

Review Article

Functions of the Hsp90 chaperone system: lifting client proteins to new heights

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Abstract: The molecular chaperone Hsp90 is an essential protein in eukaryotic organisms and is highly conserved throughout all kingdoms of life. It serves as a platform for the folding and maturation of many client proteins including protein kinases and steroid hormone receptors. To fulfill this task Hsp90 performs conformational changes driven by the hydrolysis of ATP. Further, it can resort to a broad set of co-chaperones, which fit the Hsp90 machinery to the needs of specific client proteins. During the last years the number of identified co-chaperones has been consistently rising, implying that the client spectrum of Hsp90 may be much more diverse and larger than currently known. Many cofactors contain a TPR-domain for interactions at the C-terminus of Hsp90 and in many cases their functions and client sets remain to be uncovered. Hsp90 is also a putative target to interfere with cancerous and infectious diseases. Thus the knowledge on more of its cellular functions would provide also more therapeutic options for the future. In this review we compile the current knowledge on the Hsp90 ATPase mechanism, cofactor regulation and prospects of Hsp90 inhibition.

Keywords: Hsp90, chaperone, clients, SHR, kinase, ATPase cycle

Introduction

Heat-shock protein 90 (Hsp90) is a highly conserved molecular chaperone and essential for viability in eukaryotic organisms, like *Saccharomyces cerevisiae* [1], *Caenorhabditis elegans* [2] and *Drosophila melanogaster* [3]. Initially it was discovered as a protein whose abundance increases upon heat stress, but already in unstressed cells the protein amounts to about 1% of the soluble cytosolic protein [4]. In vertebrates two distinct isoforms are encoded - the mostly inducible Hsp90 α and the constitutively expressed Hsp90 β [5, 6]. Two additional homologs of Hsp90 exist in mitochondria and in the endoplasmic reticulum - Trap1 and Grp94. All Hsp90 homologs have the same domain architecture with an N-terminal nucleotide binding domain (NTD), a middle domain (MD) and a C-terminal dimerization site (CTD). They also share the mechanism of ATP hydrolysis connected to domain movements (**Figure 1**) [7, 8]. These are initiated by a short segment of the N-domain, the ATP-lid, flapping over the ATP binding pocket [6]. This alteration induces con-

tact formation at the NTDs and further results in a twisted and closed Hsp90 dimer [7, 8] (**Figure 1**). Crystal structures point to rotation movements of the nucleotide-binding domains against the MD, which may precede the contact formation of the NTDs [9-11]. This closed conformation is essential for ATP hydrolysis, after which the N-domains separate from each other and ADP gets released. Hsp90 client proteins seem to interact with the moving Hsp90 scaffold to reach unfavorable conformations stabilized by the chaperone.

The best characterized of the many client proteins (listed at <http://www.picard.ch/>) originate from two classes: steroid hormone receptors (SHR) [12] and protein kinases [13]. In fact more than two-thirds of the protein kinases depend on Hsp90, likewise many transcription factors and, as recently reported, also many E3-ligases [14]. Most of these clients are directed to proteasomal degradation if Hsp90 fails its task [15]. It is challenging to elucidate the precise function of Hsp90 in these interactions and still many mechanistic details of client pro-

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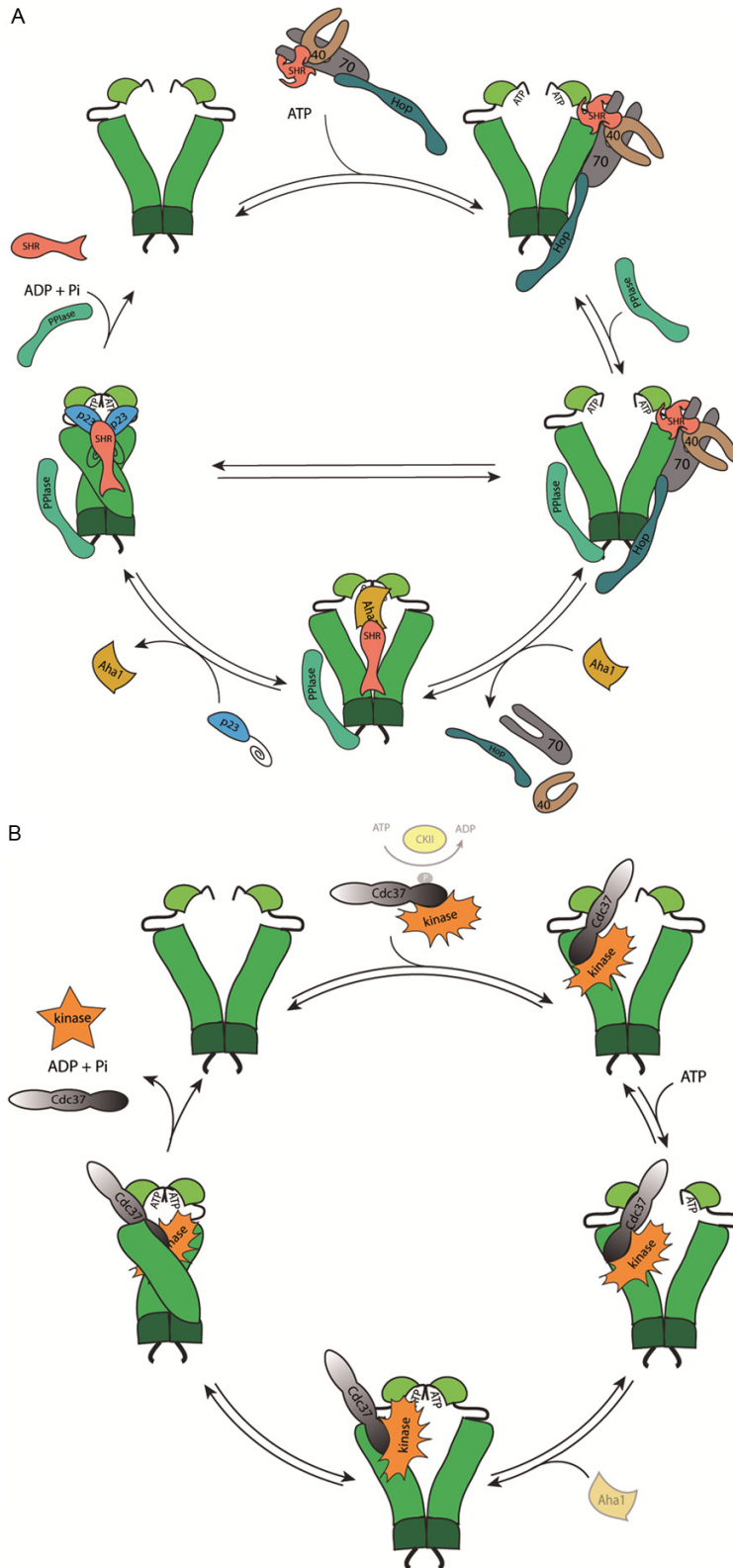


Figure 1. A: Model of the ATPase driven cycle to activate steroid hormone receptors. Different co-chaperones associate during the cycle at different stages of the SHR-activation process. B: A reaction pathway for kinase clients, which are brought into Hsp90 complexes by the kinase-specific co-chaperone Cdc37.

cessing are unclear. Do all Hsp90 clients require the same movements? How does Hsp90 recognize and interact with its clients? Does every client need its own set of co-chaperones? In this review we aim to compile the current knowledge on Hsp90 client interactions and describe the approaches to inhibit these processes by using Hsp90 inhibitory compounds.

Processing steroid hormone receptors with the help of cofactors

Complex formation between Hsp90 and its clients has been studied most extensively for SHRs (**Figure 1A**). After initial reports about Hsp90-SHR complexes in the early 1990s further proteins were identified in these assemblies [16-18] and most of these accessory proteins are helping Hsp90 during assembly of SHR complexes. At least a set of five additional proteins participate to mature SHRs in cell culture systems [19]. In the current model, the SHRs are first recognized by the Hsc70-Hsp40 chaperone complex [20, 21]. The adapter protein Hop/STIP1 (Hsp90/Hsp70 organizing protein, previously p60) is then docking this assembly to the open conformation of Hsp90. Hop/STIP1 itself consists of three TPR domains - TPR1, TPR2A and TPR2B. Hsp90 is bound to the TPR2A-domain and Hsc70 to the TPR1 or TPR2B domain enabling the client transfer between the chaperone systems [22, 23]. Another TPR-domain containing protein, like the PPlases FKBP4, FKBP5 or PPID*, binds to the second TPR-accessory motif of Hsp90 and forms an asym-

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metric complex consisting of one PPlase, Hop/STIP1, Hsp90 and the Hsc70-Hsp40 bound client protein [24, 25]. ATP-pandered conformational changes in Hsp90 lead to the closing of the N-terminal domains. This reduces the affinity of Hop/STIP1 for the assembly, leading to the exit of the adapter protein and its associated Hsc70-system (**Figure 1A**). The N-terminal dimerized conformation has high affinity for the cofactor p23, which serves to stabilize this closed complex [26]. In the p23-containing complex the steroid hormone receptor is able to bind steroid, while previous conformations were unable to do so [27]. p23 is not essential to open the hormone binding cleft of SHR but it stabilizes the heterocomplex of Hsp90 and SHR [28]. In particular the closing step can be accelerated by the Hsp90 ATPase activator Aha1 [26]. This active conformation now may exist for a certain time, until the chaperone opens up again for ADP release. Interestingly, for SHR, opening of the steroid binding cleft is the rate-limiting step on the way to a hormone binding state [29]. If steroid hormone is bound during this time, the steroid hormone receptor is released in an active form, is translocated into the nucleus where it can bind to glucocorticoid response elements on the DNA [30, 31]. If no hormone binding occurred the steroid hormone receptor is inactive and may re-enter the cycle. Apparently phosphorylation might also be relevant in this cycle, given the complex formation between Hsp90, SHR and the phosphatase PPP5 [32, 33].

It is still not clear, how the activation of SHRs by Hsp90 is achieved on a molecular level. Combining the extensive knowledge on the assembly process and on the conformational states of the co-chaperone interaction, it is likely that activation happens during the transfer from the Hop-containing Hsp90-complex to the closed state. It is well known that the p23-bound SHR is active [34] and the exclusive binding of p23 to the closed conformation of Hsp90 supports this activation model. Also, Hsp90 variants which block ATP-hydrolysis at the stage of the closed complex (like E47A-Hsp90) accumulate very stable active receptor complexes, suggesting that the important conformational changes in SHRs happen during the closing reaction of Hsp90 [35, 36].

Processing kinase clients

The activation and maturation of kinases differs from the SHR reaction cycle. A kinase-spe-

cific cofactor is involved here, the protein Cdc37 (cell division cycle 37). *In vitro* studies using reticulocyte lysate had initially shown that Raf kinase can exist in complex with Hsp90 and Cdc37 [13] but Cdc37 can also interact with kinases alone. As such it can bind to AKT (protein kinase B) [37], has an influence on Cdc28 and Cak1 [38] and on the catalytic activity of Hck [39]. Taipale and coworkers recently described hundreds of protein kinases that depend on the interaction with Cdc37 and Hsp90 [14].

Also for kinases a reaction cycle needs to be unraveled (**Figure 1B**). It is known that the N-terminal domain of Cdc37 is necessary to mediate interactions with protein kinases [40-42]. Cdc37 also binds to Hsp90 and inhibits its ATPase activity and conformational flexibility [43]. The Hsp90-Cdc37 interaction seems to involve two sites. *C. elegans* Cdc37 most strongly binds an M-domain binding site of Hsp90 whereas the human Cdc37 interacts stronger with the NTD [43-47]. It thus is likely that during complex formation with the kinase the binding features are changing and both binding sites are utilized by Cdc37 in the open and closed Hsp90 conformation. In our studies the Cdc37-Hsp90 system can be formed as an open and as a closed complex, suggesting that Cdc37 can participate in both, the open encounter complex and the stable closed complex (**Figure 1B**). It is unknown, whether the kinase also requires the help of Hsc70-Hsp40 during the transfer to Hsp90. While previous studies imply that Hsp70, Hsp40, Hop/STIP1, Cdc37 and Hsp90 are necessary to chaperone the kinase [48] direct binding studies see strong competition between Hop/STIP1 and Cdc37 for Hsp90-binding, making co-complexes of these proteins unlikely [49]. The kinase specific co-chaperone Cdc37 is putatively regulated by phosphorylation. It can be phosphorylated at Ser¹³ (Ser¹⁴ in yeast) by casein kinase 2 α (CK2) [42, 50] and gets dephosphorylated by the Hsp90-associated phosphatase PPP5 in the ternary complex of Hsp90, Cdc37 and PPP5 [42, 51]. This phosphorylation is assumed to regulate the affinity for the kinase.

Kinases seem to interact with Hsp90 and Cdc37 with structural motifs in the N-terminal lobe of the catalytic domain [52]. It has been shown that a conserved glycine-rich loop (the P-loop), necessary for ATP binding, plays an essential role in this [53, 54], but in all cases the catalytic domain of the protein kinase is

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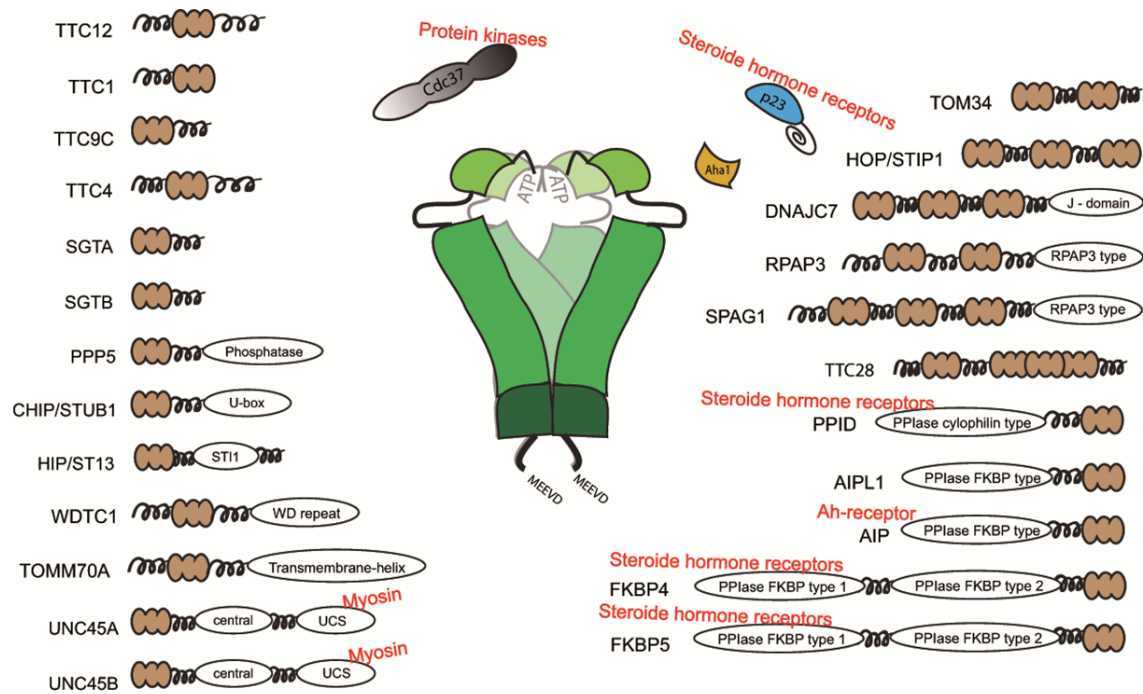


Figure 2. Overview of TPR-domain containing co-chaperones and the non-TPR containing Cdc37, p23 and Aha1. The different TPR-proteins are presumed to interact with Hsp90. The TPR domain is shown in beige.

involved [52]. For kinases, the MD and ND of Hsp90 seem to contain client binding sites [55-57]. Structural evidence from EM reconstitutions of the Hsp90-Cdc37-Cdk4 complex suggests that the kinase is located on the outside of the Hsp90 dimer [55]. Recent studies show that the chaperone system competes with ATP-binding to the kinase, with Cdc37 leaving the complex, as soon as the kinase is stabilized by nucleotide [58].

Also for kinases evidence suggests that in particular the closed conformation is the high affinity client binding state. Two-hybrid screens to detect Hsp90 clients in yeast only resulted in kinase hits, if the ATPase deficient E33A-variant was used as bait [59], implying that like with steroid hormone receptors, here also the enrichment of the closed state during the cycle results in stable complex formation.

Processing of other clients by Hsp90

Despite SHRs and kinases also the interaction site of Hsp90 with the tumor suppressor protein p53 has been studied in detail. This interaction relies mainly on electrostatic charges in the CTD and MD of Hsp90 [60]. The strong hydrophilic character of the binding may sug-

gest that Hsp90 client complexes do not go much beyond the initial encounter complex, which is usually characterized by very hydrophilic interactions [61]. This electrostatic interplay, in contrast to the more hydrophobic interaction with Hsc70, likewise has been observed in very early studies on SHR-Hsp90 complexes [34], complexes between the artificial client protein $\Delta 131\Delta$ of staphylococcus nuclease with Hsp90 [56] and between Hsp90 and citrate synthase [62].

Given the highly diverse client set of Hsp90, it may be rewarding to speculate about the nature of the client-Hsp90 interaction in the context of the potential development of this folding helper. How did these chaperone machines evolve and how did the chaperone client proteins become dependent on the chaperones? One explanation might be that any energy-driven cellular machine, which performs directed movements utilizing freely available energy sources like ATP, potentially attracts the interest of proteins in need of conformational rearrangements. In cases where this interaction is beneficial and sufficient evolutionary pressure is present on the future client, the chaperone machinery and the client protein may co-evolve

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to improve their interaction, manifesting the dependency of the client protein on Hsp90. Other clients may also utilize the same machinery, maybe even in a different way, and evolve to make maximal use of the folding helper [63, 64]. Hsp90 thus would become a scaffold, which utilizes energy to perform a more or less predictable set of movements and adaptive client proteins learned to interact with these in a beneficial way.

Like the number of client proteins, also the number of Hsp90-associated cofactors increased strongly in higher eukaryotes. This is evident from the high number of TPR-containing proteins in mammals [65]. Some of these proteins, originally devoid of a TPR-motif obtained the ability to interact with Hsp90 by adopting a TPR-domain. One such example is UNC-45, which plays an essential role - together with Hsp90 - during myosin fiber formation in muscles [49, 66, 67]. Like UNC-45, several other TPR-domain containing cofactors emerged in higher eukaryotes (**Figure 2**). For some of these proteins only limited information on their function is available, but it is to be expected that new TPR-domain containing Hsp90 cofactors enable new cellular functions (**Figure 2**). These cofactors include for example the protein Xap2/AIP, which was found in complex with the dioxin-receptor [68, 69] or the mitochondrial TOM70 import receptor. While the core system is remarkably conserved in all eukaryotes, several other proteins, which may constitute Hsp90 partner proteins, were identified by bioinformatics approaches in higher eukaryotes, including TTC28, TTC12, WDTC1 or RPAP3 [65]. This increasing amount of co-chaperones provides opportunities for new classes of clients. It therefore is not surprising that entirely new unrelated clients emerge in higher eukaryotes like the reverse transcriptase from Hepatitis B virus or the RNA-interacting domain of the Argonaut proteins [70, 71].

Hsp90 inhibitors

Some of the established Hsp90 clients play a central role in human diseases like cancer, neurodegenerative disorders and viral infections. Mutated or overexpressed kinases like Akt [72] Bcr-Abl [73] and Raf1 [74] control cancerogenic growth [75] and it was shown that especially Hsp90 is overexpressed in cancer cells [76]. In

mamma carcinomas Hsp90 helps cancer cells to obtain resistance to certain forms of chemotherapy [77, 78]. Thus it is of interest to use the chaperone as a therapeutic target.

The first identified inhibitors were the natural benzoquinone ansamycin geldanamycin (GM) and the macrolactone radicicol (RD). They show antiproliferative and antitumor effects *in vitro* and *in vivo* [74, 79, 80]. Both inhibitors bind to the N-terminal ATP binding pocket of Hsp90 with a much higher affinity than the nucleotide [79-82]. As a consequence nucleotide binding is blocked, the chaperone cycle arrested and the stable complex with closed N-terminal domains becomes unachievable, leading to degradation of the Hsp90 client [83].

GM and RD have some pharmacological disadvantages like limited solubility and off-target toxicities [84]. Therefore semisynthetic GM analogues were developed: 17-allylamino-17-demethoxygeldanamycin (17-AAG), a carbon-17 substituted derivate, is less toxic and shows similar anti-proliferative effects [85]. Also fully synthetic inhibitors have been developed employing purine scaffolds, fragment-based screening or virtual-screening methods leading to new chemical inhibitors of the Hsp90 ATPase [84, 86]. Besides N-terminal inhibitors of Hsp90, novobiocin, an amino coumarin, is a compound targeting the dimerization domain of Hsp90 [87, 88]. Several of these Hsp90 inhibitors are in clinical trials [89].

Hsp90 is also relevant for neurodegenerative and infectious diseases. It stabilizes p35, a CDK5 activating protein leading to aberrant tau phosphorylation and also directly interacts with a mutated form of tau protein [90]. It thereby influences the accumulation of toxic tau aggregates in tauopathy like Alzheimer's disease (AD) and frontotemporal dementia (FTD) [90]. In a mouse model of tauopathy, a purine-scaffold Hsp90 inhibitor leads to a decrease in relevant tau species [91]. In viral infections Hsp90 plays a central role in the folding of essential viral proteins [92]. Here it is even possible to inhibit the replication of poliovirus and paramyxovirus by Hsp90-inhibition [92, 93]. Thus the importance to understand the Hsp90 machinery in detail is also underscored by the growing list of diseases associated with this machinery and future developments to modulate its activity.

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