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The Solution to Multiple Structures

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Getting high-resolution structures of large proteins in solution has always been a challenge. In this issue of *Structure*, Krukenberg et al. have used new methods of analyzing SAXS data to reveal a novel conformation of Hsp90 in solution.

As the links between the molecular chaperone Hsp90 and a wide range of cellular processes continues to grow, along with the increasing importance of Hsp90 as a therapeutic target, so does the need for high-resolution structures which reflect the conformations that the protein adopts in its physiological and functional state in solution. Hsp90 is essential for the correct maturation and activation of a wide range of important cellular proteins, many of which are involved in signal transduction processes and linked with oncogenic processes (Pearl et al., 2008). Hsp90 is known to act in conjunction with other cochaperones and ATP binding and hydrolysis are thought to drive conformational changes which are linked to client protein activation (Figure 1).

Initial structural work on Hsp90 focused on individual domains from a number of homologs and paralogs including human and yeast Hsp90, *E. coli* HtpG, and the ER-resident Grp94 (Pearl and Prodromou, 2006). Hsp90 has three major structural domains: the N-terminal, middle, and C-terminal domains (Figure 1). Although this work revealed a great deal about nucleotide and inhibitor binding, dimerization interfaces and, in some cases, cochaperone binding, many questions on domain-domain interactions and the function of the full-length protein remained. For many years, the structure of an intact full-length and functional protein seemed elusive, then, just like buses, three came in rapid succession (Ali et al., 2006; Dollins et al., 2007; Shiau et al., 2006). These important structures highlighted a number of aspects of Hsp90 structure and function, including the fact that the protein could exist in a number of conformations. However, differences in these structures also raised questions about the true structure(s) of Hsp90 in solution.

The first structure of full-length Hsp90 was from the Pearl/Prodromou laboratory and was of an engineered variant of yeast Hsp90 in complex with AMPPNP and the cochaperone p23 (Ali et al., 2006). In this case, a mutant was used in which the flexible highly charged linker between the N-terminal and middle domain was removed and a mutation in the N-terminal domain which promoted N-terminal domain dimerization was present. In this case, the structure of Hsp90 was shown to be a compact state in which the N-terminal domains had dimerized through a domain swapping at their N termini. Shortly after this, the Agard group published an EM study and a crystal structure of the *E. coli* homolog HtpG (Shiau et al., 2006). By EM, the apo form, AMPPNP, and ADP-bound states of HtpG were found to adopt different conformations and crystal structures of the apo and ADP-bound states revealed what appeared to be very distinct conformations: the apo state adopted an open structure in which each of the three domains exposed hydrophobic surface area, while in the ADP-bound form these hydrophobic surfaces clustered to form a much more compact state. In contrast to these results on HtpG, crystal structures of AMPPNP and ADP-bound form of the ER-paralog Grp94, which came out last year, show that there is relatively little difference in conformation between the two nucleotide bound states (Dollins et al., 2007). In this case, both states exhibited a twisted V-shaped conformation that precluded the N-terminal dimerization thought to take place on ATP binding and observed in the yeast

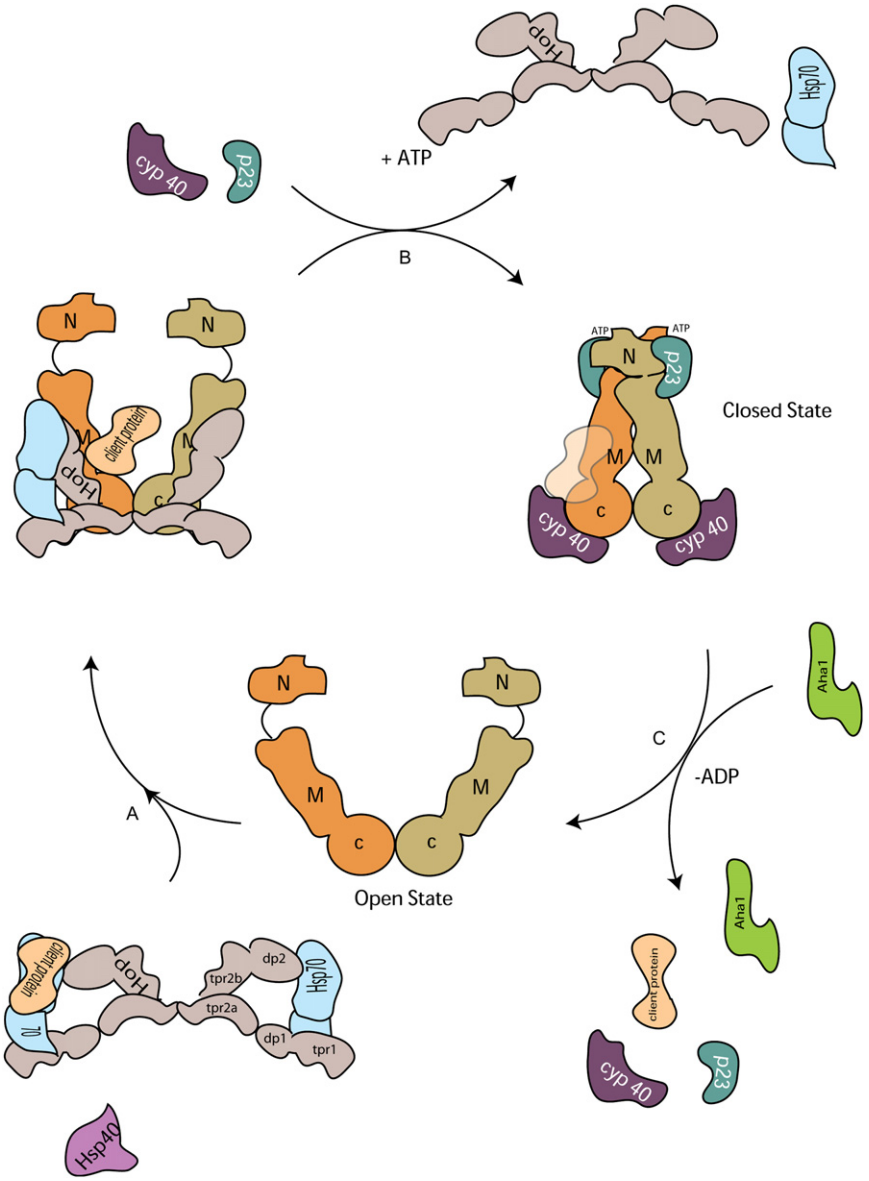

Figure 1. Model of the Conformational Cycle of Hsp90

(A) In the initial step, the client protein is transferred from the Hsp70 machinery to the open form of Hsp90 by the simultaneous binding of Hop to both Hsp70 and Hsp90.

(B) Upon ATP binding to Hsp90, the Hsp90-Hop complex is weakened and, together with the binding of other cochaperones, such as large immunophilins Cyp40 or p23, Hop is displaced from the Hsp90 complex. Binding of ATP to Hsp90 then triggers conformational changes in the N-terminal and middle domains ultimately resulting in N-terminal dimerization and formation of a closed state. This closed state is further stabilized by p23.

(C) The cycle is completed by hydrolysis of ATP which may be triggered by release of Hop, or binding of client or Aha1, resulting in the activation and subsequent release of the client protein and restoration of the open state of Hsp90.

Hsp90-AMPPNP-p23 structure (Ali et al., 2006). Recent work has been aimed at establishing the origin of these differences and the structure of the full-length protein in solution.

Small-angle X-ray scattering (SAXS) techniques have been used extensively to study the conformation of proteins and protein complexes in solution. When combined with known crystal structures, this low-resolution technique can be a powerful tool for investigating the conformation of proteins in solution. In this issue of *Structure*, Krukenberg et al. use SAXS with new modeling strategies to elucidate the structures of the apo and AMPPNP-bound forms of HtpG in solution (Krukenberg et al., 2008). Although SAXS was used some years ago to gain information on the global properties of the structure of human Hsp90 in solution (Zhang et al., 2004), the Agard study published here is the first to generate a solution structure of an Hsp90 homolog. Their studies show that apo and AMPPNP-bound states have distinct conformations, and that the AMPPNP-bound state is more compact than the apo protein. Crucially, the SAXS data for apo HtpG shows that it differs significantly from the crystal structure and is considerably more extended, highlighting the influence that crystal packing can have on protein structure, particularly a protein as dynamic as Hsp90. Using a new approach to model the solution structure of apo HtpG, the Agard group in collaboration with the Sali group, have used rigid body motions both between the middle and C-terminal domain as well as the middle and N-terminal domains and combined these with Monte Carlo and simulated annealing methods to generate a solution structure which is well defined. The novel solution structure is open and extended compared with the crystal structure.

In contrast to the single, well-defined solution structure for apo HtpG, a similar analysis on AMPPNP-bound HtpG did not generate such a good model (Krukenberg et al., 2008). A comparison of the radius of gyration, R_g, of AMPPNP-HtpG with that expected from the crystal structure of the AMPPNP-p23-yeast Hsp90 complex (Ali et al., 2006) and that measured for apo-HtpG in solution by SAXS, indicated the actual R_g is somewhere in between the two. These two things suggested that the AMPPNP-HtpG complex may be an equilibrium mix of the two conformers. Modeling of the data with an approximately 50:50 population of the two species and structures produced excellent fit with the experimental data. Thus, the SAXS data of the AMPPNP-HtpG shows unequivocally for the first time that, even in the nucleotide-bound state of HtpG, there is a dynamic conformational equilibrium.

As this elegant study on HtpG is published in *Structure*, another study using both single-particle cryo-EM and SAXS measurements on eukaryotic Hsp90 extends the information available on the

structure of Hsp90 homologs in solution (Bron et al., 2008). In their study, apo-Hsp90 is shown to be in a conformational equilibrium between two open states similar to the open state observed for apo-HtpG in Krukenberg et al. (2008). By comparing the cryo-EM maps with known crystal structures, the structural changes involved in switching between the two apo conformations are shown to require large movements of both the N-terminal domain and the middle domain around two flexible hinge regions similar to the rigid-body motions of the middle domain relative to both the N-terminal and C-terminal domains required in the study on HtpG. Both studies are reminiscent of results from an H/D mass spectrometry study on human Hsp90 in solution, which showed that cochaperone and inhibitor binding to the N-terminal domain induced conformational changes in both domain-domain interfaces (Phillips et al., 2007).

Krukenberg et al. (2008), along with other studies on Hsp90 in solution, contributes to providing strong evidence that this protein has a highly dynamic structure and that it can adopt a number of conformations in solution, which might not necessarily be similar to those seen in the crystalline form. This work illustrates the importance of combining solution studies with high-resolution structures. Having established the value of SAXS techniques to probe the conformation of Hsp90 homologs in solution (Bron et al., 2008; Krukenberg et al., 2008), these methods will almost certainly prove to be valuable in elucidating the structures of larger Hsp90-cochaperone complexes and hopefully ultimately the structure of a client protein bound to the Hsp90 assembly machine. Indeed SAXS has already been used very effectively on the complex formed between Hsp90 and the cochaperone Hop (Onuoha et al., 2008).

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Trapping Fugitive Filament Formers

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In this issue of *Structure*, Boczkowska et al. (2008) investigate the activation of the Arp2/3 complex and propose a provocative model for Arp2/3-dependent filament formation.

Eukaryotic cells must solve a difficult problem: how to establish and maintain order across cellular dimensions (typically 10–100 microns) using molecules that are several orders of magnitude smaller (typically 1–20 nanometers). They do this, in part, by assembling molecules into networks of cytoskeletal polymers. Networks of crosslinked actin filaments, for example, control the shape and mechanical properties of most cells and enable them to move. Considerable work has gone into understanding the structure and assembly of these cytoskeletal polymers and we know a lot about how pre-existing polymers elongate. We do not understand nearly so well, however, how new polymers are formed from monomeric precursors. In the particular case of actin, the nucleation of new filaments, whether spontaneous or initiated by regulatory factors, is slow compared to filament elongation. Nucleation intermediates are, therefore, rare and short lived. These properties make them poor candidates for structural studies, which generally require large quantities of stable and homogeneous species.

This problem confronted structural biologist Roberto Dominguez and his colleagues (Boczkowska et al., 2008), who wanted to understand how one important nucleator, the Arp2/3 complex, generates new actin filaments. To understand the specific questions these authors address requires a brief discussion how actin filaments form spontaneously, in the absence of a nucleation factor. An actin filament can be described as a two-stranded (two-start), right-handed helix. To move from one monomer to the next along one strand of the filament requires a translation