 5: implications for TPR-mediated protein–protein interactions, EMBO J. 17 (1998) 1192–1199.

[69] B.D. Johnson, R.J. Schumacher, E.D. Ross, D.O. Toft, Hop modulates Hsp70/Hsp90 interactions in protein folding, J. Biol. Chem. 273 (1998) 3679–3686.
[70] H.C. Chang, S. Lindquist, Conservation of Hsp90 macromolecular complexes in *Saccharomyces cerevisiae*, J. Biol. Chem. 269 (1994) 24983–24988.
[71] A.M. Silverstein, M.D. Galigniana, M.S. Chen, J.K. Owens-Grillo, M. Chinkers, W.B. Pratt, Protein phosphatase 5 is a major component of glucocorticoid receptor.hsp90 complexes with properties of an FK506-binding immunophilin, J. Biol. Chem. 272 (1997) 16224–16230.
[72] S.K. Wandinger, M.H. Suhre, H. Wegele, J. Buchner, The phosphatase Ppt1 is a dedicated regulator of the molecular chaperone Hsp90, EMBO J. 25 (2006) 367–376.
[73] A. Brychzy, T. Rein, K.F. Winklhofer, F.U. Hartl, J.C. Young, W.M. Obermann, Cofactor Tpr2 combines two TPR domains and a J domain to regulate the Hsp70/Hsp90 chaperone system, EMBO J. 22 (2003) 3613–3623.
[74] R. Srikakulam, L. Liu, D.A. Winkelmann, Unc45b forms a cytosolic complex with Hsp90 and targets the unfolded myosin motor domain, PLoS One 3 (2008) e2137.
[75] J.M. Barral, A.H. Hutagalung, A. Brinker, F.U. Hartl, H.F. Epstein, Role of the myosin assembly protein UNC-45 as a molecular chaperone for myosin, Science 295 (2002) 669–671.
[76] M.B. Cox, D.L. Riggs, M. Hessling, F. Schumacher, J. Buchner, D.F. Smith, FK506-binding protein 52 phosphorylation: a potential mechanism for regulating steroid hormone receptor activity, Mol. Endocrinol. 21 (2007) 2956–2967.
[77] J.L. Johnson, D.O. Toft, A novel chaperone complex for steroid receptors involving heat shock proteins, immunophilins, and p23, J. Biol. Chem. 269 (1994) 24989–24993.
[78] D.L. Riggs, P.J. Roberts, S.C. Chirillo, J. Cheung-Flynn, V. Prapapanich, T. Ratajczak, R. Gaber, D. Picard, D.F. Smith, The Hsp90-binding peptidylprolyl isomerase FKBP52 potentiates glucocorticoid signaling in vivo, EMBO J. 22 (2003) 1158–1167.
[79] P.K. Tai, H. Chang, M.W. Albers, S.L. Schreiber, D.O. Toft, L.E. Faber, P59 (FK506 binding protein 59) interaction with heat shock proteins is highly conserved and may involve proteins other than steroid receptors, Biochemistry 32 (1993) 8842–8847.
[80] S.C. Nair, R.A. Rimerman, E.J. Toran, S. Chen, V. Prapapanich, R.N. Butts, D.F. Smith, Molecular cloning of human FKBP51 and comparisons of immunophilin interactions with Hsp90 and progesterone receptor, Mol. Cell. Biol. 17 (1997) 594–603.
[81] T. Ratajczak, A. Carrello, Cyclophilin 40 (CyP-40), mapping of its hsp90 binding domain and evidence that FKBP52 competes with CyP-40 for hsp90 binding, J. Biol. Chem. 271 (1996) 2961–2965.
[82] C. Mayr, K. Richter, H. Lilie, J. Buchner, Cpr6 and Cpr7, two closely related Hsp90-associated immunophilins from Saccharomyces cerevisiae, differ in their functional properties, J. Biol. Chem. 275 (2000) 34140–34146.
[83] K. Richter, P. Muschler, O. Hainzl, J. Reinstein, J. Buchner, Sti1 is a non-competitive inhibitor of the Hsp90 ATPase. Binding prevents the N-terminal dimerization reaction during the atpase cycle, J. Biol. Chem. 278 (2003) 10328–10333.
[84] H. Wegele, S.K. Wandinger, A.B. Schmid, J. Reinstein, J. Buchner, Substrate transfer from the chaperone Hsp70 to Hsp90, J. Mol. Biol. 356 (2006) 802–811.
[85] H. Wegele, M. Haslbeck, J. Reinstein, J. Buchner, Sti1 is a novel activator of the Ssa proteins, J. Biol. Chem. 278 (2003) 25970–25976.
[86] A. Brinker, C. Scheufler, F. Von Der Mulbe, B. Fleckenstein, C. Herrmann, G. Jung, I. Moarefi, F.U. Hartl, Ligand discrimination by TPR domains. Relevance and selectivity of EEVD-recognition in Hsp70×Hop×Hsp90 complexes, J. Biol. Chem. 277 (2002) 19265–19275.
[87] A.M. Gaiser, F. Brandt, K. Richter, The non-canonical Hop protein from *Caenorhabditis elegans* exerts essential functions and forms binary complexes with either Hsc70 or Hsp90, J. Mol. Biol. 391 (2009) 621–634.
[88] F. Yi, I. Doudevski, L. Regan, HOP is a monomer: investigation of the oligomeric state of the co-chaperone HOP, Protein Sci. 19 (2010) 19–25.
[89] J. Li, K. Richter, J. Buchner, Mixed Hsp90-cochaperone complexes are important for the progression of the reaction cycle, Nat. Struct. Mol. Biol. 18 (2011) 61–66.
[90] S. Ghaemmaghami, W.K. Huh, K. Bower, R.W. Howson, A. Belle, N. Dephoure, E.K. O'Shea, J.S. Weissman, Global analysis of protein expression in yeast, Nature 425 (2003) 737–741.
[91] Y. Fang, A.E. Fliss, J. Rao, A.J. Caplan, SBA1 encodes a yeast hsp90 cochaperone that is homologous to vertebrate p23 proteins, Mol. Cell. Biol. 18 (1998) 3727–3734.
[92] G. Flom, J. Weekes, J.L. Johnson, Novel interaction of the Hsp90 chaperone machine with Ssl2, an essential DNA helicase in *Saccharomyces cerevisiae*, Curr. Genet. 47 (2005) 368–380.
[93] G. Flom, J. Weekes, J.J. Williams, J.L. Johnson, Effect of mutation of the tetratricopeptide repeat and asparatate-proline 2 domains of Sti1 on Hsp90 signaling and interaction in *Saccharomyces cerevisiae*, Genetics 172 (2006) 41–51.
[94] H. Kosano, B. Stensgard, M.C. Charlesworth, N. McMahon, D. Toft, The assembly of progesterone receptor–hsp90 complexes using purified proteins, J. Biol. Chem. 273 (1998) 32973–32979.
[95] V.K. Gangaraju, H. Yin, M.M. Weiner, J. Wang, X.A. Huang, H. Lin, Drosophila Piwi functions in Hsp90-mediated suppression of phenotypic variation, Nat. Genet. 43 (2011) 153–158.
[96] N.V. Marozkina, S. Yemen, M. Borowitz, L. Liu, M. Plapp, F. Sun, R. Islam, P. Erdmann-Gilmore, R.R. Townsend, C.F. Lichti, S. Mantri, P.W. Clapp, S.H. Randell, B. Gaston, K. Zaman, Hsp 70/Hsp 90 organizing protein as a nitrosylation target in cystic fibrosis therapy, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 11393–11398.
[97] A.V. Koulov, P. Lapointe, B. Lu, A. Razvi, J. Coppinger, M.Q. Dong, J. Matteson, R. Laister, C. Arrowsmith, J.R. Yates III, W.E. Balch, Biological and structural basis for Aha1 regulation of Hsp90 ATPase activity in maintaining proteostasis in the human disease cystic fibrosis, Mol. Biol. Cell 21 (2010) 871–884.
[98] A. Chadli, I. Bouhouche, W. Sullivan, B. Stensgard, N. McMahon, M.G. Catelli, D.O. Toft, Dimerization and N-terminal domain proximity underlie the function of the molecular chaperone heat shock protein 90, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 12524–12529.
[99] J.P. Grenert, B.D. Johnson, D.O. Toft, The importance of ATP binding and hydrolysis by hsp90 in formation and function of protein heterocomplexes, J. Biol. Chem. 274 (1999) 17525–17533.
[100] J.L. Johnson, T.G. Beito, C.J. Krco, D.O. Toft, Characterization of a novel 23-kilodalton protein of unactive progesterone receptor complexes, Mol. Cell. Biol. 14 (1994) 1956–1963.
[101] T. Weikl, K. Abelmann, J. Buchner, An unstructured C-terminal region of the Hsp90 co-chaperone p23 is important for its chaperone function, J. Mol. Biol. 293 (1999) 685–691.
[102] I. Grad, T.A. McKee, S.M. Ludwig, G.W. Hoyle, P. Ruiz, W. Wurst, T. Floss, C.A. Miller III, D. Picard, The Hsp90 cochaperone p23 is essential for perinatal survival, Mol. Cell. Biol. 26 (2006) 8976–8983.
[103] F.J. Echtenkamp, E. Zelin, E. Oxelmark, J.I. Woo, B.J. Andrews, M. Garabedian, B.C. Freeman, Global functional map of the p23 molecular chaperone reveals an extensive cellular network, Mol. Cell 43 (2011) 229–241.
[104] J.L. Johnson, D.O. Toft, Binding of p23 and hsp90 during assembly with the progesterone receptor, Mol. Endocrinol. 9 (1995) 670–678.
[105] W.M. Obermann, H. Sondermann, A.A. Russo, N.P. Pavletich, F.U. Hartl, In vivo function of Hsp90 is dependent on ATP binding and ATP hydrolysis, J. Cell Biol. 143 (1998) 901–910.
[106] S.H. McLaughlin, F. Sobott, Z.P. Yao, W. Zhang, P.R. Nielsen, J.G. Grossmann, E.D. Laue, C.V. Robinson, S.E. Jackson, The co-chaperone p23 arrests the Hsp90 ATPase cycle to trap client proteins, J. Mol. Biol. 356 (2006) 746–758.
[107] Y. Morishima, K.C. Kanelakis, P.J. Murphy, E.R. Lowe, G.J. Jenkins, Y. Osawa, R.K. Sunahara, W.B. Pratt, The hsp90 cochaperone p23 is the limiting component of the multiprotein hsp90/hsp70-based chaperone system in vivo where it acts to stabilize the client protein: hsp90 complex, J. Biol. Chem. 278 (2003) 48754–48763.
[108] S. Bose, T. Weikl, H. Bugl, J. Buchner, Chaperone function of Hsp90-associated proteins, Science 274 (1996) 1715–1717.
[109] B.C. Freeman, D.O. Toft, R.I. Morimoto, Molecular chaperone machines: chaperone activities of the cyclophilin Cyp-40 and the steroid aporeceptor-associated protein p23, Science 274 (1996) 1718–1720.
[110] A.J. Weaver, W.P. Sullivan, S.J. Felts, B.A. Owen, D.O. Toft, Crystal structure and activity of human p23, a heat shock protein 90 co-chaperone, J. Biol. Chem. 275 (2000) 23045–23052.
[111] A.M. Gaiser, A. Kretzschmar, K. Richter, Cdc37-Hsp90 complexes are responsive to nucleotide-induced conformational changes and binding of further cofactors, J. Biol. Chem. 285 (2010) 40921–40932.
[112] G. Siligardi, B. Panaretou, P. Meyer, S. Singh, D.N. Woolfson, P.W. Piper, L.H. Pearl, C. Prodromou, Regulation of Hsp90 ATPase activity by the co-chaperone Cdc37p/p50cdc37, J. Biol. Chem. 277 (2002) 20151–20159.
[113] J. Ferguson, J.Y. Ho, T.A. Peterson, S.I. Reed, Nucleotide sequence of the yeast cell division cycle start genes CDC28, CDC36, CDC37, and CDC39, and a structural analysis of the predicted products, Nucleic Acids Res. 14 (1986) 6681–6697.
[114] S.I. Reed, The selection of S. cerevisiae mutants defective in the start event of cell division, Genetics 95 (1980) 561–577.
[115] B. Dey, J.J. Lightbody, F. Boschelli, CDC37 is required for p60v-src activity in yeast, Mol. Biol. Cell 7 (1996) 1405–1417.
[116] J.S. Brugge, Interaction of the Rous sarcoma virus protein pp 60src with the cellular proteins pp50 and pp90, Curr. Top. Microbiol. Immunol. 123 (1986) 1–22.
[117] M. MacLean, D. Picard, Cdc37 goes beyond Hsp90 and kinases, Cell Stress Chaperones 8 (2003) 114–119.
[118] M.P. Mayer, R. Nikolay, B. Bukau, Aha, another regulator for hsp90 chaperones, Mol. Cell 10 (2002) 1255–1256.
[119] G.P. Lotz, H. Lin, A. Harst, W.M. Obermann, Aha1 binds to the middle domain of Hsp90, contributes to client protein activation, and stimulates the ATPase activity of the molecular chaperone, J. Biol. Chem. 278 (2003) 17228–17235.
[120] X. Wang, J. Venable, P. LaPointe, D.M. Hutt, A.V. Koulov, J. Coppinger, C. Gurkan, W. Kellner, J. Matteson, H. Plutner, J.R. Riordan, J.W. Kelly, J.R. Yates III, W.E. Balch, Hsp90 cochaperone Aha1 downregulation rescues misfolding of CFTR in cystic fibrosis, Cell 127 (2006) 803–815.
[121] F. Pirkl, J. Buchner, Functional analysis of the Hsp90-associated human peptidyl prolyl cis/trans isomerases FKBP51, FKBP52 and Cyp40, J. Mol. Biol. 308 (2001) 795–806.
[122] D.A. Peattie, M.W. Harding, M.A. Fleming, M.T. DeCenzo, J.A. Lippke, D.J. Livingston, M. Benasutti, Expression and characterization of human FKBP52, an immunophilin that associates with the 90-kDa heat shock protein and is a component of steroid receptor complexes, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 10974–10978.
[123] T. Ratajczak, B.K. Ward, C. Cluning, R.K. Allan, Cyclophilin 40: an Hsp90-cochaperone associated with apo-steroid receptors, Int. J. Biochem. Cell Biol. 41 (2009) 1652–1655.
[124] A.A. Duina, H.C. Chang, J.A. Marsh, S. Lindquist, R.F. Gaber, A cyclophilin function in Hsp90-dependent signal transduction, Science 274 (1996) 1713–1715.
[125] J. Fanghanel, G. Fischer, Insights into the catalytic mechanism of peptidyl prolyl cis/trans isomerases, Front. Biosci. 9 (2004) 3453–3478.

[126] B.K. Ward, P.J. Mark, D.M. Ingram, R.F. Minchin, T. Ratajczak, Expression of the estrogen receptor-associated immunophilins, cyclophilin 40 and FKBP52, in breast cancer, Breast Cancer Res. Treat. 58 (1999) 267–280.
[127] W.K. Sumanasekera, E.S. Tien, J.W. Davis 2nd, R. Turpey, G.H. Perdew, J.P. Vanden Heuvel, Heat shock protein-90 (Hsp90) acts as a repressor of peroxisome proliferator-activated receptor-alpha (PPARalpha) and PPARbeta activity, Biochemistry 42 (2003) 10726–10735.
[128] B.K. Meyer, G.H. Perdew, Characterization of the AhR–hsp90–XAP2 core complex and the role of the immunophilin-related protein XAP2 in AhR stabilization, Biochemistry 38 (1999) 8907–8917.
[129] B.K. Meyer, J.R. Petrulis, G.H. Perdew, Aryl hydrocarbon (Ah) receptor levels are selectively modulated by hsp90-associated immunophilin homolog XAP2, Cell Stress Chaperones 5 (2000) 243–254.
[130] A. Kazlauskas, S. Sundstrom, L. Poellinger, I. Pongratz, The hsp90 chaperone complex regulates intracellular localization of the dioxin receptor, Mol. Cell. Biol. 21 (2001) 2594–2607.
[131] B.N. Fukunaga, M.R. Probst, S. Reisz-Porszasz, O. Hankinson, Identification of functional domains of the aryl hydrocarbon receptor, J. Biol. Chem. 270 (1995) 29270–29278.
[132] F. Edlich, F. Erdmann, F. Jarczowski, M.C. Moutty, M. Weiwad, G. Fischer, The Bcl-2 regulator FKBP38-calmodulin-Ca2+ is inhibited by Hsp90, J. Biol. Chem. 282 (2007) 15341–15348.
[133] T. Okamoto, Y. Nishimura, T. Ichimura, K. Suzuki, T. Miyamura, T. Suzuki, K. Moriishi, Y. Matsuura, Hepatitis C virus RNA replication is regulated by FKBP8 and Hsp90, EMBO J. 25 (2006) 5015–5025.
[134] H. Kang, S.L. Sayner, K.L. Gross, L.C. Russell, M. Chinkers, Identification of amino acids in the tetratricopeptide repeat and C-terminal domains of protein phosphatase 5 involved in autoinhibition and lipid activation, Biochemistry 40 (2001) 10485–10490.
[135] C.K. Vaughan, M. Mollapour, J.R. Smith, A. Truman, B. Hu, V.M. Good, B. Panaretou, L. Neckers, P.A. Clarke, P. Workman, P.W. Piper, C. Prodromou, L.H. Pearl, Hsp90-dependent activation of protein kinases is regulated by chaperone-targeted dephosphorylation of Cdc37, Mol. Cell 31 (2008) 886–895.
[136] M.J. Austin, P. Muskett, K. Kahn, B.J. Feys, J.D. Jones, J.E. Parker, Regulatory role of SGT1 in early R gene-mediated plant defenses, Science 295 (2002) 2077–2080.
[137] M.G. Catlett, K.B. Kaplan, Sgt1p is a unique co-chaperone that acts as a client adaptor to link Hsp90 to Skp1p, J. Biol. Chem. 281 (2006) 33739–33748.
[138] Y.T. Lee, J. Jacob, W. Michowski, M. Nowotny, J. Kuznicki, W.J. Chazin, Human Sgt1 binds HSP90 through the CHORD-Sgt1 domain and not the tetratricopeptide repeat domain, J. Biol. Chem. 279 (2004) 16511–16517.
[139] Y. Kadota, B. Amigues, L. Ducassou, H. Madaoui, F. Ochsenbein, R. Guerois, K. Shirasu, Structural and functional analysis of SGT1-HSP90 core complex required for innate immunity in plants, EMBO Rep. 9 (2008) 1209–1215.
[140] A. Takahashi, C. Casais, K. Ichimura, K. Shirasu, HSP90 interacts with RAR1 and SGT1 and is essential for RPS2-mediated disease resistance in Arabidopsis, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 11777–11782.
[141] M. Zhang, Y. Kadota, C. Prodromou, K. Shirasu, L.H. Pearl, Structural basis for assembly of Hsp90-Sgt1-CHORD protein complexes: implications for chaperoning of NLR innate immunity receptors, Mol. Cell 39 (2010) 269–281.
[142] R. Zhao, Y. Kakihara, A. Gribun, J. Huen, G. Yang, M. Khanna, M. Costanzo, R.L. Brost, C. Boone, T.R. Hughes, C.M. Yip, W.A. Houry, Molecular chaperone Hsp90 stabilizes Pih1/Nop17 to maintain R2TP complex activity that regulates snoRNA accumulation, J. Cell Biol. 180 (2008) 563–578.
[143] K. Eckert, J.M. Saliou, L. Monlezun, A. Vigouroux, N. Atmane, C. Caillat, S. Quevillon-Cheruel, K. Madiona, M. Nicaise, S. Lazereg, A. Van Dorsselaer, S. Sanglier-Cianferani, P. Meyer, S. Morera, The Pih1-Tah1 cochaperone complex inhibits Hsp90 molecular chaperone ATPase activity, J. Biol. Chem. 285 (2010) 31304–31312.
[144] D.L. Riggs, M.B. Cox, H.L. Tardif, M. Hessling, J. Buchner, D.F. Smith, Noncatalytic role of the FKBP52 peptidyl-prolyl isomerase domain in the regulation of steroid hormone signaling, Mol. Cell. Biol. 27 (2007) 8658–8669.
[145] D.F. Smith, W.P. Sullivan, T.N. Marion, K. Zaitsu, B. Madden, D.J. McCormick, D.O. Toft, Identification of a 60-kilodalton stress-related protein, p60, which interacts with hsp90 and hsp70, Mol. Cell. Biol. 13 (1993) 869–876.
[146] D.F. Smith, B.A. Stensgard, W.J. Welch, D.O. Toft, Assembly of progesterone receptor with heat shock proteins and receptor activation are ATP mediated events, J. Biol. Chem. 267 (1992) 1350–1356.
[147] M.P. Hernandez, A. Chadli, D.O. Toft, HSP40 binding is the first step in the HSP90 chaperoning pathway for the progesterone receptor, J. Biol. Chem. 277 (2002) 11873–11881.
[148] N.S. Cintron, D. Toft, Defining the requirements for Hsp40 and Hsp70 in the Hsp90 chaperone pathway, J. Biol. Chem. 281 (2006) 26235–26244.
[149] F. Forafonov, O.A. Toogun, I. Grad, E. Suslova, B.C. Freeman, D. Picard, p23/Sba1p protects against Hsp90 inhibitors independently of its intrinsic chaperone activity, Mol. Cell. Biol. 28 (2008) 3446–3456.
[150] B.C. Freeman, S.J. Felts, D.O. Toft, K.R. Yamamoto, The p23 molecular chaperones act at a late step in intracellular receptor action to differentially affect ligand efficacies, Genes Dev. 14 (2000) 422–434.
[151] Y.G. Zhao, R. Gilmore, G. Leone, M.C. Coffey, B. Weber, P.W. Lee, Hsp90 phosphorylation is linked to its chaperoning function. Assembly of the reovirus cell attachment protein, J. Biol. Chem. 276 (2001) 32822–32827.
[152] B.T. Scroggins, L. Neckers, Post-translational modification of heat-shock protein 90: impact on chaperone function, Expert Opin. Drug Discov. 2 (2007).
[153] M. Duval, F. Le Boeuf, J. Huot, J.P. Gratton, Src-mediated phosphorylation of Hsp90 in response to vascular endothelial growth factor (VEGF) is required for VEGF receptor-2 signaling to endothelial NO synthase, Mol. Biol. Cell 18 (2007) 4659–4668.
[154] M. Kurokawa, C. Zhao, T. Reya, S. Kornbluth, Inhibition of apoptosome formation by suppression of Hsp90beta phosphorylation in tyrosine kinase-induced leukemias, Mol. Cell. Biol. 28 (2008) 5494–5506.
[155] M. Mollapour, S. Tsutsumi, A.C. Donnelly, K. Beebe, M.J. Tokita, M.J. Lee, S. Lee, G. Morra, D. Bourboulia, B.T. Scroggins, G. Colombo, B.S. Blagg, B. Panaretou, W.G. Stetler-Stevenson, J.B. Trepel, P.W. Piper, C. Prodromou, L.H. Pearl, L. Neckers, Swe1Wee1-dependent tyrosine phosphorylation of Hsp90 regulates distinct facets of chaperone function, Mol. Cell 37 (2010) 333–343.
[156] M. Mollapour, S. Tsutsumi, A.W. Truman, W. Xu, C.K. Vaughan, K. Beebe, A. Konstantinova, S. Vourganti, B. Panaretou, P.W. Piper, J.B. Trepel, C. Prodromou, L.H. Pearl, L. Neckers, Threonine 22 phosphorylation attenuates hsp90 interaction with cochaperones and affects its chaperone activity, Mol. Cell 41 (2011) 672–681.
[157] M. Mollapour, S. Tsutsumi, Y.S. Kim, J. Trepel, L. Neckers, Casein kinase 2 phosphorylation of Hsp90 threonine 22 modulates chaperone function and drug sensitivity, Oncotarget 2 (2011) 407–417.
[158] S.P. Lees-Miller, C.W. Anderson, The human double-stranded DNA-activated protein kinase phosphorylates the 90-kDa heat-shock protein, hsp90 alpha at two NH2-terminal threonine residues, J. Biol. Chem. 264 (1989) 17275–17280.
[159] T.B. Deb, A.H. Zuo, Y. Wang, R.J. Barndt, A.K. Cheema, S. Sengupta, C.M. Coticchia, M.D. Johnson, Pnck induces ligand-independent EGFR degradation by probable perturbation of the Hsp90 chaperone complex, Am. J. Physiol. Cell Physiol. 300 (2011) C1139–C1154.
[160] A.S. Sreedhar, E. Kalmar, P. Csermely, Y.F. Shen, Hsp90 isoforms: functions, expression and clinical importance, FEBS Lett. 562 (2004) 11–15.
[161] Y. Miyata, Protein kinase CK2 in health and disease: CK2: the kinase controlling the Hsp90 chaperone machinery, Cell. Mol. Life Sci. 66 (2009) 1840–1849.
[162] Y. Yang, R. Rao, J. Shen, Y. Tang, W. Fiskus, J. Nechtman, P. Atadja, K. Bhalla, Role of acetylation and extracellular location of heat shock protein 90alpha in tumor cell invasion, Cancer Res. 68 (2008) 4833–4842.
[163] J.J. Kovacs, P.J. Murphy, S. Gaillard, X. Zhao, J.T. Wu, C.V. Nicchitta, M. Yoshida, D.O. Toft, W.B. Pratt, T.P. Yao, HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor, Mol. Cell 18 (2005) 601–607.
[164] P. Bali, M. Pranpat, J. Bradner, M. Balasis, W. Fiskus, F. Guo, K. Rocha, S. Kumaraswamy, S. Boyapalle, P. Atadja, E. Seto, K. Bhalla, Inhibition of histone deacetylase 6 acetylates and disrupts the chaperone function of heat shock protein 90: a novel basis for antileukemia activity of histone deacetylase inhibitors, J. Biol. Chem. 280 (2005) 26729–26734.
[165] E.F. de Zoeten, L. Wang, K. Butler, U.H. Beier, T. Akimova, H. Sai, J.E. Bradner, R. Mazitschek, A.P. Kozikowski, P. Matthias, W.W. Hancock, Histone deacetylase 6 and heat shock protein 90 control the functions of foxp3+ T-regulatory cells, Mol. Cell. Biol. 31 (2011) 2066–2078.
[166] B.T. Scroggins, K. Robzyk, D. Wang, M.G. Marcu, S. Tsutsumi, K. Beebe, R.J. Cotter, S. Felts, D. Toft, L. Karnitz, N. Rosen, L. Neckers, An acetylation site in the middle domain of Hsp90 regulates chaperone function, Mol. Cell 25 (2007) 151–159.
[167] G. Garcia-Cardena, R. Fan, V. Shah, R. Sorrentino, G. Cirino, A. Papapetropoulos, W.C. Sessa, Dynamic activation of endothelial nitric oxide synthase by Hsp90, Nature 392 (1998) 821–824.
[168] A. Martinez-Ruiz, L. Villanueva, C. Gonzalez de Orduna, D. Lopez-Ferrer, M.A. Higueras, C. Tarin, I. Rodriguez-Crespo, J. Vazquez, S. Lamas, S-nitrosylation of Hsp90 promotes the inhibition of its ATPase and endothelial nitric oxide synthase regulatory activities, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 8525–8530.
[169] M. Retzlaff, M. Stahl, H.C. Eberl, S. Lagleder, J. Beck, H. Kessler, J. Buchner, Hsp90 is regulated by a switch point in the C-terminal domain, EMBO Rep. 10 (2009) 1147–1153.
[170] R.J. Sims III, D. Reinberg, From chromatin to cancer: a new histone lysine methyltransferase enters the mix, Nat. Cell Biol. 6 (2004) 685–687.
[171] D.M. Ruden, X. Lu, Hsp90 affecting chromatin remodeling might explain transgenerational epigenetic inheritance in *Drosophila*, Curr. Genomics 9 (2008) 500–508.
[172] R. Hamamoto, Y. Furukawa, M. Morita, Y. Iimura, F.P. Silva, M. Li, R. Yagyu, Y. Nakamura, SMYD3 encodes a histone methyltransferase involved in the proliferation of cancer cells, Nat. Cell Biol. 6 (2004) 731–740.
[173] R. Hamamoto, F.P. Silva, M. Tsuge, T. Nishidate, T. Katagiri, Y. Nakamura, Y. Furukawa, Enhanced SMYD3 expression is essential for the growth of breast cancer cells, Cancer Sci. 97 (2006) 113–118.
[174] R. Zhao, W.A. Houry, Molecular interaction network of the Hsp90 chaperone system, Adv. Exp. Med. Biol. 594 (2007) 27–36.
[175] A. Ziemiecki, M.G. Catelli, I. Joab, B. Moncharmont, Association of the heat shock protein hsp90 with steroid hormone receptors and tyrosine kinase oncogene products, Biochem. Biophys. Res. Commun. 138 (1986) 1298–1307.
[176] K.J. Howard, S.J. Holley, K.R. Yamamoto, C.W. Distelhorst, Mapping the HSP90 binding region of the glucocorticoid receptor, J. Biol. Chem. 265 (1990) 11928–11935.
[177] Y. Xu, S. Lindquist, Heat-shock protein hsp90 governs the activity of pp 60v-src kinase, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 7074–7078.
[178] F. Momose, T. Naito, K. Yano, S. Sugimoto, Y. Morikawa, K. Nagata, Identification of Hsp90 as a stimulatory host factor involved in influenza virus RNA synthesis, J. Biol. Chem. 277 (2002) 45306–45314.
[179] A. Mayor, F. Martinon, T. De Smedt, V. Petrilli, J. Tschopp, A crucial function of SGT1 and HSP90 in inflammasome activity links mammalian and plant innate immune responses, Nat. Immunol. 8 (2007) 497–503.
[180] S. Boulon, B. Pradet-Balade, C. Verheggen, D. Molle, S. Boireau, M. Georgieva, K. Azzag, M.C. Robert, Y. Ahmad, H. Neel, A.I. Lamond, E. Bertrand, HSP90 and its R2TP/Prefoldin-like cochaperone are involved in the cytoplasmic assembly of RNA polymerase II, Mol. Cell 39 (2010) 912–924.

[181] D. Picard, B. Khursheed, M.J. Garabedian, M.G. Fortin, S. Lindquist, K.R. Yamamoto, Reduced levels of hsp90 compromise steroid receptor action in vivo, Nature 348 (1990) 166–168.
[182] W.B. Pratt, F.C. Dalman, S. Meshinchi, L.C. Scherrer, The relationship between glucocorticoid receptor binding to Hsp90 and receptor function, Nippon Naibunpi Gakkai Zasshi 66 (1990) 1185–1197.
[183] J.C. Young, N.J. Hoogenraad, F.U. Hartl, Molecular chaperones Hsp90 and Hsp70 deliver preproteins to the mitochondrial import receptor Tom70, Cell 112 (2003) 41–50.
[184] J.E. Whittier, Y. Xiong, M.C. Rechsteiner, T.C. Squier, Hsp90 enhances degradation of oxidized calmodulin by the 20 S proteasome, J. Biol. Chem. 279 (2004) 46135–46142.
[185] Y. Guo, T. Guettouche, M. Fenna, F. Boellmann, W.B. Pratt, D.O. Toft, D.F. Smith, R. Voellmy, Evidence for a mechanism of repression of heat shock factor 1 transcriptional activity by a multichaperone complex, J. Biol. Chem. 276 (2001) 45791–45799.
[186] J. Zou, Y. Guo, T. Guettouche, D.F. Smith, R. Voellmy, Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1, Cell 94 (1998) 471–480.
[187] K. Nadeau, A. Das, C.T. Walsh, Hsp90 chaperonins possess ATPase activity and bind heat shock transcription factors and peptidyl prolyl isomerases, J. Biol. Chem. 268 (1993) 1479–1487.
[188] R. Geller, M. Vignuzzi, R. Andino, J. Frydman, Evolutionary constraints on chaperone-mediated folding provide an antiviral approach refractory to development of drug resistance, Genes Dev. 21 (2007) 195–205.
[189] H.Y. Shim, X. Quan, Y.S. Yi, G. Jung, Heat shock protein 90 facilitates formation of the HBV capsid via interacting with the HBV core protein dimers, Virology 410 (2011) 161–169.
[190] S. Ujino, S. Yamaguchi, K. Shimotohno, H. Takaku, Heat-shock protein 90 is essential for stabilization of the hepatitis C virus nonstructural protein NS3, J. Biol. Chem. 284 (2009) 6841–6846.
[191] J.J. Hung, C.S. Chung, W. Chang, Molecular chaperone Hsp90 is important for vaccinia virus growth in cells, J. Virol. 76 (2002) 1379–1390.
[192] K.M. Kampmueller, D.J. Miller, The cellular chaperone heat shock protein 90 facilitates Flock House virus RNA replication in Drosophila cells, J. Virol. 79 (2005) 6827–6837.
[193] L. Dmochewitz, M. Lillich, E. Kaiser, L.D. Jennings, A.E. Lang, J. Buchner, G. Fischer, K. Aktories, R.J. Collier, H. Barth, Role of CypA and Hsp90 in membrane translocation mediated by anthrax protective antigen, Cell. Microbiol. 13 (2011) 359–373.
[194] G. Haug, J. Leemhuis, D. Tiemann, D.K. Meyer, K. Aktories, H. Barth, The host cell chaperone Hsp90 is essential for translocation of the binary Clostridium botulinum C2 toxin into the cytosol, J. Biol. Chem. 278 (2003) 32266–32274.
[195] D.A. Hubert, Y. He, B.C. McNulty, P. Tornero, J.L. Dangl, Specific Arabidopsis HSP90.2 alleles recapitulate RAR1 cochaperone function in plant NB-LRR disease resistance protein regulation, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 9556–9563.
[196] Y. Kadota, K. Shirasu, R. Guerois, NLR sensors meet at the SGT1-HSP90 crossroad, Trends Biochem. Sci. 35 (2010) 199–207.
[197] Y. Liu, T. Burch-Smith, M. Schiff, S. Feng, S.P. Dinesh-Kumar, Molecular chaperone Hsp90 associates with resistance protein N and its signaling proteins SGT1 and Rar1 to modulate an innate immune response in plants, J. Biol. Chem. 279 (2004) 2101–2108.
[198] O.A. Toogun, D.C. Dezwaan, B.C. Freeman, The hsp90 molecular chaperone modulates multiple telomerase activities, Mol. Cell. Biol. 28 (2008) 457–467.
[199] D.C. DeZwaan, O.A. Toogun, F.J. Echtenkamp, B.C. Freeman, The Hsp82 molecular chaperone promotes a switch between unextendable and extendable telomere states, Nat. Struct. Mol. Biol. 16 (2009) 711–716.
[200] R. Li, J. Soosairajah, D. Harari, A. Citri, J. Price, H.L. Ng, C.J. Morton, M.W. Parker, Y. Yarden, O. Bernard, Hsp90 increases LIM kinase activity by promoting its homodimerization, FASEB J. 20 (2006) 1218–1220.
[201] A. Citri, D. Harari, G. Shohat, P. Ramakrishnan, J. Gan, S. Lavi, M. Eisenstein, A. Kimchi, D. Wallach, S. Pietrokovski, Y. Yarden, Hsp90 recognizes a common surface on client kinases, J. Biol. Chem. 281 (2006) 14361–14369.
[202] C.M. Gould, N. Kannan, S.S. Taylor, A.C. Newton, The chaperones Hsp90 and Cdc37 mediate the maturation and stabilization of protein kinase C through a conserved PXXP motif in the C-terminal tail, J. Biol. Chem. 284 (2009) 4921–4935.
[203] K. Terasawa, K. Yoshimatsu, S. Iemura, T. Natsume, K. Tanaka, Y. Minami, Cdc37 interacts with the glycine-rich loop of Hsp90 client kinases, Mol. Cell. Biol. 26 (2006) 3378–3389.
[204] S.F. Falsone, S. Leptihn, A. Osterauer, M. Haslbeck, J. Buchner, Oncogenic mutations reduce the stability of SRC kinase, J. Mol. Biol. 344 (2004) 281–291.
[205] K. Richter, J. Buchner, Closing in on the hsp90 chaperone–client relationship, Structure 19 (2011) 445–446.
[206] U. Jakob, H. Lilie, I. Meyer, J. Buchner, Transient interaction of Hsp90 with early unfolding intermediates of citrate synthase. Implications for heat shock in vivo, J. Biol. Chem. 270 (1995) 7288–7294.
[207] D. Walerych, G. Kudla, M. Gutkowska, B. Wawrzynow, L. Muller, F.W. King, A. Helwak, J. Boros, A. Zylicz, M. Zylicz, Hsp90 chaperones wild-type p53 tumor suppressor protein, J. Biol. Chem. 279 (2004) 48836–48845.
[208] D. Walerych, M. Gutkowska, M.P. Klejman, B. Wawrzynow, Z. Tracz, M. Wiech, M. Zylicz, A. Zylicz, ATP binding to Hsp90 is sufficient for effective chaperoning of p53 protein, J. Biol. Chem. 285 (2010) 32020–32028.
[209] S. Rudiger, S.M. Freund, D.B. Veprintsev, A.R. Fersht, CRINEPT-TROSY NMR reveals p53 core domain bound in an unfolded form to the chaperone Hsp90, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 11085–11090.
[210] S.J. Park, B.N. Borin, M.A. Martinez-Yamout, H.J. Dyson, The client protein p53 adopts a molten globule-like state in the presence of Hsp90, Nat. Struct. Mol. Biol. 18 (2011) 537–541.
[211] F. Hagn, S. Lagleder, M. Retzlaff, J. Rohrberg, O. Demmer, K. Richter, J. Buchner, H. Kessler, Structural analysis of the interaction between Hsp90 and the tumor suppressor protein p53, Nat. Struct. Mol. Biol. (2011).
[212] W. Li, Y. Li, S. Guan, J. Fan, C.F. Cheng, A.M. Bright, C. Chinn, M. Chen, D.T. Woodley, Extracellular heat shock protein-90alpha: linking hypoxia to skin cell motility and wound healing, EMBO J. 26 (2007) 1221–1233.