The mechanism of cooperation between Hsp70 and Hsp104 chaperones in the reactivation of aggregated proteins.

ABSTRACT

Hsp104 is a yeast chaperone that rescues misfolded proteins from aggregates associated with proteotoxic stress and aging. This activity enables cells to survive stress conditions.

Hsp104 consists of: N-terminal domain, M-domain and two Nucleotide Binding Domains (NBDs), assembled into a spiral-shaped hexamer. Protein disaggregation involves polypeptide extraction from an aggregate and its translocation through the central channel of Hsp104. This process relies on ATP hydrolysis by the twelve NBDs.

In this work, I studied the collaboration between the Hsp104 disaggregase and its chaperone partner Hsp70. Using biochemical approach, I revealed that Hsp104 is exceptionally sensitive to ADP and that Hsp70 enables the disaggregation activity of Hsp104 at the, otherwise limiting, ATP and ADP concentrations similar to those in yeast cytosol.

Hsp70 exerts a dual effect on Hsp104: it activates it by interacting with its M-domain and it facilitates binding to the aggregated substrate. I showed that the latter function of Hsp70 is responsible for overcoming ADP inhibition of Hsp104. To verify, if this crucial role depends on the Hsp104-Hsp70 interaction, I identified a critical residue within the M-domain responsible for binding to Hsp70. Hsp104 variant with a disrupted interaction site served to demonstrate that Hsp70 recruits Hsp104 to aggregates through the M-domain. I also showed that the support of Hsp70 in protein binding is specific to aggregates and does not involve non-aggregated, disordered protein substrates.

Furthermore, my observations suggest a mechanism of overcoming ADP inhibition. The process of polypeptide threading by Hsp104, once initiated, facilitates binding of ATP, which strongly stimulates the ATPase activity of the disaggregase in the presence of ADP. In addition, I showed that ADP predominantly affects the NBD2

domain. Thus, my findings indicate that a protein substrate plays a role of a nucleotide exchange factor for the second Nucleotide Binding Domain of Hsp104.

My results point to an additional level of Hsp104 regulation by Hsp70, which restricts the potentially toxic protein-unfolding activity of Hsp104 to the disaggregation process, providing the yeast protein-recovery system with substrate specificity and efficiency in ATP consumption.