Hsp70 regulates the activity of Hsp104 disaggregase in the response of yeast *Saccharomyces cerevisiae* to stress.

Tomasz Chamera

ABSTRACT

The Hsp104 disaggregase from *Saccharomyces cerevisiae* is a chaperone protein which plays a key role in reactivation of proteins which are trapped in aggregates. The Hsp104 monomer consists of the N-terminal domain (NTD), two AAA+ - type nucleotide binding domains: NBD1 and NBD2, NBD1-associated M-domain (MD) and short C-terminal domain (CTD). The active form of the disaggregase is a hexamer containing a channel located centrally inside the structure. During reactivation, Hsp104 releases the polypeptide chain from aggregates and translocates it through its central channel, whereby the polypeptide leaving the channel of disaggregase can be refolded to its native state. An effective disaggregation process requires cooperation between Hsp104 and Hsp70 chaperones. Hsp70 binds aggregates, which makes the recruitment of Hsp104 to aggregates possible. Additionally, the interaction of Hsp70 with the M domain of Hsp104 activates the disaggregase. The cooperation between Hsp70 and Hsp104 is crucial for the disaggregation process, but the site of interaction between these proteins has not yet been recognized.

In my doctoral thesis, I aimed to establish the role of yeast Hsp70 – Ssa1 in the regulation of Hsp104 activity. I also defined the significance of Hsp70 and Hsp104 interaction in performing effective and efficient disaggregation process. Based on the comparison of amino acid sequences of the M domain from Hsp104 homologs from different species, I identified the phenylalanine at position 508 in Hsp104 as the site of interaction with Ssa1.

The substitution of phenylalanine to alanine in this position impairs the cooperation between the disaggregase and Ssa1 which makes the disaggregase unable to bind with aggregates, which prevents the disaggregation initiation. *In vivo* analysis showed that yeast cells carring the F508A mutation in Hsp104 do not show resistance to temperature stress and at the same time Hsp104 F508A protein is unable to recognize the aggregate under conditions of cellular stress.

My results also show that the interaction between the disaggregase and Hsp70 is important to protect cells from the potentially toxic activity of Hsp104 - unfolding native proteins with unstructured regions. The interaction of Hsp104 and Hsp70 shifts the substrate

specificity of the disaggregase from non-aggregated proteins with unstructured regions towards proteins trapped in aggregates.