Closing In on the Hsp90 Chaperone-Client Relationship

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The molecular chaperone Hsp90 regulates the activity and stability of a set of client proteins. Despite progress in understanding its mechanism, the interaction of Hsp90 with clients has remained enigmatic. Now, in a recent issue of *Molecular Cell*, Street and coworkers present results that integrate the client in the Hsp90 chaperone cycle.

Hsp90 is a molecular chaperone found in the cytosol of eubacteria and eukaryotes. Unlike other promiscuous molecular chaperones, it seems to interact with a more defined set of client proteins that include many regulatory proteins. During its ATPase cycle, the Hsp90 dimer undergoes large conformational changes from an open to a closed conformation. These changes are thought to be relevant for the stabilization and activation of the client proteins. Despite progress in recent years on the structure of Hsp90, its conformational regulation, the function of cochaperones, and the growing lists of bona fide client proteins, little is known about how Hsp90 interacts with its clients. In a recent issue of *Molecular Cell*, Street et al. (2011) describe a technical tour de force to unveil the secrets of this interaction. They show that binding of a model client induces a partially closed conformation of Hsp90 and at the same time enhances the Hsp90 ATPase activity.

Hsp90, in its eukaryotic rendering, is one of the most complex chaperone machineries known, as it functions together with a large set of cochaperones. In contrast to eukaryotic Hsp90, bacterial Hsp90 (called HtpG in *E. coli*) seems to be a solo player: none of these cochaperones has been identified, yet prokaryotic Hsp90 is highly homologous in structure to its eukaryotic counterpart, suggesting that the basic mechanism is conserved (Buchner, 2010). Hsp90 proteins are homodimers, with protomers consisting of three domains each. The N-terminal domains bind ATP and, together with parts of the middle domains, form the split-ATPase site. The C-terminal domains mediate the dimerization of Hsp90. During the ATP-induced conformational cycle, the N-terminal domains dimerize and associate with the middle domains. These rearrangements are rate-limiting for the slow ATPase reaction, which is in the range of one ATP hydrolysis per minute. In eukaryotes, there are a growing number of proteins that depend on these Hsp90 conformational gymnastics to access or maintain their active state. These include many protein kinases and a number of transcription factors, such as most steroid hormone receptors, p53, and proteins as diverse as viral reverse transcriptases and viral coat proteins (see http://www.picard.ch/downloads/Hsp90interactors.pdf). Again, in contrast to its eukaryotic counterpart, prokaryotic Hsp90 has only a few known authentic clients, such as proteins from phycobilisomes (Sato et al., 2010) and the ribosomal protein L2 (Motojima-Miyazaki et al., 2010).

Few studies to date have embarked on the structural analysis of the interaction of Hsp90 with client proteins. Experimentally, this is a challenging problem, as many of the authentic or model chaperone client proteins are unstable and aggregation-prone. This severely limits the accessibility of biophysical methods. To overcome these hurdles, the Agard laboratory decided to tackle this question by using a well-behaved and well-studied model client protein, a fragment of *staphylococcal* nuclease (SN) that is known to be largely unfolded in solution and soluble even at high concentrations, making it amenable to rigorous analysis (Shortle, 2002). Addition of SN to *E. coli* Hsp90 led to the formation of a specific complex with low micromolar affinity and a stoichiometry of one client per Hsp90 dimer.

This chaperone-client complex put the authors in the unique position to apply sophisticated techniques for addressing key open questions. First, they asked how the binding of a client protein affects the conformational equilibrium of Hsp90. SAXS experiments revealed a compaction of Hsp90 in the presence of SN, suggestive of a transition from the open to a more closed state of Hsp90 (Figure 1). This effect was even more pronounced in the presence of the nonhydrolysable ATP-analog, AMP-PNP, which is known to induce N-terminal dimerization leading to the closed state of Hsp90. The preference of SN for the closed state was directly confirmed by a FRET system, which allows monitoring of this transition in Hsp90. Here, the addition of SN resulted in the acceleration of the closing kinetics. Furthermore, Hsp90 in the closed state exhibited a higher affinity for SN. Since this closing reaction is the rate-limiting step in the Hsp90 ATPase cycle, the authors also found an acceleration of ATP hydrolysis consistent with previous reports for human and *E. coli* Hsp90 (McLaughlin et al., 2002; Motojima-Miyazaki et al., 2010), supporting the notion that this is an evolutionarily conserved effect.

The authors also present new information on the client-binding site of Hsp90. Surprisingly, locations of the client-binding sites are still an open question, with published evidence for their localization in each of the domains of Hsp90 (see Buchner, 2010). Using SAXS experiments together with Hsp90 variants, Street et al. (2011) suggest that SN binds to the

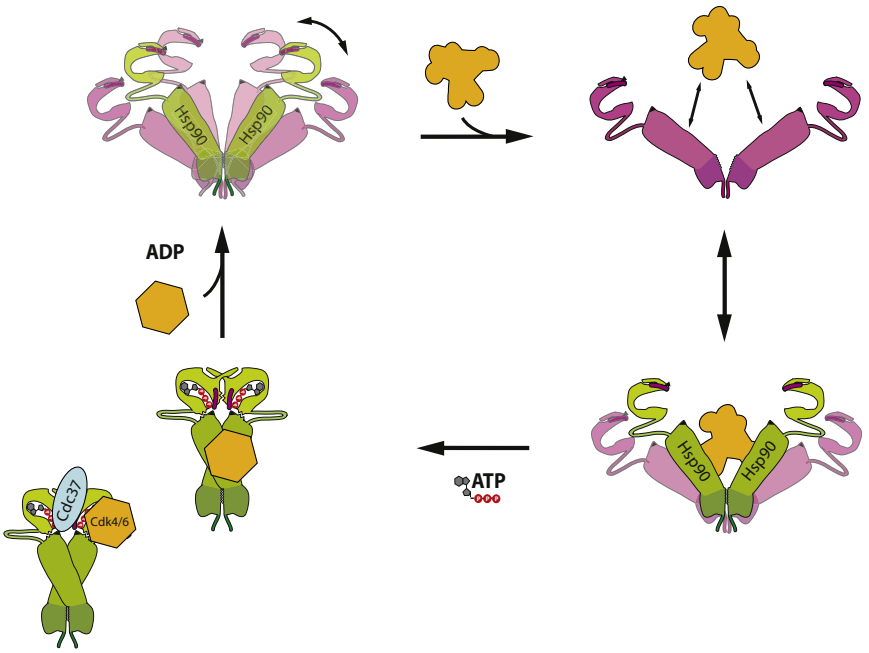

Figure 1. The Conformational Cycle of Hsp90 in the Presence of a Client Protein
Hsp90 is a dimeric chaperone that can adopt several conformations (pink, open; green, more closed). It binds to structurally labile client proteins (orange) via its middle domains. The client induces changes in Hsp90 that help to overcome the slow conformational rearrangements prior to the ATP hydrolysis reaction. The closing reaction of Hsp90 is initiated by ATP binding. Release of the folded client protein (orange hexagon) may occur after the hydrolysis reaction. A structurally distinct Hsp90 complex containing the co-chaperone Cdc37 and the client kinase Cdk4/6 (Vaughan et al., 2006) is shown on the left for comparison.

middle domain of Hsp90, which was also implicated as the main interaction site for an authentic client of bacterial Hsp90 (Sato et al., 2010). The resolution obtained so far does not allow discrimination between the symmetric or asymmetric binding of the client to Hsp90. This is an interesting and mechanistically important question, as it was recently shown that some cochaperones influence the Hsp90 dimer in an asymmetric manner (Retzlaff et al., 2010; Li et al., 2011).

Finally, NMR spectroscopy was employed to gain insight into the interaction site on SN. As mentioned, the larger part of this SN variant is natively unfolded, but it also contains a helical region. The structure of the client protein did not change strongly when bound to Hsp90. Specifically, the signals of the helical region became weaker in the Hsp90-client protein complex, suggesting that this region interacts with Hsp90. This finding is consistent with the general notion that Hsp90 interacts with folded, native-like and potentially metastable structures (Jakob et al., 1995), defining the position of Hsp90 in the chaperone network of the cell downstream of other chaperones such as Hsp70.

The study by the Agard lab on the analysis of an Hsp90-client complex is a huge step forward in our understanding of how this chaperone turns over its client proteins. It shows that the client is a kinetic accelerator of the initial conformational changes in Hsp90, possibly facilitating this transition by favorable interactions with Hsp90. At least for eukaryotic Hsp90, some clients seem to form very stable complexes with Hsp90 in vivo, which even survive coimmunoprecipitation (Pratt and Toft, 1997). The affinity determined here and previously in vitro (McLaughlin et al., 2002) seems to be in a different range. It will be exciting to see to what extent the observed effects can be transferred to high-affinity binding endogenous client proteins in the future and whether there is a unifying mechanism for the interaction of Hsp90 with its many structurally distinct client proteins. For an Hsp90 kinase-cochaperone complex, cryo-electron microscopy and image processing suggested an interaction of the native-like kinase with the N- and M-domains of Hsp90 (Figure 1) (Vaughan et al., 2006). It remains to be seen how the differences in interaction can be related to client-specific effects and how the interaction surfaces in Hsp90 may be differentially used.

In the future, it will also be important to consider how Hsp90 affects the conformation of the client protein. Hsp90 had been proposed to facilitate conformational changes, which are energetically unfavorable but important for the activation of client proteins. In the context of this study, it seems that the client primes Hsp90 for conformational changes in response to nucleotide binding, and it is reasonable to assume that these conformational changes may in some cases also affect the conformation of the client protein (Figure 1). The results presented by Street et al. (2011) are therefore an important step forward in understanding the chaperone mechanism of Hsp90. They place the client in the Hsp90 chaperoning cycle and set the stage for further experiments to elucidate the underlying mechanism.

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