The Hsp90 Chaperone Machinery*

Sebastian Karl Wandinger1, Klaus Richter, and Johannes Buchner2
From the Department of Chemistry and the Center for Integrated Protein Science, Technische Universität München, 85747 Garching, Germany

Hsp90 was originally identified as one of several conserved heat shock proteins. Like the other major classes of heat shock proteins, Hsp90 exhibits general protective chaperone properties, such as preventing the unspecific aggregation of non-native proteins (1). However, Hsp90 seems to be more selective than the other promiscuous general chaperones, as it preferentially interacts with a specific subset of the proteome (2). Another specific feature of Hsp90 is its regulatory role of inducing conformational changes in folded, native-like substrate proteins that lead to their activation or stabilization (3). Recently, the three-dimensional structures of full-length Hsp90 from Escherichia coli, yeast, and the endoplasmic reticulum were solved (4–7). Together with sequence data, these showed that, although Hsp90 maintained its general domain structure from bacteria to man, distinct changes seem to have adapted Hsp90 to the more complex protein environment of the eukaryotic cell. Concomitant with the occurrence of a long charged linker connecting the N-3 and M-domains, the eukaryotic protein exhibits an extension of the C-terminal domain, which includes the conserved amino acid motif MEEVD at the C terminus (8). This region serves as the major interaction site for a cohort of co-chaperones (Table 1) (9), which apparently support Hsp90 activity in the folding and activation of its substrate proteins in eukaryotes. In this review, we summarize the current knowledge on the functional principles of this molecular machine, including the ATP-driven chaperone cycle of Hsp90 and its regulation by co-chaperones and post-translational modifications.

Structure and ATPase Activity

Hsp90 is a flexible dimer. Each monomer consists of three domains: the N-domain, connected by a long linker sequence (in eukaryotes) to an M-domain, which is followed by a C-terminal dimerization domain (Fig. 1). The N-domain possesses a deep ATP-binding pocket (10), where ATP is bound in an unusual kinked manner. ATP hydrolysis by Hsp90 is rather slow: Hsp90 from yeast hydrolyzes one molecule of ATP every 1 or 2 min (11, 12), and human Hsp90 hydrolyzes one molecule of ATP every 20 min (0.04 min⁻¹) (13). The ATPase activity is essential for the function of Hsp90 in yeast (11, 14). The slow hydrolysis suggests that complex conformational rearrangements of Hsp90 are coupled to the ATPase reaction and that these represent the rate-limiting step of the enzyme. The first steps of these conformational changes were elucidated recently in detail (15): upon ATP binding, a short segment of the N-domain called the “ATP lid” changes its position and flaps over the binding pocket (Fig. 1, steps 2 and 3). This releases a short N-terminal segment from its original position (16). In a subsequent reaction, this segment binds to the respective N-domain of the other subunit in the dimer, producing a strand-swapped, transiently dimerized N-terminal conformation (step 3) (5, 15).

These N-terminal rearrangements result in further conformational changes throughout the entire Hsp90 dimer leading to a twisted and compacted dimer, in which N- and M-domains associate and the distance between M-domains is shortened by 40 Å (5). The association of N- and M-domains completes the active site of this “split ATPase” (step 4). Recently, a similar progression of steps was shown to occur also for the endoplasmic homolog Grp94 (17), mitochondrial TRAP1 (6, 18), and human Hsp90 (19). Therefore, the scenario outlined above seems to be the ubiquitously conserved ATPase mechanism for Hsp90.

Interestingly, the unusual way in which ATP is bound by Hsp90 is perfectly mimicked by some natural compounds, such as geldanamycin and radicicol. These are highly specific and potent inhibitors of the Hsp90 ATPase (20), blocking the maturation of substrate proteins and eventually resulting in their degradation (21). As several Hsp90 substrate proteins are kinases, which can be deregulated in the development of cancer, derivatives of Hsp90 inhibitors are currently being investigated as anticancer therapeutics at the stage of clinical trials (22).

Hsp90 Cofactors Involved in Substrate Maturation

Current models assume that the conformational changes associated with ATP hydrolysis are required for reaching or maintaining an activated state of a substrate protein. In well-studied examples such as the SHRs, several cofactors interact with Hsp90 in a sequential manner to assemble a functional chaperone machinery (23, 24). The basis for this ordered succession of different assemblies can now be rationalized, as it turned out that several Hsp90 cofactors display a strong binding preference for specific Hsp90 conformations. The loading of an SHR onto Hsp90 requires the cooperation of Hsp90 with the chaperone Hsp70 and its cofactor Hsp40 (25). Moreover, both chaperones become physically linked by an adaptor protein called Hop/Sti1 (Table 1). This co-chaperone binds via small helical TPR domains to the C-terminal ends of Hsp70 and Hsp90 (26). It seems that Hsp70 stabilizes the SHR in a conformation that can be recognized and bound by Hsp90. However, experimental evidence for this notion is largely lacking. How the substrate in this complex is transferred from Hsp70 to Hsp90 is also still unclear. It might be that the bridging by Hop/Sti1 selects for Hsp90 molecules in a conformation competent for substrate binding in addition to increasing the local concen-

* This work was supported by grants from the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie (to K.R. and J.B.). This minireview will be reprinted in the 2008 Minireview Compendium, which will be available in January, 2009.

1 Present address: Kinaxo Biotechnologies GmbH, 82152 Martinsried, Germany.
2 To whom correspondence should be addressed. E-mail: johannes.buchner@ch.tum.de.
3 The abbreviations used are: N-domain, N-terminal domain; M-domain, middle domain; SHR, steroid hormone receptor; TPR, tetratricopeptide repeat; PPlase, peptidylprolyl cis/trans-isomerase; eNOS, epithelial nitric-oxide synthase.
TABLE 1
Selected Hsp90 cofactors

| Co-factor | Function | K_D value |
|-----------|----------|-----------|
| Hop       | Interaction with substrate maturation (steroid hormone receptors), Adapter for Hsp90/Hsp70 ATPase regulator (yeast) | 32 nM (70) |
| FKBP51    | Participation in substrate maturation (steroid hormone receptors), Chaperone properties | 174 nM (77) |
| FKBP52    | Participation in substrate maturation (steroid hormone receptors), Chaperone properties | 55 nM (77) |
| Cyp40     | Participation in substrate maturation (steroid hormone receptors), Chaperone properties | 226 nM (77) |
| Xap2      | Complex formation with arylhydrocarbon receptor and Hbx | n.d. |
| Ubiquitin ligase | Tagging proteins for degradation, L4: ubiquitin ligase | n.d. |
| Hsp90 phosphatase | Dephosphorylation of Hsp90, Optimizing Hsp90 substrate maturation | 670 nM (62) |
| Other TPR co-factors | | |
| Unc45     | Assembly of myosin fibers | |
| Tic4      | Essential in yeast | 5.1 µM (37) |
| Tom70     | Mitochondrial protein import | n.d. |
| Eos64     | Chlamydia protein import | n.d. |
| Sgt1      | Interacts with Hsp90 through its CSD domain in a nucleotide dependent manner. Forms complexes with Hsp90 and Skp1p/Cdc53p/F box protein ubiquitin ligase complex | n.d. |
| Non TPR Co-factors | | |
| p23       | Participation in substrate maturation (steroid hormone receptors), Binding to the N-terminal closed conformation, ATPase inhibitor, chaperone properties | 140 nM (closed ATPase-Hsp90 variant) (28) |
| Aha1      | ATPase activator | 670 nM (41) |
| Cdc37     | Kinase-specific Hsp90 co-factor, ATPase inhibitor, chaperone properties | 9.8 nM (31) |

Up to now, more than a dozen distinct Hsp90 cofactors have been identified (9). Their large number is not paralleled by other chaperone systems. Most bind Hsp90 with submicromolar affinities (Table 1). The major class of these is the TPR domain-containing cofactors, which include the proteins Hop/Sti1, PP5/Ppt1, and the large PPIases, among others (Table 1). Some of these cofactors could specifically facilitate the activation of a certain set of substrate proteins. In this context, the cofactor Unc45 has been shown to participate in early muscle development during the assembly of myosin filaments (32), and Xap2/AIP has been found in complex with the protein HbX from the hepatitis B virus (33) or the endogenous aryl hydrocarbon receptor (34). It remains to be seen whether these cofactors are really highly specialized or whether they are just the first substrates identified. Interestingly, in yeast, only two cofactors of the Hsp90 system are essential for viability (in addition to Hsp90). These are Cdc37 and Cns1 (29, 35, 36). Cns1 has been shown to associate with both Hsp90 and Hsp70; however, due to the presence of a single TPR domain, no ternary complexes can be formed (37). The function of Cns1 remains elusive.

Layers of Regulation

Hsp90 is embedded into several control mechanisms that influence its activity. As mentioned above, the ATPase activity is intrinsically decelerated due to slow conformational changes of the lid segment within the N-domains. In addition to this, the ATPase activity of Hsp90 is regulated by several cofactors. Another set of cofactors modulates substrate processing without changing the ATPase activity. Finally, the activity of Hsp90 is also regulated by post-translational modifications.

ATPase-modulating Cofactors

Several of the cofactors of Hsp90 modulate its ATPase activity by interacting preferentially with a specific conformation of Hsp90. p23/Sba1 binds to the ATPase domain and stabilizes the N-terminally dimerized conformation at the late stage of the ATPase cycle (Fig. 1) (28, 38, 39). This position p23/Sba1 to be part of the Hsp90 complex at the moment of hydrolysis and appears to be the reason for the decrease in the ATP turnover rate of Hsp90 in the presence of p23/Sba1.

A second site of cofactor interaction resides in the M-domain, to which Aha1, the only known activator of the Hsp90 ATPase, binds. This interaction stimulates the weak ATPase activity of Hsp90 by >10-fold (40). The stimulatory interaction of Aha1 with the M-domain of Hsp90 suggests a participation of this domain in the rate-limiting step of the ATPase reaction. Structural studies show that Aha1 binding remodels the M-domain around the catalytically active Arg380 and shifts the domain to a conformation resembling the closed conformation, committed for ATP hydrolysis (compare Fig. 1, step 3) (7, 41).

Another option for modulating the ATPase activity of Hsp90 is implemented by the co-chaperone Sti1 in yeast. Sti1 binds to the C-terminal end of Hsp90 via its TPR domain. In addition, there is a second binding site in the N- or M-domain (42). Binding to this second site allows Sti1 to inhibit the ATPase of Hsp90 completely (42, 43). Interestingly, ATP binding is not affected (42). As in the case of p23/Sba1, Sti1 selects a specific conformation of Hsp90 for binding. Biochemical analysis sug-
suggests that Sti1 binding prevents the N-terminal dimerization and association of the N- and M-domains. (Fig. 1, step 3) (42). In consequence, the $K_m$ value of ATP hydrolysis is not affected, but $k_{cat}$ is effectively reduced. This is the classical behavior of a noncompetitive inhibitor. The open state propagated by Sti1 is the acceptor state for substrate, as outlined above.

Cdc37 also inhibits the ATPase activity of Hsp90 (31, 43). Details of the respective mechanisms are still unclear, but crystallographic data provide a model in which Cdc37 binds to the ATP lid in the N-terminal nucleotide-binding site of Hsp90 and prevents the N-terminal dimerization by inserting as a dimer in between the two N-domains (44). Because Cdc37 is involved in the loading of Hsp90 with kinases, it is consistent with notions about the acceptor state of Hsp90 that Cdc37 also keeps Hsp90 in an open state. The deceleration of the ATPase activity probably permits this state to persist for an extended period of time. Together, these cofactors allow the adjustment of the basic conformational changes of Hsp90 that can be viewed as substrate processing steps to the specific needs of certain clients concerning binding to Hsp90 but also, in the case of p23/Sba1, its release.

**Post-translational Modifications**

A further level of regulation that has gained increasing attention is covalent modifications of Hsp90, such as acetylation (45), S-nitrosylation (46), and phosphorylation (47–49). These modifications lead to alterations in the maturation of Hsp90 substrates (50). Although some of the modified amino acid positions have already been identified (mostly by incorporation of the corresponding radiolabeled groups and/or mass spectrometry), it is a challenging task to obtain a quantitative picture of the modifications.

For nitrosylation, an interesting feedback loop between human Hsp90 and its substrate eNOS was discovered (46, 51). On the one hand, eNOS activity depends on the chaperone activity of Hsp90; on the other hand, the nitrosylating agent NO modifies human Hsp90 at Cys$^{597}$ (which is part of the C-terminal domain). As a consequence, the Hsp90 ATPase activity is inhibited (51), which inhibits in turn the up-regulation of eNOS activity by Hsp90. This might facilitate a tight regulation of cellular NO production in a negative feedback loop (51).

For acetylation, a similar scenario emerges. Histone deacetylase inhibitors, which result in the hyperacetylation of Hsp90, lead to a reduced interaction with and maturation of several of its substrate proteins, such as p53, Raf1, Bcl-Abl, and the glucocorticoid receptor (52–55). As a consequence, an increase in proteasomal degradation of some Hsp90 substrates was found. HDAC6 was identified as the enzyme deacetylating Hsp90 (52).

In vivo, Hsp90 is acetylated at least at two sites; one was identified as Lys$^{294}$ (in human Hsp90α) (45). Furthermore, besides the ability to interact with its substrate proteins, the binding of
ATP by Hsp90 was also shown to be compromised upon acetylation of Hsp90 (55, 56).

Phosphorylation of Hsp90 has already been known for 30 years (47). By two-dimensional gel electrophoresis, it was found that at least four differently phosphorylated isoforms of Hsp90 exist (57, 58). For mammalian Hsp90 proteins, the majority of Hsp90 molecules contain on average three phosphates/monomer (59). The Hsp90 phosphorylation level is high under physiological conditions (60). Heat shock conditions induce an increased Hsp90 phosphorylation turnover (61). All known sites reside in the N-domain of Hsp90, but so far, no specific effect can be attributed to a particular phosphorylation event.

Kinases suggested to phosphorylate Hsp90 comprise casein kinase II, Akt, DNA-dependent protein kinase, and, in yeast cells, the Akt homolog Sch9 (reviewed in Ref. 50). Interestingly, one of the cofactors associating with Hsp90 via a TPR domain is PP5/Ppt1, a bona fide phosphatase. The yeast homolog dephosphorylates Hsp90 in a specific manner only when bound to Hsp90 (62). Dephosphorylation of Hsp90 is important for its in vivo function, as in a ppt1 deletion strain, the maturation of several substrate proteins was found to be impaired (62).

It will be a highly rewarding task to understand Hsp90 regulation at the level of post-translational modifications in detail, as this allows the cell to exert fine-tuned control over the Hsp90 chaperone machinery. The situation is even more complicated than described, as numerous co-chaperones of Hsp90 are regulated by modifications such as phosphorylation as well (63, 64).

Substrate Selection

In this context, a long standing open question is the extent of substrate specificity of Hsp90. Compared with other chaperones, a large number of substrate proteins are known due to their stable interactions that allowed isolation of the respective complexes. Many of the identified Hsp90 substrate proteins fall into two classes: transcription factors (such as SHRs and p53) and signaling kinases. The interactions with important regulatory proteins apparently allow Hsp90 to influence seemingly unrelated processes, such as evolutionary events (65, 66), mitochondrial homeostasis (67), and the propagation of RNA viruses (68). Surprisingly, the known substrate proteins are different in structure, leaving several possibilities as to which determinants are important for interaction with Hsp90. A good example is Src kinase. This protein exists in two variants, a cellular form (c-Src) and a viral form (v-Src). Both forms are almost identical (95%), with just a few amino acid changes. But although v-Src is strictly Hsp90-dependent in its activity, c-Src is largely independent of Hsp90 (69). A first step toward understanding the basis for the differences in the Hsp90 dependence of v-Src and c-Src is the finding that the intrinsic stabilities of the two Src forms differ, with v-Src being a highly unstable protein (70). Systematic variations of Hsp90-interacting kinases have further added to the notion that it is not a specific binding element but rather the stability of the protein that seems to be important for binding (71). Whether this is the general scheme remains to be seen, as there is evidence that a specific loop (“activation loop”) in the kinase might participate in governing the interaction with Hsp90 (72).

Regarding identification of the substrate-binding site, Hsp90 is certainly lagging behind other chaperones such as Hsp70 and GroE, for which we have a clear idea of this region. For Hsp90, experimental evidence exists for binding sites in all three domains of the protein (12, 73, 74). However, recent advances have begun to shed light on substrate binding. Determination of full-length Hsp90 crystal structures of open and closed conformations is a major achievement in this context (4–6). In analogy to chaperones like GroE and derived from homology of Hsp90 to DNA-binding topoisomerases (75), it was speculated that the substrate protein may be encapsulated by the Hsp90 dimer. This notion is not in agreement with the aforementioned structures, in which there is not enough space to accommodate a substrate protein between the two Hsp90 monomers. The first direct view of the structure of an Hsp90-substrate complex comes from electron microscopy and image processing of Hsp90 in complex with the kinase CdK4 and the co-chaperone Cdc37. In this assembly, the substrate kinase is bound in a highly asymmetric fashion to the M-domain and probably the N-domain of one subunit, whereas the co-chaperone Cdc37 resides between the N-domains (74). Whether this association is the same for all kinases and whether this can be generalized for other substrate proteins remain to be determined.

Perspectives

Although the basic features of ATP turnover and associated conformational changes of Hsp90 appear to be solved now, other characteristics of the Hsp90 machinery are not understood to date. These include substrate turnover and requirements for formation of particular substrate-co-chaperone-Hsp90 complexes. A combination of in vivo and in vitro approaches will be required to resolve these questions. Once we obtain a more detailed view of the processive power of this molecular machine, we can aim to integrate variations concerning specific substrate proteins, cofactors, and particular mechanisms of regulation and post-translational modifications into the picture.

Acknowledgments—We thank Heike Eberl and Emma Simpson for critical comments on the manuscript.

REFERENCES

1. Wiech, H., Buchner, J., Zimmermann, R., and Jakob, U. (1992) Nature 358, 169–170
2. Picard, D. (2002) CMLS Cell. Mol. Life Sci. 59, 1640–1648
3. Jakob, U., Lilie, H., Meyer, I., and Buchner, J. (1995) J. Biol. Chem. 270, 7288–7294
4. Shiau, A.-K., Harris, S. F., Southworth, D. R., and Agard, D. A. (2006) Cell 127, 329–340
5. Ali, M. M., Roe, S. M., Vaughan, C. K., Meyer, P., Panaretou, B., Piper, P. W., Prodomou, C., and Pearl, L. H. (2006) Nature 440, 1013–1017
6. Dollins, D. E., Warren, J. J., Immormino, R. M., and Gewirth, D. T. (2007) Mol. Cell 28, 41–56
7. Pearl, L. H., and Prodomou, C. (2006) Annu. Rev. Biochem. 75, 271–294
8. Chen, S., Sullivan, W. P., Toft, D. O., and Smith, D. F. (1998) Cell Stress Chaperones 3, 118–129
9. Richter, K., Meinschmidt, B., and Buchner, J. (2005) in Protein Folding Handbook (Buchner, J., and Kiefhaber, T., eds) pp. 768–829, Wiley-VCH Verlag GmbH & Co., Weinheim, Germany
10. Prodomou, C., Roe, S. M., O’Brien, R., Ladbury, J. E., Piper, P. W., and Pearl, L. H. (1997) Cell 90, 65–75
