Structures of GRP94-Nucleotide Complexes Reveal Mechanistic Differences between the Hsp90 Chaperones

Life depends on the biochemical activity of the thousands of proteins that inhabit and decorate the surface of every one of our cells. Proteins themselves, although simple linear combinations of the twenty amino acids, derive their remarkable properties from the complex three-dimensional structures into which they fold. In this way, enzyme active sites are created, protein-protein recognition surfaces are formed, and the chemistry of life is set in motion. Although in principle the precise three-dimensional structure for each protein is encoded in its linear chain of amino acids, in practice it is often difficult or impossible for a protein to achieve this final fold on its own in the context of a cellular environment that is packed to the gills with millions of other proteins, nucleic acids, carbohydrates, lipids, and other small molecules. As a result, cells have evolved a corps of proteins known as molecular chaperones that assist newly synthesized proteins as they adopt their active fold. One such family of chaperones is known as the hsp90 family (Pratt and Toft, 2003). "Client" proteins of the hsp90 family are diverse, and their functions range from signal transduction to immune response. Specific inhibitors of hsp90 chaperones exhibit potent anti-tumor activity (Chiosis et al., 2006; Sharp and Workman, 2006), showing that preventing the proper folding of client proteins, many of which are implicated in cancer, can have profound therapeutic implications.

The mechanism by which hsp90 chaperones act to mature their client proteins is not yet established. Hsp90s exist as dimers and it has been shown that chaperoning activity is closely tied to their ability to hydrolyze ATP (Obermann et al., 1998; Panaretou et al., 1998; Chadli et al., 2000). In order to understand how these are related, we used diffraction data collected at beamlines 11-1 of SSRL and 8.2.1 of ALS to determine the high resolution X-ray crystal structure of mammalian GRP94, the hsp90 chaperone that is found in the endoplasmic reticulum of cells. GRP94 is a particularly intriguing member of the hsp90 family. Earlier studies had suggested that GRP94 did not hydrolyze ATP, and thus was mechanistically different from other hsp90s (Nicchitta, 1998). The structure that we solved helped explain these observations (Dollins et al., 2007). In particular, we saw that in the presence of an ATP analog the GRP94 dimer adopted a structure that resembled a "twisted V" (Figure 1). This conformation prevented the proper alignment of the residues thought to be

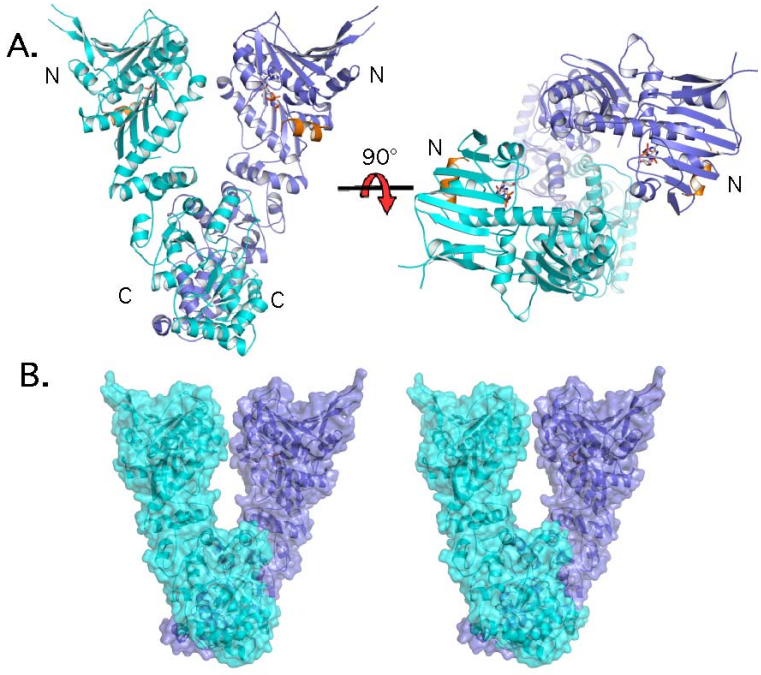

Figure 1. Overview of the GRP94 structure. The two protomers of the GRP94 dimer are shown in blue and cyan. (A) Ribbon drawing of side and top views. The two N-terminal domains of the dimer do not interact, causing the misalignment of ATP hydrolysis residues. (B) Stereo surface view of the GRP94 dimer. The twisted V shape is readily apparent.

required for ATP hydrolysis. Surprisingly, however, the X-ray structure also showed that a simple 90 degree rotation of one of the domains of GRP94 could lead to the productive alignment of the catalytic residues. Prompted by this structural insight, we carried out a series of careful biochemical measurements that showed that in fact GRP94 had a very weak but reproducible ATPase activity. These experiments suggested that the transition from the "twisted V" conformation to one that aligns the catalytic residues was likely to be a key step in the regulation of GRP94 activity (Figure 2). This insight was important not only for our understanding of GRP94 but also for understanding other hsp90s. In particular, unlike its counterparts in yeast or bacteria, cytoplasmic human Hsp90 also exhibits unusually weak ATPase activity, and thus may bear a strong structural resemblance to GRP94. Together these observations have succeeded in establishing the place of GRP94 in the hsp90 family and, together with our earlier studies of the isolated domains of GRP94 (Soldano et al., 2003; Immormino et al., 2004; Dollins et al., 2005), opens the door to the design of inhibitors that specifically target this chaperone.

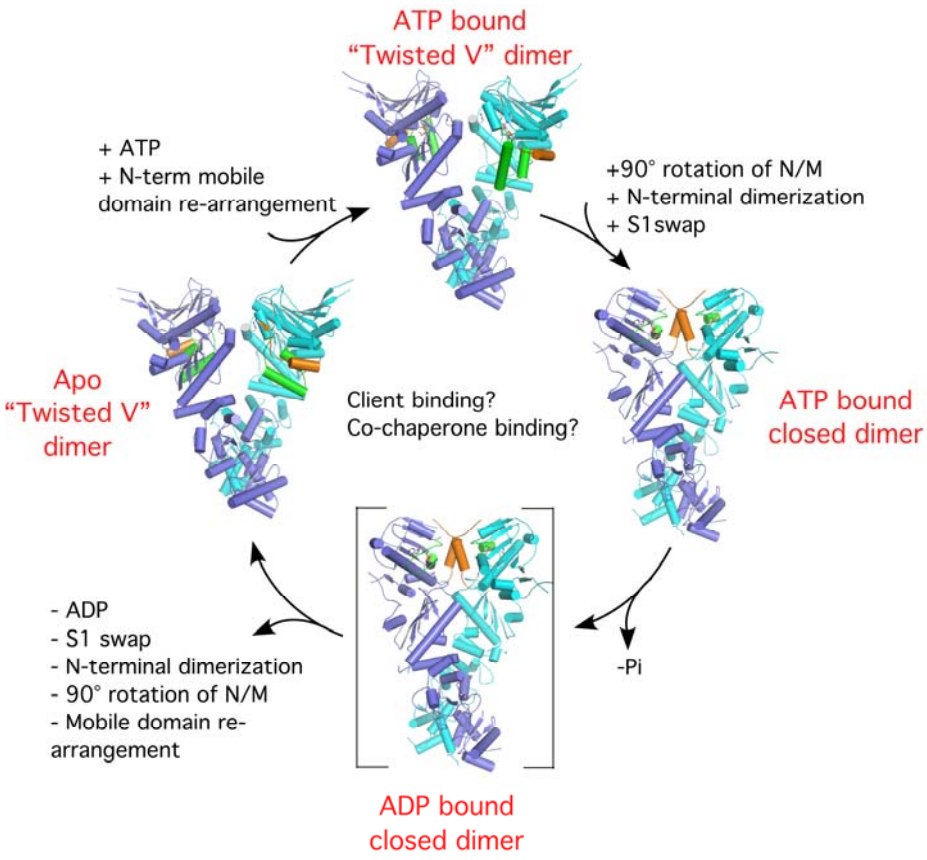

Figure 2. Model of the GRP94 ATP hydrolysis mechanism. The conformational changes that lead to the alignment of ATP-catalytic residues are shown. Such rearrangements are likely to allow for the binding and release of client proteins from the chaperone.

Primary Citation

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