Structure, Function and Regulation of the Hsp90 Machinery

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Heat shock protein 90 (Hsp90) is an ATP-dependent molecular chaperone which is essential in eukaryotes. It is required for the activation and stabilization of a wide variety of client proteins and many of them are involved in important cellular pathways. Since Hsp90 affects numerous physiological processes such as signal transduction, intracellular transport, and protein degradation, it became an interesting target for cancer therapy. Structurally, Hsp90 is a flexible dimeric protein composed of three different domains which adopt structurally distinct conformations. ATP binding triggers directionality in these conformational changes and leads to a more compact state. To achieve its function, Hsp90 works together with a large group of cofactors, termed co-chaperones. Co-chaperones form defined binary or ternary complexes with Hsp90, which facilitate the maturation of client proteins. In addition, posttranslational modifications of Hsp90, such as phosphorylation and acetylation, provide another level of regulation. They influence the conformational cycle, co-chaperone interaction, and inter-domain communications. In this review, we discuss the recent progress made in understanding the Hsp90 machinery. *(Biomed J 2013;36:106-117)*

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Heat shock protein 90 (Hsp90), one of the most abundant and conserved molecular chaperones, is essential in eukaryotic cells.[1,2] Different from other well-known molecular chaperone like Hsp70 and GroEL/ES, Hsp90 is not required for *de novo* folding of most proteins but facilitates the final maturation of a selected clientele of proteins.[3] Hsp90 clients include protein kinases, transcription factors such as p53, and steroid hormone receptors (SHRs).[4-7] Therefore, Hsp90 does not only function in protein folding but also contribute to various cellular processes including signal transduction, intracellular transport, and protein degradation.

Interestingly, while bacteria possess an Hsp90 protein, called HtpG in *Escherichia coli*, no Hsp90 gene has been found in archea.[8-10] However, bacterial Hsp90 is not essential and its precise function remains to be investigated. Recent studies suggest that it collaborates with the DnaK (Hsp70) system in substrate remodeling and may function against oxidative stress.[11,12] In yeast, there are two Hsp90 isoforms in the cytosol, Hsc82 and Hsp82, of which Hsp82 is up-regulated up to 20 times under heat stress.[2] Hsp90α and Hsp90β are the two major isoforms in the cytoplasm of mammalian cells. Hsp90α is inducible under stress conditions, while Hsp90β is constitutively expressed.[13] Hsp90 analogues also exist in other cellular compartments such as Grp94 in the endoplasmic reticulum, Trap-1 in the mitochondrial matrix, and ch-Hsp90 in the chloroplast.[14-16] Interestingly, Hsp90 can be secreted as well and it promotes tumor invasiveness. Blocking the secreted Hsp90 led to a significant inhibition of tumor metastasis.[17]

Structure of Hsp90

Structurally, Hsp90 is a homodimer and each protomer contains three flexibly linked regions, an N-terminal ATP-binding domain (N-domain), a middle domain (M-domain), and a C-terminal dimerization domain (C-domain) [Figure 1].[18] Except for the charged linker located

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between the N- and M-domains in eukaryotic Hsp90, this domain organization is conserved from bacteria to man. Hsp90 is a member of a special class of structurally related, evolutionarily conserved split ATPases, the so-called Gyrase, Hsp90, Histindine Kinase, MutL (GHKL) domain ATPases, which contain a Bergerat ATP-binding fold.[19] Another interesting feature of the ATP binding region is that several conserved amino acid residues form a "lid" that closes over the nucleotide binding pocket in the ATP-bound state but is open during the ADP-bound state.[18] The M-domain of Hsp90 is involved in ATP hydrolysis, as it contains crucial catalytic residues for forming the composite ATPase site. Moreover, the M-domain contributes to the interaction sites for client proteins and some co-chaperones.[20] The C-domain is essential for the dimerization of Hsp90. Interestingly, in eukaryotic Hsp90, the opening of the C-domains is anti-correlated to the closing of the N-domain.[21] A conserved MEEVD motif at the C-terminal end serves as the docking site for the interaction with co-chaperones which contain a tetratricopeptide repeat (TPR) clamp.[22]

Conformational dynamics of Hsp90

Hsp90 is a weak ATPase and the turnover rates are very low, with 1 min^{-1} for yeast Hsp90 and 0.1 min^{-1} for human Hsp90.[23-25] Structural studies revealed that Hsp90 spontaneously adopts structurally distinct conformations, which seem to be in a dynamic equilibrium [Figure 1].[9,26] Nucleotide binding induces directionality and a conformational cycle.[9,27,28] In the apo state, Hsp90 adopts a "V"-shaped form, termed "open conformation" [Figure 1]. ATP binding triggers a series of conformational changes including repositioning of the N-terminal lid region and a dramatic change in the N-M domain orientation. Finally, Hsp90 reaches a more compact state, termed "closed conformation" in which the N-domains are dimerized [Figure 1].[9,18] Recent biophysical studies using ensemble and single molecule fluorescence resonance energy transfer (FRET) assays allowed to further dissect the ATP-induced conformational changes [Figure 2].[26,28] After fast ATP binding, Hsp90 slowly reaches the first intermediate state (I1), in which the ATP lid is closed but the N-domains are still open. The N-terminal dimerization leads to the formation of the second intermediate state (I2), in which the M-domain repositions and interacts with the N-domain. Then Hsp90 reaches a fully closed state in which ATP hydrolysis occurs. After ATP is hydrolyzed, the N-domains dissociate, release ADP as well as inorganic phosphate (Pi), and Hsp90 returns to the open conformation again.[28]

Notably, nucleotide binding is not the only determinant for Hsp90 conformation. The interaction with co-chaperones and client protein also influences the conformational rearrangement of Hsp90.[29,30] These results suggest that there may be a dynamic equilibrium between the different conformations of Hsp90 and this conformational plasticity is functionally important since it may allow Hsp90 to adapt to different client proteins.

Co-chaperone regulation of Hsp90

Co-chaperone regulation is a conserved feature of the eukaryotic Hsp90 system. To date, more than 20 co-chaperones have been identified.[1,31] They regulate the function of Hsp90 in different ways such as inhibition and activation of the ATPase of Hsp90 as well as recruitment of specific client

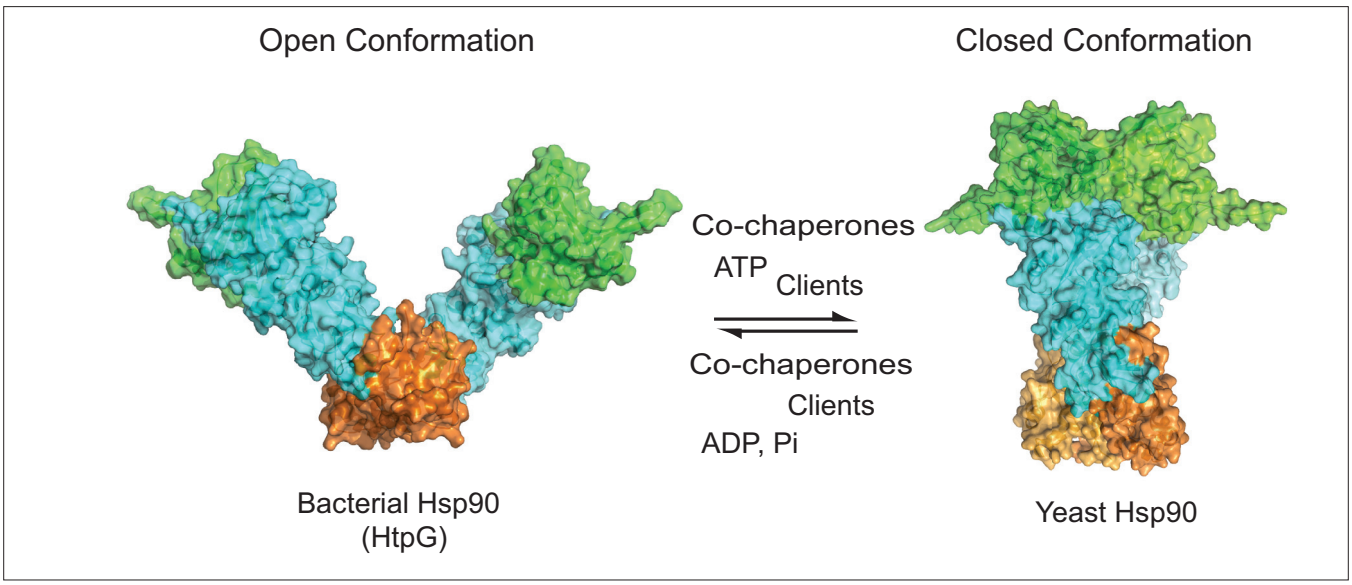

Figure 1: Open and closed conformation of Hsp90. Crystal structures of full-length Hsp90 from *E. coli* (HtpG) in the open conformation (left, PDB 2IOQ) and nucleotide-bound yeast Hsp90 in the closed conformation (right, PDB 2CG9). The N-domain is depicted in green, the M-domain in blue, and the C-domain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

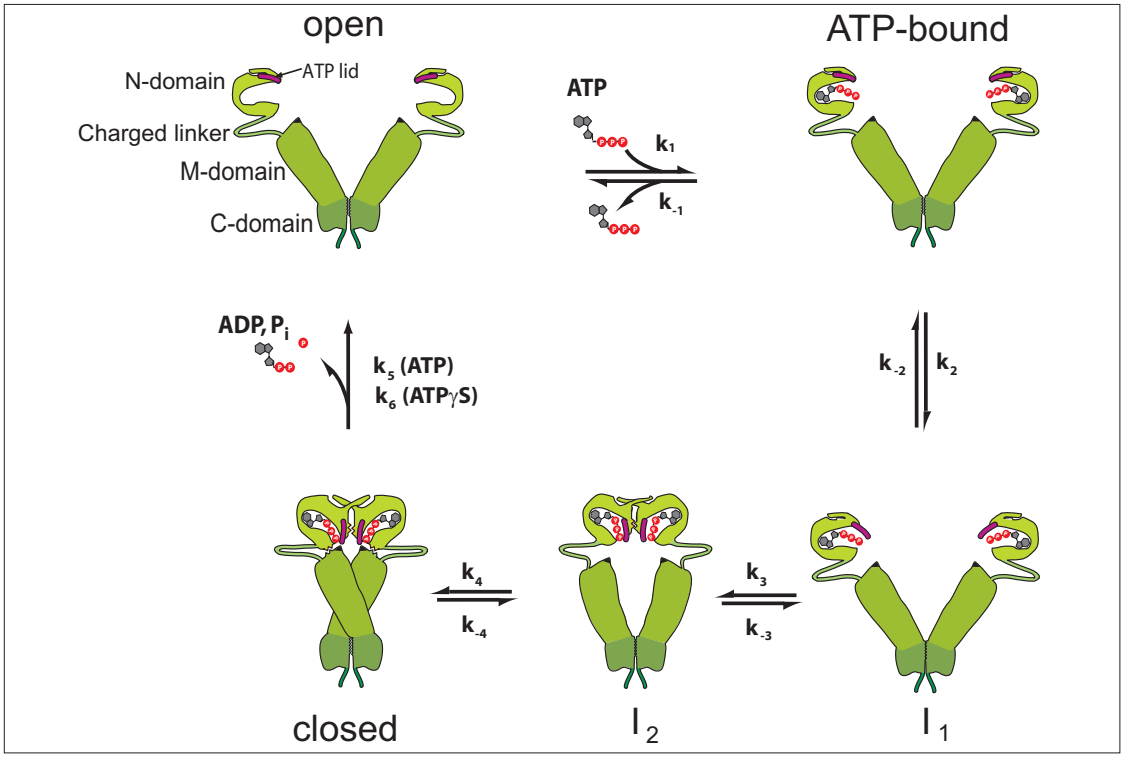

Figure 2: Conformational cycle of Hsp90. After fast ATP binding, Hsp90 slowly reaches the first intermediate state (I1), in which the ATP lid is closed but the N-domains are still open. Then, the N-terminal dimerization leads to the formation of the second intermediate state (I2), in which the M-domain repositions and interacts with the N-domain. Then, Hsp90 reaches a fully closed state in which ATP hydrolysis occurs. After ATP is hydrolyzed, the N-domains dissociate, release ADP and Pi, and Hsp90 returns to the open conformation.

proteins to the cycle. Interestingly, different co-chaperones work together to facilitate the maturation of Hsp90 clients.[32] The composition of co-chaperone complexes seems to depend to some degree on the presence of a specific client protein.

The chaperone cycle for SHRs

Early work on Hsp90 mainly focused on the co-chaperone requirement for the activation of SHRs.[32,33] The maturation of most SHRs strictly depends on the interaction with Hsp90. Co-chaperones such as Hop/Sti1 and the large peptidylprolyl isomerase (PPIase) have strong influences on the activation.[32,34] Research on the assembly of Hsp90 with SHRs had shown that several distinct complexes are formed during the maturation processes.[32,35,36] According to reconstitution experiments, SHRs must pass through three complexes with different co-chaperone compositions chronologically to reach their active conformation. Hsp70/Hsp40 were identified as components in the "early complex."[32] After association with Hsp90 through the adaptor protein Hop, the "intermediate complex" is formed.[37,38] In addition to the intermediate complex, a third complex that contains a PPIase and the co-chaperone p23 had been found as the last step of the cycle.[39-41] Notably, similar heterocomplexes can be found from yeast to man even in the absence of client protein.[32] Recent studies [using FRET, analytical ultracentrifugation (aUC), nuclear magnetic resonance (NMR) spectroscopy, and electron microscopy] provided insight into how the exchange of co-chaperones is regulated.[42-44] Based on these results, a new model of the chaperone cycle emerges [Figure 3A], in which first one Hop/Sti1 binds to the Hsp90 dimer and stabilizes its open conformation. As a result, the Hsp90 ATPase activity is inhibited. 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 final complex together with Hsp90 and p23/Sba1. Following ATP hydrolysis, p23/Sba1, PPIase, and the folded client are released from Hsp90.[42]

Hop/Sti1 serves as an adaptor protein between Hsp70 and Hsp90 and facilitates the transfer of client protein.[37,38] Therefore, it is indispensable for maintaining the hormone binding activity of the glucocorticoid receptor (GR) and progesterone receptor (PR).[45,46] Notably, Hop/Sti1 is a member of the large group of TPR co-chaperones. They contain a specialized conserved TPR-clamp domain, which consists of three TPR motifs and recognizes the C-terminal

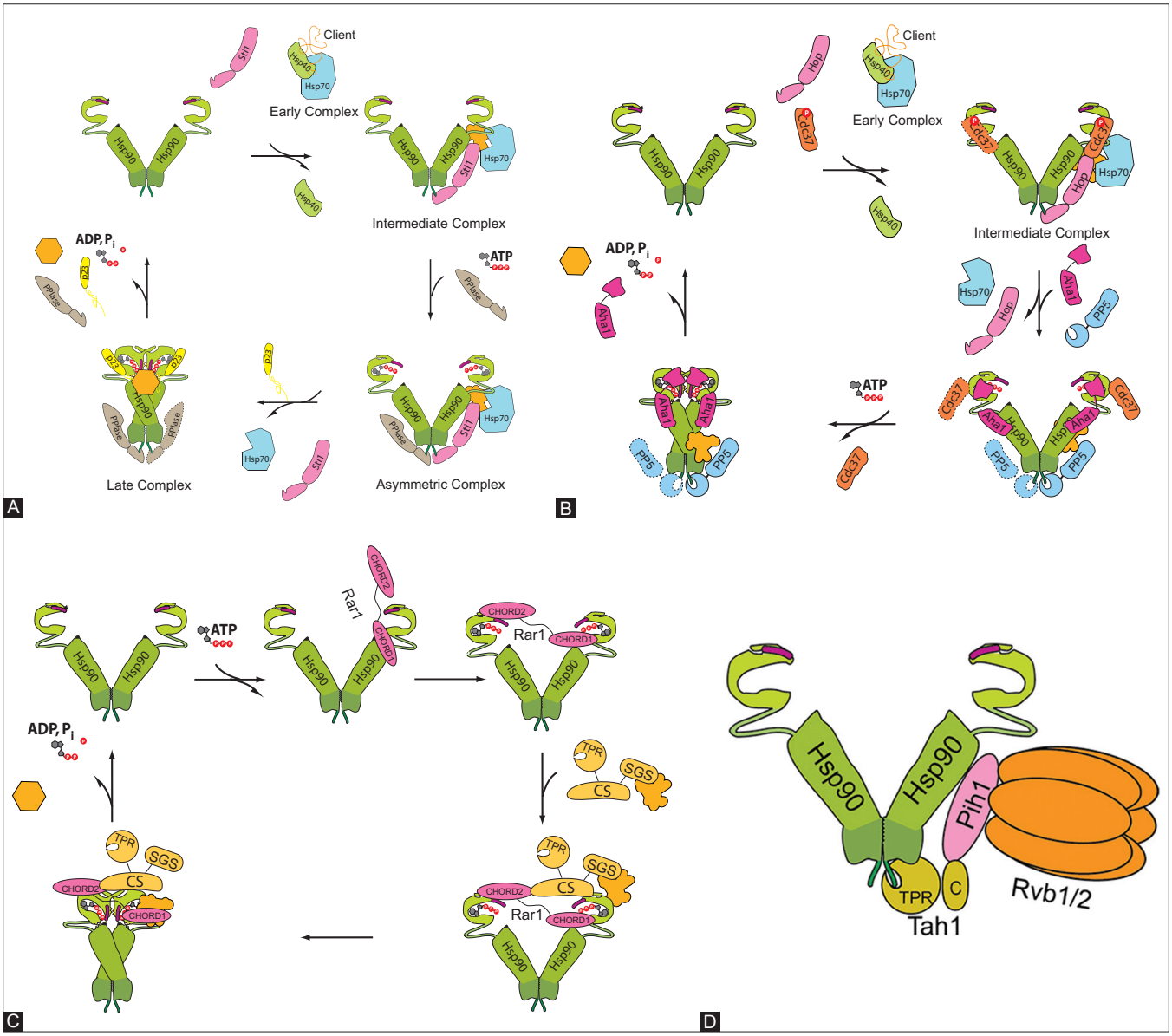

Figure 3: Hsp90 chaperone cycles. (A) Hsp90 chaperone cycle for SHRs. Hsp70, Hsp40, and a client protein form an "early complex." The client protein is transferred from Hsp70 to Hsp90 through the adaptor protein Hop/Sti1. One Hop/Sti1 bound is sufficient to stabilize the open conformation of Hsp90. The 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 the hydrolysis of ATP, p23/Sba1 and the folded client are released from Hsp90. (B) Hsp90 chaperone cycle for kinases. In the early stage, Hsp70 and Hsp40 interact with newly synthesized kinases. Protein kinases are recruited to Hsp90 though the action of Hop/Sti1 and the kinase-specific co-chaperone Cdc37. Both are able to stabilize the Hsp90/kinase complex. Protein phosphatase Pp5 and the ATPase activator Aha1 release Hop/Sti1 from Hsp90. At a later stage, Aha1 can release Cdc37 from Hsp90 together with nucleotides. (C) Hsp90 chaperone cycle for NLRs. Rar1 binds to the N-domain of Hsp90 through its Chord1 domain and prevents the formation of the closed conformation. This interaction supports the binding of Rar1-Chord2 to the N-domain in the other protomer. With the association of Rar1-Chord2, Sgt1 interacts with Hsp90 as well as with an NLR protein. In the stable ternary complex, the lid segment promotes ATP hydrolysis. Once ATP is hydrolyzed, Rar1, Sgt1, and the NLR protein may dissociate from Hsp90. (D) Hsp90–R2TP complex. Model of the R2TP complex in yeast. Pih1 interacts with Rvb1/2, with the M-domain of Hsp90, and the C-domain of Tah1. Tah1 binds to the C-terminal MEEVD motif of Hsp90 through its TPR domain.

MEEVD motif in Hsp90.[22] Besides Hop/Sti1, the protein phosphatase PP5 (yeast homologue Ppt1), and members of the PPIase family, like Fkbp52, Fkbp51, and Cyp40 (yeast homologues Cpr6/Cpr7), belong to this group.

The TPR-containing PPIases contain a PPIase domain, which catalyzes the interconversion of the cis–trans isomeriza-

tion of peptide bonds prior to proline residues[47] and a TPR domain for the interaction with Hsp90. Most of these large PPIases show independent chaperone activity.[48-50] 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 estrogen receptor (ER) complexes[51] and Fkbp52 mediates potentiation of GR through increasing GR hormone-binding affinity.[34] Interestingly, the potentiation effects do not strictly depend on the PPIase activity of Fkbp52 as PPIase-deficient mutants are also able to potentiate GR transactivation, which suggests a noncatalytic role of PPIases in the regulation of SHR signaling.[52]

In contrast to Hop/Sti1 and the TPR-PPIases, p23 is a conformation-specific co-chaperone which binds exclusively to the closed conformation of Hsp90.[53,54] This small acidic protein contains an unstructured C-terminal tail, which is essential for its intrinsic chaperone activity.[55,56] p23 was identified as a component in SHR complexes, together with Hsp90 and a PPIase.[57] It facilitates the maturation of client proteins by stabilizing the closed conformation of Hsp90.[58] As a result, the ATP hydrolysis, which is indispensable for the release of the client protein,[59-61] is partially inhibited in the presence of p23/Sba1.[41,62]

Chaperone cycle for protein kinases

Similar to SHRs, the maturation of protein kinases also requires the Hsp70 chaperone machinery [Figure 3B].[63] In the early stage, Hsp70 and Hsp40 interact with newly synthesized kinases. Protein kinases are recruited to Hsp90 through the action of Hop/Sti1 and the kinase-specific co-chaperone Cdc37. Both are able to stabilize the Hsp90/kinase complex.[64] At a later stage, the ATPase activator Aha1 can release Cdc37 from Hsp90, together with nucleotides,[65] which leads to the final activation of protein kinases.

Cdc37 is specific for chaperoning kinases.[66,67] It was originally identified in *Saccharomyces cerevisiae* as a gene essential for cell cycle progression.[68,69] Cdc37 interacts with kinases through its N-terminal domain and binds to the N-domain of Hsp90 via its C-terminal part. Similar to Hop/Sti1, the interaction of Cdc37 with Hsp90 leads to the stabilization of the open conformation and the inhibition of Hsp90 ATPase activity.[70]

In contrast to the co-chaperones discussed above, Aha1 is the most powerful ATPase activator of Hsp90.[71] It binds the N- and M-domains of Hsp90.[20,30] Binding of Aha1 induces a partially closed Hsp90 conformation and accelerates the progression of the ATPase cycle dramatically.[28,30] The presence of Aha1 enables Hsp90 to bypass the I1 state and to directly reach I2 in the ATPase cycle.[28] The activation of specific clients such as viral Src kinase (v-Src) and SHRs is severely affected in Aha1 knockout cells.[72] Moreover, Aha1 plays a critical role in the inherited misfolding disease cystic fibrosis (CF) through participating in the quality control pathway of the cystic fibrosis transmembrane conductance regulator (CFTR). Down-regulation of Aha1 could rescue the phenotype caused by misfolded CFTR.[73] Recent research highlighted the function of Aha1 in the progression of the Hsp90 cycle. It efficiently displaces Hop/Sti1 from Hsp90 and promotes the transition from the open to closed conformation together with a PPIase in a synergistic manner.[74]

Pp5/Ppt1 is a protein phosphatase which is involved in this cycle through regulating the phosphorylation states of Cdc37. It associates with Hsp90 through its N-terminal TPR domain. Binding to Hsp90 results in the abrogation of the intrinsic inhibition of Pp5/Ppt1.[75] Pp5/Ppt1 specifically dephosphorylates Hsp90 and Cdc37 in Hsp90 complexes.[76,77] In Ppt1 knockout strains, the activity of Hsp90-specific clients is significantly reduced, which implies that the tight regulation of the Hsp90 phosphorylation state is necessary for the efficient processing of client proteins.[76]

Chaperone cycle for nucleotide-binding site and leucine-rich repeat domain containing (NLR) proteins

NLRs are conserved immune sensors which recognize pathogens.[78] Accumulating evidence indicates that Hsp90 and its co-chaperones Sgt1 and Rar1 are involved in the maturation of these proteins.[79] Sgt1 interacts with the N-domain of Hsp90 through its CS domain, which is structurally similar to p23/Sba1.[80,81] However, Sgt1 has no inherent Hsp90 ATPase regulatory activity due to differences in interaction.[81] Interestingly, although a TPR domain is present in Sgt1 as well, it is not involved in the interaction with Hsp90.[82] Functionally, Hsp90 and Sgt1 form a ternary complex with the co-chaperone Rar1, which acts as a core modulator in plant immunity.[78]

During the recruitment and activation of NLRs, Rar1 binds to the N-domain of Hsp90 through its Chord1 domain and prevents the formation of the closed conformation [Figure 3C]. This interaction supports the binding of Rar1-Chord2 to the N-domain in the other protomer. With the association of Rar1-Chord2, Sgt1 is promoted to interact with Hsp90 as well as with an NLR protein. In the stable ternary complex, the lid segment is very flexible, thus permitting access by a catalytic arginine residue of the M-domain to the ATP binding site and promoting ATP hydrolysis. Once ATP is hydrolyzed, Rar1, Sgt1, and the NLR protein may dissociate from Hsp90.[83]

Hsp90 complexes in RNA processing

Recent studies showed that Hsp90 is also involved in the assembly of small nucleolar ribonucleoproteins (snoRNPs) and RNA polymerase.[84-86] The chaperone cycle is not com-

pletely understood yet. However, the central player in this process, the R2TP complex (consisting of Tah1, Pih1, and the AAA+ ATPase Rvb1 and Rvb2) has been extensively investigated [Figure 3D].[86,87]

The co-chaperone Tah1 interacts with Hsp90 through its TPR domain and its C-terminal region binds Pih1, an unstable non-TPR co-chaperone of Hsp90 [Figure 3D]. During the maturation of snoRNP, the Hsp90–Tah1 complex stabilizes Pih1 *in vivo* and prevents its aggregation *in vitro*.[84] The Tah1–Pih1 heterodimer is able to inhibit the ATPase activity of Hsp90.[88] Tah1 and Pih1 are then transferred to the Rvb1/2 complex leading to the formation of the R2TP complex [Figure 3D]. Together, Hsp90 and the R2TP complex are involved in the biogenesis and assembly of snoRNPs. Notably, neither Hsp90 nor R2TP are components of the mature snoRNP complex. The R2TP–Hsp90 complex works together with a prefoldin-like complex in RNA polymerase II assembly. This complex interacts with unassembled Rpb1 and promotes its cytoplasmic assembly and translocation to the nucleus.[85]

In addition to the activation of client protein, co-chaperones are also involved in other physiological processes, such as mitochondrial/chloroplast protein import (Tom70/Toc64),[89,90] nuclear migration (NudC),[91] and melanoma progression (TTC4).[92] The above examples provide a glimpse on Hsp90 co-chaperone cycles. For some cycles, we have obtained a full picture with detailed information; for others, we just start to understand their contributions to client protein activation.

Regulation of Hsp90 by posttranslational modifications

Posttranslational modifications are another important regulatory element of the Hsp90 machinery. Different posttranslational modifications such as phosphorylation, acetylation, nitrosylation, and methylation tightly control the function of Hsp90 and thus influence the maturation of client proteins.[93]

Phosphorylation

Phosphorylation is the most frequently detected post-translational modification of Hsp90. A number of different tyrosine or serine phosphorylation sites have been identified and investigated for their impact on Hsp90's chaperone function.[94] For example, only phosphorylated Hsp90 stimulates the activity of the Hsp90 client protein heme-regulated inhibitor kinase (HRI); dephosphorylation eliminated the ability of Hsp90 to activate this client protein.[95] Interestingly, hyperphosphorylation also leads to a decreased Hsp90 activity. In yeast, the protein phosphatase Ppt1 deletion compromised the activation of specific clients.[76] Therefore, the phosphorylation states of Hsp90 must be precisely regulated in order to maintain the proper function of Hsp90. In addition, phosphorylation also modulates the interaction with co-chaperones and thus exerts further influence on the Hsp90 machinery.[96] For example, tyrosine phosphorylation on Hsp90 disrupts the interaction with Cdc37 and promotes the recruitment of Aha1.[97] C-terminal phosphorylation of Hsp90 regulates alternate binding to co-chaperones Chip and Hop, which determine cellular protein folding/degradation balances.[98] Furthermore, phosphorylation affects the conformational cycle of Hsp90, such as formation of the active sites, general flexibility, and inter-domain communication.[96,99]

A number of different kinases can phosphorylate Hsp90, such as double-stranded DNA protein kinase, c-Src kinase, protein kinase A (PKA), CK2 protein kinase, and Swe1Wee1 kinase.[100-103] Interestingly, many of them are at the same time Hsp90 client proteins. This indicates that the change of phosphorylation states of Hsp90 may influence the folding and activation of certain groups of client proteins.

Acetylation

Acetylation is a reversible modification mediated by opposing actions of acetyltransferases and deacetylases.[104] Hsp90 acetylation and its influence on the chaperone machinery have been extensively investigated in recent years. In the case of Hsp90, p300 was reported to be the acetyltransferase and HDAC6 acts as a deacetylase which removes the acetyl group from the protein.[105,106] Deacetylation of Hsp90 drives the formation of Hsp90 client complexes and promotes the maturation of the client protein GR. Hsp90 can be acetylated at different sites.[107] A study from Necker's lab pointed out that K294, an acetylation site in the M-domain, strongly influences the binding between Hsp90 and its client protein. In general, acetylation weakens Hsp90–client interaction, and thus, Hsp90 fails to support the activation of the client protein.[108]

Nitrosylation

S-nitrosylation is a reversible covalent modification of reactive cysteine thiols in proteins by nitric oxide (NO).[109,110] Mammalian Hsp90 is a target of S-nitrosylation mediated by NO produced by its client protein, endothelial nitric oxide synthase (eNOS).[111] S-nitrosylation was reported as a negative regulator which inhibits the ATPase activity of Hsp90.[111] In addition, the activation of its client protein, eNOS, was also reduced consistent with the notion thatHsp90 acts as an NO sensor.[111] This provides a feedback mechanism to inhibit further eNOS activation. Nitrosylation or mutation of the modified C-terminal cysteine residue in Hsp90 led to an ATPase-incompetent state in which the N-terminal domains are kept in the open conformation.[112] The result indicates that nitrosylation has a profound impact on the inter-domain communication in the Hsp90 dimer.

Hsp90 client protein recognition

To date, more than 200 Hsp90 client proteins have been identified (see http://www.picard.ch/downloads/Hsp90interactors.pdf). Besides the well-studied clients such as protein kinases and SHRs, many others related to, for example, viral infection, innate immunity, and RNA modification, have been discovered in recent years.[84,113,114] The interaction with the Hsp90 machinery enables their correct folding, activation, transport, and even degradation.[89,115-117]

Our understanding of the Hsp90 machinery has been greatly advanced by research of the last decades. However, some fundamental questions related to client proteins still remained unanswered, such as the location of the client-binding sites on Hsp90. Current evidence suggests that binding sites could be localized in each of the domains of Hsp90.[8] Another intriguing question unsolved so far is how Hsp90 recognizes its clients. Hsp90 clients belong to different families and do not share common sequences or structural motifs. Although some regions were identified which are important for the recognition of certain group of clients, for example, the αC–β4 loop in kinases,[118,119] this is not the only determinant for the interaction as other regions adjacent to the kinase domain also influence the binding to Hsp90.[120,121] It is reasonable to assume that Hsp90 recognizes certain conformations or the stability of the client protein rather than its primary sequence. Src kinase is a prominent example here. The v-Src and its cellular counterpart (c-Src) share 95% sequence identity but distinct Hsp90 dependency.[122] The activation of v-Src strictly depends on Hsp90, while c-Src is largely independent of Hsp90.[122] Notably, v-Src is an aggregation-prone protein and much more sensitive to thermal and heat denaturation than c-Src.[123] In the case of p53, biochemical experiments suggest that p53 interacts with Hsp90 in a rather folded state.[124-126] However, recent results imply that p53 may be destabilized by Hsp90,[127] and NMR-based approaches suggested that for heat-treated p53, Hsp90 binds the largely unfolded protein.[128] Park *et al*., proposed that Hsp90-bound p53 is in a molten globule state.[129] In contrast, Hagn *et al*. reported a native-like structure of p53 interaction with Hsp90.[130] Further analysis seems to be required to resolve this conundrum and to determine the molecular mechanism for client recognition.

Hsp90 and protein degradation

Although in general, Hsp90 stabilizes and promotes the correct folding of its client proteins, it was also found to facilitate protein degradation. In addition to soluble cytosolic proteins, several reports have shown that Hsp90 is also required for the degradation of ER membrane proteins such as cytochrome p450 2E1, mutant CFTRΔF508, and apolipoprotein B.[131-133] Another aspect which supports the idea that Hsp90 may be involved in the ubiquitin–proteasome pathway is the discovery of a protein called carboxyl terminus of Hsp70-interacting protein (CHIP).[134] As an E3 ubiquitin ligase, CHIP can ubiquitinate unfolded proteins. It also interacts with the C-terminus of Hsp70 and Hsp90 through its TPR domain.[135,136] The CHIP knockdown is known to stabilize some Hsp90 clients, while its overexpression promotes their degradation.[137-139] Recently, more E3 ligases have been found to be associated with Hsp90, such as Ubr1 and Cul5 which are involved in the quality control or degradation of different client proteins.[140,141] However, aspects such as the selection of different ligases remain to be further elucidated.

Hsp90 inhibitors and human diseases

As many proteins which control cell survival, proliferation, and apoptosis are client proteins of Hsp90, Hsp90 function is closely related to human health. A number of reports have suggested that Hsp90 plays a crucial role in the progression of diseases.[142-145] For example, the expression of Hsp90 is 2- to 10-fold higher in tumor cells than in normal cells.[146] Therefore, Hsp90 has emerged as an important target in several diseases. Numerous natural and synthetic Hsp90 inhibitors have been developed in recent years, some of which exhibit excellent antitumor activities and have entered clinical trials.[141,144,147,148]

Geldanamycin (GA), a benzoquinone ansamycin antibiotic, is the first discovered Hsp90 inhibitor. It binds competitively to the ATP binding site in the N-domain of Hsp90 and thus prevents the conformational change of Hsp90.[149,150] GA exhibits potent antitumor effects; however, due to the poor solubility and high toxicity, it cannot be used as a drug candidate.[151] Different derivatives have been synthesized, such as 17-AAG, which is more hydrophilic and already showed success in preclinical and clinical studies.[152,153]

Radicicol (also known as monorden) is another natural product inhibitor of Hsp90, which is a 14-membered macrolide originally isolated from *Monosporium bonorden*.[154] Radicicol also acts as a nucleotide-mimicking compound and occupies the ATP binding pocket of Hsp90, but with a much higher affinity than ATP.[154] *in vitro* studies have shown that radicicol has potent anti-proliferation effects.[155] However, *in vivo* studies did not reveal anti-tumor activities, which is probably due to its low biological stability.[156]

Besides these two, many new inhibitors have been found or synthesized. For example, radanamycin amide (radamide) was designed based on the co-crystallization structures of the GA/Hsp90 N-domain and the Radiciol/Hsp90 N-domain.[149,157] This chimeric compound contains both radicicol's resorcinol ring and the quinine ring from GA. It shows potent inhibition effects of Hsp90 in a low

micromolar range in breast cancer cells.[157] PU3, a representative of purine-based inhibitor, binds to Hsp90 and inhibits the growth of breast cancer cells.[158] Novobiocin, a coumarin antibiotic, was also identified as an Hsp90 inhibitor.[159] Interestingly, novobiocin targets the C-terminal domain of Hsp90.[160] More compounds with better inhibitory activity and less toxicity are currently designed and synthesized.[161]

Currently, there are more than 10 different Hsp90 inhibitors in various stages of clinical development, like IPI504, NVP-AUY922, and STA-9090.[162] The results obtained so far look promising, but still several key questions need to be addressed. Like in basic research on Hsp90, significant progress has been made in recent years, but important questions remain to be solved. Prospects are very good that major breakthroughs will be achieved in the near future.

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