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Hsp90 and co-chaperones twist the functions of diverse client proteins

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Abstract

Hsp90 molecular chaperones are required for the stability and activity of a diverse range of client proteins that have critical roles in signal transduction, cellular trafficking, chromatin remodeling, cell growth, differentiation and reproduction. Mammalian cells contain three types of Hsp90s: cytosolic Hsp90, mitochondrial Trap-1, and Grp94 of the endoplasmic reticulum. Each of the Hsp90s, as well as the bacterial homolog, HtpG, hydrolyze ATP and undergo similar conformational changes. Unlike the other forms of Hsp90, cytosolic Hsp90 function is dependent on a battery of co-chaperone proteins that regulate the ATPase activity of Hsp90 or direct Hsp90 to interact with specific client proteins. This review will summarize what is known about Hsp90's ability to mediate the folding and activation of diverse client proteins that contribute to human diseases, such as cancer and fungal and viral infections.

Hsp90 structure and ATPase cycle

Molecular chaperones assist the folding of newly synthesized or misfolded proteins, preventing their aggregation. The highly conserved molecular chaperone Hsp90 (heat shock protein, 90 kDA) is a global cellular regulator critical for the folding and regulation of a wide array of cellular proteins, termed clients. Hsp90 interacts with clients in a dynamic ATP-dependent cycle to ensure client folding, transport and/or assembly into multiprotein complexes 1‚2. Hsp90 is abundant in eukaryotic cells and its expression increases when cells are exposed to a variety of stresses. Two isoforms of Hsp90 are expressed in the cytosol of yeast and mammalian cells, and expression of one form is essential for viability. Higher eukaryotes also contain the related proteins Trap-1 of mitochondria and Grp94 of the endoplasmic reticulum. Genome-wide studies in *Saccharomyces cerevisiae* suggest that up to 10% of all proteins are directly or indirectly dependent on Hsp90 for function 3‚4. A current list of Hsp90-interacting proteins is thoughtfully maintained by Didier Picard (http://www.picard.ch/downloads/downloads.htm). This list includes both clients, which are dependent on Hsp90 for folding, stability and/or activity, as well as co-chaperones, which regulate Hsp90 and/or client function.

Hsp90 contains three conserved domains: an N-terminal ATP-binding domain, a middle domain and a carboxy-terminal domain. All examined forms of Hsp90 bind and hydrolyze ATP. Recent structural evidence supports a unified model for the conformational changes of Hsp90 that contains numerous distinct steps (Reviewed in5¯7). A simplified model is presented in Figure 1. In the absence of nucleotide (**A**), Hsp90 is dimerized at the carboxy-terminus resulting in an open conformation. Nucleotide binding (**B**) induces the closing of a lid over bound nucleotide (**C**) followed by association of N-terminal domains. In the closed

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conformation, portions of the amino-terminal domain 'cross-over' to associate with the other protomer (**D**). The Hsp90 dimer then forms a compact twisted structure (**E**) that results in association of a flexible loop from the middle domain with the nucleotide-binding pocket. This structure is capable of ATP hydrolysis. After hydrolysis Hsp90 returns to the open conformation. The rates of ATP hydrolysis and conformational changes are variable, with differences observed between Trap-1, Grp94 and HptG as well as cytosolic Hsp90 isolated from different species. In addition, some forms of Hsp90 adopt the 'closed' conformation even in the absence of nucleotide 8. Thus, in contrast to early results that suggest the conformational changes were strictly dependent on nucleotide, more recent studies suggest that nucleotide shifts the equilibrium between distinct conformations 7.

Assembly of cytosolic Hsp90 with client proteins is dependent on co-chaperone proteins

Beginning in the early 1990s, a number of additional proteins, or co-chaperones, that co-purified with Hsp90 in complex with steroid receptors or protein kinases were identified (Table 1) 2,9,10. Although Hsp90 is frequently described as a 'molecular chaperone machine', there is no evidence that Hsp90 exists as one large complex containing Hsp90 and all co-chaperones. Instead, Hsp90 and co-chaperone proteins interact with client proteins in an ordered pathway that involves sequential ATP-dependent interactions of the client protein with Hsp70 and Hsp90. In a model of interaction with steroid hormone receptors, the newly synthesized or misfolded client first interacts with Hsp40 and Hsp70. Transfer of a client protein from Hsp70 to Hsp90 is facilitated by the tetratricopeptide repeat (TPR) containing protein Hop/Sti1, which is able to simultaneously bind Hsp70 and Hsp90 through separate TPR domains. Displacement of Hop/Sti1 by other TPR-containing co-chaperones promotes nucleotide-binding and formation of the ATP-bound closed form of Hsp90. P23 interaction further stabilizes the closed conformation characterized by dimerized amino-termini. ATP hydrolysis, which may be accelerated by Aha1, leads to client dissociation 2,7. The pattern of interacting co-chaperones is likely client-specific 11,12. For example, Cdc37, which inhibits N-terminal dimerization and ATPase activity, likely substitutes for Hop during folding of protein kinases 13. Similarly, Sgt1, which is homologous to p23 and also binds the dimerized amino terminus of Hsp90 14, may replace p23 function for clients such as the Polo kinase 7,15.

Most of the Hsp90 co-chaperones were first identified by virtue of their co-purification with Hsp90 complexes isolated out of yeast or mammalian cell extracts (Hop, PP5, p23, Sgt1, FKBP51/52, Cyp40 and Cdc37). Additional co-chaperones were identified by virtue of physical or genetic interactions with Hsp90 of *S. cerevisiae* (Tah1, Aha1, Sse1 and Cns1). Table 1 lists the co-chaperones that function as part of Hsp90 complexes 13,14,16–36. Co-chaperone binding varies as Hsp90 undergoes conformational changes. For instance, p23 preferentially binds the ATP-bound closed state. TPR-containing co-chaperones compete for binding to the conserved carboxy-terminal MEEVD sequence of Hsp90, but some TPR containing-co-chaperones preferentially bind Hsp90 in the presence of nucleotide 37. Co-chaperones that do not regulate the ATPase activity of Hsp70 or Hsp90, likely have specialized *in vivo* functions, such as localization or trafficking. Even closely related co-chaperones such as FKBP51 and FKBP52, may have differential effects on client function, suggesting that they may contact clients directly or otherwise affect Hsp90 function in a client-specific manner 12. A dramatic demonstration of both the importance and differing roles of co-chaperones in client folding came from a recent study of the mutant cystic fibrosis transmembrane conductance regulator (CFTR) 38. The stability of the mutant CFTR was specifically enhanced in the absence of Aha1, but not p23, suggesting that co-chaperones play a critical role in the quality control process that lead to degradation of misfolded Hsp90 clients.

All forms of Hsp90 are presumed to have similar functions in promoting client folding and activation in order to support general biosynthetic and homeostatic needs of the cell and respond to a variety of stress conditions. However, co-chaperones that regulate the ATPase activity of Trap-1, Grp94 or HtpG have not been identified. It is unclear whether the co-chaperone requirement for cytosolic Hsp90 reflects differences in the mechanics of Hsp90 function or differences in the types of proteins that require Hsp90 for function. The overall composition of the Hsp90 molecular chaperone machine appears to vary in diverse eukaryotic organisms, and genes encoding co-chaperones that stimulate (Aha1) or inhibit (Hop/Sti1 and Cdc37) the essential ATPase activity of Hsp90 are not absolutely conserved 39. Thus, the function of some co-chaperones may be restricted to specific subsets of client proteins, be required for client protein activation in a species-dependent manner, or be redundant with other co-chaperones.

Hsp90 and client protein function

The main classes of Hsp90 clients include transcription factors, such as nuclear receptors for steroid hormones, kinases, including representatives from all branches of the kinome, and a third class of unrelated proteins such as telomerase and proteins required for viral replication. Unlike molecular chaperones such as Hsp70 that bind polypeptides in an extended, unfolded conformation, Hsp90 maintains client proteins in a nearly-completely folded conformation poised to respond to an activation signal, such as ligand binding or phosphorylation. The naturally occurring small-molecule geldanamycin and its derivatives bind the ATP binding site of Hsp90 and inhibit the ATP-dependent chaperone activities of Hsp90. These compounds disrupt formation of the ‘closed’ Hsp90 complex characterized by dimerized amino-termini and p23 interaction. This alters the folding pathway, resulting in reduced client activity, prolonged interaction of the client with Hsp70 and targeting of the client to the ubiquitin pathway for degradation. Mutation of Hsp90 in yeast or pharmacological inhibition in a wide range of tissues results in loss of client activity and/or stability. In the case of steroid hormone receptors, geldanamycin prevents formation of the high affinity hormone binding state, resulting in reduced hormone binding and transcriptional activation as well as receptor degradation. Similarly, the accumulation and activity of numerous protein kinases, such as v-src, ErbB2, and Raf-1 is disrupted. The Hsp90 requirement for folding varies from client to client: Hsp90 is continually required for the maintenance of steroid hormone receptor activity but is required only during synthesis of some protein kinases, such as p56(lck) 1‚2‚9‚40. Similarly, Hsp90 client proteins have varying sensitivity to Hsp90 inhibitors 41.

Hsp90 clients do not share any obvious sequence or structural homology and the location of the client-binding site(s) remains unclear. Over the years, each of the three different domains of Hsp90 have been implicated in client binding 9, and it is possible that clients have different binding sites and/or that the client binding site changes as Hsp90 undergoes conformational changes. Recent evidence suggests that clients bind the amino-terminal and/or middle domains on the ‘outside’ of the dimer 1‚7. In particular, the single-particle electron microscopy structure of a Hsp90-Cdc37-Cdk4 kinase complex 42 structure shows bivalent interactions of Cdk4 with the N-terminal and middle domains. The specific conformation of Hsp90 that is active in client folding is not known, although one conformation of HtpG was recently shown to be highly active in suppressing aggregation of a model client protein 43. Clarification of the mechanism by which Hsp90 interacts with and mediates the folding of diverse clients remains one of the biggest challenges in understanding Hsp90 function.

Functions of HtpG, Trap-1 and GRP94

What is known about the functions of other forms of Hsp90? In contrast to an essential role for cytosolic Hsp90 in eukaryotes, deletion of HtpG affects bacterial growth only at very high temperatures. There is, however, some evidence that HtpG facilitates protein folding in stressed *E. Coli* 44. A more recent study suggests that HtpG has a specific role in regulation of uroporphyrinogen decarboxylase, an enzyme required for tetrapyrrole biosynthesis 45, but further work is needed to clarify this interaction. Mitochondrial Trap-1 plays a critical role in regulating mitochondrial integrity and Hsp90 inhibitors targeted to mitochondria cause selective tumor cell death 46. Grp94 is an abundant component of the endoplasmic reticulum and the ATPase activity of GRP94 is essential for development in mice 47. Grp94 helps to protect cells from a variety of stresses and plays a role in degradation of misfolded proteins by the ER-associated degradation pathway 48. Some of the specific proteins known to require Grp94 for function are insulin-like growth factor- II, immunoglobulins and some toll-like receptors.

Buffering capabilities of Hsp90

Research in *Drosophila melanogaster* and *Arabidopsis thaliana* demonstrated that Hsp90 is able to buffer phenotypic abnormalities. Reduction of Hsp90 activity, either through mutation or pharmacologic inhibition of Hsp90, led to the accumulation of a variety of phenotypic abnormalities. The abnormalities are believed to be the result of variations in proteins that function within morphogenic signaling pathways. Hsp90 presumably binds and stabilizes proteins containing alterations that would otherwise result in reduced or altered function. Consistent with the role of Hsp90 in protein folding, the observed abnormal phenotypes were enhanced at higher and lower temperatures. These exciting results suggest that Hsp90 may play a role in evolution by allowing accumulation of polymorphic variants of critical signaling pathways. As organisms are exposed to stresses during natural selection, these hidden genetic variations may play a critical role in enhancing the survival of distinct genotypes within a population 49›50.

One of the interesting aspects of these experiments is that these Hsp90-dependent phenotypic variations are heritable. Whether this mechanism for phenotypic transmission is genetic, epigenetic, or both, is still under debate 50–53. In an epigenetic model, DNA methylation and chromatin modification leads to gene silencing or activation that is inherited without any change to the genome. In support of this model, Hsp90 and co-chaperones interact physically or genetically with multiple proteins in chromatin remodeling complexes containing Ino80 and/or SWR-C 3. Hsp90 may also directly or indirectly influence the activity of CARM1 (coactivator associated arginine methyltransferase 1) and SMYD3, a human histone methyltransferase, resulting in altered patterns of DNA methylation 51.

Role of Hsp90 in cancer

Hsp90 is responsible for the chaperoning and maintenance of several oncogenic kinases such as Raf-1, Bcr-Abl, and ErbB2. Because it affects the activity of clients critical for multiple steps in tumor progression, such as immortalization (telomerase), impaired apoptosis (AKT), angiogenesis (HIF-1α) and invasion/metastasis (matrix metalloproteinase 2, MMP2), it is an important target of cancer therapeutics 1›40. Hsp90 is upregulated 10-fold in tumor cells suggesting that it helps maintain tumor cell growth and/or survival. The Hsp90 protein may also be altered in tumor cells, with reports of altered co-chaperone interactions and post-translational modifications that result in an activated state that facilitates malignant progression 54›55. Hsp90's buffering capabilities may contribute to its involvement in maintaining tumors, since it may allow tumor cells to tolerate genetic

alterations that may otherwise be lethal to the cell 40. Another role for Hsp90 in the maintenance of tumor cells is its ability to inhibit apoptosis. Mitochondrial Trap-1, as well as cytosolic Hsp90, which localizes to mitochondria in tumor cells, plays critical roles in regulating mitochondrial integrity. As evidence of this critical function, Hsp90 inhibitors targeted to mitochondria cause selective tumor cell death 46.

Several small molecule inhibitors that destabilize clients and induce apoptosis have been used in clinical trials to treat a range of tumor types. The best studied of these compounds is a derivative of geldanamycin, 17-allylamino-17-demethoxygeldanamycin (17-AAG), now called tanespimycin 1. Some of the most promising results with Hsp90 inhibitors in clinical trials are as part of combination therapies for breast cancer, lung cancer and some leukemias 56,57.

Role of Hsp90 in pathogenesis

Recent studies suggest that in addition to their promise in treating a variety of cancers, Hsp90 inhibitors may be combined with antifungals to treat infections with pathogenic fungi such as *Candida albicans* or *Aspergillus umigates* 58. The class of antifungals most widely used in clinical settings is azoles, which target ergosterol, a component of fungal membranes. In the presence of Hsp90, fungi are able to adapt to antifungals though alteration of various signaling pathways, such as upregulating the ergosterol biosynthetic pathway. Hsp90 inhibitors disrupt the signaling pathways required to adapt to the drug, thus preventing drug resistance 59.

Hsp90 is also required for the replication of many viruses, such as hepatitis B and C viruses, flock house virus, cytomegalovirus, influenza A and others (reviewed in 1). The folding and maturation of picornavirus capsid proteins is also dependent on Hsp90. Inhibition of Hsp90 impaired capsid production and viral replication without allowing the development of drug-resistant viruses 60. These results emphasize the key role of Hsp90 in allowing cells to adapt to a variety of stresses and suggest that Hsp90 inhibitors may be used in combination with other anti-virals to treat a large number of infectious diseases.

Future Directions

Hsp90 and co-chaperones regulate the activity of a large number of clients in a variety of biological processes, including reproduction, the immune response, cell growth and differentiation. More recent studies point to a critical role for Hsp90 and co-chaperones in plant development and resistance to pest and disease 1,59,61. Despite these advances, it is remarkable that little is known about how Hsp90 actually mediates client protein folding. The location of the client-binding site(s) within Hsp90 remains unknown, and it is unknown whether different client proteins have different interactions with Hsp90. In addition, despite recent advances in understanding the dynamic conformational changes that occur within Hsp90 as it binds and hydrolyzes ATP, the conformation of Hsp90 that is capable of promoting client folding is not known.

Another question about Hsp90 is how it is able to promote client protein folding either in the absence of co-chaperones (as in the case of HtpG, Trap-1 and Grp94), or with a set of co-chaperones that appears to vary in a species-specific manner 39. Since some of the co-chaperones are known to directly regulate the ATPase activity of Hsp90 (Hop, Aha1 and Cdc37), part of the requirement for co-chaperones could be due to differing intrinsic ATPase activities of the different forms of Hsp90 5. At this time it remains unclear how alterations in the ATPase cycle may affect the folding of particular clients. It is possible that a fast ATPase cycle is required for rapid folding of signaling molecules whereas other clients with more complex folding pathways may require slower ATPase cycles in order to productively

interact with Hsp90. As more is learned about the specificity of co-chaperone interactions it may be possible to distinguish whether specific clients must have access to the same co-chaperones in disparate species. Alternatively it is possible, given the role of Hsp90 in buffering the effect of alterations in proteins, that a client that requires one set of co-chaperones in mammalian cells may be dependent on a different set of co-chaperones in another species or even be completely independent of Hsp90 for function in another species.

The availability of a growing list of small-molecule inhibitors of Hsp90 will continue to promote rapid advancements in our understanding of how Hsp90 interacts with client proteins. Of particular promise are clinical studies that indicate that a range of tumor cells respond favorably to Hsp90 inhibitors such as tanespimycin, particularly when used in combination of other drugs. Future clinical studies will hopefully show similar promise in targeting Hsp90 to treat fungal and viral infections.

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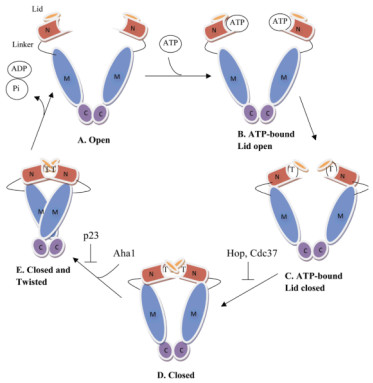

Figure 1. Hsp90 ATPase cycle

In the absence of ATP (**A**), Hsp90 is dimerized at its C-terminus in the 'open' conformation. Upon ATP binding (**B**), the N-terminal domains undergo subsequent conformational changes that result in closing of a 'lid' over the bound nucleotide (**C**) and formation of a second dimerization interface between the amino-termini (**D**, the 'closed' conformation). Continued rearrangements of the closed conformation allow interaction of the N-terminal and middle domains resulting in the 'closed and twisted' conformation which is able to hydrolyze ATP (**E**). After ATP hydrolysis the lid opens and the N-terminal domains release from one another returning to the open conformation (**A**). Co-chaperones bind Hsp90 and modulate the ATPase cycle. Hop/Sti1 and Cdc37 inhibit the ATPase activity by keeping the Hsp90 in the open conformation. Aha1 stimulates ATP hydrolysis by promoting formation of the closed conformation, while Sba1 stabilizes the closed and twisted conformation.

Table 1

Co-chaperones and co-activators that function with Hsp90 during client protein folding.

Co-chaperone (vertebrates)	Co-chaperone (yeast)	Essential in Yeast?	Effect on Hsp90 ATPase activity	Hsp90 binding site	Characteristics	Refs. (reviewed in 2,9,10)
Hop	Sti1	No	Inhibits (Sti1)	C-terminus	TPR domains, binds Hsp70 and Hsp90	35,36
PP5	Ppt1	No	None	C-terminus	TPR domain, phosphatase domain	22
FKBP51, 52	-	-	None	C-terminus	TPR domain, peptidyl-prolyl isomerase	33
Cyp40	Cpr6, Cpr7	No	None	C-terminus	TPR domain, peptidyl-prolyl isomerase	34
-	Tah1	No		C-terminus	TPR domain	16
TTC4	Cns1	Yes		C-terminus	TPR domain, activates ATPase activity of Hsp70	31,32
XAP2	-	-		C-terminus	TPR domain	30
AIPL1	-	-		C-terminus	TPR domain	17
Tpr2	-	-		C-terminus	TPR domain and J domain	19
GCUNC-45	-	-		N-terminus	TPR domain	20
p23	Sba1	No		N-terminus	Stabilizes closed conformation	28,29
P50/Cdc37	Cdc37	Yes	Inhibits	N-terminus	Binds kinase clients	13
Sgt1	Sgt1	Yes		N-terminus	TPR domain and domain with homology to p23	14, 15
Aha1	Aha1, Hch1	No	Stimulates	Middle domain	Potent activator of Hsp90 ATPase activity	27
Hdj2	Ydj1	No	None	None	Hsp40 molecular chaperone, stimulates ATPase activity of Hsp70	25,26
Hip	-	-		None	TPR domain, inhibits ATPase activity of Hsp70	18
Chip	-	-		None	TPR domain, ubiquitin ligase, binds Hsp70	24
Hsp110	Sse1	No		None	Nucleotide exchange factor for Hsp70	21,23