The Hsp70 system interactions with substrate protein and the role of these interactions in Lon-dependent proteolysis of substrate.

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Hsp70 and its obligatory co-chaperones J-domain proteins (JDP) are the most ubiquitous chaperone systems involved in protein homeostasis, both under physiological conditions and during stress and pathology. Less known, however, are specialized functions of these systems, such as the involvement of JDP/Hsp70 in biogenesis of iron-sulfur clusters (FeS) – cofactors required for activity of many proteins. Regardless of whether the JDP/Hsp70 system controls proteostasis, or is involved in the FeS biogenesis, its function depends on reversible binding of a substrate protein. Substrate binding cycle is driven by conformational changes of Hsp70 controlled by hydrolysis of ATP. The ATPase activity of Hsp70 is allosterically stimulated by binding of J-domain and substrate. The J-domain and substrate function synergistically – together they stimulate the ATPase many fold more efficiently than alone. Little is known however about the molecular mechanisms underlying progression of the substrate binding cycle.

Research conducted by our group aims at revealing these mechanisms using as a model JDP/Hsp70 systems specialized in the FeS biogenesis. These systems interact with only a single substrate – a scaffold protein on which FeS are assembled and from which, upon interaction with chaperones, they are transferred onto target proteins.

In this thesis I described the biochemical reconstruction of a tripartite complex consisting of Hsp70-JDP-substrate, which constitutes a key intermediate in the substrate binding cycle by chaperones involved in the FeS biogenesis. I show, using pull-down and bio-layer interferometry (BLI) assays that formation of this complex is a two-step process: (1) JDP binds substrate and subsequently, (2) JDP-substrate complex recruits Hsp70 via JDP-Hsp70 and substrate-Hsp70 interactions. However, disruption of any of these individual interactions prevents the tripartite complex formation. The mechanism of the Hsp70 binding to the JDP-substrate complex, described in this thesis, can also explain the mechanism behind synergetic activation of the Hsp70's ATPase by the JDP and substrate.

I also characterized the effect of bacterial JDP/Hsp70 system involved in the FeS biogenesis on a Lon protease dependent degradation of a scaffold protein. I show that, like in the yeast system, also in bacteria scaffold protein is proteolyzed by Lon and its degradation is inhibited by interaction with chaperones. The obtained results indicate that JDP/Hsp70 systems functioning in the FeS biogenesis not only facilitate the FeS transfer from the scaffold protein onto the target proteins but also control the cellular level of the scaffold by protecting it against degradation.