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The bacterial Hsp90 chaperone: Cellular functions and mechanism of action

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ABSTRACT

Heat shock protein 90 (Hsp90) is a molecular chaperone that folds and remodels proteins, thereby regulating the activity of a large number of client proteins. Hsp90 is widely conserved across species and is essential in all eukaryotes and some bacteria under stress conditions. To carry out protein remodeling, bacterial Hsp90 collaborates with the Hsp70 molecular chaperone and its cochaperones. The mechanism of protein remodeling carried out by eukaryotic Hsp90 is more complex, involving more than 20 Hsp90 cochaperones in addition to Hsp70 and its cochaperones. In this review, we focus on recent progress toward understanding the basic mechanisms of bacterial Hsp90-mediated protein remodeling and the collaboration between Hsp90 and Hsp70. We describe the universally conserved structure and conformational dynamics of these chaperones and their interactions with each other and with client proteins. The physiological roles of Hsp90 in *Escherichia coli* and other bacteria are also discussed. We anticipate that the information gained from exploring the mechanism of the bacterial chaperone system will provide a framework for understanding the more complex eukaryotic Hsp90 system and its modulation by Hsp90 co-chaperones.

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1. INTRODUCTION

Proteome quality control, called proteostasis, depends on a delicate balance between the synthesis, folding, aggregation and degradation of cellular proteins. One important group of proteins involved in proteostasis is comprised of molecular chaperones, which

facilitate protein folding, unfolding and remodeling. These molecular machines modulate the kinetic partitioning of polypeptides between pathways leading to active proteins with native conformations, protein aggregates or degraded proteins [reviewed in (7; 25; 44; 56; 98; 109; 122) (Fig. 1).

Hsp90, Heat shock protein 90, is a highly conserved ATP-dependent molecular chaperone that functions in protein homeostasis by promoting protein folding, remodeling, activation and inactivation (13; 64; 126; 139) (Fig. 1). Additionally, Hsp90 holds client proteins, to protect them from aggregation and degradation (Fig. 1). Hsp90 is a highly abundant protein in both bacteria and eukaryotes under normal non-stress conditions and its level is further increased during stress conditions (9; 82). Hsp90 is essential in some bacteria under stress conditions (58; 141), but not in others, including *Escherichia coli* (9). In eukaryotes, Hsp90 is an essential protein. It is known to promote the remodeling of hundreds of client proteins and to participate in many cellular functions, such as protein trafficking, signal transduction, and receptor maturation (13; 116; 126). Moreover, some Hsp90 clients are oncogenic proteins and their stabilization by Hsp90 can lead to cancer progression, making Hsp90 a potential drug target (18; 97; 126).

To perform protein remodeling functions, bacterial Hsp90 requires the collaborative activity of another ATP-dependent molecular chaperone, DnaK, the bacterial homolog of Hsp70 (41; 44; 104). Hsp70 chaperones, like Hsp90s, are important in protein homeostasis and function in protein folding and remodeling (45; 91; 122). They act with two cochaperones, a J-domain protein and a nucleotide exchange factor. J-domain proteins deliver substrates to Hsp70 and stimulate Hsp70 ATPase

activity (23; 45; 66). Nucleotide exchange factors promote ADP release and ATP binding by Hsp70 (14; 45). Eukaryotic Hsp90 collaborates with Hsp70 and its cochaperones in some, but not all, protein remodeling activities (13; 100; 116; 126). In addition, protein remodeling of specific clients by eukaryotic Hsp90 is regulated by one or several of the more than 20 Hsp90 cochaperones (64; 126). In contrast, bacteria lack homologs of the eukaryotic Hsp90 cochaperones and bacterial Hsp90 cochaperones have yet to be identified (16).

2. DOMAIN ORGANIZATION, STRUCTURE AND CONFORMATIONAL DYNAMICS OF HSP90

2.1. Domain organization

Hsp90 is a highly conserved protein; for example, there is about 55% similarity between *E. coli* Hsp90 and human Hsp90 α . Bacteria generally possess only one Hsp90 isoform, whereas eukaryotes have several. In humans, Hsp90s are found in the cytosol (Hsp90 α and Hsp90 β), the endoplasmic reticulum (GRP94) and the mitochondria (TRAP1). In yeast, two cytosolic Hsp90 isoforms are found, the constitutively expressed Hsc82 and the stress induced Hsp82. (The nomenclature for Hsp90 isoforms is shown in Table 1.)

Hsp90 is a homodimer under most conditions (13), although yeast mitochondrial Hsp90, TRAP1, has been observed to tetramerize under some conditions (65). The Hsp90 protomer is composed of three domains, the N-terminal domain (NTD), the middle domain (MD) and the C-terminal domain (CTD) (13; 77; 114; 139). The NTD is responsible for ATP binding and hydrolysis. The ATP binding site is structurally related to the GHKL (Gyrase, Hsp90, Histidine Kinase, MutL) family of ATPases, which are

characterized by having a “split ATPase.” As such, Hsp90 residues essential for ATP hydrolysis are located in the MD, away from the ATP binding pocket in the NTD and conformational rearrangements are required for ATP hydrolysis (24; 95; 129). The MD is the principal domain involved in client binding (6; 43; 49; 70; 84; 103; 134; 146). Hsp70 also interacts with a region in the Hsp90 MD (30; 42; 74)}(12). Hsp90 dimerization occurs through CTD interactions.

Although the domain organization is conserved, differences exist among the Hsp90s. One difference is that bacterial Hsp90 and mitochondrial Hsp90, TRAP1, lack a flexible charged linker of about 50 residues that connects the NTD and MD in eukaryotic cytosolic Hsp90. It has been suggested that the flexible linker modulates Hsp90 conformational changes that are important for chaperone function and regulation by cochaperones (50; 143). The second major difference is that eukaryotic cytosolic Hsp90 homologs possess a C-terminal MEEVD motif that is absent in bacterial Hsp90, mitochondrial TRAP1 and endoplasmic reticulum (ER) Hsp90, GRP94 (13; 126). This region is responsible for binding to many Hsp90 co-chaperones that contain tetratricopeptides repeat domains (TPR) (64; 81; 126). The absence of this C-terminal extremity in bacterial Hsp90, as well as in TRAP1 and GRP94, is consistent with the observation that homologs of Hsp90 containing cochaperones have not been identified in bacteria, mitochondria and the ER (64; 81; 126) (28).

2.2. Structure of Hsp90 and conformational dynamics

The structure of Hsp90 has been extensively studied by a combination of approaches including X-ray crystallography, SAXS, negative-stain EM and FRET (13; 77; 81; 88; 94;

113; 114; 126; 145). Structures of truncated and full-length Hsp90 from various species with and without nucleotides, cochaperones, or clients have been obtained. Altogether they show that Hsp90 is a highly dynamic and flexible protein, and that it cycles through large conformational changes to perform chaperone activity.

The crystal structure of *E. coli* Hsp90 in the absence of nucleotide (apo form) was solved by Agard and coworkers. It shows the Hsp90 dimer adopting an open V-shape conformation with dimerization occurring through the CTD (129) (Figure 2A). However, in solution, apo Hsp90 presents a more extended conformation than observed in the crystal structure (76; 131; 132). Multiple V-shaped and extended conformations of eukaryotic Hsp90 have also been visualized in solution, highlighting the considerable flexibility of the apo form of Hsp90 (15; 75).

A very different structure is seen when Hsp90 binds nucleotide. When bound to the non-hydrolyzable ATP analog, AMP-PNP, and stabilized by the cochaperone p23/Sba1, Pearl and colleagues observed yeast Hsp82 in a closed conformation with a subdomain in the NTD, referred to as the lid, covering the bound nucleotide (4) (Fig. 2C). In this conformation the two NTDs rotate and contact one another. Additionally, elements of the NTD and the MD become closer than in the apo conformation, a prerequisite for ATP hydrolysis by the “split ATPase”. Recently, structural studies by Agard and coworkers of mitochondrial TRAP1 bound to AMP-PNP have revealed the existence of a slightly different closed form with a strained asymmetric conformation (79) (Fig. 2D). In this structure, one protomer is in a conformation similar to the one found in the closed structure of yeast Hsp82 (4), while the other protomer shows a helix swap at the interface between the MD and the CTD. Functional importance of this

conformational asymmetry has been suggested (32; 102). In contrast, structural studies of the ER Hsp90 paralog, GRP94, show that in the presence of AMP-PNP, GRP94 adopts a symmetric closed twisted dimer, with dimerization in the NTD being controlled by a pre-N domain extension that is not present in other Hsp90s (60) (Fig. 2E). Structures of ADP-bound Hsp90 reveal an even more compact conformation than that seen with AMPPNP (129; 131) (Fig. 2B).

The conformations observed by structural and biochemical studies describe an ATP cycle starting with an apo, open V-shaped, conformation of Hsp90 (Fig. 2). ATP binding causes the NTD to rotate and dimerize, with the lid closing over the nucleotide. At this point Hsp90 is in the closed conformation that is competent to hydrolyze ATP. Following ATP hydrolysis, ADP and phosphate are released, and the dimer again adopts the open apo conformation as the cycle is completed. However, differences exist among the different Hsp90 isoforms. The bacterial Hsp90 ATP hydrolytic cycle is driven in a deterministic fashion by a mechanical ratchet mechanism (46; 117). In contrast, eukaryotic Hsp90 seems to be more flexible than bacterial Hsp90, functioning in a non-deterministic manner by populating all the conformations independent of the nucleotide (96).

In bacteria, the cycle is further modulated by the Hsp70 chaperone and its cochaperones and by clients (42; 44; 133; 134) (Fig. 2F). In eukaryotes, Hsp90 cochaperones and post-translational modifications, as well as Hsp70, Hsp70 cochaperones and clients, play roles in regulation (5; 13; 64; 126).

2.3. Client binding by Hsp90

Hsp90 binds to a large number of clients with highly diverse structural characteristics or folding statuses spanning from intrinsically disordered to partially or natively folded (94; 116; 120; 126; 139). In addition, no consensus motif has been found in the clients that bind Hsp90. In bacteria very few natural Hsp90 clients have been identified (see section 5), while in eukaryotes, many Hsp90 clients have been reported, including kinases, steroid hormone receptors, transcription factors and many other proteins. For an up-to-date list of Hsp90 clients, visit the page maintained by Didier Picard, <https://www.picard.ch/downloads/Hsp90interactors.pdf>.

A region on *E. coli* Hsp90 comprising residues in the MD and CTD important for client binding has been identified by Genest et al. by a genetic screen (43). Mutations in this region strongly affect client binding, therefore reducing the chaperone function. The same region was identified by Street et al. using a structural approach (133-135). The residues in the MD and CTD important for client binding define a large binding surface in a cleft between the two protomers of the dimer. This binding surface is rearranged in the different conformations of Hsp90, suggesting a possible mechanism to regulate client binding and release.

Eukaryotic yeast Hsp82 has also been shown to interact with clients through residues in the MD and CTD (43; 105; 134). More recently, structural studies of Hsp90 in complex with the ligand binding domain of the glucocorticoid receptor (GR-LBD) (70; 84) or with the Cdk4 kinase and Cdc37 co-chaperone (146), confirm the MD as the main interaction site in Hsp90 with additional contacts in other domains. However, for some clients, the binding region is different. For example, the Tau protein principally interacts with an extended region in the NTD of Hsp90 (67).

3. THE DnaK CHAPERONE: AN HSP90 PARTNER

3.1. Structure of DnaK

Hsp90 collaborates with Hsp70, another ATP-dependent molecular chaperone, in a variety of protein remodeling activities. However, in the absence of Hsp90, Hsp70 functions independently in a wide array of cellular processes that involve protein folding and remodeling (7; 122). In addition to promoting *de novo* protein folding and activation, Hsp70s participate in the prevention of aggregation and the disaggregation and refolding of misfolded and aggregated proteins. They also act with proteases to rid cells of irreversibly misfolded proteins (21; 29; 35; 91; 98; 107). There is high conservation between human Hsp70 and bacterial Hsp70, referred to as DnaK.

DnaK, like all Hsp70s, is comprised of an N-terminal nucleotide-binding domain (NBD) connected by a short flexible linker to the substrate-binding domain (SBD) (17; 21; 44; 90-92; 122; 160) (Fig. 3A). The NBD is divided into four subdomains, which form two lobes with a deep cleft between them where nucleotide binds. The SBD is made up of two subdomains: SBD β , which contains the substrate binding pocket and is composed of β -strands, and SBD α , which is a helical lid domain. In addition, there is an unstructured C-terminal tail. Hsp70 undergoes large conformational transitions that are dependent on the nucleotide-bound state. In the ADP-bound state, the NBD and SBD are spatially separated, connected only by the linker. Hsp70 is considered to be “closed” in the ADP conformation, with the helical lid subdomain of the SBD covering the substrate binding pocket in the SBD β (Fig. 3A) (11; 21; 89-91; 158; 159). Although the SBD α closes when a peptide is bound to the substrate-binding site, it remains partially

open when the substrate is a large polypeptide, suggesting the importance of lid mobility in accommodating diverse substrates (87; 124; 144). In the ATP-bound state, the lid is open and SBD β and SBD α bind to different faces on the NBD, leaving the substrate-binding site accessible for rapid, low affinity substrate interactions (Fig. 3A) (72; 115). In addition, the SBD α rotates to interact with the NBD, which causes the interdomain linker to dock into an exposed groove in the NBD and induce conformational changes in the NBD (Fig. 3A) (33; 72; 115). Altogether, the structures of the ADP-bound closed conformation and ATP-bound open conformation of Hsp70 DnaK reveal large structural rearrangements dependent on nucleotide binding state.

3.2. Conformational dynamics of DnaK and the role of DnaK cochaperones

Hsp70 chaperone activity is coupled to large conformational transitions between the ATP- and ADP- bound state of Hsp70. These conformational changes are regulated by two Hsp70 cochaperones, a J-domain protein (JDP) and a nucleotide exchange factor (NEF) (45; 53; 66; 72; 78; 85; 90; 92). JDPs recognize and bind substrates thus targeting them for Hsp70 action. They also stimulate ATP hydrolysis by Hsp70 and promote high affinity substrate binding (23; 45; 66; 71). In *E. coli*, DnaJ is the principal JDP, although there are several others, including CbpA (45; 66). Eukaryotes generally have a large number of JDPs and it has been suggested that JDPs are responsible for imparting substrate specificity to Hsp70s (10; 23; 45; 66). NEFs interact with the NBD of DnaK and accelerate nucleotide exchange by DnaK by promoting ADP release and ATP binding (14; 17; 45; 53). GrpE is the sole *E. coli* NEF. Eukaryotes generally possess multiple NEFs, including Hsp110, Bag domain proteins and HspBP1 (14; 111;

127; 130; 140). While eukaryotic NEFs are functionally similar to GrpE, they do not share structural homology.

The mechanism for protein remodeling and reactivation by *E. coli* DnaK is the summation of work from many groups, particularly those of Bukau, Mayer and Hartl, and is shown in Fig. 3B (7; 90; 91; 122). First, a substrate containing non-native regions interacts with a JDP and is delivered to DnaK (Fig. 3B, step 1) (92; 119; 144; 160; 161). The JDP stimulates ATP hydrolysis by DnaK, which promotes a conformational transition to the closed ADP-bound state of DnaK with bound substrate (Fig. 3B, step 2) (1; 23; 45; 66; 71; 78). GrpE accelerates nucleotide exchange by DnaK (Fig. 3B, step 3), which in turn facilitates substrate release, returning DnaK to the open ATP-bound state (14; 17; 45; 53). The released non-native substrate refolds spontaneously or is rebound by Hsp70 or another molecular chaperone for additional cycles of binding and release (Fig. 3B, step 4).

4. REMODELING ACTIVITY OF HSP90: COLLABORATION BETWEEN HSP90 AND THE DNAK CHAPERONE

4.1. Physical and functional interaction between Hsp90 and the DnaK chaperone

Hsp90s are required to activate clients and reactivate inactive proteins, however, ATP-dependent chaperone activity by Hsp90 alone has not been demonstrated in vitro with purified proteins using either bacterial or eukaryotic Hsp90 isoforms. In vitro, Hsp90 alone possesses holdase activity, the ability to bind and stabilize client proteins and prevent their aggregation independent of ATP, as first shown by Jakob and Buchner (Fig. 5A). The holdase activity is a conserved activity of Hsp90 proteins that has been

reported in bacteria and eukaryotes (6; 27; 58; 62; 104; 123; 128; 152; 154; 156). However, it remains to be established if holdase activity is a physiologic activity of Hsp90.

To promote remodeling activity in vitro, bacterial Hsp90 requires DnaK and its cochaperones, and eukaryotic Hsp90 requires Hsp90 cochaperones and in addition some reactions additionally require Hsp70 and its cochaperones. For example, *E. coli* Hsp90 acts with DnaK, DnaJ/CbpA and GrpE to reactivate inactivated model clients (41; 99). Biochemical experiments have shown that DnaK, with its cochaperones, acts first, likely by binding and stabilizing non-native regions of the client. Then in a second step, Hsp90 and DnaK, with its cochaperones, act together to complete client remodeling and reactivation (41; 42). Recent in vitro experiments by Moran Luengo et al. have shown that when the concentration of DnaK is high, DnaK blocks client refolding by shifting the client on-off equilibrium toward the on state (99). Under these conditions, Hsp90 relieves the block to promote productive client folding. This Hsp90 function appears to be conserved across species since similar in vitro results were obtained when human chaperones and co-chaperones were used. These in vitro reactivation experiments require ATP hydrolysis by both DnaK and Hsp90, indicating that protein remodeling is occurring during both phases of the reaction (41; 42; 99). In a similar fashion, eukaryotic Hsp70, in combination with a JDP, acts prior to Hsp90 in protein reactivation reactions in vitro (70; 99; 101; 142; 151). In contrast to protein reactivation by bacterial Hsp90 and DnaK, in vitro protein reactivation by eukaryotic Hsp90 and Hsp70 is stimulated by Hop (Hsp90-Hsp70 organizing protein), an Hsp90 cochaperone, in addition to a JDP (70; 99; 101; 142; 151). However, a recent study by Picard and colleagues indicates that human

Hsp70 and Hsp90 function more efficiently in the absence of Hop in vivo and in vitro, suggesting their mechanism of action is similar to that of bacterial Hsp90 and Hsp70 (12).

In studies to explore the mechanism of collaboration between bacterial Hsp90 and Hsp70, a direct interaction between the two chaperones was discovered (41; 42; 73; 104). The interaction has been observed both in vivo using a bacterial two hybrid assay and in vitro using purified proteins (41; 42; 73). Although the interaction is weak, it is stabilized by both client protein and JDP (42). Hsp90 residues important for the interaction with DnaK were identified using mutagenesis, bacterial two hybrid and reactivation assays. The residues are located in a region of the MD of Hsp90 (42) (Fig. 4A). Further work showed that, in vivo and in vitro, Hsp90 interacts with the DnaK NBD (Fig. 4B) and, more specifically, with a region of DnaK that overlaps with the JDP binding region (73), which had been previously defined (3; 40; 71; 93; 136). Crosslinking experiments demonstrated that the residues shown to be important for the Hsp90-DnaK interaction were indeed the sites of the direct interaction (30), and together with a molecular docking study suggested a model for the interaction (Fig. 4C). The significance of the overlapping sites on DnaK for Hsp90 and DnaJ is currently unknown. Additionally, many of the residues in the shared Hsp90- and JDP-binding site of DnaK are buried when DnaK is in the ATP-bound conformation, indicating that the ADP-bound form of DnaK interacts with Hsp90 (72; 92; 115).

Eukaryotic Hsp90s and Hsp70s also directly interact, albeit weakly. For example, the yeast Hsp90 and Hsp70 homologs, Hsp82 and Ssa1, have been shown to interact both in vivo and in vitro (19; 36; 37; 74; 101; 118). Moreover, mutational analysis of

Hsp82 suggested and crosslinking studies confirmed that the site of interaction involves the region of Hsp82 homologous to that of bacterial Hsp90 (30; 74). The demonstration that yeast Hsp90 and Hsp70 directly interact was unexpected because yeast possess a Hop homolog, Sti1, that binds to both Hsp90 and Hsp70 and provides a bridge connecting the two chaperones (55; 63; 125). However, while yeast Hsp90 and Hsp70 interact directly, Sti1 stabilizes the weak interaction both *in vitro* (74) and *in vivo* (19; 37). It has recently been demonstrated that human Hsp90 and Hsp70 proteins also interact directly in the absence of the Hop co-chaperone, and as with bacterial chaperones, binding involves the region on Hsp90 homologous to the region on *E. coli* Hsp90 important for DnaK binding (12). Similarly, mouse ER GRP94 and human mitochondrial TRAP1 directly bind their respective Hsp70 chaperones, BiP and mortalin (137; 138).

4.2. Working model for the synergistic action of Hsp90 and Hsp70 in protein remodeling

A working model for the pathway of protein refolding by bacterial Hsp90 and DnaK has evolved from structural, biochemical and biophysical studies from many groups and is consistent with the current knowledge, but still speculative (44; 100) (Fig. 5B). First, a client is bound by a JDP (referring to either DnaJ or CbpA in the model for the mechanism in *E. coli*) (Fig. 5B, Step 1) and transferred to DnaK (Fig. 5B, Step 2). As the client undergoes repeated cycles of binding to and release from DnaK partial or complete refolding of some clients occurs (Fig. 5B, Step 3). The cycle is driven by conformational changes of DnaK resulting from ATP binding, hydrolysis and nucleotide exchange and is promoted by the JDP and GrpE. Some clients that do not reach their

native state by the action of DnaK and its cochaperones are targeted to Hsp90 through a transient interaction between DnaK and Hsp90 (Fig. 5B, red arrow). The JDP is likely involved in facilitating and stabilizing the interaction between Hsp90, DnaK and client (Fig. 5B, Step 4). With ATP hydrolysis, Hsp90 and DnaK interact with each other and the client (Fig. 5B, Step 5). Binding of ATP by DnaK leads to the transfer of client to Hsp90 and release of DnaK (Fig. 5B, Step 6). The client may undergo repeated cycles of binding and release from Hsp90 and eventually reach its native active conformation (Fig. 5B, Step 7).

Models for the mechanism of action of Hsp90 and Hsp70 in eukaryotes have recently been reviewed (13; 68; 94; 100; 116; 120; 126; 145). While it is likely that the basic mechanism of the collaboration between Hsp70 and Hsp90 is conserved, the eukaryotic systems require the participation of many Hsp90 cochaperones and thus are significantly more complex than the bacterial system.

5. PHYSIOLOGIC CLIENTS OF HSP90 AND THE FUNCTION OF HSP90 IN CELLULAR PROCESSES

In bacteria, Hsp90 is a very abundant protein under non-stress conditions and its level significantly increases under heat and other stress conditions (8; 22; 54; 106). However, *htpG* the gene coding for Hsp90 in *E. coli*, is not essential for the growth of *E. coli* and many other bacteria. Nevertheless, *E. coli* deleted for *htpG* grow slightly slower and have a modest increase in aggregated protein at elevated temperatures compared to wild-type strains (8). In addition, Hsp90 is essential for swarming in *E. coli* (61). Another

study indicates that biofilm formation is slightly reduced in *E. coli* in the absence of Hsp90 at elevated temperature (48).

Similarly, cells lacking Hsp90 exhibit modest growth phenotypes at elevated temperatures in *Actinobacillus actinomycetemcomitans* and *Bacillus subtilis* (148; 155). In contrast, in *Shewanella oneidensis* and some cyanobacteria, Hsp90 is essential under heat stress (34; 58; 141). Hsp90 also participates in cold tolerance in *S. oneidensis* and *Vibrio vulnificus* (20; 38). In *Mycobacterium tuberculosis*, Hsp90 is essential in stress conditions, but only in strains with a deletion of ClpB, a Clp/Hsp100 chaperone (51), revealing an important role of Hsp90 in this bacterium and suggesting an overlap in functions of Hsp90 and ClpB.

5.1. Role of Hsp90 in assembly of complex cellular structures

A role for Hsp90 in the assembly and modulation of large cellular complexes is emerging. For example, a recent study indicates that Hsp90 is one of the many regulators of the *E. coli* cell division apparatus. It modulates cell division through interactions with FtsZ, the bacterial homolog of eukaryotic tubulin (6). During cell division, FtsZ assembles at mid-cell forming the Z-ring, and constriction of this ring leads to the separation of the two daughter cells. When Hsp90 is overproduced, Z-ring formation and cell division are inhibited, leading to cell filamentation. In cells lacking Hsp90, shorter cells are observed compared to wild-type. These observations, together with the finding that Hsp90 directly interacts with FtsZ to prevent its assembly in vitro, suggest that Hsp90 holds FtsZ, thereby regulating the amount of free FtsZ available to

assemble into Z-rings. Interestingly, the ATPase activity of Hsp90 is not required for these functions, suggesting that the holdase activity of Hsp90 is sufficient (6).

Nakamoto and coworkers found that Hsp90 participates in the process of photosynthesis in cyanobacteria. They observed that in the absence of Hsp90 *Synechococcus elongatus* cell coloration changed from blue-green to yellow-green, suggesting effects on phycobilisomes (141), highly-ordered large protein complexes that are essential for photosynthesis and work as light-harvesting antenna. It was shown that the level of the rod linker (L_R^{30}) protein, important for the assembly of the phycobilisome (2; 83), was greatly reduced in the absence of Hsp90 (123). It was found that Hsp90 directly interacts with L_R^{30} and suppresses its thermal aggregation in vitro (123). These observations suggest that Hsp90 participates in complex formation by stabilizing a protein required for phycobilisome assembly.

In another study, a large-scale phylogenetic analysis looking at genes that coevolved with Hsp90 in bacteria suggested that Hsp90 is involved in motility and chemotaxis (112). These results were confirmed by showing that *E. coli* Hsp90 interacts with the flagellar protein FliN and the chemotaxis kinase CheA, both proteins associated with large protein complexes. These results suggested that one role of Hsp90 could be promoting complex assembly.

5.2. Role of Hsp90 in virulence

Hsp90 is involved in the virulence of some pathogenic bacteria (26; 39; 47; 69; 80; 147; 149; 153). For example, Hsp90 is required for maximal virulence of extraintestinal pathogenic *E. coli* (ExPEC). It has been shown that in ExPEC strains, the synthesis of

two virulence factors, the genotoxin colibactin and the siderophore yersiniabactin, was abolished in the absence of Hsp90 (39). Hsp90 is also involved in the biosynthesis of the antibiotic albicidin in *Xanthomonas albilineans* (149) and the biosurfactant arthrofactin in *Pseudomonas sp. MIS38* (150). Colibactin, yersiniabactin, albicidin and arthrofactin belong to the family of hybrid polyketide/nonribosomal peptides and are produced by complex biosynthesis pathways (59; 108; 110; 121), suggesting that these pathways share a common protein remodeling mechanism that depends on Hsp90.

There are other observations implicating a role of Hsp90 in virulence of many bacteria, including *Salmonella enterica Typhimurium*, *Edwardsiella tarda*, *Leptospira interrogans*, *Francisella tularensis subsp. novicida* and *Pseudomonas aeruginosa* (26; 47; 69; 80; 86; 147; 153). Altogether, these studies suggest a prominent role of Hsp90 in bacterial virulence.

5.3. Hsp90 and proteolysis

There is interplay between proteolysis and Hsp90 chaperone activity in bacteria as well as eukaryotes (6; 31; 39; 57; 157). For example, in *S. oneidensis*, Hsp90 has been shown to remodel and stabilize TilS, an essential tRNA modifying enzyme required for translation at elevated temperatures (57; 58). In the absence of Hsp90, TilS activity is reduced, and translation of proteins whose mRNA sequence contains AUA codons is therefore compromised. Interestingly, HsIVU protease has been shown to degrade TilS in the absence of Hsp90 (57; 58). Since growth of *S. oneidensis* under heat stress relies on TilS activity, no growth is observed in the absence of Hsp90 (TilS

degradation), however growth is restored in a strain lacking both Hsp90 and the HslVU protease (TilS stabilization).

As explained above (Section 5.3), the absence of Hsp90 abolishes the production of colibactin in ExPEC strains (39). Moreover, in cells lacking both HslVU protease Hsp90, production of the toxin is restored by expression of Hsp90. These results suggest that an unknown client of Hsp90 responsible for colibactin production is degraded in the absence of Hsp90, but stabilized when the protease is absent, such that there is a sufficient amount of the client needed for colibactin production.

In another example, *E. coli* FtsZ, an Hsp90 client (Section 5.2), has a longer half-life in vivo in wild-type cells than in cells lacking Hsp90. In addition, in vitro FtsZ is degraded by ClpXP protease and Hsp90 protects it from degradation, although the physiological protease responsible for FtsZ degradation has not been determined (6).

E. coli Hsp90 has also been shown to be required for the CRISPR-Cas system, an adaptive immunity system that provides resistance to foreign genetic elements, such as those present in plasmids and phages (157). Moreover, production of the Cas3 protein in a strain that does not produce Hsp90 restores CRISPR-Cas activity, suggesting that Cas3 is a client of *E. coli* Hsp90. Further experiments showed that Hsp90 protects the Cas3 client from degradation (157).

Therefore, we can hypothesize that the antagonist action of the Hsp90 chaperone and the protease on the client could serve as a post-translational regulatory mechanism to fine-tune the level of a client protein in some growth conditions. In another model, Hsp90 could target misfolded proteins to HslVU, ClpXP and other proteases for degradation.

6. CONCLUDING REMARKS

As the mechanism of client remodeling and activation by the ATP-dependent Hsp90 and Hsp70 chaperones becomes clearer, the highly elaborate nature of the collaboration between these two molecular machines also becomes more evident. Compared to the bacterial proteins, an additional layer of complexity is found in eukaryotes with the numerous Hsp90 cochaperones and posttranslational modifications that modulate Hsp90 chaperone activity. However, the bacterial Hsp90 and Hsp70 chaperones provide an exquisite simpler model to decipher the mechanism of action of the Hsp90-Hsp70 collaboration. Interestingly, since Hsp90 and Hsp70 are highly conserved from bacteria to eukaryotes, advances made with bacterial proteins can directly be translated to the eukaryotic proteins, as exemplified by the identification of the client and Hsp70 binding sites in *E. coli*. Future progress is needed to provide a more precise understanding of how clients are remodeled by the chaperone teamwork and to define the contribution of each chaperone in this process. To gain a global view of the involvement of Hsp90 in bacterial physiology, it becomes crucial now to comprehensively identify its clients in bacteria as it has been done in eukaryotes. Importantly, given the unexpected importance of Hsp90 in bacterial virulence, Hsp90 inhibitors specifically targeting the bacterial Hsp90 isoform would be a valuable tool to limit bacterial virulence and provide new means to combat multidrug-resistant bacteria. The tremendous wealth of knowledge on Hsp90 inhibitors from studies of eukaryotic Hsp90 provides a strong foundation for developing novel treatments targeting Hsp90 in bacterial infections.

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Table 1. Main Hsp90 isoforms found in bacteria, yeast and humans.

Species	Gene name	Protein name	Compartment	Amino acids number	UniProt ID
<i>Escherichia coli</i>	<i>htpG</i>	Hsp90 _{Ec} (HtpG) (C62.5)	Cytosol	624	P0A6Z3
<i>Saccharomyces cerevisiae</i>	<i>HSP82</i>	Hsp82	Cytosol	709	P02829
	<i>HSC82</i>	Hsp82	Cytosol	705	P15108
Human	<i>HSP90AA1</i>	Hsp90 α	Cytosol	732	P07900
	<i>HSP90AB1</i>	Hsp90 β	Cytosol	724	P08238
	<i>HSP90B1</i>	GRP94	Endoplasmic reticulum	803	P14625
	<i>TRAP1</i>	TRAP1	Mitochondria	704	Q12931

FIGURE LEGENDS

Figure 1. The interplay of chaperones and proteases in proteostasis. Some polypeptides emerging from the ribosome are cotranslationally folded by chaperones, while others reach their native conformations spontaneously. However, many non-native and unfolded protein require the assistance of chaperones, including Hsp60s, Hsp70s and Hsp90s, to promote folding (step 1). Chaperones are responsible for binding to and preventing the aggregation of non-native polypeptides, including Hsp90 and Hsp70s (Step 2). Remarkably, chaperones are known to disaggregate aggregated proteins and restore their activity, including Clp/Hsp100s in collaboration with Hsp70s (Step 3). Chaperones, including the Clp/Hsp100 chaperone components of proteases, also facilitate degradation of irreversibly damaged and misfolded proteins (Step 4). Chaperones also prevent degradation by binding to non-native proteins (Step 4).

Figure 2. Hsp90 chaperone. A. Model of the crystal structure of the *E. coli* Hsp90 dimer in the apo form (PDB: 2IOQ) (129) with the C-terminal domains aligned to the crystal structure of the isolated C-terminal domain (PDB: 1SF8) (52). B. Dimer structure of *E. coli* Hsp90 in the ADP-bound conformation (PDB: 2IOP) (129). C. Dimer structure of *S. cerevisiae* Hsp82 in the AMP-PNP-bound conformation (PDB: 2CG9) showing the location of the unstructured linker region and contacts between the NTDs (4). D. Dimer structure of *Danio rerio* (zebrafish) mitochondrial Hsp90, TRAP1, in the AMP-PNP-bound conformation (PDB: 4IPE), showing the location of the helix swap region (79). E. Dimer structure of *Canis lupus familiaris* endoplasmic reticulum Hsp90, GRP94, in the

AMP-PNP-bound conformation (PDB: 5ULS), showing the pre-N domain in green (60).

F. Model of the cycle of Hsp90 conformational dynamics regulated by ATP, cochaperones and clients. All images were prepared using PyMOL, and the domains are colored as in A. B-E, bound nucleotide is shown in black as CPK models.

Figure 3. DnaK chaperone. A. The structure of *E. coli* DnaK is shown on the left in the closed ADP-bound conformation (PDB: 2KHO) (11) and on the right in the open ATP-bound conformation (PDB: 4B9Q) (72). The DnaK NBD (red) is connected by a flexible linker to the SBD (blue) that is made up of two subdomains, an α -helical subdomain, the “lid”, and a β -sheet subdomain that contains the substrate-binding site. ATP is shown in black as a CPK model. B. Cartoon representation of substrate interaction and remodeling by DnaK and its cochaperones. The JDP (DnaJ or CbpA) interacts with a client (Step 1). Then, the JDP with bound client interacts with DnaK (Step 2). ATP is hydrolyzed and GrpE accelerates nucleotide exchange by DnaK (Step 3). The client is released from DnaK and either refolds spontaneously (Step 4) or undergoes repeated cycles of ATP-dependent binding and release.

Figure 4. Region of interaction between *E. coli* Hsp90 and DnaK. A. surface-rendered model of *E. coli* Hsp90 dimer in the apo conformation, constructed from the biological assembly 1 of PDB code 2IOP (129) using CHARMM to build in missing atoms (73) and showing one protomer in gray and the other in light blue. The site of DnaK interaction on the MD is shown in red (42) and the site of client-binding in the MD and CTD is shown in green (43). B. Cartoon model of DnaK in the ADP-bound conformation (PDB code

2KHO) (11) is shown in orange. The site of Hsp90 interaction on the NBD is shown in dark blue as CPK models (73). C. Docked surface-rendered model of the apo structure of Hsp90 (shown and colored as in A) and a cartoon model of ADP-bound form of DnaK (shown and colored as in B) (73). All images were prepared using PyMOL.

Figure 5. A. Working model for the holding activity of Hsp90. First, in an ATP independent reaction, the aggregation prone client or folding intermediate is recognized by Hsp90 and bound stoichiometrically (Step 1). The client undergoes on and off cycles of ATP independent binding and release by Hsp90. Next, for some clients, release occurs transiently, without the need for nucleotide, but for other clients, ATP binding, hydrolysis, and/or release may be necessary (Step 2). After multiple cycles, the client is released and refolds in its native conformation (Step 3). B. Working model for protein remodeling by *E. coli* Hsp90 in collaboration with DnaK. First, the JDP (DnaJ or CbpA) interacts with a client (Step 1). Then, the JDP with bound client interacts with DnaK (Step 2). The client undergoes repeated cycles of binding to and release from DnaK with partial or complete refolding of some clients occurring (Step 3). Some clients that do not reach their native state by the action of DnaK and its cochaperones, are targeted to Hsp90 through a transient interaction between DnaK and Hsp90 (red arrow). The JDP is involved in facilitating and stabilizing the interaction between Hsp90, DnaK and the client (Step 4). With ATP hydrolysis, Hsp90 and DnaK interact with each other and the client (Step 5). The client is transferred to Hsp90 and may undergo repeated cycles of binding and release from Hsp90 (Step 6). Lastly, the partially folded client is eventually released from Hsp90 and reaches its native active conformation (Step 7).

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Figure 1

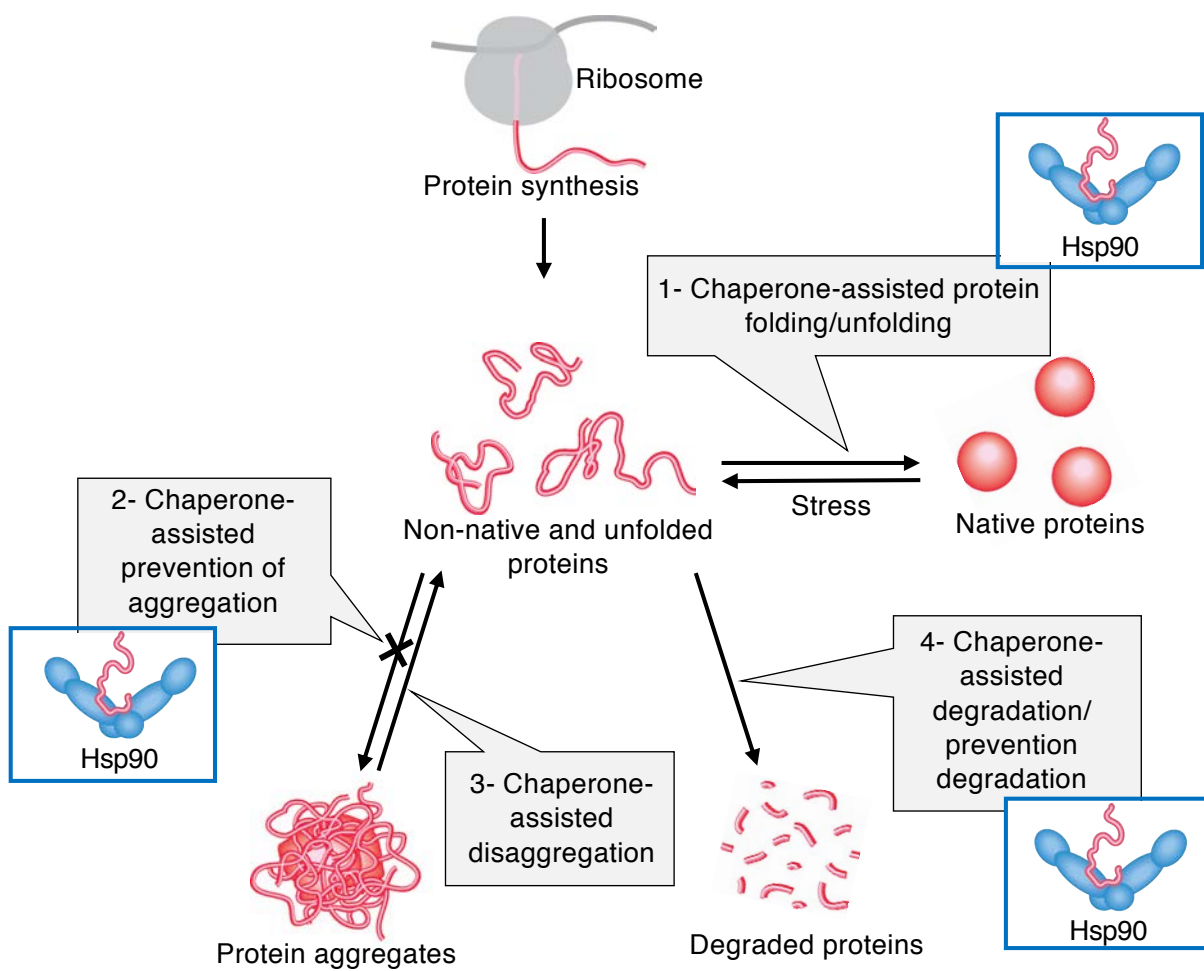


Figure 2

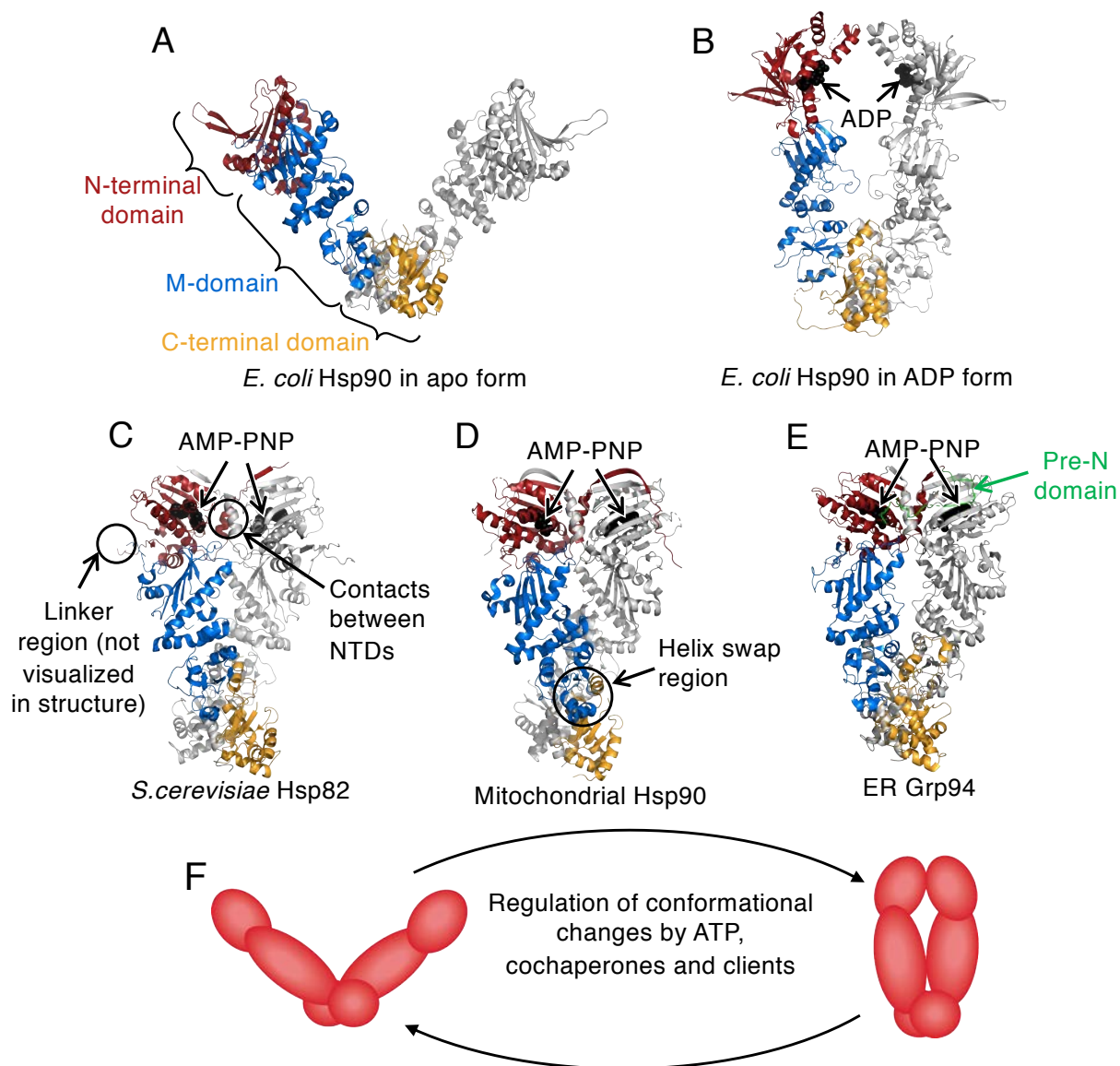


Figure 3

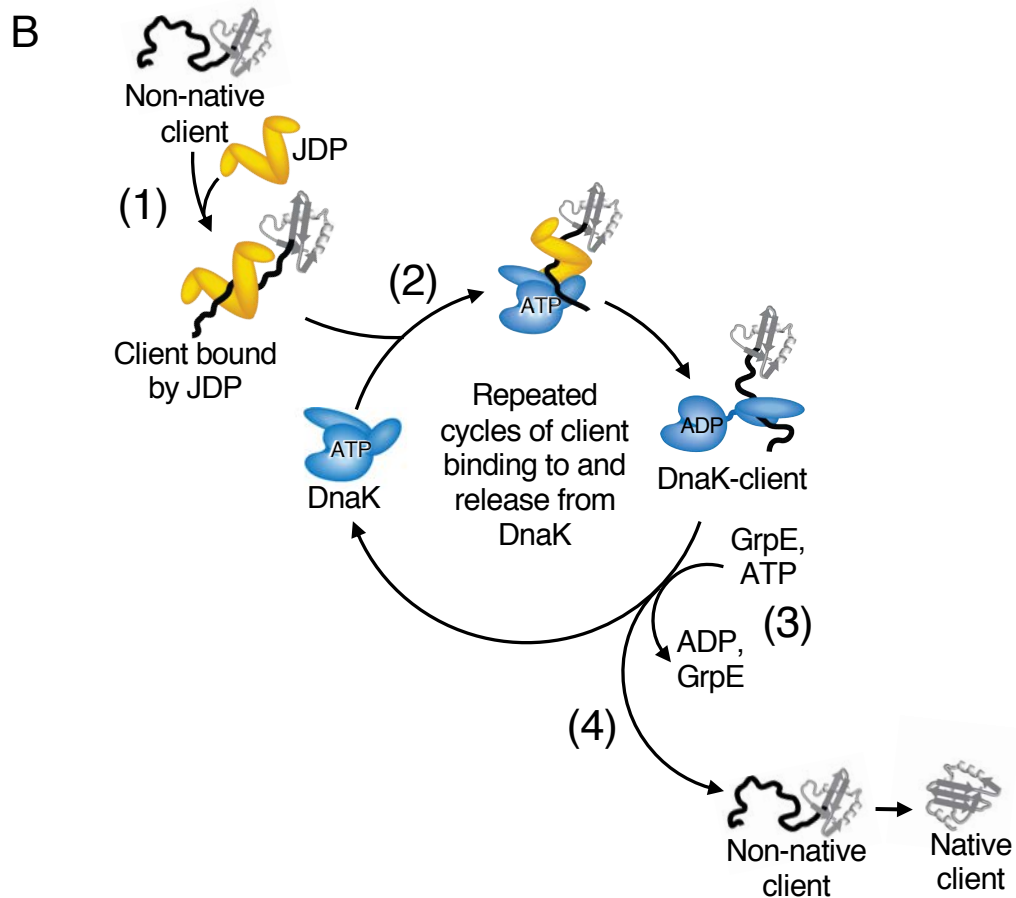
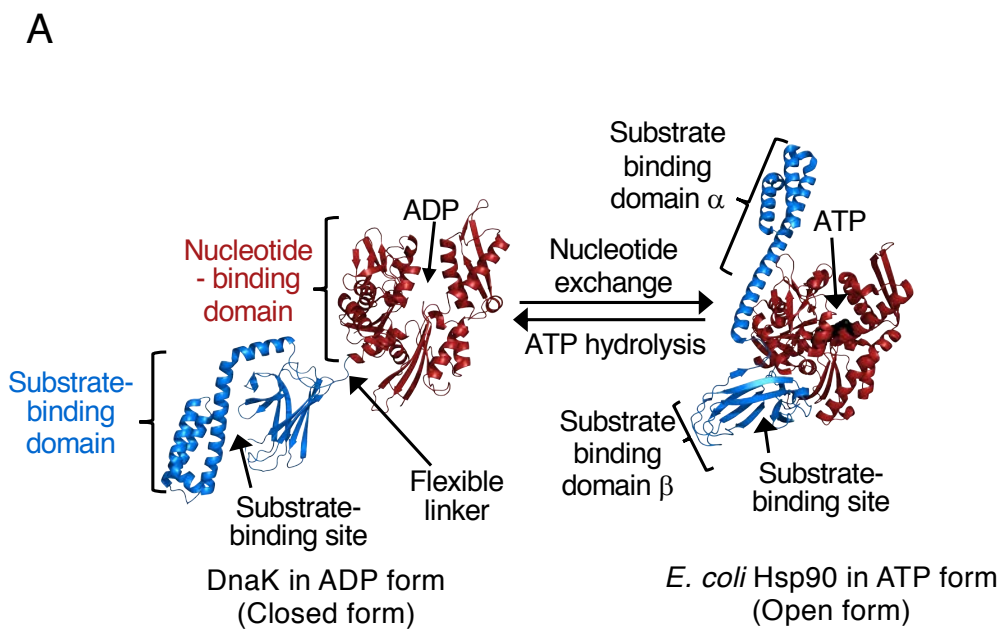


Figure 4

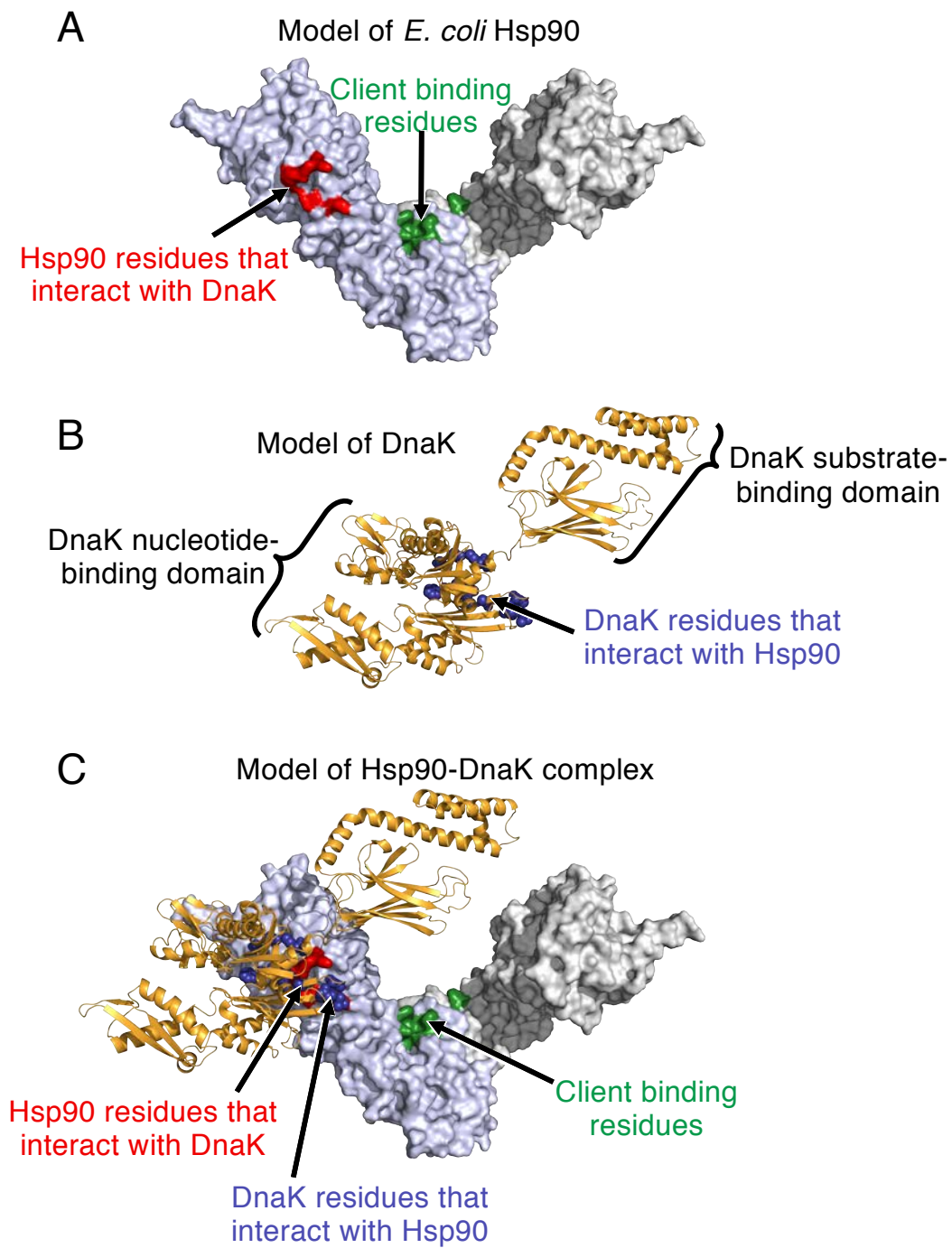


Figure 5

