

Hsp90: a specialized but essential protein-folding tool

Jason C. Young, Ismail Moarefi, and F. Ulrich Hartl

Cellular Biochemistry, Max Planck Institute for Biochemistry, Martinsried D-82152, Germany

Hsp90 is unique among molecular chaperones. The majority of its known substrates are signal transduction proteins, and recent work indicates that it uses a novel protein-folding strategy.

Cells are equipped with several classes of structurally unrelated molecular chaperones to ensure both the proper folding of proteins upon synthesis and their refolding under conditions of denaturing stress. Different chaperones follow distinct strategies to achieve the general goal of preventing protein misfolding and aggregation in the highly crowded cellular environment. For example, the monomeric Hsp70 chaperone proteins promiscuously recognize short hydrophobic peptide segments such as those exposed in nascent polypeptide chains. In contrast, the so-called chaperonins form oligomeric double ring structures capable of enclosing entire proteins or protein domains to promote folding in a sequestered environment. Chaperone proteins often cooperate in folding: a polypeptide that is bound by Hsp70 at an early stage of folding can be transferred to a chaperonin to reach its final native state (Hartl, 1996; Bukau et al., 2000). For certain client polypeptides in the eukaryotic cytosol, an alternate pathway has been proposed in which binding to Hsp70 is followed by an interaction with the dimeric chaperone protein Hsp90 (Smith et al., 1992; Smith, 1993; Hutchison et al., 1994). Recent advances in understanding the structure and function of Hsp90 now indicate that it uses a protein-folding strategy different from that of either Hsp70 or the chaperonins. Here, we discuss the distinct biochemical mechanisms by which Hsp90 handles its substrate polypeptides and the unique biological functions of this curious protein-folding tool.

Unlike Hsp70, eukaryotic cytosolic Hsp90 does not act generally in nascent protein folding (Nathan et al., 1997). Hsp90 is distinguished from other chaperones in that most of its known substrates are signal transduction proteins, the classical examples being steroid hormone receptors and signaling kinases (Picard et al., 1990; Xu and Lindquist, 1993). Because Hsp90 is essential for maintaining the activity of nu-

merous signaling proteins, it plays a key role in cellular signal transduction networks. At a molecular level, Hsp90 binds to substrate proteins, which are in a near native state and thus at a late stage of folding (Jakob et al., 1995) poised for activation by ligand binding or interaction with other factors. In fulfilling its role, Hsp90 operates as part of a multichaperone machinery in the cytosol, which includes Hsp70 as mentioned above but also peptidyl-prolyl isomerases and other cochaperones (Bose et al., 1996; Freeman et al., 1996). This complexity of function has made Hsp90 more difficult to investigate than the Hsp70 or chaperonin proteins. For example, Hsp90 has only been established recently as an ATP-dependent chaperone (Prodromou et al., 1997; Obermann et al., 1998; Panaretou et al., 1998), and the exact functions of the Hsp90 ATPase activity are still being worked out. In the following sections, we will first describe the structure and mechanism of the Hsp90 protein, then focus on its regulation by cochaperones, and finally discuss the biological contribution of Hsp90 to cellular processes including signal transduction, protein degradation, and morphological evolution.

Structure and ATPase cycle of Hsp90

The highly conserved Hsp90 chaperone family includes the eponymous Hsp90 (90 kD heat shock protein) of the eukaryotic cytosol, termed variously Hsp90 α and β in humans (corresponding to a major and minor isoform), Hsp86 and Hsp84 in mice, Hsp83 in *Drosophila*, and Hsc82 and Hsp82 in yeast. Other family members are HtpG in the bacterial cytosol, Grp94/gp96 in the endoplasmic reticulum of eukaryotes, and the recently discovered Hsp75/TRAP1 in the mitochondrial matrix (Argon and Simen, 1999; Felts et al., 2000; Thomas and Baneyx, 2000). All of these proteins share a common structural plan (Fig. 1 A) and are thus expected to have a similar mechanism of action. Hsp90 is a constitutive homodimer with its main intersubunit contacts within the COOH-terminal 190 residues (Nemoto et al., 1995). The highly conserved 25 kD NH₂-terminal domain of Hsp90 is the binding site for ATP and for geldanamycin (GA),* a representative of the ansamycin drugs, which specifically target Hsp90 (Whitesell et al., 1994). GA inhibits the Hsp90 ATPase with nanomolar affinity, and crystallography has shown that the drug occupies the nucleotide-binding

Address correspondence to Ulrich Hartl, Dept. of Cellular Biochemistry, Max-Planck-Institut für Biochemie, Am Kopferspitz 18a, Martinsried D-82152, Germany. Tel.: 49-89-8578-2233. Fax: 49-89-8578-2211. E-mail: uhartl@biochem.mpg.de

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*Abbreviations used in this paper: AMP-PNP, 5'-adenylylimidodiphosphate; GA, geldanamycin; HSF, heat shock transcription factor; TPR, tetratricopeptide repeat.

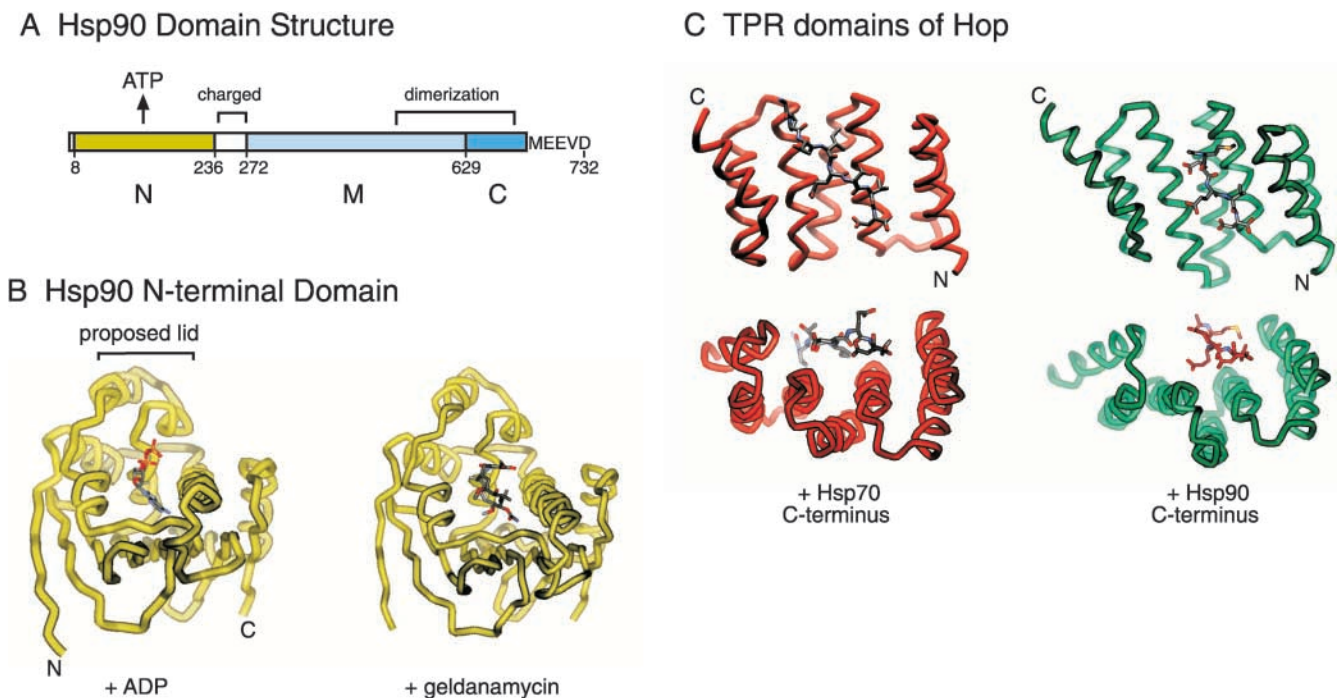


Figure 1. Structural basis for the interaction of Hsp90 with nucleotides, ansamycin drugs, and TPR domain cochaperones. (A) Domain organization of Hsp90. Amino acid numbers of domain boundaries for human Hsp90 α are marked. See text for references. (B) Crystal structures of the Hsp90 NH₂-terminal domain complexed with ADP and with the ansamycin drug GA (Prodromou et al., 1997; Stebbins et al., 1997). The proposed lid sequence over the nucleotide-binding pocket is indicated. (C) Crystal structures of the Hsp70-binding NH₂-terminal TPR domain of Hop complexed with the Hsp70 COOH-terminal peptide GPTIEVD (left) and the Hsp90-binding central TPR domain of Hop complexed with the Hsp90 COOH-terminal peptide MEEVD (right). Structures in the bottom panel are rotated 90° from the top panel. The peptides are oriented with the COOH-terminal carboxyl groups at the bottom of the figure (top) and towards the viewer (bottom) (Scheufler et al., 2000).

cleft within the NH₂-terminal domain (Fig. 1 B) (Prodromou et al., 1997; Stebbins et al., 1997; Obermann et al., 1998; Panaretou et al., 1998). A divergent charged sequence separates the NH₂-terminal domain from a conserved though structurally flexible 35 kD middle domain and the 12 kD COOH-terminal domain required for dimerization (Stebbins et al., 1997; Maruya et al., 1999). Both the NH₂- and COOH-terminal domains of Hsp90 have been implicated in binding of substrate polypeptides, and substrate binding at the NH₂-terminal site is affected by nucleotides, GA, and the adjacent charged sequence of Hsp90 (Young et al., 1997; Scheibel et al., 1998, 1999).

In vitro, Hsp90 from *Saccharomyces cerevisiae* has a slow but clearly measurable ATPase activity. The affinity of yeast Hsp90 for ATP ($K_D > 100 \mu\text{M}$) is about 10 times poorer than that of the chaperonins and the catalysis rate comparable to that of Hsp70 without cochaperone or peptide stimulation ($\sim 0.5 \text{ min}^{-1}$). Interestingly, recent data indicates that as for Hsp70 the ATPase activity of Hsp90 is stimulated markedly to a biologically relevant rate by the binding of a putative substrate protein (Prodromou, C., and L.H. Pearl, personal communication). In vivo, Hsp90 that has been mutated to abolish the ATPase activity cannot substitute for the essential wild-type protein in yeast (Obermann et al., 1998; Panaretou et al., 1998). As for the other ATP-dependent chaperones, the ATPase cycle is expected to regulate the interaction of Hsp90 with substrate polypeptides.

The ATP-binding domain of Hsp90 is structurally related to a superfamily of homodimeric ATPases, comprising in ad-

dition to Hsp90 DNA gyrase and topoisomerase II, the DNA mismatch repair protein MutL, and the histidine kinases CheA and EnvZ (Bergerat et al., 1997; Dutta and Inouye, 2000). It has been suggested that Hsp90 shares the structural mechanism of these proteins in which ATP binding induces intersubunit contacts between the nucleotide-binding domains in the homodimer (shown schematically in Fig. 2) (Dutta and Inouye, 2000). This dimerization of the nucleotide-binding domains may be critical for ATP hydrolysis to occur. Indeed, in the absence of nucleotide Hsp90 dimers have an extended appearance in the electron microscope with the NH₂-terminal ATP-binding domains at either end but can adopt circular structures in the presence of ATP (Maruya et al., 1999). Following removal of the COOH-terminal dimerization domain, transient homodimers of yeast Hsp90 are observed by cross-linking in the presence of the nonhydrolyzable analogue 5'-adenylylimidodiphosphate (AMP-PNP), and fluorescence probes attached to the NH₂ termini of full-length Hsp90 interact most strongly with each other when AMP-PNP is bound (Prodromou et al., 2000). Intriguingly, a fraction of ATP bound to intact Hsp90 cannot be competed by free nucleotide and is committed to hydrolysis (Weigl et al., 2000). It is tempting to suggest that formation of the transient NH₂-terminal dimer results in sequestration of the bound nucleotide.

In the gyrase and MutL proteins, the γ -phosphate of the bound ATP is coordinated by a flexible "lid" sequence within the ATP-binding domains (Fig. 1 B), and closing of the lid exposes a binding face that stabilizes the transient dimer

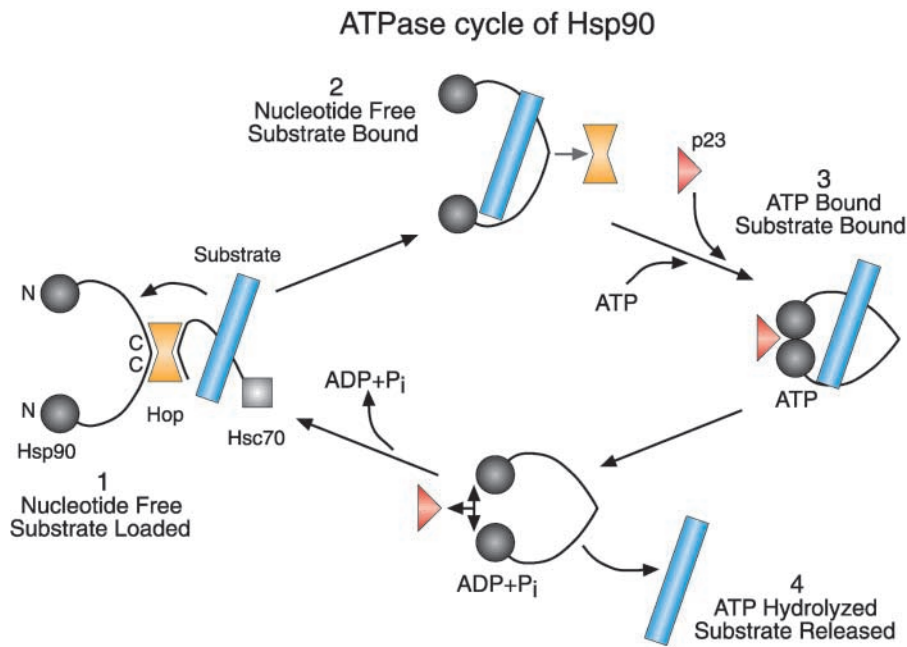


Figure 2. **The substrate-binding ATPase cycle of Hsp90** (Chadli et al., 2000; Prodromou et al., 2000; Young and Hartl, 2000). (1) Polypeptide substrate may be transferred from Hsc70 to the nucleotide-free state of Hsp90 induced by Hop. (2) Hsp90 in the open nucleotide-free state can bind substrate polypeptide. (3) ATP binding induces dimerization of the NH₂-terminal domains of Hsp90 (circles) and permits p23 binding; substrate is bound by Hsp90 clamp. (4) Hydrolysis of bound ATP releases substrate by opening clamp or by inducing some other conformational change.

(Dutta and Inouye, 2000). A point mutation in the putative lid sequence favored ATP-dependent dimerization of Hsp90 and also increased its ATPase rate, consistent with the proposed model (Prodromou et al., 2000). However, other interpretations cannot yet be ruled out, since part of the Hsp90 lid sequence can take on an alternate conformation where a loop partially blocks the ATP-binding pocket (Stebbins et al., 1997). This may suggest a more complex mechanism to regulate ATP binding. Also, in MutL the γ -phosphate of ATP contacts a lysine residue in a segment of the protein structurally unrelated to Hsp90 (Ban et al., 1999), and there may be further differences between Hsp90 and the other superfamily members in details of the ATPase mechanism.

How does the ATPase regulate polypeptide binding by Hsp90? One possible mechanism is suggested by the nucleotide-driven cycle of DNA gyrase. The ATP-bound state of DNA gyrase forms a “molecular clamp,” which is proposed to close around a DNA strand, and ATP hydrolysis releases the DNA after the supercoiling reaction (Berger et al., 1996; Ban et al., 1999). In analogy, the ATP-bound state of Hsp90 binds stably to substrate polypeptides, whereas substrate release is achieved through ATP hydrolysis. Isolated complexes of mammalian Hsp90 bound to a model substrate are dissociated with ATP but not with the nonhydrolyzable nucleotide AMP-PNP. Furthermore, point mutations in Hsp90, which reduce the ATP hydrolysis rate but not ATP binding also reduce the efficiency of ATP-dependent complex dissociation (Young and Hartl, 2000). Overall, a mechanism is outlined in which a substrate polypeptide is held by the closed internally dimerized ATP-bound clamp of Hsp90 (Fig. 2). Hydrolysis of bound ATP releases polypeptide by opening up the Hsp90 dimer or by some other conformational change.

One interesting question raised by this model is the manner in which substrate proteins are held by Hsp90 in the ATP state. The extended Hsp90 homodimer was estimated to be \sim 28-nm long and 7-nm wide (Maruya et al., 1999)

and in a circular clamp conformation could enclose a central space perhaps 3–4 nm in diameter. Consistent with this estimate, a homodimerized 92-kD fragment of topoisomerase II provides a hole between 2.5- and 5.5-nm wide (Berger et al., 1996). For comparison, the cavity of the bacterial chaperonin GroEL, which encapsulates nonnative proteins, is 6.5 by 8.0 nm (Hartl, 1996). Thus, although a space encircled by the Hsp90 dimer is probably too small for an entire protein substrate to fit inside, a sizable domain could be accommodated. It is also possible that Hsp90 does not enclose its substrate but rather that a substrate-binding face is optimally exposed by Hsp90 in the ATP clamp state. Evidence for multiple substrate-binding sites along the length of the Hsp90 protein has been presented, consistent with either model (Young et al., 1997; Scheibel et al., 1998), but the structural features of a substrate recognized by Hsp90 have not yet been determined. Once the precise substrate-binding mode of Hsp90 has been established, the larger question of how it aids in folding can be addressed.

Regulatory cochaperones of cytosolic Hsp90

Although the bacterial and endoplasmic reticulum forms of Hsp90 appear to operate independently, Hsp90 in the eukaryotic cytosol interacts with a variety of cochaperone proteins that assemble into a multichaperone complex and regulate the function of Hsp90 and Hsp70. The largest class of cochaperones bind to Hsp90 via a modular domain containing typically three 34 amino acid, helix-turn-helix tetratricopeptide repeat (TPR) motifs (Fig. 1 C). These TPR domains are found fused to a series of different functional domains, for example peptidyl-prolyl isomerase domains (the cyclophilin Cyp40 and the immunophilins FKBP52 and FKBP51) (Johnson and Toft, 1994) or a protein phosphatase (PP5) (Chen et al., 1996). Hsp90-binding TPR domains have also been identified fused to other TPR domains, which recognize Hsp70 and then connect Hsp70 to Hsp90 (p60/Hop/Sti1) (Chen and Smith, 1998; Johnson et

al., 1998) or may recruit Hsp90 to the mitochondrial import machinery (Tom34) (Young et al., 1998). The TPR cochaperone Cns1 in yeast can suppress cyclophilin defects and may also connect Hsp90 to Hsp70 (Dolinski et al., 1998; Marsh et al., 1998). Most recently, the TPR cofactor CHIP was shown to link Hsp90 with the ubiquitination apparatus, controlling protein degradation by the proteasome (Connell et al., 2001; see below).

The TPR domain cochaperones compete with each other for a binding site at the COOH terminus of Hsp90 (Chen et al., 1998; Young et al., 1998). In the case of the cochaperone Hop, the TPR domain containing the main Hsp90-binding site recognizes the five COOH-terminal residues MEEVD of Hsp90. The isolated TPR domain binds to the pentapeptide with specificity and affinity comparable to the interaction between the full-length proteins. Although this may seem surprising, the crystal structure of the TPR domain bound to a synthetic peptide shows that the stacked α -helices of the TPR motifs form a groove, which makes considerable contacts with the peptide (Fig. 1 C). In particular, the peptide is anchored by interactions with several conserved residues in the TPR domain that form a “two-carboxylate clamp” to hold the side chain and carboxyl group of the terminal aspartate residue in place. Interestingly, Hsc70 is bound by a separate TPR domain of Hop in a similar fashion with the last eight residues of Hsc70 (GPTIEEVD) held in the groove of the TPR domain and anchored by an identical carboxylate clamp (Fig. 1 C). Hydrophobic interactions with the divergent NH₂ termini of the peptides account for the specificity of binding (Scheufler et al., 2000).

The amino acid residues of Hop, which form the carboxylate clamp, are absolutely conserved in the TPR domains of other Hsp90-binding cochaperones but not in functionally unrelated TPR proteins. Also, the COOH-terminal sequences of Hsp90 and Hsc70 are conserved within the eukaryotic cytosolic forms of these chaperones (Scheufler et al., 2000). Because both Hsp90 and Hsc70 share a terminal EEVD sequence, there should be TPR domains, which can bind either chaperone at the same site. This has now been observed with the single TPR domain of the cochaperone CHIP (Connell et al., 2001; Meacham et al., 2001).

In addition to linking Hsp90 with the Hsc70 chaperone system, Hop acts as an inhibitor of the Hsp90 ATPase by preventing access to the nucleotide-binding site of Hsp90. This inhibitory activity of Hop has been proposed to be part of a substrate-loading mechanism for Hsp90, where an Hsp90-Hop-Hsc70 complex permits transfer of substrate polypeptide from Hsc70 to the nucleotide-free state of Hsp90 (Prodromou et al., 1999) (Fig. 2). Binding of ATP onto Hsp90 should then displace the Hop-Hsc70 loading system and simultaneously close the substrate-binding clamp of Hsp90 (Fig. 2). Although such a loading mechanism remains to be demonstrated directly, it is consistent with earlier time course experiments, showing that initial binding of Hsc70, Hop, and Hsp90 to progesterone receptor was followed by the dissociation of Hsc70 and Hop, leading to “mature” Hsp90 complexes (Smith et al., 1992; Smith, 1993; Hutchison et al., 1994).

Mature complexes of Hsp90 with bound substrate are also characterized by binding of the cochaperone p23 (Johnson

and Toft, 1994), which is unrelated to the TPR domain proteins. Although p23 can itself act as a chaperone in binding unfolded polypeptides (Bose et al., 1996; Freeman et al., 1996), p23 and its yeast homologue Sba1 recognize specifically the ATP-bound state of Hsp90 (Sullivan et al., 1997; Fang et al., 1998), implying a more specialized function of p23 in regulating the Hsp90 ATPase cycle. The binding site for p23 is located within the NH₂-terminal half of Hsp90 and is supported by induced dimerization of Hsp90 (Chadli et al., 2000). This is consistent with the structural model of the Hsp90 ATPase cycle discussed above. Although p23 does not affect the ATPase activity of Hsp90, the cochaperone significantly stimulates the ATP hydrolysis-dependent dissociation of Hsp90–substrate complexes. One possible mechanism is that p23 more stringently couples the nucleotide state of Hsp90 with conformational changes throughout the dimer (Young and Hartl, 2000). The active core domain of p23 has an immunoglobulin-like fold with several highly conserved residues exposed in one cluster, forming the probable contact site for Hsp90 (Weaver et al., 2000). The core domain of p23 is sufficient for its ATP-dependent action on Hsp90 (Young and Hartl, 2000), whereas the chaperone activity of p23 resides in a separate COOH-terminal domain (Weickl et al., 1999). The recently reported Hsp90-independent effects of p23 on steroid hormone receptor signaling (Freeman et al., 2000) may arise from this chaperone domain.

The cochaperone protein p50/Cdc37 interacts with Hsp90 and certain protein kinases and is proposed to target Hsp90 to kinases that are dependent on the chaperone for maturation (Dai et al., 1996; Stepanova et al., 1996). Cdc37 has some properties of a chaperone and may itself contribute to the folding reaction (Kimura et al., 1997). Cdc37 also interacts functionally with androgen receptor but not glucocorticoid receptor (Rao et al., 2001). There may be further cochaperones of Hsp90 yet to be identified. For example, the proteasome activator PA28 has been reported recently to stimulate the Hsc70-mediated refolding of luciferase bound to Hsp90 *in vitro* (Minami et al., 2000).

Hsp90 and signal transduction

A growing set of signal transduction proteins constitute the majority of known Hsp90 substrates. These proteins are critically dependent on Hsp90 for their maturation and conformational maintenance. Disruption of Hsp90 function by mutations or treatment with inhibitors such as the ansamycins led to multiple physiologic defects in live cells, consistent with a contribution of Hsp90 throughout the cellular signaling network. For example, in the regulation of cell division alone disruption of Hsp90 affects multiple stages of the mitogenic signal cascade, cyclin-dependent progression through both G1 and G2, and centrosome function during mitosis (Fisher et al., 2000; for review see Helmbrecht et al., 2000; Lange et al., 2000).

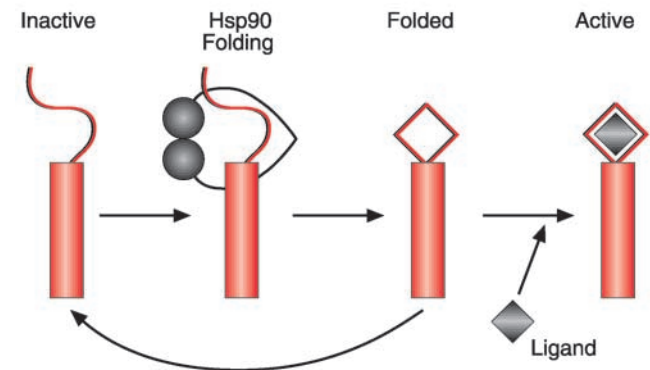
An *in vivo* requirement for Hsp90 has been established for some steroid hormone receptors (Picard et al., 1990; for review see Pratt and Toft, 1997), several tyrosine and serine/threonine kinases such as pp60/v-src, Wee-1, Cdk4, and Raf (Xu and Lindquist, 1993; Aligue et al., 1994; Stepanova et al., 1996; van der Straten et al., 1997), and disparate proteins such as nitric oxide synthase and calcineurin (Gar-

cia-Cardena et al., 1998; Imai and Yahara, 2000). These proteins are characteristically large and multidomain and require stabilizing interactions with other factors for their function such as steroid ligands for the steroid hormone receptors or cyclins for the cyclin-dependent kinases. Because signaling proteins with multiple regulatory states often undergo a conformational switch, the structural flexibility needed for these steps may render them inherently less stable and thus more likely to be recognized by Hsp90. On the other hand, under stress conditions such as heat shock Hsp90 contributes more generally to the refolding of denatured proteins (Nathan et al., 1997). Although the exact structural features recognized by Hsp90 are not yet understood, the exposure of these features to Hsp90 is likely a result of intrinsic or stress-induced structural flexibility. Thus, interactions of substrate proteins with Hsp90 arise from structural properties at the molecular level rather than biological function.

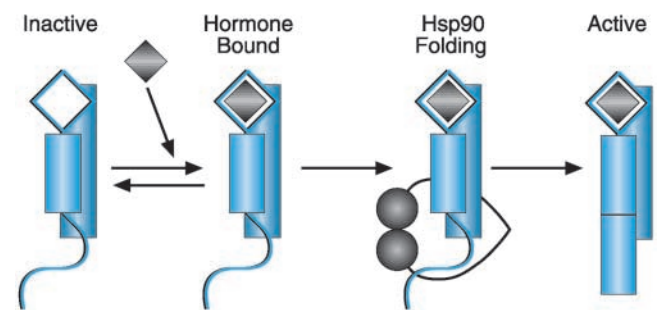
The best characterized example of an Hsp90-dependent signaling pathway is that of the steroid hormone receptors (Pratt and Toft, 1997) (Fig. 3 A). Interaction of the glucocorticoid receptor with Hsp90 is essential for its activity (Picard et al., 1990), and the unstable ligand-binding domain of the receptor is sufficient for this interaction (Young and Hartl, 2000). Monomeric glucocorticoid receptor and the closely related progesterone receptor are loaded onto Hsp90 by the Hsp70/Hop-dependent mechanism described above and attain their hormone-binding conformation after binding to Hsp90. Once the folded monomeric receptor has been released from the chaperones, it either binds the appropriate steroid hormone, resulting in dimerization and activation, or remains unstable and is recognized again by the chaperone machinery (Smith, 1993). Recently, progesterone receptor and glucocorticoid receptor have been used to reconstitute the typical multichaperone complexes with purified mammalian Hsp90, Hsc70, Hop, and p23. Although the requirement for cochaperones *in vitro* is still controversial (Morishima et al., 2000; Rajapandi et al., 2000), the ATP-dependent function of Hsp90 in steroid receptor maturation has been demonstrated (Grenert et al., 1999).

In the case of *v-src* and other kinases, Hsp90 is thought to stabilize the exposed catalytic domains before assembly of the kinases into the final signaling complex, a mechanism conceptually related to the steroid receptor pathway (Xu and Lindquist, 1993; Aligue et al., 1994; Stepanova et al., 1996). A variation of the steroid receptor signaling pathway has been reported for the *Drosophila* ecdysone receptor, a member of the heterodimeric retinoid X receptor family (Fig. 3 B). The isolated receptor together with its partner protein USP is already capable of binding ecdysone but not DNA, and the Hsp90 chaperone machinery converts the hormone-bound receptor to the active DNA-binding state (Arbeitman and Hogness, 2000). Another mechanism allows human Hsp90 to regulate its own expression by sequestering the monomeric inactive form of heat shock transcription factor (HSF) 1 under nonstress conditions (Fig. 3 C). Misfolded proteins produced by stress can compete with HSF1 for binding to Hsp90, liberating the transcription factor for trimerization and initiating the heat shock response (Zou et al., 1998).

A Steroid Hormone Receptor



B Ecdysone Receptor + USP



C HSF1

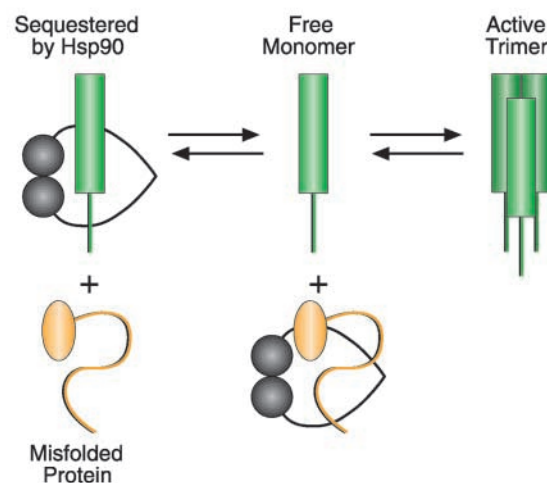


Figure 3. **Different modes of Hsp90 action in signaling pathways.**

(A) Inactive steroid hormone receptors are folded by the Hsp90 machinery. Folded receptors either are stabilized and activated by hormone binding or remain unstable and are rebound by chaperones (Smith, 1993; Pratt and Toft, 1997). (B) Ecdysone receptor with its partner USP can bind hormone but requires folding by the Hsp90 machinery to become active for DNA binding (Arbeitman and Hogness, 2000). (C) Monomeric HSF1 is sequestered by Hsp90. Under stress conditions, misfolded proteins compete for Hsp90, and HSF1 is displaced from Hsp90 and can form the active trimer (Zou et al., 1998).

Hsp90 and protein degradation

The hallmark of ansamycin activity in live cells is induced degradation of Hsp90 substrate proteins by the ubiquitin-dependent proteasome pathway (Whitesell et al., 1994; Schulte et al., 1995). Although ansamycins were identified histori-

cally as tyrosine kinase inhibitors, they are now known to specifically target Hsp90 family members (Whitesell et al., 1994). Ansamycins can inhibit the chaperone-mediated folding of Hsp90 substrates by blocking their ATP-dependent dissociation from Hsp90 (Schneider et al., 1996; Segnitz and Gehring, 1997; Stancato et al., 1997; Young and Hartl, 2000). On the other hand, certain substrate proteins are released from Hsp90 by ansamycins. For these proteins, either drug-induced degradation is not observed as in the case of HSF1 (Zou et al., 1998) or the interaction with Hsp90 may be indirect as in the case of cystic fibrosis transmembrane regulator (Loo et al., 1998; Meacham et al., 2001).

How are Hsp90-bound polypeptides targeted for degradation? The TPR cochaperone CHIP, which recognizes both Hsp90 and Hsc70 (see above), contains a U-box domain homologous to those of the E4 ubiquitination factors and may present chaperone-bound misfolded proteins for ubiquitination. Consistent with this possibility, overexpression of CHIP in cultured cells caused increased ubiquitination and degradation of glucocorticoid receptor and cystic fibrosis transmembrane regulator, known substrates of Hsp90 and Hsc70, respectively (Connell et al., 2001; Meacham et al., 2001). In terms of the kinetic model of protein folding and degradation (Wickner et al., 1999), the activity of CHIP suggests that substrates, which reside longer on Hsp90, for example when its ATPase is inhibited by GA, would be more likely to be ubiquitinated. The existence of CHIP also suggests that like protein folding, protein targeting to the degradation machinery is regulated by additional factors whose functions are not anticipated by a simple kinetic-partitioning model.

Hsp90 and morphological evolution

Defects in cell physiology caused by Hsp90 disruption can lead to defects at the level of tissue and organism. Interestingly, recent work connects Hsp90 function with morphological evolution, a process that often requires the effects of independent genetic changes (Rutherford and Lindquist, 1998). Hsp90-null mutants are lethal in eukaryotes, but surprisingly partial disruption of Hsp90 in *Drosophila* by a temperature-sensitive mutation or low amounts of GA shows a wide assortment of heritable phenotypic variations. The variations may arise from alleles of proteins, which depend on the full function of Hsp90 as a conformational buffer to maintain “wild-type” activity but whose phenotypes can be stabilized by other genes following selection. By extension, polymorphisms in all of the proteins participating in an Hsp90-dependent signaling pathway should be buffered by Hsp90 function. In the wild, overloading of the Hsp90 “capacitor” with denatured proteins under environmental stress could similarly increase the phenotypic diversity on which natural selection and ultimately evolution acts (Rutherford and Lindquist, 1998). Hsp90 with its connection to the cellular signaling network may be particularly suited to such a function. Thus, the mechanisms of chaperone-mediated protein folding at a molecular level can be integrated with cellular processes and with the development of organisms and species.

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References

- Aligue, R., H. Akhavan-Niak, and P. Russell. 1994. A role for Hsp90 in cell cycle control: Wee1 tyrosine kinase activity requires interaction with Hsp90. *EMBO J.* 13:6099–6106.
- Arbeitman, M.N., and D.S. Hogness. 2000. Molecular chaperones activate the *Drosophila* ecdysone receptor, an RXR heterodimer. *Cell.* 101:67–77.
- Argon, Y., and B.B. Simen. 1999. Grp94, an ER chaperone with protein and peptide binding properties. *Semin. Cell. Dev. Biol.* 10:495–505.
- Ban, C., M. Junop, and W. Yang. 1999. Transformation of MutL by ATP binding and hydrolysis: a switch in DNA mismatch repair. *Cell.* 97:85–97.
- Berger, J.M., S.J. Gamblin, S.C. Harrison, and J.C. Wang. 1996. Structure and mechanism of DNA topoisomerase II. *Nature.* 379:225–232.
- Bergerat, A., B. de Massy, D. Gabelle, P.-C. Varoutas, A. Nicolas, and P. Forterre. 1997. An atypical topoisomerase II from archaea with implications for meiotic recombination. *Nature.* 386:414–417.
- Bose, S., T. Weikl, H. Bugl, and J. Buchner. 1996. Chaperone function of Hsp90-associated proteins. *Science.* 274:1715–1717.
- Bukau, B., E.E. Deuring, C. Pfund, and E. Craig. 2000. Getting newly synthesized proteins into shape. *Cell.* 101:119–122.
- Chadli, A., I. Bouhouche, W. Sullivan, B. Stensgard, N. McMahon, and M.G. Caletti. 2000. Dimerization and N-terminal domain proximity underlie the function of the molecular chaperone heat shock protein 90. *Proc. Natl. Acad. Sci. USA.* 97:12524–12529.
- Chen, S., and D.F. Smith. 1998. Hop as an adaptor in the heat shock protein 70 (Hsp70) and Hsp90 chaperone machinery. *J. Biol. Chem.* 273:35194–35200.
- Chen, M.S., A.M. Silverstein, W.B. Pratt, and M. Chinkers. 1996. The tetratricopeptide repeat domain of protein phosphatase 5 mediates binding to glucocorticoid receptor heterocomplexes and acts as a dominant negative mutant. *J. Biol. Chem.* 271:32315–32320.
- Chen, S., V. Prapapanich, R.A. Rimerman, B. Honoré, and D.F. Smith. 1998. Differential interactions of p23 and the TPR-containing proteins Hop, Cyp40, FKBP52 and FKBP51 with Hsp90 mutants. *Cell Stress Chaperones.* 3:118–129.
- Connell, P., C.A. Ballinger, J. Jiang, Y. Wu, L.J. Thompson, J. Höhfeld, and C. Patterson. 2001. The co-chaperone CHIP regulates protein triage decisions mediated by heat-shock proteins. *Nat. Cell Biol.* 3:93–96.
- Dai, K., R. Kobayashi, and D. Beach. 1996. Physical interaction of mammalian CDC37 with CDK4. *J. Biol. Chem.* 271:22030–22034.
- Dolinski, K.J., M.E. Cardenas, and J. Heitman. 1998. CNS1 encodes an essential p60/Sti1 homolog in *Saccharomyces cerevisiae* that suppresses cyclophilin 40 mutations and interacts with Hsp90. *Mol. Cell. Biol.* 18:7344–7352.
- Dutta, R., and M. Inouye. 2000. GHKL, an emergent ATPase/kinase superfamily. *Trends Biochem. Sci.* 25:24–28.
- Fang, Y., A.E. Fliss, and A.J. Caplan. 1998. SBA1 encodes a yeast hsp90 cochaperone that is homologous to vertebrate p23. *Mol. Cell. Biol.* 18:3727–3734.
- Felts, S.J., B.A.L. Owen, P. Nguyen, J. Trepel, D.B. Donner, and D.O. Toft. 2000. The hsp90-related protein TRAP1 is a mitochondrial protein with distinct functional properties. *J. Biol. Chem.* 275:3305–3312.
- Fisher, D.L., E. Mandart, and M. Dorée. 2000. Hsp90 is required for c-Mos activation and biphasic MAP kinase activation in *Xenopus* oocytes. *EMBO J.* 19:1516–1524.
- Freeman, B.C., D.O. Toft, and R.I. Morimoto. 1996. Molecular chaperone machines: chaperone activities of the cyclophilin Cyp-40 and the steroid aporeceptor-associated protein p23. *Science.* 274:1718–1720.
- Freeman, B.C., S.J. Felts, D.O. Toft, and K.R. Yamamoto. 2000. The p23 molecular chaperones act at a late step in intracellular receptor action to differentially affect ligand efficacies. *Genes Dev.* 14:422–434.
- Garcia-Cardena, G., R. Fan, V. Shah, R. Sorrentino, G. Cirino, A. Papapetropoulos, and W.C. Sessa. 1998. Dynamic activation of endothelial nitric oxide synthase by Hsp90. *Nature.* 392:821–824.
- Grenert, J.P., B.D. Johnson, and D.O. Toft. 1999. The importance of ATP binding and hydrolysis by hsp90 in formation and function of protein heterocomplexes. *J. Biol. Chem.* 274:17525–17533.
- Hartl, F.U. 1996. Molecular chaperones in cellular protein folding. *Nature.* 381:571–579.
- Helmbrecht, K., E. Zeise, and L. Rensing. 2000. Chaperones in cell cycle regulation and mitogenic signal transduction: a review. *Cell Prolif.* 33:341–365.
- Hutchison, K.A., K.D. Dittmar, M.J. Czar, and W.B. Pratt. 1994. Proof that hsp70 is required for assembly of the glucocorticoid receptor into a heterocomplex with hsp90. *J. Biol. Chem.* 269:5043–5049.
- Imai, J., and I. Yahara. 2000. Role of Hsp90 in salt stress tolerance via stabilization

- and regulation of calcineurin. *Mol. Cell. Biol.* 20:9262–9270.
- Jakob, U., H. Lilie, I. Meyer, and J. Buchner. 1995. Transient interaction of Hsp90 with early unfolding intermediates of citrate synthase. Implications for heat shock *in vivo*. *J. Biol. Chem.* 270:7288–7294.
- Johnson, J.L., and D.O. Toft. 1994. A novel chaperone complex for steroid receptors involving heat shock proteins, immunophilins, and p23. *J. Biol. Chem.* 269:24989–24993.
- Johnson, B.D., R.J. Schumacher, E.D. Ross, and D.O. Toft. 1998. Hop modulates Hsp70/Hsp90 interactions in protein folding. *J. Biol. Chem.* 273:3679–3686.
- Kimura, Y., S.L. Rutherford, Y. Miyata, I. Yahara, B.C. Freeman, L. Yue, R.I. Morimoto, and S. Lindquist. 1997. Cdc37 is a molecular chaperone with specific functions in signal transduction. *Genes Dev.* 11:1775–1785.
- Lange, B.M.H., A. Bachi, M. Wilm, and C. González. 2000. Hsp90 is a core centrosomal component and is required at different stages of the centrosome cycle in *Drosophila* and vertebrates. *EMBO J.* 19:1252–1262.
- Loo, M.A., T.J. Jensen, L. Cui, Y. Hou, X.B. Chang, and J.R. Riordan. 1998. Perturbation of Hsp90 interaction with nascent CFTR prevents its maturation and accelerates its degradation by the proteasome. *EMBO J.* 17:6879–6887.
- Marsh, J.A., H.M. Kalton, and R.F. Gaber. 1998. Cns1 is an essential protein associated with the hsp90 chaperone complex in *Saccharomyces cerevisiae* that can restore cyclophilin 40-dependent functions in *cp17Δ* cells. *Mol. Cell. Biol.* 18:7353–7359.
- Maruya, M., M. Sameshima, T. Nemoto, and I. Yahara. 1999. Monomer arrangement in Hsp90 dimer as determined by decoration with N and C-terminal region specific antibodies. *J. Mol. Biol.* 285:903–907.
- Meacham, G.C., C. Patterson, W. Zhang, J.M. Younger, and D.M. Cyr. 2001. The Hsc70 co-chaperone CHIP targets immature CFTR for proteasomal degradation. *Nat. Cell Biol.* 3:100–105.
- Minami, Y., H. Kawasaki, M. Minami, N. Tanahashi, K. Tanaka, and I. Yahara. 2000. A critical role for the proteasome activator PA28 in the Hsp90-dependent protein refolding. *J. Biol. Chem.* 275:9055–9061.
- Morishima, Y., P.J. Murphy, D.P. Li, E.R. Sanchez, and W.B. Pratt. 2000. Stepwise assembly of a glucocorticoid receptor-hsp90 heterocomplex resolves two sequential ATP-dependent events involving first hsp70 and then hsp90 in opening of the steroid binding pocket. *J. Biol. Chem.* 275:18054–18060.
- Nathan, D.F., M.H. Vos, and S. Lindquist. 1997. *In vivo* functions of the *Saccharomyces cerevisiae* Hsp90 chaperone. *Proc. Natl. Acad. Sci. USA.* 94:12949–12956.
- Nemoto, T., Y. Ohara-Nemoto, T. Takagi, and K. Yokoyama. 1995. Mechanism of dimer formation of the 90-kDa heat-shock protein. *Eur. J. Biochem.* 233:1–8.
- Obermann, W.M.J., H. Sondermann, A.A. Russo, N.P. Pavletich, and F.U. Hartl. 1998. *In vivo* function of Hsp90 is dependent on ATP binding and ATP hydrolysis. *J. Cell Biol.* 143:901–910.
- Panaretou, B., C. Prodromou, S.M. Roe, R. O'Brien, J.E. Ladbury, P.W. Piper, and L.H. Pearl. 1998. ATP binding and hydrolysis are essential to the function of the Hsp90 molecular chaperone *in vivo*. *EMBO J.* 17:4829–4836.
- Picard, D., B. Khursheed, M.J. Garabedian, M.G. Fortin, S. Lindquist, and K.R. Yamamoto. 1990. Reduced levels of hsp90 compromise steroid receptor activation *in vivo*. *Nature.* 348:166–168.
- Pratt, W.B., and D.O. Toft. 1997. Steroid receptor interactions with heat shock protein and immunophilin chaperones. *Endocr. Rev.* 18:306–360.
- Prodromou, C., S.M. Roe, R. O'Brien, J.E. Ladbury, P.W. Piper, and L.H. Pearl. 1997. Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. *Cell.* 90:65–75.
- Prodromou, C., G. Siligardi, R. O'Brien, D.N. Woolfson, L. Regan, B. Panaretou, J.E. Ladbury, P.W. Piper, and L.H. Pearl. 1999. Regulation of hsp90 ATPase activity by tetratricopeptide repeat (TPR)-domain co-chaperones. *EMBO J.* 18:754–762.
- Prodromou, C., B. Panaretou, S. Chohan, G. Siligardi, R. O'Brien, J.E. Ladbury, S.M. Roe, P.W. Piper, and L.H. Pearl. 2000. The ATPase cycle of Hsp90 drives a molecular 'clamp' via transient dimerization of the N-terminal domains. *EMBO J.* 19:4383–4392.
- Rajapandi, T., L.E. Greene, and E. Eisenberg. 2000. The molecular chaperones Hsp90 and Hsc70 are both necessary and sufficient to activate hormone binding by glucocorticoid receptor. *J. Biol. Chem.* 275:22597–22604.
- Rao, J., P. Lee, S. Benzeno, C. Cardozo, J. Albertus, D.M. Robins, and A.J. Caplan. 2001. Functional interaction of human Cdc37 with the androgen receptor but not the glucocorticoid receptor. *J. Biol. Chem.* 276:5814–5820.
- Rutherford, S.L., and S. Lindquist. 1998. Hsp90 as a capacitor for morphological evolution. *Nature.* 396:336–342.
- Scheibel, T., T. Weikl, and J. Buchner. 1998. Two chaperone sites in Hsp90 differing in substrate specificity and ATP dependence. *Proc. Natl. Acad. Sci. USA.* 95:1495–1499.
- Scheibel, T., H.I. Siegmund, R. Jaenicke, P. Ganz, H. Lilie, and J. Buchner. 1999. The charged region of Hsp90 modulates the function of the N-terminal domain. *Proc. Natl. Acad. Sci. USA.* 96:1297–1302.
- Scheufler, C., A. Brinker, G. Bourenkov, S. Pegoraro, L. Moroder, H. Bartunik, F.U. Hartl, and I. Moarefi. 2000. Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine. *Cell.* 101:199–210.
- Schneider, C., L. Sepp-Lorenzino, E. Nimmesgern, O. Ouerfelli, S. Danishefsky, N. Rosen, and F.U. Hartl. 1996. Pharmacologic shifting of a balance between protein refolding and degradation mediated by Hsp90. *Proc. Natl. Acad. Sci. USA.* 93:14536–14541.
- Schulte, T.W., M.V. Blagosklonny, C. Ingui, and L. Neckers. 1995. Disruption of the Raf-1-Hsp90 molecular complex results in destabilization of Raf-1 and loss of Raf-1-Ras association. *J. Biol. Chem.* 270:24585–24588.
- Segnitz, B., and U. Gehring. 1997. The function of steroid hormone receptors is inhibited by the hsp90-specific compound geldanamycin. *J. Biol. Chem.* 272:18694–18701.
- Smith, D.F. 1993. Dynamics of heat shock protein 90-progesterone receptor binding and the disactivation loop model for steroid receptor complexes. *Mol. Endocrinol.* 7:1418–1429.
- Smith, D.F., B.A. Stensgard, W.J. Welch, and D.O. Toft. 1992. Assembly of progesterone receptor with heat shock proteins and receptor activation are ATP mediated events. *J. Biol. Chem.* 267:1350–1356.
- Stancato, L.F., A.M. Silverstein, J.K. Owens-Grillo, Y.H. Chow, R. Jove, and W.B. Pratt. 1997. The hsp90-binding antibiotic geldanamycin decreases Raf levels and epidermal growth factor signaling without disrupting formation of signaling complexes or reducing the specific enzymatic activity of Raf kinase. *J. Biol. Chem.* 272:4013–4020.
- Stebbins, C.E., A.A. Russo, C. Schneider, N. Rosen, F.U. Hartl, and N.P. Pavletich. 1997. Crystal structure of an Hsp90-geldanamycin complex: targeting of a protein chaperone by an antitumor agent. *Cell.* 89:239–250.
- Stepanova, L., X. Leng, S.B. Parker, and J.W. Harper. 1996. Mammalian p50/Cdc37 is a protein kinase-targeting subunit of Hsp90 that binds and stabilizes Cdk4. *Genes Dev.* 10:1491–1502.
- Sullivan, W., B. Stensgard, G. Caucutt, B. Bartha, N. McMahon, E.S. Alnemri, G. Litwack, and D.O. Toft. 1997. Nucleotides and two functional states of hsp90. *J. Biol. Chem.* 272:8007–8012.
- Thomas, J.G., and F. Baneyx. 2000. ClpB and HtpG facilitate de novo protein folding in stressed *Escherichia coli* cells. *Mol. Microbiol.* 36:1360–1370.
- van der Straten, A., C. Rommel, B. Dickson, and E. Hafen. 1997. The heat shock protein 83 (Hsp83) is required for Raf-mediated signalling in *Drosophila*. *EMBO J.* 16:1961–1969.
- Weaver, A.J., W.P. Sullivan, S.J. Felts, B.A. Owen, and D.O. Toft. 2000. Crystal structure and activity of human p23, a heat shock protein 90 co-chaperone. *J. Biol. Chem.* 275:23045–23052.
- Weikl, T., K. Abelmann, and J. Buchner. 1999. An unstructured C-terminal region of the Hsp90 co-chaperone p23 is important for its chaperone function. *J. Mol. Biol.* 293:685–691.
- Weikl, T., P. Muschler, K. Richter, T. Veit, J. Reinstein, and J. Buchner. 2000. C-terminal regions of Hsp90 are important for trapping the nucleotide during the ATPase cycle. *J. Mol. Biol.* 303:583–592.
- Whitesell, L., E.G. Mimnaugh, B. De Costa, C.E. Myers, and L.M. Neckers. 1994. Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. *Proc. Natl. Acad. Sci. USA.* 91:8324–8328.
- Wickner, S., M. Maurizi, and S. Gottesman. 1999. Posttranslational quality control: folding, refolding and degrading proteins. *Science.* 268:1888–1893.
- Xu, Y., and S. Lindquist. 1993. Heat-shock protein hsp90 governs the activity of pp60v-src kinase. *Proc. Natl. Acad. Sci. USA.* 90:7074–7078.
- Young, J.C., and F.U. Hartl. 2000. Polypeptide release by Hsp90 involves ATP hydrolysis and is enhanced by the co-chaperone p23. *EMBO J.* 19:5930–5940.
- Young, J.C., C. Schneider, and F.U. Hartl. 1997. *In vitro* evidence that hsp90 contains two independent chaperone sites. *FEBS Lett.* 418:139–143.
- Young, J.C., W.M.J. Obermann, and F.U. Hartl. 1998. Specific binding of tetratricopeptide repeat proteins to the C-terminal 12-kDa domain of Hsp90. *J. Biol. Chem.* 273:18007–18010.
- Zou, J., Y. Guo, T. Guertouche, D.F. Smith, and R. Voellmy. 1998. Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. *Cell.* 94:471–480.