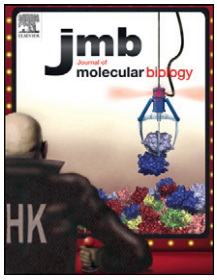

Hsp70 and Hsp90 of *E. coli* Directly Interact for Collaboration in Protein Remodeling

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Abstract

Hsp90 is a highly conserved molecular chaperone that remodels hundreds of client proteins, many involved in the progression of cancer and other diseases. It functions with the Hsp70 chaperone and numerous cochaperones. The bacterial Hsp90 functions with an Hsp70 chaperone, DnaK, but is independent of Hsp90 cochaperones. We explored the collaboration between *Escherichia coli* Hsp90 and DnaK and found that the two chaperones form a complex that is stabilized by client protein binding. A J-domain protein, CbpA, facilitates assembly of the $Hsp90_{Ec}$–DnaK–client complex. We identified *E. coli* Hsp90 mutants defective in DnaK interaction *in vivo* and show that the purified mutant proteins are defective in physical and functional interaction with DnaK. Understanding how Hsp90 and Hsp70 collaborate in protein remodeling will provide the groundwork for the development of new therapeutic strategies targeting multiple chaperones and cochaperones.

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Introduction

Proteins belonging to the Hsp90 family are present in nearly all organisms and comprise a highly conserved class of ATP-dependent molecular chaperones [1–4]. In eukaryotes, Hsp90 is essential for cell viability. It is required for remodeling and activation of hundreds of client proteins involved in many crucial cell processes, such as cell signaling and response to stress. Protein remodeling by Hsp90 requires the assistance of the Hsp70 chaperone and numerous Hsp90 cochaperones.

Hsp90 is a homodimer with each protomer containing an N-terminal ATP binding domain [nucleotide-binding domain (NBD)], a middle domain (M-domain) that participates in client binding [5–7] and a C-terminal domain (C-domain) that is involved in dimerization and client binding [4,5]. Eukaryotic Hsp90 also contains a linker region of about 50 amino acids between the NBD and the M-domain and a C-terminal extension of 35 amino acids that interacts with several cochaperones. Hsp90 undergoes multiple conformational changes in response to ATP binding and hydrolysis [8–14]. In the absence of ATP, the Hsp90 dimer adopts an open V-shaped structure with the protomers interacting via the C-terminal dimerization domain [13]. When ATP is bound, the protein adopts a closed conformation with the two N-domains of the dimer interacting and a portion of the N-domain, the "lid", closing over the nucleotide in each protomer [15]. Additional conformational changes occur upon ATP hydrolysis [8–12], and after ADP release, Hsp90 reverts back to the open conformation [2,11]. Additionally, client protein binding and cochaperone interactions cause changes in the conformation of Hsp90 and affect the residence time in the various conformations [1,14,16,17].

The Hsp70 chaperone and more than 20 cochaperones, including Hop/Sti1, Aha1/Hch1, p23/Sba1, Cdc37 and Sgt1, collaborate with Hsp90 to remodel and activate the diverse group of client proteins in the eukaryotic cytosol [1]. The cochaperones regulate the Hsp90 ATPase activity and recruit specific client proteins. Some cochaperones direct the chaperone cycle by stabilizing specific

conformations such as the open or the closed state of Hsp90. For example, Hop/Sti1 interacts simultaneously with Hsp70 and Hsp90 through its multiple tetratricopeptide repeat domains and facilitates substrate transfer from Hsp70 to Hsp90 by stabilizing the open conformation of Hsp90 [18,19].

The bacterial homolog of Hsp90 in *Escherichia coli*, the product of *htpG* and referred to as $Hsp90_{Ec}$, is a very abundant protein under nonstress conditions and is further induced under stress conditions [1]. It shares about 50% sequence similarity with human Hsp90. $Hsp90_{Ec}$ is not an essential protein under laboratory conditions [20]. However, when cells carry mutations in $Hsp90_{Ec}$ they grow more slowly at high temperature [20], exhibit a slight increase in aggregated proteins at high temperature [21], lose adaptive immunity conferred by the CRISPR system [22] and show a subtle defect in motility [23]. Additionally, when $Hsp90_{Ec}$ is overexpressed in *E. coli*, cells filament and become sensitive to SDS [5].

Both eukaryotic Hsp90 and $Hsp90_{Ec}$ have been shown to remodel proteins *in vitro*. For example, eukaryotic Hsp90 reactivates denatured luciferase in conjunction with Hop/Sti1, Hsp70 and Hsp40 [19,24,25]. Similarly, $Hsp90_{Ec}$ has the ability to reactivate heat-denatured luciferase *in vitro* [26]. This reaction requires ATP hydrolysis by $Hsp90_{Ec}$ and also requires DnaK, the *E. coli* homolog of Hsp70, and the DnaK cochaperone, DnaJ (or a DnaJ homolog, CbpA) [26]. GrpE, the prokaryotic nucleotide exchange factor, stimulates the rate of reactivation, although it is not essential [26]. $Hsp90_{Ec}$ and DnaK physically interact to mediate protein reactivation independent of a Hop/Sti1-like cochaperone [26]. *E. coli* Hsp90 is not unique [3,4,27]; recently, it has been reported that Hsp90 and Hsp70 contact one another directly in complexes containing Hop and client protein [28–30]. Moreover, Hsp90 from *Synechococcus elongatus*, *Neurospora crassa* and *Plasmodium falciparum* have also been shown or suggested to interact with their cognate Hsp70 system [31–34]. Biochemical experiments using *E. coli* proteins suggest that DnaK and DnaJ/CbpA act first on the client protein and then $Hsp90_{Ec}$ and the DnaK system collaborate synergistically to complete remodeling of the client protein [26].

In this paper, we explored the mechanism of collaboration between $Hsp90_{Ec}$ and DnaK both *in vivo* and *in vitro*. We show that $Hsp90_{Ec}$ and DnaK form a binary complex and $Hsp90_{Ec}$, DnaK and client protein form a ternary complex. CbpA promotes assembly of the ternary complex. We identified $Hsp90_{Ec}$ mutants defective in DnaK interaction *in vivo* and show that the purified mutant proteins are defective in DnaK interaction *in vitro* and impaired in protein reactivation with DnaK and its cochaperones. Together, these findings provide a better understanding of how these two important chaperones collaborate in client remodeling.

Results

Formation of an $Hsp90_{Ec}$–DnaK–client protein complex is facilitated by CbpA

We previously showed that $Hsp90_{Ec}$ functions synergistically with DnaK and its cochaperones, a J-domain protein (CbpA or DnaJ) and GrpE in client protein reactivation *in vitro* [26]. In addition, we showed that $Hsp90_{Ec}$ and DnaK interact *in vivo* in a bacterial two-hybrid assay and *in vitro* using purified proteins [26]. To shed light on the mechanism of protein remodeling by $Hsp90_{Ec}$ and DnaK, we sought to dissect the multiprotein reaction pathway into intermediates and partial reactions.

We explored the interaction between $Hsp90_{Ec}$ and DnaK by testing whether binding of client protein or DnaK cochaperones affects the stability of the previously observed $Hsp90_{Ec}$–DnaK complex [26]. We used an *in vitro* protein–protein interaction assay (pull-down assay) in which DnaK was labeled with biotin, DnaK D45C-biotin, and incubated with various pure proteins in the presence of ATP (Fig. 1 and Supplemental Fig. S1a). Biotinylated DnaK, along with DnaK-associated proteins, was then captured on neutravidin agarose beads, the beads were washed, proteins were eluted and the eluted proteins were analyzed by SDS-PAGE. When biotinylated DnaK and $Hsp90_{Ec}$ were incubated together, $Hsp90_{Ec}$ weakly associated with DnaK (Fig. 1a, lane 2), as observed previously by an ultrafiltration assay [26]. When the two chaperones were incubated with ribosomal protein L2, a client protein known to interact with $Hsp90_{Ec}$ [5,16], we observed significantly more $Hsp90_{Ec}$ associated with biotinylated DnaK and L2, suggesting formation of a more stable ternary $Hsp90_{Ec}$–DnaK–L2 complex (Fig. 1a, lane 3). CbpA further stimulated assembly or stabilization of the DnaK–$Hsp90_{Ec}$–L2 complex (Fig. 1a, lane 4, and Supplemental Fig. S1b) and the stimulatory effect of CbpA required L2 (Fig. 1a, lane 6). Additional experiments indicated that DnaK could associate with L2 and CbpA alone, as well as L2 and CbpA together (Supplemental Fig. S1c). In contrast, DnaJ did not affect assembly of the DnaK–$Hsp90_{Ec}$–L2 complex (Supplemental Fig. S1b). We do not understand why CbpA and DnaJ behaved differently in these experiments and in protein reactivation [26]. However, they differ in that DnaJ contains a cysteine-rich Zn^{2+} binding region (Type I J-domain protein) and CbpA lacks this region (Type II J-domain protein). GrpE had no detectable effect on the association of $Hsp90_{Ec}$ with DnaK and L2 in the presence of CbpA and was not detected in association with the complex (Fig. 1a, lane 5). In a control experiment, $Hsp90_{Ec}$, L2, CbpA and GrpE did not bind detectably to the neutravidin agarose (Fig. 1a, lane 8). Together, these results suggest that the DnaK–$Hsp90_{Ec}$ complex is strengthened