BioMol Concepts, Vol. 3 (2012), pp. 79–97 • Copyright © by Walter de Gruyter • Berlin • Boston. DOI 10.1515/BMC.2011.054

Review

The universe of Hsp90

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Abstract

Molecular chaperones are key components in the maintenance of cellular homeostasis and survival, not only during stress but also under optimal growth conditions. Among the ATP-dependent chaperones, heat shock proteins (Hsp90) proteins play a special role. While Hsp90s can interact with unfolded and misfolded proteins, their main (and in eukaryotic cells essential) function appears to involve interactions with a limited number of protein clients at late steps of maturation or in 'alter-native' conformations for regulating their stability and activity. Because Hsp90 clients are hubs of diverse signaling networks and participate in nearly every cellular function, Hsp90s interconnect many regulatory circuits and link them to environmental impacts. The availability and activity of Hsp90 may thus influence complex physiological and pathophysiological processes, such as differentiation, development, aging, cancer, neurodegeneration, and infectious diseases. Furthermore, through homeostatic effects on differentiation and development, Hsp90s act as capacitors of phenotypic evolution. In this review, we discuss recent insights in the structure and chaperone cycle of Hsp90s, the mechanisms underlying Hsp90 binding to clients, and potential reasons why client proteins specifically require the assistance of Hsp90s. Moreover, the current views on Hsp90-cochaperone interactions and regulation of Hsp90 proteins *via* posttranslational modifications are summarized. The second half of this article is devoted to the role of Hsp90 proteins in health and disease, aging, and evolution.

Keywords: aging; chaperones; evolution; protein folding; regulation by cochaperones; regulation by posttranslational modifications.

Introduction

The 90-kDa heat shock proteins (Hsp90s) are highly conserved, ubiquitously expressed in eukaryotic cells, and belong to the most abundant proteins in the cytosol. In eukaryotes, Hsp90s are essential for housekeeping functions and induced under various stress conditions, whereas in many prokaryotes, Hsp90s appear to be dispensable for most growth conditions. Hsp90s are homodimeric ATPases (1) and the ATPase activity of Hsp90s is essential *in vivo* (2, 3). The function of Hsp90s, in contrast to other chaperones, has remained an enigma for several decades. While the other ATP-dependent chaperones were shown to refold denatured model proteins *in vitro* (Hsp60 and Hsp70), or unfold proteins (Hsp100), no such activities could be found for Hsp90s (4, 5). Furthermore, Hsp90s do not appear to make an important contribution to *de novo* folding of proteins, another pivotal task of molecular chaperones (6). The observation that Hsp90s form complexes with certain nuclear receptors and protein kinases finally led to the hypothesis that these proteins act as activity regulators of a limited number of protein substrates, designated as 'clients.' To date, almost 300 *bona fide* Hsp90 clients have been identified. Among these, two dominant groups have been distinguished, specifically, transcription factors and kinases, most of which participate in signal transduction pathways of cell growth and differentiation. The 'other' clients form a very diverse group and include DNA- and RNA-binding proteins (including polymerases), ribosomal proteins, small GTPases, cytoskeletal proteins, and ion channels. Many viruses hijack Hsp90s for maturation of their proteins. Overall, Hsp90 clients are not related with regard to sequence or structure, and the mechanisms by which Hsp90s bind to these proteins and regulate their activity, turnover, trafficking, cofactor insertion, membrane insertion, ligand binding, or covalent modification are yet to be established. In eukaryotic cells, the ATPase cycle of Hsp90 is intimately coupled to the Hsp70 chaperone machine. Both systems rely on a large number of specific cochaperones, which enter and leave the machine in a defined order, thus regulating client maturation. The main aim of this article is to provide an overview of the intricacy of Hsp90 machinery and complexity of cytosolic Hsp90 function.

Structure and ATPase cycle of Hsp90

Hsp90 consists of an N-terminal nucleotide-binding domain (ND), a middle domain (MD) and a C-terminal dimerization domain (DD) (Figure 1). Both ND and MD are involved in ATP hydrolysis. All three domains interact with different cochaperones and substrates, although the middle domain appears to play a central role in these interactions (Figure 2). Based on the structure of ND, Hsp90 is classified as a member of a superfamily containing DNA mismatch repair protein MutL, DNA Gyrase B, and histidine kinases (1). The ND is also the binding site of the specific inhibitors, geldanamycin and radicicol (7).

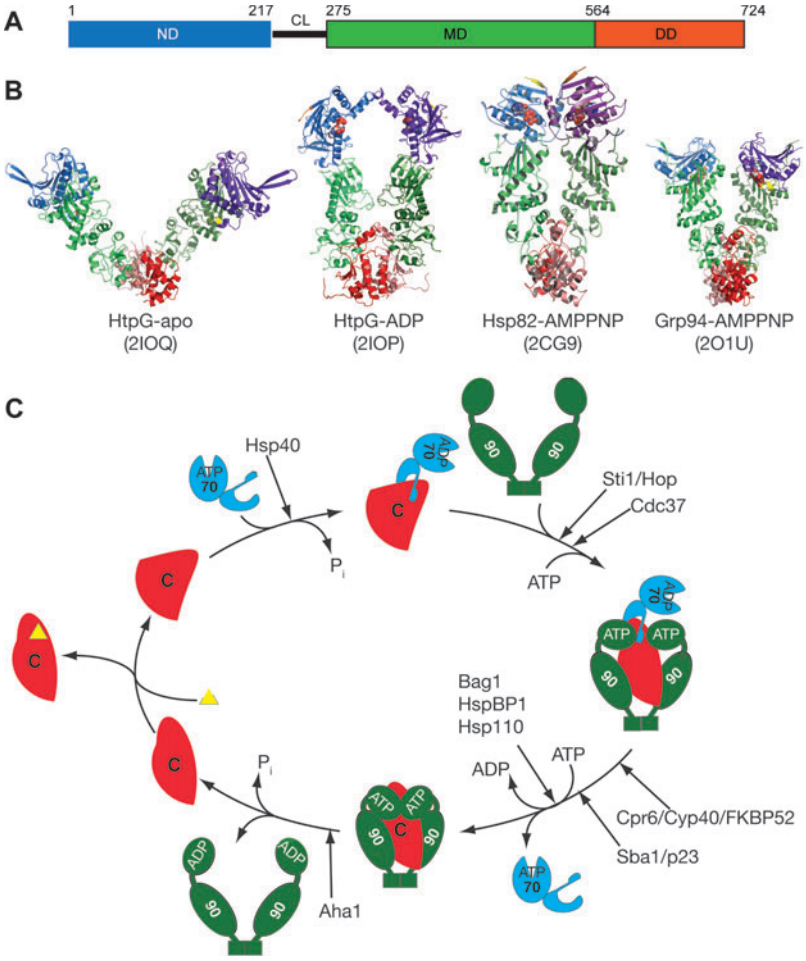

Figure 1 The Hsp90 domain organization, structures, and chaperone cycle.
(A) Domain organization of Hsp90. ND, nucleotide-binding domain; MD, middle domain; DD, C-terminal dimerization domain; CL, charged linker connecting ND and MD. Numbers indicate the domain boundaries in human Hsp90β. (B) Secondary structure representations of crystal structures of full-length Hsp90 proteins. HtpG-apo, dimeric model of *Escherichia coli* Hsp90 crystallized in the absence of nucleotides; HtpG-ADP, dimer of *E. coli* Hsp90 crystallized in the presence of ADP as an intercalating dimer of dimers; Hsp82-AMPPNP, structure of *Saccharomyces cerevisiae* Hsp90 crystallized in the presence of the cochaperone Sba1 (not shown) and AMPPNP; Grp94-AMPPNP, crystal structure of the *Canis familaris* endoplasmic reticulum Hsp90 in the presence of AMPPNP. The Protein Data Bank (PDB) entry codes are given below the structures. ND, blue/purple; MD, green; DD, red. The resolved N-terminal part of the structures, corresponding to five residues, are shown in yellow and orange to indicate the position of the N-termini. (C) Hsp90 chaperone cycle. Clients (C) initially interact with Hsp70 (70) mediated by J-domain cochaperones (Hsp40), which facilitate client binding by stimulating Hsp70 ATPase activity. The Hop/Sti1 scaffold cochaperone assembles the Hsp70-Hsp90-client complex. Hop/Sti1 is displaced by peptidyl-prolyl-cis/trans-isomerases (e.g., Cpr6, Cyp40, FKBP52) and p23/Sba1, which is coupled to ATP binding to Hsp90. Hsp70 release requires ADP dissociation and ATP binding probably catalyzed by nucleotide exchange factors (e.g., Bag1, HspBP1, Hsp110). Aha1-stimulated ATP hydrolysis by Hsp90 induces client release. The client may subsequently rebind Hsp70. Clients exit the chaperone cycle *via* ligand binding or posttranslational modifications (yellow triangle).

The Hsp90 dimer displays a large degree of conformational freedom, as evident from crystal structures, electron microscopy (EM) and small angle X-ray scattering (SAXS) data for different homologs (8–11) (Figure 1B). The subdomains show significant differences in their relative orientations, and the protomers perform wide shearing and twisting motions around the DD, ranging from wide open to very compact closed states with dimerized NDs. Furthermore, Hsp90 appears to fluctuate continuously between two open and two closed conformations in the apo, ATP- and ADP-bound states, as shown for the yeast homolog, Hsp82 (12). Nucleotide binding changes the relative abundance of the different conformations by altering the forward and back transition rates. The distribution of conformations also varies between species (13), explaining the different ATPase rates, since only the closed, N-terminally dimerized conformation can hydrolyze ATP. The C-terminal DD opens and closes as well with rapid kinetics and in reciprocal correlation to ND (14). The crystal structure of Hsp82 in the presence of β,γ-imidoadenosine 5′-triphosphate (AMPPNP) and the cochaperone, Sba1, represents a closed conformation with dimerization of NDs and close contacts between ND and MD. In this conformation, the first β-strand (residues

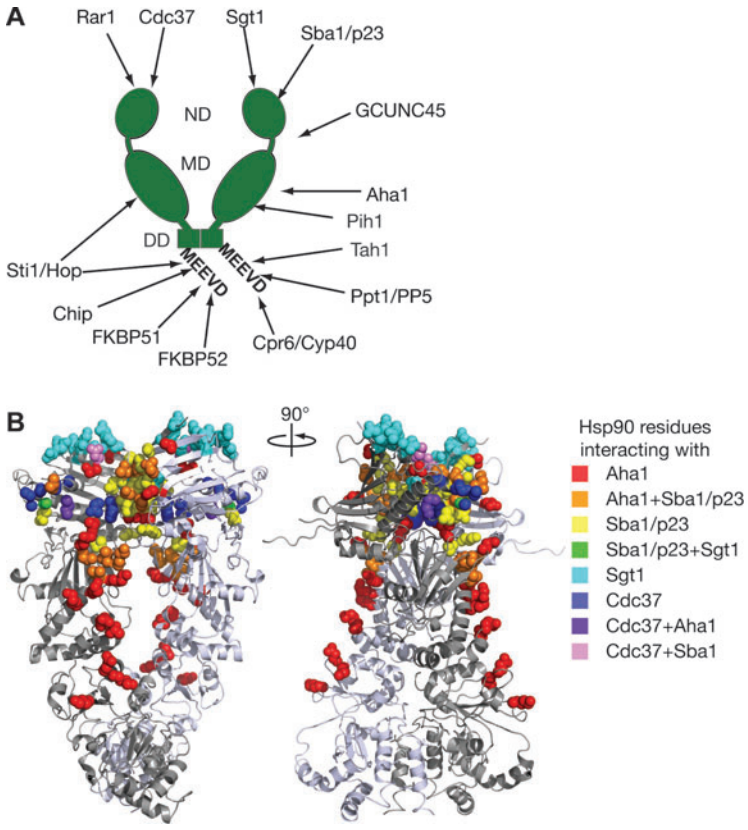

Figure 2 Interaction of cochaperones with Hsp90.
(A) Primary interaction area for selected cochaperones on a schematic illustration of Hsp90. (B) Cartoon representation of the crystal structure of yeast Hsp82 (PDB entry code 2CG9) with cochaperone-interacting residues shown as spheres. Coloring: Aha1 interacting residues, red, orange, and purple (44, 62); Sba1/p23 interacting residues, yellow, orange, and green (8); Sgt1 interacting residues, cyan and green (204); Cdc37 interacting residues, blue, purple, and pink (205). Since some of the structures only contained isolated domains of Hsp90, additional interaction sites in other domains are possible.

1–8 in Hsp82) of one protomer crosses over to the ND of the second protomer completing a β-sheet, a lid structure closes over the nucleotide-binding pocket, and the catalytic loop of the MD contacts the nucleotide-binding pocket to initiate ATP hydrolysis. Multiple mutual contacts between ND and MD regions of both protomers account for the cooperativity of ATP hydrolysis (15–17). Similarly, for the *Escherichia coli* homolog, HtpG, the most compact conformation prevails in the ATP-induced state (10, 18, 19), although conflicting data have also been reported (13). Graf and coworkers showed that ATP binding induces slow stepwise conformational changes in the secondary structure, which start at the nucleotide-binding pocket, proceed with dimerization of NDs, and finally lead to the docking of ND and MD. In their study, addition of ADP did not afford as high a degree of protection as ATP, indicating that for HtpG, conformational changes are coupled more strongly to ATP binding and hydrolysis, in contrast to Hsp82. Prokaryotic and eukaryotic Hsp90s show several structural differences, indicative of mechanistic differences. In eukaryotes, Hsp90s possess a charged flexible linker between the ND and MD. Hainzl and coworkers showed that the linker is largely dispensable for ATPase activity, but necessary for cochaperone regulation and client activation. The linker may provide additional freedom for the relative orientations of the N and M domains, which could be required for the mechanism of regulation through cochaperones, and also create a flexible interface for client interactions (20). Furthermore, eukaryotic Hsp90s have a conserved glutamate-glutamate-valine-aspartate (EEVD) motif at the C-terminus. This sequence is part of a binding site for cochaperones containing tetratricopeptide repeat (TPR) domains. As only two substrates and no cochaperones are known for HtpG, it is possible that these motifs (among other characteristics) evolved in eukaryotes to create a flexible system able to meet the requirements of a growing protein clientele.

Interactions of Hsp90 with clients

The first clients of Hsp90 identified were nuclear steroid hormone receptors (SHRs) and oncogenic kinases (21–23). Association with Hsp90 and Hsp70 was shown to be necessary for hormone binding of glucocorticoid receptor (GR) and progesterone receptor (24, 25) and for kinase activity (26). The function of Hsp90 is specifically inhibited by ansamycin antibiotics, such as geldanamycin. The drug competitively

inhibits ATP binding to Hsp90 and leads to the dissociation of nearly all clients, generally resulting in their inactivation and degradation (27). This tool provides a rapid and effective means to identify Hsp90 client proteins. A growing list of these proteins is maintained by Didier Picard (http://www.picard.ch/downloads/Hsp90interactors.pdf).

Which client properties are recognized by Hsp90?

In vitro, Hsp90s bind hydrophobic peptides and prevent the aggregation of several denatured model substrates (5, 28, 29), maintaining these substrates in a state that can be refolded by the Hsp70 system (4). These findings indicate that Hsp90 can recognize misfolded or partially unfolded polypeptides. This so-called holdase activity of Hsp90 is nucleotide-independent, and its relevance *in vivo* is not clear, as yeast shows no increase in the level of aggregated proteins following heat shock treatment when Hsp90 function is compromised (6). Furthermore, as the ATPase activity of Hsp90 is essential *in vivo*, nucleotide-independent interactions with clients do not constitute the indispensible functions of Hsp90 (2, 3).

ATP-dependent prevention of aggregation activity of Hsp90 also exists, as has been demonstrated with Aβ as the substrate (30). However, it is not clear whether monomers or small oligomers of Aβ are bound by Hsp90. In another study, Zhao and colleagues provided evidence that Hsp90 autonomously resolves small aggregates of Pih1 in an ATP-dependent manner *in vitro* (31). Albeit, the issue of whether aggregated Pih1 is in an unfolded conformation remains to be established. In these cases, exposed hydrophobic motifs cannot be the only determinant of binding, since Hsp90 exerts this ATP-dependent activity on a limited number of proteins.

On the other hand, Buchner and coworkers (32) demonstrated that luciferase can be in an active conformation when bound to Hsp90. However, binding required initial interactions of denatured luciferase with Hsp70 and Hsp40, and cochaperone-mediated transfer of refolded luciferase to Hsp90. In contrast, checkpoint kinase (Chk) 1 is chaperoned posttranslationally by Hsp70 and Hsp90, with no induced unfolding step (33). Yet, the chaperoning reaction was performed with immobilized Chk1, and spontaneous unfolding cannot be excluded. Similarly, GR and progesterone receptors are immunoadsorbed and washed with high salt before conversion into a hormone-binding-competent conformation by Hsp70 and Hsp90 (24, 34). To our knowledge, reconstitution of this process free in solution has not been achieved, suggesting a largely unfolded and aggregation-prone conformation of client proteins. Interactions of p53 with Hsp90 have also been observed under conditions where p53 exhibits a mostly unstructured conformation (35). These data suggest that Hsp90 has various modes of interaction with clients. On the one hand, Hsp90 preferentially interacts with at least partially unfolded polypeptides, and on the other, the folding process *in vivo* may require complex and varying interaction motifs composed of flexible and folded protein parts.

The complexity and specificity of recognition motifs has been extensively characterized for kinases (26, 36). Dependency on Hsp90 is substantially altered with single amino acid substitutions, often in correlation with an increase in intrinsic instability. The best known examples are proto-oncogenic kinases and their mutated counterparts (26, 37–41). Interactions of the same kinase at different stages of maturation can be governed by different determinants, as demonstrated for the receptor tyrosine kinases ErbB1 and ErbB2 of the epidermal growth factor receptor (EGFR) family (42). An extensive study of 105 kinases provided evidence that interactions with Hsp90 are determined by surface electrostatics, rather than contiguous sequence motifs (43). However, so far, no common determinants of binding have been discovered that discriminate client from non-client kinases.

For most client proteins investigated to date, interactions with Hsp90 require Hsp70 and a host of cochaperones, indicating that Hsp90 exerts its essential effects only in the context of a complex chaperone machinery. The issue of why Hsp90 needs the assistance of Hsp70 for client binding is still an enigma. It is likely that the key to this question lies in the ability of Hsp70 to interact with linear hydrophobic motifs and refold denatured proteins to their native state. Hsp70, in cooperation with its J-domain cochaperone (Hsp40), is considerably more efficient in preventing aggregation of denatured proteins and converts the client into a conformation that presents the complex surface pattern recognized by Hsp90.

How does Hsp90 bind clients?

Since interactions of Hsp90 with clients are mostly transient and require multiple factors, no high-resolution structures are currently available for Hsp90-client complexes. Original models proposed that clients are bound in a clamp-like fashion between the Hsp90 protomers at a 1:2 stoichiometry (44, 45). However, in the crystal structure of yeast Hsp82 complexed with AMPPNP and the cochaperone, Sba1, there is not enough space between the protomers to accommodate the folded client protein.

This dilemma was solved by Vaughan and coworkers, who managed to purify a complex of Hsp90 with the cell cycle dependent kinase Cdk4 and the cochaperone Cdc37 from Sf9 cells and achieved the first low-resolution structure of this complex by negative-stain EM and single-particle analysis (46). Albeit conclusive evidence is still lacking that the observed complex is a functional intermediate of the Hsp90-client activation cycle. Cdk4 was bound in a 1:2 stoichiometry, but in an asymmetric manner to one protomer. Although this study did not resolve detailed interactions at the protein-protein interfaces, their findings show that the N- and C-terminal lobes of the kinase interact with the N and M domains of Hsp90 in an extended conformation. As the nucleotide-binding cleft in Cdk4 is located between the lobes, Hsp90 could regulate the activity of the kinase by positioning the lobes in close proximity relative to each other. The electron density attributed to Cdk4 covers a hydrophobic patch in Hsp90, which was previously identified as important for substrate interactions *via* mutagenesis analyses. Recently, Agard and colleagues provided evidence that a model client containing folded and unfolded parts binds between the two protomers of a V-shape conformation of *E. coli* Hsp90 (47). Interestingly, the client-stimulated Hsp90

ATPase activity, consistent with earlier observations for GR ligand-binding domain and human Hsp90 (48), and SAXS and fluorescence resonance energy transfer (FRET) data, suggested that the client induces structural changes in Hsp90, leading to adoption of a more closed conformation. The final conformation of the Hsp90-client complex also appeared asymmetric, as in the case of Cdk4. The significant conformational changes in Hsp90 during the ATPase cycle may provide the plastic interaction surface required to integrate different states of one client and the ensemble of all clients.

What is the effect of Hsp90 on clients?

In contrast to Hsp70 and Hsp60 chaperones, Hsp90 cannot refold misfolded proteins. For many clients, interactions with Hsp90 are essential for prevention of degradation and activity, suggesting that Hsp90 prevents unfolding. The majority of client proteins are believed to be inactive while in complex with Hsp90 but maintained in a state from which rapid activation is possible. For example, heat shock factors (HSF1) of *Drosophila* and vertebrates are found in unstimulated cells in the cytoplasm as inactive monomers in complex with Hsp90 (49). Upon stress induction, HSF1 is released, trimerizes, and translocates to the nucleus for DNA binding. In yeast, HSF is constitutively trimeric and bound to DNA, but still interacts with Hsp90 and thus maintained in an inactive state (50). Inhibition of Hsp90 leads to higher basal activity of HSF (49).

A detailed structural interpretation of chaperone action has been proposed for glucocorticoid receptor. In the crystal structures of the ligand-binding domain of SHRs, an α-helix occludes the entrance of the steroid-binding pocket. According to the model Hsp70 opens the steroid binding pocket by removing this helix and Hsp90 subsequently keeps the binding pocket in an open conformation to allow steroid binding (51). This model suggests a more static function for Hsp90 and appears inconsistent with the finding that the influence of Hsp90 on clients depends on its ATP hydrolysis-coupled conformational cycle (52). Moreover, most Hsp90 client complexes are believed to be dynamic, as shown for steroid hormone receptors (53).

The fact that Hsp90 does not only play a static function but also can substantially influence a client's activity is demonstrated by Hsp90's interaction with glycogen synthase kinase GSK3β. While bound to Hsp90, GSK3β acts as an autophosphorylating tyrosine kinase, after release, as a transphosphorylating serine/threonine kinase (54). Therefore, Hsp90-bound clients may perform specific functions that differ from those of the unbound client.

The data collectively indicate that Hsp90 stabilizes defined conformations of clients, thereby preventing unfolding and degradation and preserving functionality, even in the context of destabilizing mutations.

Interactions of Hsp90 with cochaperones

Prokaryotic Hsp90s may only interact with the Hsp70 system, as no sequences with similarity to eukaryotic genes encoding Hsp90-specific cochaperones have been discovered in prokaryotic genomes to date. In contrast, in eukaryotes, Hsp90-client complexes contain many accessory proteins, which assemble together with Hsp70 and Hsp90 into a complex machinery. The interactions of this Hsp70-Hsp90 machinery with client proteins are believed to follow a dynamic chaperone cycle originally proposed for interactions with the nuclear progesterone receptor (Figure 1) (53). The client initially interacts with Hsp70 in a J domain protein-assisted process and is subsequently transferred through an intermediate Hsp70-Hsp90-client complex to Hsp90. The mature Hsp90-client complex is believed to dissociate with a client-specific half-life (5 min for progesterone receptor), and the client re-enters the cycle by binding to Hsp70. Progression through this cycle requires ATP hydrolysis by Hsp70 and Hsp90 and is regulated by the transient association of several cochaperones. New cochaperones of the Hsp70-Hsp90 chaperone machine have been discovered over the last few years, and the total number is currently at about 30 (Table 1). Cochaperones regulate progression through the cycle by inhibiting (Sti1/Hop, Sba1/p23, Cdc37) or stimulating [Aha1 (3)] the ATPase activity of Hsp90 or by competing for binding to the EEVD motifs at the C-termini of Hsp70 and Hsp90 [TPR-containing proteins, such as Sti1/Hop, FKBP51, FKBP52, Cyp40, Cpr6, PP5/Ppt1, Tah1, CHIP (55)]. Some cochaperones act as a scaffold for the early Hsp70-Hsp90 complex (Sti1/Hop) or as client-specific adaptors and targeting factors (e.g., Cdc37 for protein kinases, androgen receptor, and hepatitis B virus reverse transcriptase (56), GCUNC45 for myosin and SHRs, Tah1 for snoRNA maintenance machinery). Others have enzymatic activity, such as peptidyl-prolyl-isomerases (e.g., FKBP52, Cpr6) and the protein phosphatase, PP5/Ppt1, or recruit enzymatic activities like the cochaperones CHIP and Sgt1 (recruiting ubiquitination machineries). Cofactors additionally influence intracellular localization. For instance, FKBP52 enhances nuclear transport of the GR-Hsp90 complex after stimulation with glucocorticoids (57). Consistent with the diverse functions cochaperones have and with their different effects on Hsp90, cochaperones are believed to stabilize Hsp90 in defined conformations by interacting with specific parts as illustrated in Figure 2.

Eukaryotic genomes differ substantially in their set of cochaperones (58). The number of cochaperones has increased with the diversification of clientele proteins to augment the plasticity of the Hsp70/Hsp90 system and meet their specialized requirements. As prokaryotes have no cochaperones and minimalistic eukaryotic systems appear to require Hsp70 and Hsp90 only (59), this pair possibly represents the necessary and sufficient core. The compositions and amounts of cochaperones recovered with different substrates vary. Some proteins, like Sti1/Hop and Sba1/p23, are considered general cochaperones, owing to their presence in many Hsp90-client complexes, while others have a more restricted client cohort. Table 1 lists the currently known cochaperones, their presumptive targets, and loss-of-function phenotypes.

Consistent with their role in fine tuning diverse client proteins, cochaperones show a substantial degree of redundancy, with only Cdc37, Sgt1, and Cns1 identified as essential in

Table 1 Cochaperones of Hsp90.

Cochaperone	Effect on ATPase	Substrates/specialization	Interaction motif	Knockout/down phenotype	References
Aha1	Stimulates	General		Stress sensitivity[a]	(3)
AIPL1		Retina specific	TPR	Blindness	(61, 161)
Cdc37	Inhibits	Kinases		Essential[a]	(162, 163)
CHIP		General	TPR	Heat shock sensitivity	(144, 164)
CHORDC1 (Chp1)		ROCK I and II, ubiquitously expressed	CHORD, CS	Semisterility and embryonic lethality[b] Cell death in response to stress[c] Centrosome amplification and lethality[d,e]	(165–168)
Cpr7		SHR	TPR	Heat sensitivity[a]	(169)
Cyp40/Cpr6		SHR	TPR	Heat sensitivity[a]	(169, 170)
DYX1C1		Neuronal migration	TPR, CS	Dyslexia	(171, 172)
FKBP51		SHR	TPR	–	(173)
FKBP52		SHR	TPR	Infertility[e]	(174–176)
GCUNC-45		Myosin, PR	TPR	Knockout lethal, mutants defective in thick filament and contractile ring assembly[b,d]	(177–179)
Hop/Sti1	Inhibits	General	TPR	Stress sensitivity[a]	(180, 181)
Melusin		Muscle specific; localized at actin membrane junctions, signal transduction in response to mechanical stretch in cardiomyocytes	CHORD, CS	Increased heart dilation[c]	(182, 183)
NASP	Stimulates	H1	TPR	G_1-/S-phase progression affected	(184, 185)
NudCL2/NUDC		LIS1/dynein regulation	CS	Dispersion of Golgi apparatus, perinuclear accumulation of microtubules, displaced centrosome[c]	(186)
p23/Sba1	Inhibits	General	CS	Perinatal lethality[e]	(187, 188)
PP5/Ppt1		SHR, kinases	TPR	Hsp90 hyperphosphorylation[a] Normal growth, fertile[e], G2/M DNA damage checkpoint defect[c]	(82, 181)
Rar1 (plants)		Innate immunity with Sgt1	CHORD	No resistance induction	(189)
Sgt1		cell cycle (Polo kinase), innate immunity	CS	Essential[a]	(189, 190)
SGTA		AR, apoptosis	TPR	Mitotic arrest[c]	(191–193)
SIP		SSTK	CS	?	(194)
Tah1, Pih1		Rvb1/2, Rrp43, Nop58; RNA processing	TPR	Heat sensitivity[a]	(31, 195)
Tel2, Tti1, Tti2		PI3K-like kinases, ATM, ATR, mTOR, DNA-PKcs		Reduced kinase levels[c]	(196)
TPR2		General	TPR	Decreased SHR response[c]	(197)
TTC4/Cns1		DNA replication/?	TPR	Essential[a]	(198–200)
WISp39		P21	TPR	Cell cycle arrest after radiation challenge[c]	(201)
XAP2		AhR, PPARα	TPR	?	(202, 203)

[a]In *S. cerevisiae*, [b]in an invertebrate animal model, [c]in cell culture, [d]in a vertebrate animal model, [e]In a mammalian animal model.

yeast. In higher eukaryotes, several cochaperones have become essential for the function of specific organs over time, such as AIPL1 for the retina and FKBP52 for reproductive organs (60, 61). Evidently, specialized cochaperones have evolved to adapt the Hsp90 machine to the requirements of sophisticated clients needed in specific differentiated cells.

In addition to clients, several cochaperones bind asymmetrically to Hsp90 (46, 55, 62, 63). Asymmetry may be an important feature of the cycling chaperone machine that ensures directionality, whereby binding of a factor to one protomer primes the other protomer for binding of the next factor.

Posttranslational modifications of Hsp90

Early on, Hsp90 was discovered as a phosphoprotein (64). Two-dimensional gel electrophoresis revealed the existence of several isoforms of Hsp90, which differed in apparent pI. To date, more than 40 modified sites have been identified in human Hsp90 mainly using mass spectrometry. These sites are distributed over the entire protein in all three domains (Figure 3). Phosphorylation, acetylation, methylation, *S*-nitrosylation, and ubiquitination have been reported. Certain modifications are isotype-specific for human Hsp90α or Hsp90β. Altered modification patterns contribute to or occur as a consequence of pathological processes (65–68). The emerging picture of the role of posttranslational modifications in Hsp90 is rather sketchy. A number of major issues remain to be resolved. For instance, what is the impact on client and cochaperone binding and, thus, contribution to the complex regulation of the system? How dynamic is the global pattern of modification in the cellular Hsp90 pool? Are there discrete pools with defined modification codes, and does each class of substrates have its own modification requirements?

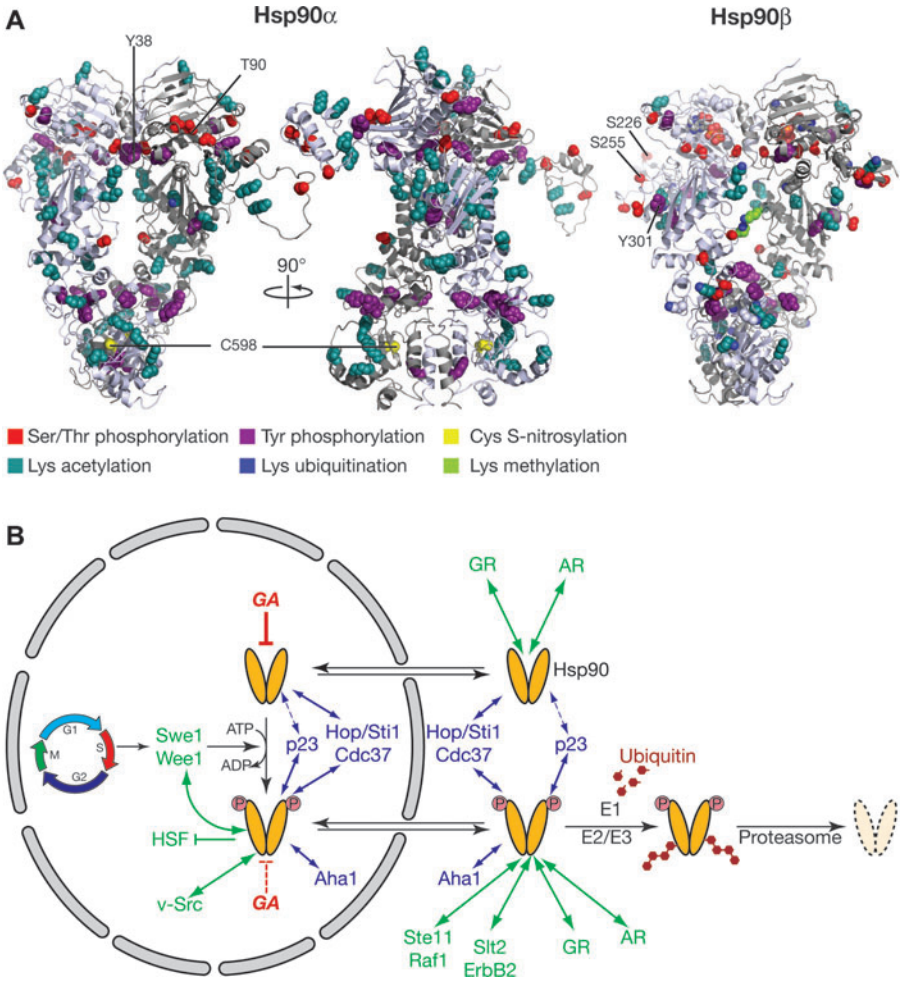

Figure 3 Posttranslational modifications of Hsp90.
(A) Cartoon representations of homology models of human Hsp90α (two structures on the left) and human Hsp90β (right structure). Modified residues are depicted as spheres colored as indicated. Residues specified in the text are labeled. (B) An example of the consequences of Hsp90 phosphorylation. The Hsp90 client, Swe1, a yeast ortholog of the mammalian cell cycle regulating serine/threonine- and tyrosine-specific protein kinase Wee1, phosphorylates Hsp90 during the S-phase at a single conserved tyrosine in the nucleotide-binding domain. Phosphorylated and unphosphorylated Hsp90 differentially interact with cochaperones (shown in blue) and clients (shown in green). Geldanamycin (GA) binds unphosphorylated Hsp90 with higher apparent affinity. Double arrows indicate interactions; dashed lines represent weak interactions [reprinted with modifications from (206) with permission from Elsevier].

Several studies have shown a negative impact of phosphorylation and acetylation on client activity and cochaperone binding (66, 67, 69–73), indicating that these modifications regulate substrate release or prevent client transfer from Hsp70 to Hsp90. As different clients deviate in their temporal and mechanistic requirements for Hsp90 chaperoning, it is likely that the impact of the same modification on different clients varies as well. For example, phosphorylation of S226 (human Hsp90β) and S255 (human Hsp90β) leads to dissociation and inactivation of aryl hydrocarbon receptor (AhR) (67), dissociation and activation of the apoptotic protease activating factor (Apaf)-1 (70) and activation of heme-sensitive eIF-2α kinase (74).

Regulation of the endothelial nitric oxide synthase (eNOS) provides the best known example of how different signaling events can induce different Hsp90 modifications and alter activity toward the same client. Proto-oncogene tyrosine-protein kinase Src-mediated phosphorylation of Hsp90 at Y301 (human Hsp90β) induced by vascular endothelial growth factor (VEGF) signaling leads to eNOS binding and activation (75), phosphorylation at T90 (human Hsp90α) by protein kinase A (PKA) leads to eNOS dissociation and inactivation (66), and *S*-nitrosylation at C598 (human Hsp90α) by eNOS itself leads to eNOS inactivation (76).

Modifications differ significantly in their dynamics *in vivo*. While Casein kinase II (CKII) phosphorylation, especially at S226 and S255, persists constitutively *in vivo* (77), others occur dynamically only during signaling events. Many Hsp90-modifying enzymes are clients of Hsp90 (Src, serine/threonine/tyrosine-protein kinase Wee1, serine/threonine-protein kinase Akt/Sch9, eNOS), creating negative and positive feedback loops. The Hsp90 inhibitor, geldanamycin, which leads to the dissociation and degradation of many client proteins, has been shown to reduce overall phosphorylation of Hsp90, particularly at tyrosines (78, 79). Clients involved in negative feedback loops include Akt/Sch9 (80) and eNOS (76). Wee1 is the most well-characterized example of a kinase involved in a positive feedback loop. Wee1 kinase phosphorylates Y38 of human Hsp90α, and the homologous kinase, Swe1, acts on the homologous site, Y24, in yeast Hsp82. This event alters cochaperone binding, the dynamics of N-terminal dimerization in Hsp90, and shifts client specificity. The activities of kinases, including Swe1, and the Hsp90-mediated inhibition of yeast HSF depend on phosphorylation of this site. In contrast, signaling *via* steroid hormone receptors appears to occur independently, since Swe1 has no effect on GR activity in the yeast model system. However, a phosphomimetic variant does not support growth of yeast, indicating that chaperoning of other essential clients is blocked (81) (Figure 3B). This is the only known example of a modification essential for one client with dynamics crucial for viability.

Removal of posttranslational modifications in Hsp90 is still poorly understood. Knockout of HDAC6, which leads to hyperacetylation of Hsp90, has no impact on viability or development in mice (63), indicating that higher acetylation levels can be tolerated without apparent detrimental effects. Mice with knockout of the gene encoding the protein phosphatase, PP5, that dephosphorylates Hsp90, are viable as well, indicating that PP5 is redundant (82). Similarly, knockout of the PP5 homolog in yeast, Ppt1, leads to no apparent phenotype (83). With the exception of yeast Ppt1, which dephosphorylates CKII-phosphorylated Hsp90, no phosphatases have been identified that act on Hsp90. Interestingly, Mollapour and coworkers showed that yeast Hsp82 phosphorylated at Y24 is ubiquitinated and degraded. However, this does not appear to be a general mechanism, since under heat shock conditions, the phosphate turnover on Hsp90 (84) increases dramatically, demonstrating the dynamic nature of the system. Further studies are essential to determine the enzymes participating in the dynamics of posttranslational modifications of Hsp90.

Hsp90 in health and disease

Regulation of Hsp90 expression in health

Mammals possess in the cytosol two Hsp90 isoforms with 86% identity, Hsp90α and Hsp90β, encoded by the genes, *HSPC1* (*HSP90AA1*) and *HSPC3* (*HSP90AB1*), respectively. These proteins are largely redundant in adults, exert comparable effects on clients, and display similar interaction patterns with cochaperones (85). In general, isoform β is constitutively expressed and essential at the very early stages of embryonic differentiation (86), suggesting that at least during this period, Hsp90β performs a highly specific function that cannot be substituted by Hsp90α. Isoform α expression displays profound fluctuations under diverse conditions and, most importantly, is induced by different stresses, such as heat shock. The protein shows a dynamic expression pattern in mitosis and meiosis and increased steady-state levels during development and in the adult animal in brain and testis. Hsp90α is involved in muscle cell development (87) and myosin chaperoning together with the cochaperone GCUNC45 (88), which, interestingly, has fivefold higher affinity for the α-isoform than the β-isoform (89). However, in mice, Hsp90α is only essential for the completion of meiosis in spermatocytes. Both isoforms are down regulated in non-dividing differentiating cells (osteoblasts and promyelocytic leukemia cells) (90). Conversely, the α-isoform is predominantly induced by diverse proliferation-stimulating extracellular signals (91).

The presence of an inducible Hsp90 gene ensures flexibility of regulation and enables cells to transiently recruit additional chaperone power, in particular, processes such as stress, major changes in cellular structure (meiosis, mitosis), complex protein machines under strain (muscles), or tissues with high-energy demand (brain). In mammals, Hsp90β can accomplish most of these functions, with the exception of meiosis in spermatocytes (92).

Under specific conditions, Hsp90 accumulates in the nucleus. Starving yeast retains Hsp90α (Hsp82) in the nucleus, which may be related to its function in sporulation (93). Amphibians show nuclear transfer of Hsp90β in oocytes and early embryogenesis (94). Major changes in transcriptional programs may require extremely high levels of Hsp90 due to its role in transcription and/or chromatin remodeling (95).

Cancer

Several chaperones, among them Hsp90, are overexpressed in cancer cells (96–99), and high overexpression of chaperones correlates with poor prognosis (100–102). Cancer cells appear to depend on chaperone activity, to a higher extent, than normal cells (103). First, malignant transformation depends on overexpression or mutational activation of proto-oncogenes, many of which are clients of Hsp90. Activated oncoproteins are inherently unstable and depend even more on Hsp90 than their proto-oncogenic counterparts (26, 37, 39, 41, 104). Second, many cancer cells exhibit genomic instability and mutator phenotypes creating mutant proteins or fusion constructs that are unstable and depend on chaperones. Third, chaperones are required to cope with extreme stress conditions that cancer cells have to survive, such as hypoxia and acidosis, and eventually, damage induced by chemotherapy and radiation. Numerous proto-oncogenic clients of Hsp90 are involved in all six hallmarks of cancer proposed by Hanahan and Weinberg (105). Remarkably, Hsp90 is not only involved in chaperoning members of intracellular pathways but also acts on the cell surface. Hsp90α is secreted to the extracellular space through the exosome pathway (106, 107). Although it remains to be established, if and how it can function outside the cell as a chaperone, the presence of Hsp90α is associated with the activity of several extracellular proteins, such as metalloprotease 2, which is important for tumor invasion and metastasis. Notably, targeting Hsp90α on the surface of tumor cells using membrane-impermeable geldanamycin derivatives specifically decreases their invasiveness (108).

Membrane-permeable Hsp90 inhibitors accumulate in tumor cells (109) and have higher affinity for Hsp90 *in vivo* than *in vitro* (103, 110, 111). Several mechanisms may contribute to this increased affinity of Hsp90 for inhibitors, including posttranslational modifications (68, 81), patterns of overexpression of cochaperones (98, 103), and in the case of drugs with a quinone group (geldanamycin derivatives), the NADPH/quinone oxidoreductase 1 level (112). Thus, Hsp90 represents a promising multimodal target for cancer therapy. Targeting Hsp90 inhibits the growth of many tumors driven by mutated oncoprotein clients, including mast/stem cell growth factor receptor tyrosine kinase Kit in gastrointestinal stromal tumors, epidermal growth factor receptor tyrosine kinase (EGFR) in non-small cell lung cancer, nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) in anaplastic large cell lymphomas, BCR-ABL fusion tyrosine-protein kinase in chronic myelogenous leukemia, receptor-type tyrosine-protein kinase FLT3 in acute myelogenous leukemia, tyrosine-protein kinase ZAP-70 in chronic lymphocytic leukemia, ErbB-2 in breast cancer, and serine/threonine-protein kinase B-Raf in myeloma (113). An overview on clinical efforts to develop Hsp90 inhibitors as anticancer drugs can be found in Trepel et al. (114).

On the other hand, some tumors do not respond to Hsp90 inhibition and develop resistance or even show enhanced progression. The main reason seems to be that Hsp90 inhibition induces the heat shock response, leading to overexpression of Hsp27 or Hsp70 chaperones, which substitute for Hsp90 function. Moreover, Hsp27 and Hsp70 actively prevent apoptosis. Indeed, concomitant inhibition of Hsp70 increases the efficacy of the geldanamycin derivative, 17-*N*-Allylamino-17-demethoxygeldanamycin (17-AAG) (115). In addition, client proteins can acquire mutations that decrease their dependency on Hsp90 (37, 42). Sensitivity to geldanamycin (GA) depends on the specific isoform (116). Most tumors selectively overexpress the α-isoform, while others also express the β-isoform. Although Hsp90β appears more responsive to inhibitors, it also supports chemoresistance (117).

Subcellular organelles contain specific Hsp90 paralogs. Changes in organellar homeostasis in cancer cells also promote addiction to the resident Hsp90. Mitochondrial Hsp90 homolog Trap1 inhibits apoptosis through interactions with cyclophilin D. Altieri and colleagues observed selective apoptosis of cancer cells upon targeting Hsp90 inhibitors to mitochondria (118). Moreover, overexpression of the endoplasmic reticulum (ER) Hsp90 homolog Grp94, is correlated with resistance to radiotherapy in some cancers (119). Grp94 is involved in the stress response as well as antigen processing and presentation. Grp94-peptide complexes represent tumor-specific antigens, and researchers have explored their potential as vaccines against cancer cells (107).

Neurodegenerative diseases

The hallmark of many neurodegenerative diseases is the formation of large extra- and intracellular protein deposits. The main constituents of these deposits are characteristic for individual diseases. Not the deposits themselves but intermediates generated during the pathway of deposit formation are believed to be the toxic species driving the disease. This process is regulated differentially by a variety of chaperones. Hsp70 and Hsp40 have been shown to rescue cells from toxicity and neuronal loss in models of several diseases (120) by mediating proteasomal degradation of the inducing agent (121) or interfering with the formation of toxic intermediates (122, 123). In contrast, other studies have reported that Hsp90 stabilizes the toxic intermediates and promotes inclusion formation (124, 125). In addition, in cases when aberrant signaling *via* deregulated kinases plays a role in disease onset, as in tauopathies (126, 127), Hsp90 contributes indirectly to the process by stabilizing the respective kinases. On the other hand, several Hsp90 cochaperones rescue Tau microtubule binding (128). Hsp90 also inhibits Aβ fibril formation *in vitro* (30). Despite these diverse and partly counteracting effects of Hsp90, treatment of model systems with Hsp90 inhibitors have disclosed promising results (126, 129–131). Hsp90 inhibition not only abrogates the aforementioned effects but also induces the heat shock response, which promotes the levels of the 'good' chaperones, Hsp70 and Hsp40.

One important feature of neurodegeneration is loss of neurons caused by deregulated apoptosis. As during development of the brain many more neurons are created than survive in the final structure, neurons, probably more than other non-dividing cells in the organism, may live constantly on the verge of cell death. For their survival, neurons seem to rely on the cytoprotective functions of chaperones like Hsp70 and Hsp27, which

are highly active in preventing apoptosis. The mitochondrial Hsp90 chaperone Trap1 may also contribute to survival of neurons. (118) Trap1 prevents the release of cytochrome c from mitochondria through interactions with cyclophilin D (118). Trap1 is also linked to neurodegenerative diseases. Mutational inactivation of the Trap1-phosphorylating kinase PINK1 is a known cause of early onset familial Parkinson's disease (132). PINK1-mediated phosphorylation of Trap1 was shown to be protective by oxidative stress and important for prevention of apoptosis. PINK1 could only protect neuronal cells when Trap1 levels were not reduced by RNAi. It is therefore conceivable that other Trap1-affecting impacts, including mutations in Trap1 itself, could be causative in Parkinson's disease and potentially other neurodegenerative diseases. Moreover, brain cells, like many tumor cells and in contrast to cells in most other organs, contain cytosolic Hsp90 in mitochondria and targeting of an Hsp90 inhibitor to mitochondria-induced apoptosis in tumor cells (has not been tested in neurons) (118). In contrast, geldanamycin at concentrations used in most trials does not induce apoptosis in healthy neurons, which might be due to the induction of the heat shock response and to compensatory action of Hsp70 and Hsp27. However, in some pathological cases, Hsp90 may be the sole protector against apoptosis (118, 133). Therefore, the size of the therapeutic window for Hsp90 inhibitor-based therapy may vary substantially in individual pathologies.

Infections

Hsp90 inhibition has therapeutic potential in many infectious diseases, a few of which are mentioned below. Intracellular pathogens abuse the host chaperone machinery for the completion of their life cycle (134, 135). Viral proteins have apparently evolved such a strong addiction to Hsp90 that despite high mutation rates, they are unable to find alternative folding pathways (136). Extracellular pathogens also rely strongly on their own Hsp90 system to cope with the hostile environment of the host. Interestingly, parasites express Hsp90 on the cell surface, and these homologs are sufficiently divergent from human Hsp90 to serve as antigens for the immune system. A monoclonal antibody retrieved from an immunized squirrel monkey with protective immunity against malaria recognized *Plasmodium* Hsp90 (137, 138), and a preclinical study has revealed protection against *Candida albicans* by the recombinant human antibody Mycograb (139). Small-molecule inhibitors are additionally a current focus of medical research. Downregulation of Hsp90 relieves the fungal burden in mice (140), and selective inhibitors are effective against *Plasmodium falciparum* (141).

Hsp90 and aging

Organismic aging is caused by compromised cellular homeostasis, fitness, and plasticity, leading to degeneration and cell death in vital organs. According to the 'garbage catastrophe' hypothesis, aged differentiated cells lose the capacity to dispose of damaged and malfunctioning proteins and organelles (142). Damaged or misfolded proteins can assume cytotoxic properties, and their constant removal is thus essential for cell survival. Chaperones preserve cellular homeostasis *via* multiple mechanisms. First, chaperones are involved in quality control and protein triage decisions. Misfolded proteins are ubiquitinated by the Hsp70 and Hsp90 cochaperone and U-box E3 ligase CHIP, which effectively targets Hsp70-client complexes (143). Indeed, CHIP knockout mice display several aging phenotypes (144). Second, researchers have concordantly observed a decline of the heat shock response with age. Hsp90, Hsp70, CHIP, and other cochaperones regulate the stability and activity of HSF1, which is the main mediator of the heat shock response. Hsp90 is associated with inactive HSF1, maintaining its stability under non-stress conditions, and is also important for termination of the heat shock response (49). In this manner, Hsp90 contributes to the ability of the cell to overcome periods of stress. Third, chaperone-mediated autophagy and macroautophagy, which contribute to the clearance of damaged proteins and organelles, are compromised in aging cells (145, 146). Fourth, Hsp90 suppresses transcriptional noise and preserves the plasticity of cellular responses. Transcriptional noise increases with age, leading to a decline in the functional integrity of cellular collectives (147).

The apoptosis-inhibiting potential of chaperones directly antagonizes their age-counteracting roles. Senescent cells acquire resistance to apoptosis, which results in accumulation of unfit cells that corrupt organ function (148). These cells continuously accumulate malfunctioning proteins and organelles but fail to undergo programmed cell death. Hsp90 counteracts this vicious cycle by preserving cellular homeostasis. However, Hsp90 also acts as an inhibitor of apoptosis. Therefore, higher levels of Hsp90 may have the potential to aggravate aging in differentiated tissues.

In contrast to differentiated cells, those with proliferating potential maintain the ability of self-renewal and disposal of damaged inventory by asymmetric distribution during cell division (149). However, they accumulate mutations and the probability to undergo malignant transformation increases with age. As high levels of Hsp90 are associated with proliferative potential and inhibition of apoptosis, in dividing cells, Hsp90 performs an ambivalent role as well. On the one hand, it supports the renewal of tissues and chaperones DNA repair enzymes (150, 151), and on the other hand, Hsp90 chaperones oncoproteins supporting malignancy.

Given this complexity and ambivalence of function, the physiological roles of these versatile chaperones, in particular, Hsp90, in different cellular contexts of aging organisms, remain to be elucidated.

Hsp90 and evolution

Over the course of evolution, phenotypes are selected through dynamic interactions with changing environments. The translation of genotype to phenotype is an extremely complex process. Changes in genetic information as well as fluctuations in the complex network, which regulates the expression of genetic information, act on the phenotype. Since Hsp90

controls many hubs of this network, it is not surprising that it is a multifaceted player in evolution. The organism exists in a dynamic balance between stabilizing factors conferring network robustness and destabilizing factors contributing to DNA instability and fluctuations in gene expression. Instability is evident as a motor of evolvability, whereas robustness ensures stable developmental homeostasis and reproduction of a persistent phenotype. Genetic networks possess intrinsic stochastic noise, which allows genetically identical daughter cells of a single mother to interact differently with the environment. Thus, one genotype is able to dynamically switch between different phenotypes in response to rapid environmental changes. This plasticity is believed to be important for differentiation. One phenotypic state can be fixed at the epigenetic level (152) and potentially also the genetic level by a process known as genetic assimilation (153). Transitions between stable phenotypes often require multiple mutations, whereby each mutation is likely to cause reduced fitness. The most feasible mechanism, which allows the accumulation of these mutations simultaneously bypassing iterative periods of reduced fitness, is a phenotypic buffer function also known as canalization (154). This mechanism would impede the manifestation of a phenotype until an environmental challenge or intrinsic change, such as mutation in the buffer itself, compromises its activity. The architecture of genetic networks possesses a robustness originating from functional redundancy, which can buffer loss-of-function mutations (155). However, the optimal candidates for environmentally responsive buffers, which allow accumulation of polymorphisms, are molecular chaperones, in particular, Hsp90. Hsp90 is involved in buffering of every aspect of variability, including stochastic variation (156), chromatin states (157), and genetic polymorphism. Compromised Hsp90 or stress leads to manifestation of pleiotropic phenotypes, many originating from hidden polymorphisms (158). After a period of selection, altered phenotypes can persist even under restored Hsp90 function, indicating that the enrichment of polymorphisms overcomes the buffering capacity of Hsp90 at some point. In this way, a new phenotype may become fixed and heritable. Clients and pathways differ in their dependency on Hsp90, and therefore, buffer capacity will depend on the pathways involved in shaping a phenotype. This applies to genetic variation as well as plasticity (156). Clients can alter dependency on Hsp90 by acquiring single mutations to recruit Hsp90 buffer capacity to newly formed unstable pathways or render pathways independent and robust (154). The canalizer Hsp90 supports the accumulation of mutations. Conversely, it also suppresses the generation of mutations by chaperoning several DNA repair proteins, including the high-fidelity translesion DNA polymerase-η (151), members of the Fanconi anemia

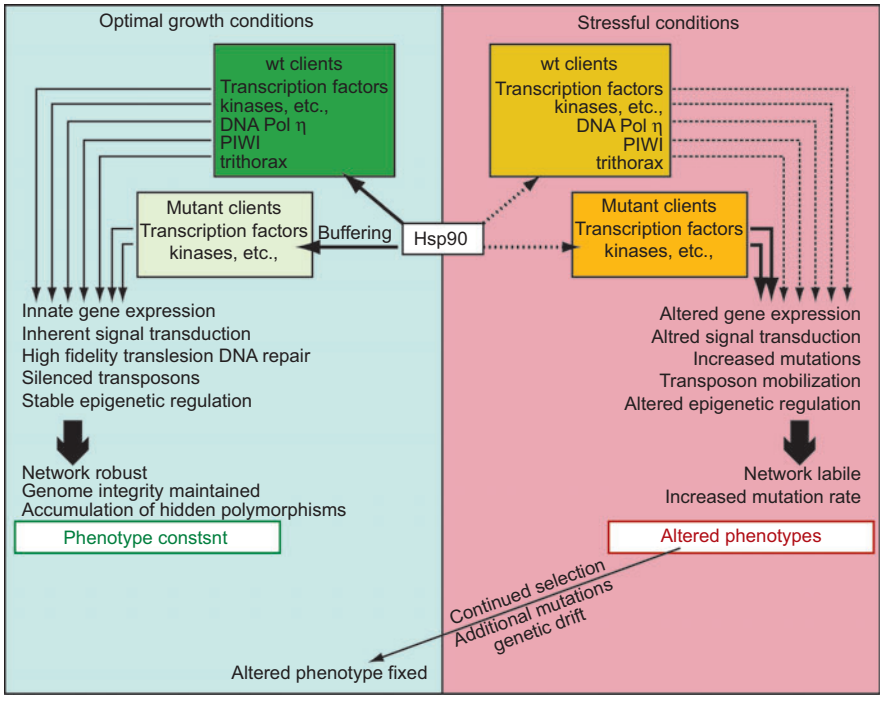

Figure 4 The role of Hsp90 in evolution.
Under optimal growth conditions, Hsp90 contributes to network robustness, genome and epigenome integrity, and consequently a stable phenotype, *via* control of the activity and stability of wild-type clients and its buffering effect on mutated clients. The capacitor function of Hsp90 promotes the accumulation of phenotypically silent polymorphisms. Under stress conditions, the buffering capability of Hsp90 is exhausted, and clients have reduced or altered activities, which may lead to altered phenotypes. Moreover, high-fidelity DNA repair (DNA polymerase η) and transposon maintenance (Piwi) are compromised, leading to an increased mutation rate. Continued selection of an altered phenotype can result in stabilization of this phenotype to allow expression, even in the absence of stress.

DNA damage response pathway (150), and the transposon-downregulating Piwi proteins (159). The observation that inhibition of Hsp90 by 17-AAG increases the frequency of mutations and chromosomal abnormalities is consistent with its proposed role as a guardian of genome integrity. Therefore, during stress phases, preexisting mutations manifest and plasticity increases, and mutation rates rise, enhancing genetic assimilation and fixation of a trait (Figure 4).

The role of epigenetics in the generation of phenotypic variations is also multifaceted. Chromatin states are dynamic, and their fluctuations may contribute to plasticity, an important factor in dynamic adaptation to fast changes in the environment. Hsp90 chaperones trithorax proteins, which suppress phenotypic variations possibly *via* balanced interplay within the complex (157, 160). Both Hsp90 and chromatin remodeling proteins are involved in the regulation of transcription, which may contribute to the robustness of gene expression and phenotypic stability. Interestingly, several members of the chromatin remodeling SWI/SNF complex buffer fluctuations in the gene expression network (155). However, epigenetic regulation also has the potential to fix one plastic phenotype and thus contribute to limited heritability of a trait, which could support long-term genetic assimilation (152).

Expert opinion

The unique feature of Hsp90 that makes it a focus of research is the overwhelming complexity of interactions linking the protein to almost every cellular function. In addition, its cochaperones often possess their own Hsp90-independent interactome. Considering the minimal number of interactors of bacterial HtpG, the steep increase in complexity is indicative of intimate coupling to evolution in eukaryotes. While lower eukaryotes have a limited number of cochaperones and, in some cases, not even those that are essential in most eukaryotes, the number of cochaperones has increased steadily in metazoans and plants to facilitate adaptation of the Hsp90 chaperone machine to the need of new specialized clients that are essential for a subset of highly specialized cells. Multicellular organisms have evolved complicated signaling networks, and many hubs of these networks are particularly dependent on Hsp90. Accordingly, this extremely versatile protein is the key player in fundamental and complex aspects of life, such as ontogenesis, aging, and evolution (Figure 5). Eukaryotic organelles additionally depend on Hsp90 homologs. The ER resident protein, Grp94, is involved in the quality control of protein complexes and antigen presentation, while mitochondrial Trap1 participates in protection against oxidative stress and regulation of apoptosis. Importantly, many pathological processes display an addiction to Hsp90 chaperone machines. Therefore, the ongoing development of small molecule inhibitors of Hsp90, partly in combination with other therapies, represents an important aspect of pharmacological research, and provides a promising perspective for the treatment of major diseases, such as cancer, neurodegenerative disorders, and infections.

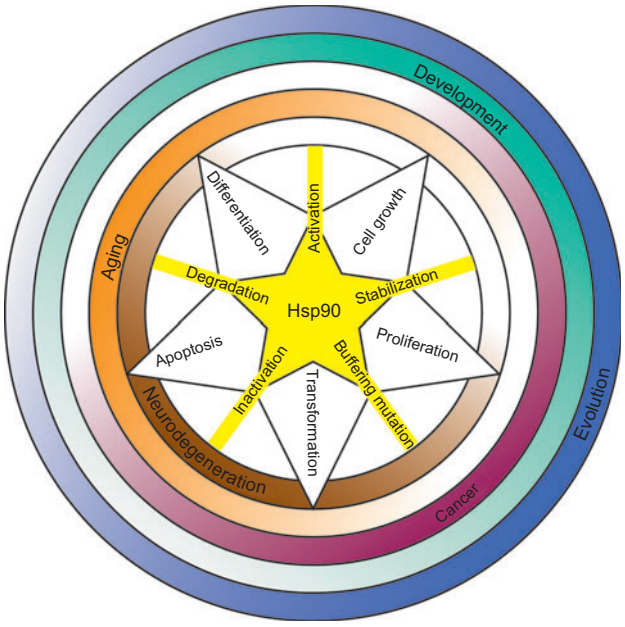

Figure 5 The universe of Hsp90.
Hsp90 activates and inactivates clients, influences their stability and degradation, and buffers mutations. Since the client proteins are involved in cell growth, proliferation, differentiation, apoptosis, and transformation, Hsp90 affects many physiological and pathophysiological processes, such as development, aging, neurodegeneration, and cancer. The influence of Hsp90 on key hubs of cellular networks affects the plasticity and adaptability of the organism, supporting the theory that the protein is an important evolutionary factor.

Outlook

Despite significant progress in the Hsp90 field, many important questions remain unanswered. For instance, how does the conformational dynamics of Hsp90 contribute to its chaperone function? How do posttranslational modifications and cochaperones regulate the conformational dynamics? How does conformational adaptability contribute to the recognition of the multitude of client proteins that are not related in sequence or structure? What does Hsp90 provoke in its client proteins? How does Hsp90 coordinate diverse signaling pathways with environmental impacts?

Further research using improved experimental techniques should aid in resolving the complexity of the Hsp90 chaperone machinery. Quantitative mass spectrometry techniques allow evaluation of the Hsp90 modification atlas under different conditions with increasing sensitivity, which facilitate our understanding of how Hsp90 is fine tuned for the benefit of specific clients and adapted to undertake certain tasks. Posttranslational modifications may positively or negatively regulate the cross-talk between different Hsp90-dependent processes. Improvement of structural methods should further enhance our understanding of the conformational dynamics of Hsp90. Hydrogen-deuterium exchange mass spectrometry has the potential to resolve conformational changes in space and time and allow clarification of the structural consequences of

posttranslational modifications and interactions with cochaperones. Sophisticated single molecule spectroscopy has facilitated investigation of the behavior of single Hsp90 dimers to link microscopic stochastic processes with macroscopic deterministic behavior. Comparison of the dynamics of different Hsp90 homologs and mutants in future analyses may help to determine the structure-function relationship. Improved cryo-EM techniques will also be instrumental in elucidating the structures of Hsp90-cochaperone and Hsp90-client complexes. The discovery of more specialized cochaperones should further uncover the adaptability and plasticity of the Hsp90 machine. Elucidation of Hsp90-client interactions at the structural level remains the most significant challenge due to intricate assembly mechanisms, the dynamic nature of Hsp90-client complexes, and high aggregation propensity of Hsp90 clients. One possible strategy is the purification of preassembled complexes from eukaryotic cells. Alternatively, one could study minimalistic systems, such as bacterial Hsp90 that does not depend on cochaperones or eukaryotic substrates, such as p53, that binds to Hsp90 *in vitro* without cochaperones.

Highlights

In eukaryotes, Hsp90 has evolved into an intricate highly dynamic machine regulated by multiple posttranslational modifications and a host of cochaperones to chaperone critical nodes of the cellular interaction network. The high degree of flexibility and plasticity of the Hsp90 system is ideally suitable for binding of a diverse set of clients that are not related in terms of sequence or structure and multidomain proteins composed of folded and unstructured regions. The suppleness and adaptability of the Hsp90 machine may thus have been a driving force in the evolution of more complex and highly regulated proteins, as well as protein assemblies.

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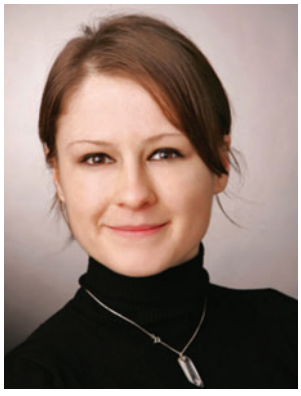
Received July 26, 2011; accepted November 2, 2011



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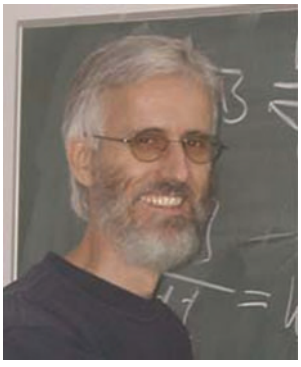

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