J. Biochem. **137**, 443–447 (2005)
DOI: 10.1093/jb/mvi056

Constantly Updated Knowledge of Hsp90

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Received December 27, 2004; accepted December 27, 2004

Although protein folding, in principle is a spontaneous process which depends only upon the amino-acid sequence, the assistance of molecular chaperones is required for many proteins to achieve their final conformation *in vivo*. While Hsp90 is one of the major molecular chaperones, it has long been the most mysterious among them. Recent advances in our knowledge regarding Hsp90 structure and function, owing to both detailed biochemical and genetic characterizations of Hsp90 co-chaperones, as well as eminent structural studies have established Hsp90 as an ATPase-dependent chaperone, and have provided a paradigm of the Hsp90 chaperone cycle, which is sequentially tuned and coordinated by a variety of co-chaperones. Here we summarize the current knowledge regarding the structure and essential activities of Hsp90, which certainly promises a deeper understanding of the functions of Hsp90 *in vivo*.

Key words: ATP, co-chaperone, Hsp90, molecular chaperone, protein folding.

Heat shock protein (Hsp) 90 is an abundant molecular chaperone (*i.e.*, ~1% of cytosolic protein) that is highly conserved from prokaryotes to eukaryotes (*1–5*). Hsp90 is involved in the folding and conformational regulation of numerous client proteins (*1–5*) (Table 1) and also mediates the refolding of stress-denatured proteins (*6–8*). The *in vivo* substrates of Hsp90 are a defined set of proteins; however, many of these are implicated in cellular signaling networks [*e.g.*, steroid hormone receptors, transcription factors and protein kinases (*1*, *4*, *5*)]. For instance, Hsp90 is essential for viability in yeast, *Caenorhabditis elegans*, and *Drosophila melanogaster* (*1–4*). Hsp90 has also been shown to act as a capacitor for morphological evolution in the fruit fly and plants (*9*, *10*), where it most probably buffers phenotypic variation affecting morphogenesis by chaperoning a variety of signal transducers, such as the aforementioned client proteins.

In addition to cytosolic Hsp90, multicellular organisms possess distinct paralogs localized in the endoplasmic reticulum and mitochondria, denoted Grp94/gp96 and Trap1/Hsp75, respectively. While there are two isoforms with 85% identity (Hsp90α and Hsp90β) in higher eukaryotes, no difference in properties between them has been reported except for mouse Hsp90β, which is inevitably required for placental development with its knock-out causing embryonic lethality even in the presence of Hsp90α (*11*). Hsp90 exists as a homodimer (*12*), namely α/α and β/β, *via* its C-terminal region (*13–16*). Hsp90 exhibits molecular chaperone activity by conformational cycling between opening and closing a molecular clamp, which is dependent upon its ATPase activity (*17–19*). This ATP-driven conformational cycle is sequentially and multiply regulated by a set of co-chaperones that assemble into a multicomponent complex (*1–5*). Antitumor drugs, such as geldanamycin and radicicol, which were originally supposed to decrease the activity of oncogenic protein kinases, are competitive inhibitors of ATP's binding to the N-terminal nucleotide pocket of Hsp90 (*2*, *20*). The drugs selectively kill tumor cells, since tumor Hsp90 is maintained in an activated multichaperone complex, which is more susceptible to inhibitors than the uncomplexed Hsp90 present in normal cells (*21*).

Hsp90 structure

Hsp90 consists of three highly conserved domains: a 25 kDa N-terminal ATP-binding domain; a 35 kDa middle domain; and, a 12 kDa C-terminal dimerization domain (Fig. 1A). In eukaryotic cytosolic Hsp90, a charged region intervenes between the N-terminal domain and the rest of the molecule (Fig. 1A), which diverges among species with respect to both length and composition, and is dispensable for viability in yeast (*22*). Although the overall structure of Hsp90 has yet to be reported, the crystal structures of each domain have been resolved.

The three-dimensional structures of the isolated N-terminal domains of both yeast and human Hsp90 were the first to be determined (*17*, *20*, *23*). These crystallographic studies determined that the N-terminal domain contains the binding site for ATP, which simultaneously serves as the binding site for the Hsp90 inhibitor, ansamycin antibiotic geldanamycin. Furthermore the ATP-binding domain of Hsp90 was found to exhibit structural similarity to DNA gyrase B, an ATP-dependent DNA topoisomerase, and is now grouped, along with histidine kinase and MutL into the GHKL superfamily with a novel ATP-binding fold (*24*). Mutations resulting in a deficiency in either ATP binding or hydrolysis ability abolish Hsp90 function both *in vivo* and *in vitro* (*25–27*). The transient association of two N-terminal domains has been demonstrated to occur in an ATP-dependent man-

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Table 1. **Hsp90 substrates.**

Transcriptional factor:	
	Steroid hormone receptors, HSF-1, p53.
Protein kinase:	
	Akt/PKB, CKII, Cdk4, Chk1, eIF-2α kinase, IKK, Plk, Raf-1, $pp60^{v\text{-}src}$, Wee1.
Others:	
	actin, Apaf-1, Calmodulin, eNOS, Proteasome, survivin, SV40 large T-antigen.

Notes: A number of substrates other than those listed above are known. Further information is available in Refs. *4* and *5*.

ner (*16*, *18*). Furthermore, biochemical studies and mechanistic analogy with related dimeric GHKL member proteins suggest that Hsp90 contains an ATPase-driven molecular clamp mechanism, similar to that of DNA gyrase B and MutL (*18*, *19*) (Fig. 1B).

The second structure reported for Hsp90 was that of the middle domain of yeast Hsp90 (*28*). Since the isolated N-terminal domain of Hsp90 has no detectable ATPase activity (*26*), another region of the Hsp90 molecule was assumed to be necessary for its full activity. This is also suggested from the structural homology which exists between Hsp90 and the other members of the GHKL superfamily of proteins (*24*). *Via* structural and functional analysis, it has been determined that the middle domain of Hsp90 contains a catalytic loop which could serve as an acceptor for the γ-phosphate of ATP (*28*) that was disordered in crystals of the N-terminal domain of Hsp90 complexed with ATP (*23*, *26*) (Fig. 1B). Thus, Hsp90, along with other GHKL proteins, is classified as a "split" ATPase, which performs its ATP-hydrolyzing activity in concert with a domain adjacent to the nucleotide-binding domain (*24*, *28*). Whereas a continuously increasing number of Hsp90 substrate proteins are listed (Table 1), their structural and/or conformational determinants for binding to Hsp90 remain elusive. Nevertheless, two chaperone sites have been mapped to the N- and C-terminal domains of Hsp90 (*29–32*). In addition, the middle domain of Hsp90 has been implicated in binding to Akt, eNOS and p53 (*33–35*) (Fig. 1A). The architectural and mechanistic similarities between Hsp90 and DNA gyrase B also suggest that the middle domain of Hsp90 is involved in the binding of client proteins (*28*).

Recently, the crystal structure of the C-terminal dimerization domain of HtpG, *Escherichia coli* Hsp90 was determined (*36*) (Fig. 1A). The reported structure is consistent with the extended antiparallel appearance of the Hsp90 dimer, previously shown by electron microscopic analysis (*16*). This dimeric nature of Hsp90 is crucial for its *in vivo* function and ATP-dependent clamp mechanism. C-terminal truncations of Hsp90 not only compromise yeast viability (*13*, *22*) but also abrogate ATP hydrolysis (*18*). These findings indicate that the otherwise weak association of the two N-terminal domains of Hsp90, which is a prerequisite for ATP hydrolysis, is strengthened by the C-terminal dimerization. Eukaryotic cytosolic Hsp90 has a conserved pentapeptide (MEEVD) at the C-terminus (Fig. 1A), which is recognized by co-chaperones containing multiple copies of the tetratricopeptide repeat (TPR) (*37–40*), however is dispensable for viability (*22*). Interestingly, the C-terminal eight residues of Hsp70 (GPTIEEVD) interact with TPR domains in a manner similar to that of Hsp90 (*40*).

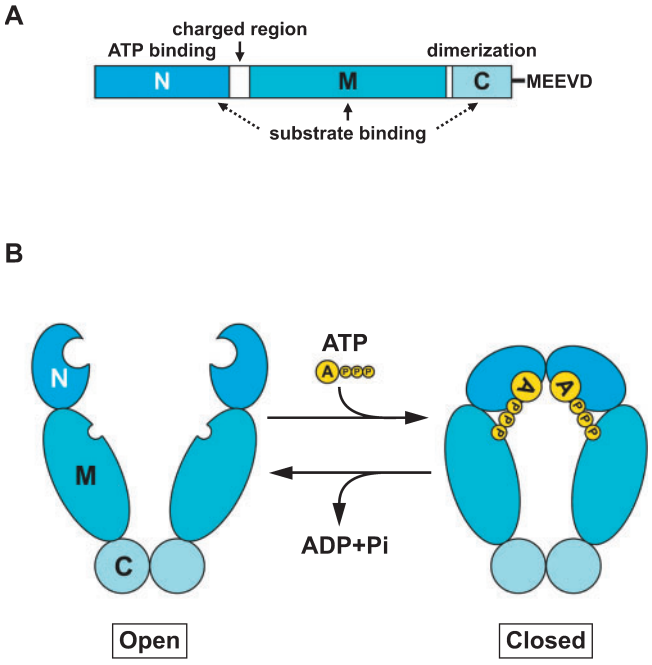

Fig. 1. **Domainal structure of Hsp90 and its ATP-driven molecular clamp.** A, schematic drawing of human Hsp90α with 732 amino acids. Hsp90 consists of three domains: an N-terminal ATP-binding domain (N); a middle domain (M); and a C-terminal dimerization domain (C) with the pentapeptide MEEVD sequence. A charged region exists between the N and M domains. All three domains are reported to interact with substrate proteins. B, ATPase cycle of Hsp90. Nucleotide-free Hsp90 is in an open state with its C-terminal domains being constitutively dimerized (left panel). Binding of ATP in the N-terminal domain induces conformational changes, resulting in the closed state of Hsp90 (right panel).

ATPase-dependent chaperone cycle

Following a long-standing controversy concerning the ATP-dependence of Hsp90 function, it has been established that Hsp90 is an ATP-dependent chaperone, largely owing to a remarkable set of crystallographic studies (*23*, *25*, *26*). The nucleotide-free state, corresponding to the "open" state of Hsp90 can capture client proteins, with the two N-terminal domains in the dimer being separate (Fig. 1B, left panel). Upon binding of ATP, conformational changes promote the transient association of the N-terminal domains, resulting in a "closed" state that is consistent with the observed toroidal structure (*16*) (Fig. 1B, right panel), thereby clamping client proteins. Even though an accumulating amount of evidence reinforces this proposed mechanism for the ATPase-coupled molecular clamp (*18*, *19*), it has become appreciated that the coordinated assistance of a range of co-chaperones as well as Hsp70 is required to accomplish the ATP-driven chaperone cycle of Hsp90 (*1–5*, *41*, *42*). These proteins assemble into a multichaperone complex, which is activated and more sensitive to Hsp90 drugs than the free form of Hsp90 (*21*), in order to assist the loading and release of client proteins, thereby leading to the progression of the chaperone cycle.

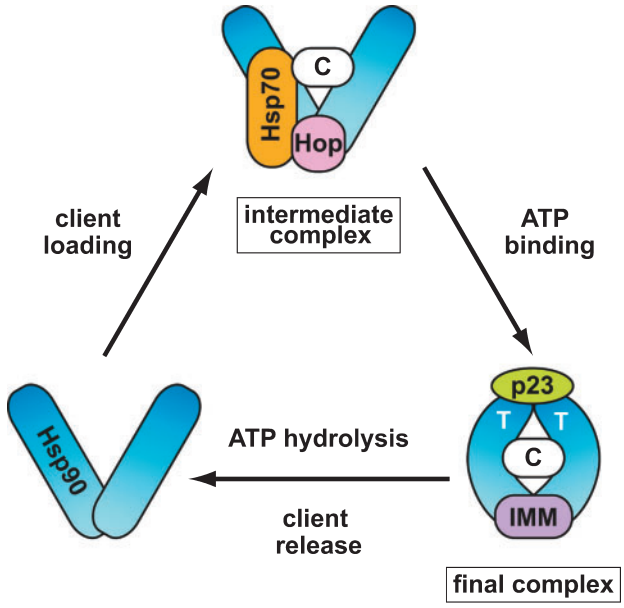

Fig. 2. **The Hsp90 chaperone cycle.** In concert with the co-chaperones [Hop, p23, and immunophilins (IMM)] as well as Hsp70, Hsp90 mediates the maturation of client proteins (C), such as steroid receptors, in an ATP-dependent manner (T: ATP). An intermediate complex is observed at an early stage of this pathway, with the final complex appearing at a later stage to finish the folding of Hsp90 client proteins.

The multichaperone system has been most intensively studied by deconvoluting the maturation pathway of steroid hormone receptors, eventually providing a paradigm of the Hsp90 chaperone cycle (*5*) (Fig. 2). In particular, steroid receptors must be kept in an activatable state that is inherently labile in structure in the absence of steroid hormones. Hsp90 fulfills this requirement by assisting maintenance of the receptors in a conformation ready for ligand binding.

Steroid receptors initially bound to Hsp70 (Hsc70) are passed to nucleotide-free Hsp90 (an open form), which is induced by the TPR domain co-chaperone Hop/Sti1 (Fig. 2, intermediate complex). Hop can bind simultaneously to Hsp90 and Hsp70 through separate TPR domains as mentioned above (*40*) and mediates the association of the two molecular chaperones as a scaffold protein. In addition to binding to the C-terminal MEEVD motif of Hsp90, Hop is likely to physically interact with the Hsp90 N-terminal domain, resulting in the inhibition of the Hsp90 ATPase activity. This inhibition is achieved by preventing the association of the N-terminal domains of Hsp90 required for ATP hydrolysis, rather than by blocking of nucleotide binding (*39*, *43*). Given that this inhibitory action of Hop is significant in accomplishing the client transfer from Hsp70 to Hsp90, the roles for the other two classes of co-chaperones, p23/Sba1 and the large TPR-containing immunophilins (FKBP51, FKBP52, cyclophilin-40/Cpr6) are intriguing, since they enter the Hsp90 complex instead of Hop (Fig. 2, final complex). p23 interacts with Hsp90 in the ATP-bound state (*44*, *45*) through the Hsp90 N-terminal domain (*46*), and probably stabilizes the closed state of Hsp90 (Fig. 1B) in order to prolong client binding, by depressing the ATPase activity of Hsp90 (*47*, *48*). Recruitment of p23 inevitably requires the N-terminal association of Hsp90 (*18*, *46*, *47*); therefore, it is counteracted by Hop because it prevents the interaction of the N-terminal domains of Hsp90 (*2*, *41*). In this context, it is noteworthy that FKBP52, which exhibits peptidylprolyl isomerase activity, potentiates the maturation of glucocorticoid receptor (*5*, *49*). Since the immunophilins take part in the Hsp90 chaperone complex simultaneously with p23 in the chaperone cycle (*1*, *41*, *42*) (Fig. 2), and Hop is displaced from Hsp90 by immunophilins competing for the same binding site at the C-terminus of Hsp90 (*39*), they likely serve as a converter from the intermediate complex to the final complex in the Hsp90 chaperone cycle (*1*, *41*) (Fig. 2). While immunophilins exhibit a small stimulatory effect on the ATPase activity of Hsp90 (*47*, *48*), the recently discovered co-chaperone member, Aha1 explicitly enhances the intrinsic ATPase activity of Hsp90 (*48*, *50*) and activation by both co-chaperones is synergistic (*48*). By binding to the middle domain of Hsp90, Aha1 promotes a conformational alteration in the catalytic loop within the middle domain and facilitates its interaction with ATP in the N-terminal domain (*50*, *51*) (Fig. 1B). Finally, upon ATP hydrolysis, the client proteins and co-chaperones dissociate from Hsp90, which can then enter a new round of the chaperone cycle (Fig. 2).

Protein kinases are the largest class of client proteins (Table 1), and require stabilization by Hsp90 prior to receiving appropriate signals. During this time, they are sustained in an inactive form that is prone to denaturation, in a manner reminiscent of the steroid hormone receptors (*1*, *3*). However, in contrast to the steroid receptors, in which Hop recruits Hsp70-bound client proteins to Hsp90 as described above, the loading of protein kinases to Hsp90 is carried out autonomously by another co-chaperone, p50/Cdc37 (*1–5*, *41*, *42*), since Cdc37 is able to interact directly and simultaneously with both kinase clients and Hsp90 (*52–54*). Cdc37 reportedly binds to client kinases *via* its N-terminal region (*53*, *54*), which seems to be controversial (*55*, and Terasawa, K., unpublished data), and is also reported to suppress protein aggregation *in vitro* (*56*). Although Cdc37 contains no TPR motif, it competes with Hop for binding to Hsp90, but not *via* a TPR domain fragment (*57*). This apparent discrepancy has been elucidated by the crystal structure of the Hsp90–Cdc37 core complex (*58*), which revealed that, unlike Hop's interaction with the C-terminus of Hsp90, the C-terminal domain of Cdc37 binds to the open face of the Hsp90 N-domain, which apparently prevents the critical conformational alterations prerequisite for its ATPase activity (*58*). Since Cdc37 and Hop share the capability of inhibiting the ATPase activity of Hsp90 (*57*), they are suggested to commonly function as the client-loading factor for Hsp90 by holding the two N-domains of the Hsp90 clamp apart, thus facilitating client loading. It has furthermore been recently suggested that their functions are not necessarily incompatible, but rather are cooperative in the Hsp90 chaperone system (*4*, *42*, *59*).

Perspectives

At the earliest stage in the history of the Hsp90 field, glucocorticoid/progesterone receptor and $pp60^{v\text{-}src}$ were the only client proteins available for study. Together,

these provided an insufficient basis for extracting their commonality as Hsp90 substrate (*e.g.,* conformational properties and binding mechanism). Nevertheless, long term intensive studies have resulted in the accumulation of a plethora of knowledge regarding Hsp90, and have culminated in the establishment of the present view of the Hsp90 chaperone cycle, largely owing to remarkable concurrent developments (*e.g.*, identification of a variety of client proteins and co-chaperones, and especially determination of the crystal structures of the Hsp90 domains). Although our knowledge of Hsp90 is constantly increasing, the present understanding appears to be sufficient to recapitulate the entirety of overall Hsp90 function *in vivo*. Nevertheless, substantial challenges remain, which necessitate a comprehensive proteomic investigation, to visualize the whole spectrum of Hsp90 client proteins, as well as further, in-depth analyses of the mechanism of Hsp90-client and Hsp90-co-chaperone interaction.

The authors would like to thank Dr. John A. Rose for critical reading of the manuscript and apologize to all colleagues whose papers could not be cited owing to space limitations. This work was supported by grants-in-aid for Scientific Research on Priority Areas (to Y. M.) and the Special Coordination Funds for Promoting Science and Technology (to K. T. and Y. M.), from Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES

1. Buchner, J. (1999) Hsp90 & Co.—a holding for folding. *Trends Biochem. Sci.* **24**, 136–141
2. Pearl, L.H. and Prodromou, C. (2000) Structure and *in vivo* function of Hsp90. *Curr. Opin. Struct. Biol.* **10**, 46–51
3. Young, J., Moarefi, I., and Hartl, F.U. (2001) Hsp90: a specialized but essential protein-folding tool. *J. Cell Biol.* **154**, 267–273
4. Picard, D. (2002) Heat-shock protein 90, a chaperone for folding and regulation. *Cell. Mol. Life Sci.* **59**, 1640–1648
5. Pratt, W.B. and Toft, D.O. (2003) Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. *Exp. Biol. Med.* **228**, 111–133
6. Yonehara, M., Minami, Y., Kawata, Y., Nagai, J., and Yahara, I. (1996) Heat-induced chaperone activity of Hsp90. *J. Biol. Chem.* **271**, 2641–2645
7. Freeman, B.C. and Morimoto, R.I. (1996) The human cytosolic molecular chaperones hsp90, hsp70 (hsc70) and hdj-1 have distinct roles in recognition of a non-native protein and protein refolding. *EMBO J.* **15**, 2969–2979
8. Minami, Y., Kawasaki, H., Minami, M., Tanahashi, N., Tanaka, K., and Yahara, I. (2000) A critical role for the proteasome activator PA28 in the Hsp90-denpendent protein refolding. *J. Biol. Chem.* **275**, 9055–9061
9. Rutherford, S.L. and Lindquist, S. (1998) Hsp90 as a capacitor for morphological evolution. *Nature* **396**, 336–342
10. Queitsch, C., Sangster, T.A., and Lindquist, S. (2002) Hsp90 as a capacitor of phenotypic variation. *Nature* **417**, 618–624
11. Voss, A.K., Thomas, T., and Gruss, P. (2000) Mice lacking HSP90β fail to develop a placental labyrinth. *Development* **127**, 1–11
12. Minami, Y., Kawasaki, H., Miyata, Y., Suzuki, K., and Yahara, I. (1991) Analysis of native forms and isoform compositions of the mouse 90-kDa heat shock protein, HSP90. *J. Biol. Chem.* **266**, 10099–10103
13. Minami, Y., Kimura, Y., Kawasaki, H., Suzuki, K., and Yahara, I. (1994) The carboxy-terminal region of mammalian HSP90 is required for its dimerization and function in vivo. *Mol. Cell. Biol.* **14**, 1459–1464
14. Nemoto, T., Ohara-Nemoto, Y., Ota, M., Takagi, T., and Yokoyama, K. (1995) Mechanism of dimer formation of the 90-kDa heat-shock protein. *Eur. J. Biochem.* **233**, 1–8
15. Meng, X., Devin, J., Sullivan, W.P., Toft, D., Baulieu, E.-E., and Catelli, M.-G. (1996) Mutational analysis of Hsp90α dimerization and subcellular localization: dimer disruption does not impede 'in vivo' interaction with estrogen receptor. *J. Cell Sci.* **109**, 1677–1687
16. Maruya, M., Sameshima, M., Nemoto, T., and Yahara, I. (1999) Monomer arrangement in HSP90 dimer as determined by decoration with N and C-terminal region specific antibodies. *J. Mol. Biol.* **285**, 903–907
17. Prodromou, C., Roe, S.M., Piper, P.W., and Pearl, L.H. (1997) A molecular clamp in the crystal structure of the N-terminal domain of the yeast Hsp90 chaperone. *Nat. Struct. Biol.* **4**, 477–482
18. Prodromou, C., Panaretou, B., Chohan, S., Siligardi, G., O'Brien, R., Ladbury, J.E., Roe, S.M., Piper, P.W., and Pearl, L.H. (2000) The ATPase cycle of Hsp90 drives a molecular 'clamp' via transient dimerization of the N-terminal domains. *EMBO J.* **19**, 4383–4392
19. Richter, K., Reinstein, J., and Buchner, J. (2003) N-terminal residues regulate the catalytic efficiency of the Hsp90 ATPase cycle. *J. Biol. Chem.* **277**, 44905–44910
20. Stebbins, C.E., Russo, A.A., Schneider, C., Rosen, N., Hartl, F.U., and Pavletich, N.P. (1997) Crystal structure of an Hsp90-geldanamycin complex: targeting of a protein chaperone by an antitumor agent. *Cell* **89**, 239–250
21. Kamal, A., Thao, L., Sensintaffar, J., Zhang, L., Boehm, M.F., Fritz, L.C., and Burrow, F.J. (2003) A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. *Nature* **425**, 407–410
22. Louvion, J.-F., Warth, R., and Picard, D. (1996) Two eukaryote-specific regions of Hsp82 are dispensable for its viability and signal transduction functions in yeast. *Proc. Natl Acad. Sci. USA* **93**, 13937–13942
23. Prodromou, C., Roe, S.M., O'Brien, R., Ladbury, J.E., Piper, P.W., and Pearl, L.H. (1997) Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. *Cell* **90**, 65–75
24. Dutta, R. and Inouye, M. (2000) GHKL, an emergent ATPase/kinase superfamily. *Trends Biochem. Sci.* **25**, 24–28
25. Panaretou, B., Prodromou, C., Roe, S.M., O'Brien, R., Ladbury, J.E., Piper, P.W., and Pearl, L.H. (1998) ATP binding and hydrolysis are essential to the function of the Hsp90 molecular chaperone *in vivo*. *EMBO J.* **17**, 4829–4836
26. Obermann, W.M.J., Sondermann, H., Russo, A.A., Pavletich, N.P., and Hartl, F.U. (1998) In vivo function of Hsp90 is dependent on ATP binding and ATP hydrolysis. *J. Cell Biol.* **143**, 901–910
27. Grenert, J.P., Johnson, B.D., and Toft, D.O. (1999) The importance of ATP binding and hydrolysis by Hsp90 in formation and function of protein heterocomplexes. *J. Biol. Chem.* **274**, 17525–17533
28. Meyer, P., Prodromou, C., Hu, B., Vaughan, C., Roe, S.M., Panaretou, B., Piper, P.W., and Pearl, L.H. (2003) Structural and functional analysis of the middle segment of Hsp90: implications for ATP hydrolysis and client protein and cochaperone interactions. *Mol. Cell* **11**, 647–658
29. Young, J.C., Schneider, C., and Hartl, F.U. (1997) In vitro evidence that hsp90 contains two independent chaperone sites. *FEBS Lett.* **418**, 139–143
30. Scheibel, T., Weiki, T., and Buchner, J. (1998) Two chaperone sites in Hsp90 differing in substrate specificity and ATP dependence. *Proc. Natl Acad. Sci. USA* **95**, 1495–1499
31. Johnson, B.D., Chadli, A., Felts, S.J., Bouhouche, I., Catelli, M.G., and Toft, D.O. (2000) Hsp90 chaperone activity requires the full-length protein and interaction among its multiple domains. *J. Biol. Chem.* **275**, 32499–32507

32. Minami, M., Nakamura, M., Emori, Y., and Minami, Y. (2001) Both the N- and C-terminal chaperone sites of Hsp90 participate in protein refolding. *Eur. J. Biochem.* **268**, 2520–2524
33. Sato, S., Fujita, N., and Tsuruo, T. (2000) Modulation of Akt kinase activity by binding Hsp90. *Proc. Natl Acad. Sci. USA* **97**, 10832–10837
34. Fontana, J., Fulton, D., Chen, Y., Fairchild, T.A., McCabe, T.J., Fujita, N., Tsuruo, T., and Sessa, W.C. (2002) Domain mapping studies reveal that the M domain of hsp90 serves as a molecular scaffold to regulate Akt-dependent phosphorylation of endothelial nitric oxide synthase and NO release. *Circ. Res.* **90**, 866–873
35. Müller, L., Schaupp, A., Walerych, D., Wegele, H., and Buchner, J. (2004) Hsp90 regulates the activity of wild type p53 under physical and elevated temperatures. *J. Biol. Chem.* **279**, 48846–48854
36. Harris, S.F., Shiau, A.K., and Agard, D.A. (2004) The crystal structure of the carboxy-terminal dimerizaion domain of htpG, the *Escherichia coli* Hsp90, reveals a potential substrate bindig site. *Structure* **12**, 1087–1097
37. Chen, S., Sullivan, W.P., Toft, D.O., and Smith, D.F. (1998) Differential interactions of p23 and the TPR-containing proteins Hop, Cyp40, FKBP52 and FKBP51 with Hsp90 mutants. *Cell Stress Chaperones* **3**, 1118–129
38. Young, J.C., Obermann, W.M.J., and Hartl, F.U. (1998) Specific binding of tetratricopeptide repeat proteins to the C-terminal 12-kDa domain of hsp90. *J. Biol. Chem.* **273**, 18007–18010
39. Prodromou, C., Siligardi, G., O'Brien, R., Woolfson, D.N., Regan, L., Panaretou, B., Ladbury, J.E., Piper, P.W., and Pearl, L.H. (1999) Regulation of Hsp90 ATPase activity by tetratricopeptide repeat (TRP)-domain co-chaperones. *EMBO J.* **18**, 754–762
40. Scheufler, C., Brinker, A., Bourenkov, G., Pegoraro, S., Moroder, L., Bartunik, H., Hartl, F.U., and Moarefi, I. (2000) Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine. *Cell* **101**, 199–210
41. Wegele, H., Müller, L., and Buchner, J. (2004) Hsp70 and Hsp90—a relay team for protein folding. *Rev. Physiol. Biochem. Pharmacol.* **151**, 1–44
42. Young, J.C., Agashe, V.R., Siegers, K., and Hartl, F.U. (2004) Pathways of chaperone-mediated protein folding in the cytosol. *Nat. Rev. Mol. Cell. Biol.* **5**, 781–791
43. Richter, K., Muschler, P., Hainzl, O., Reinstein, J., and Buchner, J. (2003) Sti1 is a non-competitive inhibitor of the Hsp90 ATPase. *J. Biol. Chem.* **278**, 10328–10333
44. Sullivan, W., Stensgard, B., Caucutt, G., Bartha, B., McMahon, N., Alnemri, E.S., Litwack, G., and Toft, D. (1997) Nucleotides and two functional states of Hsp90. *J. Biol. Chem.* **272**, 8007–8012
45. Fang, Y., Fliss, A.E., Rao, J., and Caplan, A.J. (1998) *SBA1* encodes a yeast Hsp90 cochaperone that is homologous to vertebrate p23 proteins. *Mol. Cell. Biol.* **18**, 3727–3734
46. Chadli, A., Bouhouche, I., Sullivan, W., Stensgard, B., McMahon, N., Catelli, M.G., and Toft, D.O. (2000) Dimerization and N-terminal domain proximity underlie the function of the molecular chaperone heat shock protein 90. *Proc. Natl Acad. Sci. USA* **97**, 12524–1252
47. McLaughlin, S.H., Smith, H.W., and Jackson, S.E. (2002) Stimulation of the weak ATPase activity of human Hsp90 by a client protein. *J. Mol. Biol.* **315**, 787–798
48. Panaretou, B., Siligardi, G., Meyer, P., Maloney, A., Sullivan, J.K., Singh, S., Millson, S.H., Clarke, P.A., Naaby-Hansen, S., Stein, R., Cramer, R., Mollapour, M., Workman, P., Piper, P.W., Pearl, L.H., and Prodromou, C. (2002) Activation of the ATPase activity of Hsp90 by the stress-regulated cochaperone Aha1. *Mol. Cell* **10**, 1307–1318
49. Riggs, D.L., Roberts, P.J., Chirillo, S.C., Cheung-Flynn, J., Prapapanich, V., Ratajczak, T., Gaber, R., Picard, D., and Smith, D.F. (2003) The Hsp90-binding peptidylprolyl isomerase FKBP52 potentiates glucocorticoid signaling *in vivo*. *EMBO J.* **22**, 1158–1167
50. Lotz, G.P., Lin, H., Harst, A., and Obermann, W.M.J. (2003) Aha1 binds to the middle domain of Hsp90, contributes to client protein activation, and stimulates the ATPase activity of the molecular chaperone. *J. Biol. Chem.* **278**, 17288–17235
51. Meyer, P., Prodromou, C., Liao, C., Hu, B., Roe, S.M., Vaughan, C.K., Vlasic, I., Panaretou, B., Piper, P.W., and Pearl, L.H. (2004) Structural basis of recruitment of the ATPase activator Aha1 to the Hsp90 chaperone machinery. *EMBO J.* **23**, 511–519
52. Silverstein, A.M., Grammatikakis, N., Cochran, B.H., Chinkers, M., and Pratt, W.B. (1998) $p50^{cdc37}$ binds directly to the catalytic domain of Raf as well as to a site on hsp90 that is topologically adjacent to the tetratricopeptide repeat binding site. *J. Biol. Chem.* **273**, 20090–20095
53. Grammatikakis, N., Lin, J.-H., Grammatikakis, A., Tsichlis, P.N., and Cochran, B.H. (1999) $p50^{cdc37}$ acting in concert with Hsp90 is required for Raf-1 function. *Mol. Cell. Biol.* **19**, 1661–1672
54. Shao, J., Irwin, A., Hartson, S.D., and Matts, R.L. (2003) Functional dissection of Cdc37: characterization of domain structure and amino acid residues critical for protein kinase binding. *Biochemistry* **42**, 12577–12588
55. Mort-Bontemps-Soret, M., Facca, C., and Faye, G. (2002) Physical interaction of Cdc28 with Cdc37 in *Saccaromyces cerevisiae*. *Mol. Genet. Genomics* **267**, 447–458
56. Kimura, Y., Rutherford, S.L., Miyata, Y., Yahara, I., Freeman, B.C., Yue, L., Morimoto, R.I., and Lindquist, S. (1997) Cdc37 is a molecular chaperone with specific functions in signal transduction. *Genes Dev.* **11**, 1775–1785
57. Siligardi, G., Panaretou, B., Meyer, P., Singh, S., Woolfson, D.N., Piper, P.W., Pearl, L.H., and Prodromou, C. (2002) Regulation of Hsp90 ATPase activity by the co-chaperone Cdc37/$p50^{cdc37}$. *J. Biol. Chem.* **277**, 20151–20159
58. Roe, S.M., Ali, M.M.U., Meyer, P., Vaughan, C.K., Panaretou, B., Piper, P.W., Prodromou, C., and Pearl, L.H. (2004) The mechanism of Hsp90 regulation by the protein kinase-specific cochaperone $p50^{cdc37}$. *Cell* **116**, 87–98
59. Lee, P., Shabbir, A., Cardozo, C., and Caplan, A.J. (2004) Sti1 and Cdc37 can stabilize Hsp90 in chaperone complexes with a protein kinase. *Mol. Biol. Cell* **15**, 1785–1792