Molecular Cell, Vol. 20, 493–501, November 23, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.molcel.2005.11.007

An Essential Connection: Link between Hsp70's Domains At Last

Communication between the ATPase and substrate binding domains of Hsp70 is critical for regulated interaction between this molecular chaperone and its client proteins. In this issue of *Molecular Cell*, Jiang et al. (2005) report the structure of an intact Hsp70, revealing critical interactions between the two domains.

Hsp70s are arguably the most ubiquitous and versatile molecular chaperones, promoting folding, transport across membranes, and proteolysis of client proteins. The biochemical basis of these talents is the ability of Hsp70 to reversibly interact with client protein in a manner precisely regulated by cycles of binding, hydrolysis, and release of adenine nucleotides. It has been long appreciated that the two-domain structure of Hsp70 is critical for these regulated interactions: the ~44 kDa N-terminal nucleotide binding domain (NBD) that binds and catalyzes the hydrolysis of ATP, and the ~27 kDa C-terminal substrate binding domain (SBD) that interacts with polypeptide targets. The activities of these two domains are coupled with bidirectional ligand-induced conformational changes transmitted from one domain to the other. The affinity for client proteins is increased by several orders of magnitude upon hydrolysis of ATP to ADP and inorganic phosphate. On the other hand, substrate binding to the SBD stimulates ATP hydrolysis.

For nearly a decade, researchers have pored over the structures of the individual domains (Flaherty et al., 1990; Zhu et al., 1996), trying to predict how they interact and, consequently, how they communicate with each other, while waiting for a more complete structure. The wait is now over. In this issue of *Molecular Cell*, Jiang et al. (2005) present a structure of the nonnucleotide form of intact bovine Hsc70 (bHsc70) determined by X-ray crystallography at a resolution of 2.6 Å. The group, led by Rui Sousa, was able to overcome the technical problems that plagued previous attempts to obtain well-diffracting crystals by modifying several charged surface residues and by deleting 100 amino acids from the C terminus. The structures of the individual domains within the "full-length" structure do not hold any major surprises: (1) the two-lobed NBD having two subdomains, IA and IIA, forming the base of the deep nucleotide binding cleft; and (2) the SBD consisting of a compact β sandwich composed of two β sheets forming a deep pocket in which peptide substrate binds, plus a distal α-helical portion, which, according to the previously published structure (Zhu et al., 1996), forms a lid covering the substrate binding pocket (see Figure 1).

The novelty of the structure lies in the interactions between the two domains that are revealed. Several contacts between the base of the NBD and helix A and the proximal part of helix B of the SBD were identified: electrostatic interactions involving salt bridges between residues near the junction of helices A and B of SBD and those in region IA and IIA of NBD (blue shading in Figure 1) as well as hydrophobic interactions between residues within the proximal part of helix A of SBD and region IIA of NBD (yellow shadings in Figure 1). In addition, the covalent connection between the two domains, a 10 amino acid flexible linker, is exposed on the surface of the structure. If propagation of conformational changes indeed takes place via such contact sites, regions around them should structurally change upon ligand binding. Indeed, recent multidimensional NMR studies of Hsp70 from *Thermus thermophilus* (Revington et al., 2005) revealed significant chemical shifts within the residues of the interface between regions IA, IB, and IIA of NBD, as well as in close proximity to the linker region, upon binding of an ATP analog. Similarly, binding of peptide to the SBD brings global conformational changes within the SBD, which extend to the NBD region, notably at the interface between regions IA and IIA. Thus, in both the ATP and ADP states, the regions of contact indicated by the new structure are implicated in interdomain communication.

These pieces of information provide the first phase of a structural picture of the adenine nucleotide-dependent cycle of interaction between Hsp70 and a client protein. Binding of ATP generates the conformational state that, due to the very rapid on and off rate of substrate, not only causes release of any bound substrate but also generates the form that productively initiates another round of interaction with client polypeptides. Both the linker itself and contact between the helices and NBD are known to be important in generating the ATP conformation. Amino acid alterations within the linker significantly decrease the rate of release of substrate upon ATP binding to the NBD (Laufen et al., 1999). A truncation lacking the helical region of the SBD fails to undergo a shift in tryptophan fluorescence upon ATP binding, a hallmark of global Hsp70 conformational changes (Moro et al., 2003; Slepenkov and Witt, 2002). ATP binding may lead to a tighter conformation of the NBD, disrupting the salt bridges and hydrophobic contacts with the SBD, allowing invasion of the linker region into an interface between two domains, consistent with its protease sensitivity when Hsp70 is in the nucleotide-free or ADP bound state, compared to the ATP bound form. This conformational change may also result in movement of the α helices, thus destabilizing substrate interaction with Hsp70 due to a shifting of the helices away from the peptide binding pocket. Upon ATP hydrolysis, conformational changes would occur, reestablishing the electrostatic and hydrophobic between the NBD and SBD reported by Jiang et al. (2005), repositioning the lid over the peptide binding pocket, and thus completing the cycle.

However, the story of Hsp70 conformational changes is far from closed. It is intriguing that the identified interface between the NBD and SBD is very close to the site of interaction of the J domain of the obligate Hsp70 co-chaperone, J proteins, that stimulate its ATPase activity

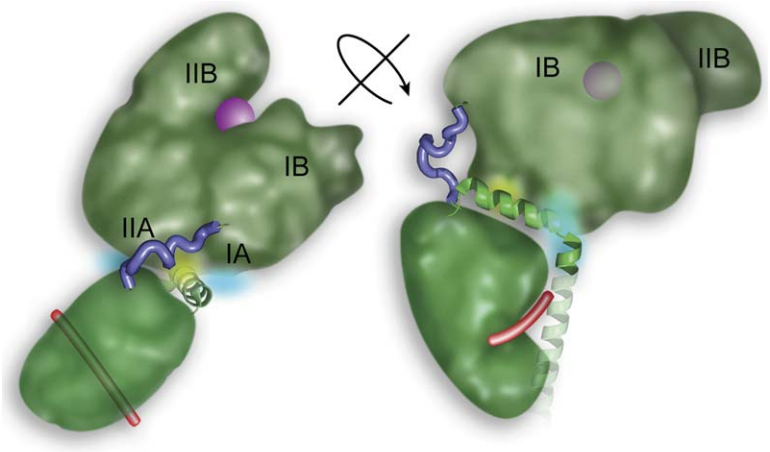

Figure 1. Schematic Representation of the Intact bHsc70 Structure

The nucleotide binding domain (NBD) and β sheet region of the substrate binding domain (SBD) are depicted without detail. Although the data presented in Jiang et al. (2005) (PDB coordinates 1YUW) was obtained from protein crystallized in the absence of nucleotide and peptide substrate, the positions of bound adenine nucleotide (pink ball) in the NBD and bound peptide in the SBD (red rod) are shown. Helices A and B at the distal part of the SBD are shown in green. The faded part of helix B is positioned according to the previously published structure of SBD alone (Zhu et al. [1996]; PDB coordinates 1DKZ). Electrostatic interactions involving salt bridges between residues K524 (helix A) and D152 (region IA of NBD) as well as E530 (helix B) and K325 (region IIA NBD) are indicated by highlighting in blue. Hydrophobic interactions between residues I515 and V519 of helix A and I216 of region IIA of the NBD are highlighted in yellow. The flexible 10 amino acid linker is shown in violet.

(Gassler et al., 1998; Suh et al., 1998). How are the conformational changes initiated by J proteins transmitted? In addition, some aspects of interdomain communication still exist even when the entire helical region of the SBD is deleted (Pellecchia et al., 2000). Does the β sandwich domain itself play some yet-to-be-defined role, perhaps in the ATP bound conformation? Structural information regarding full-length Hsp70 bound to ATP, peptide bound, and/or J proteins, coupled with analysis of mutant proteins, will be required for a full appreciation of the conformational gymnastics that Hsp70s perform. However, the information presented in Jiang et al. (2005) represents a giant step forward, and testing predictions based on this new structural information will keep workers in the field productively occupied in the meantime.

Patrick D'Silva,[1] Jaroslaw Marszalek,[1,2] and Elizabeth A. Craig[1]
[1]Department of Biochemistry
433 Babcock Drive
University of Wisconsin, Madison
Madison, Wisconsin 53726
[2]Department of Molecular and Cellular Biology
Faculty of Biotechnology
University of Gdansk
24 Kladki
80-822 Gdansk
Poland

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