Comparison of bacterial and eukaryotic Hsp70 systems functioning in the biogenesis of iron-sulfur clusters

mgr Małgorzata Kleczewska

Iron sulfur clusters are prosthetic groups of proteins crucial for their function. They consist of iron and sulfur atoms. Biogenesis of iron sulfur clusters is a complexed biochemical process, which we can divide into two steps. Firstly, (i) the iron sulfur cluster is synthesized on a specialized protein called molecular scaffold (ii) in the second step formed cluster is transferred to the target protein. The pathway requires the involvement of several different proteins which form protein multicomplexes.

In Laboratory of Biochemical Evolution, we are working on a well-known *Saccharomyces cerevisiae* yeast system. In this system at the first step Fe-S cluster is formed on a protein Isu (molecular scaffold). Several different proteins are involved in this process including Nfs1(Isd11) (cysteine desulfurase complex), Yfh1(frataxin), Yah1(ferredoxin) and its reductase Arh1. In the next step Isu1 with Fe-S cluster is transferred to the Hsp70 protein via Hsc20 protein. Hsc20 protein has a C-terminal domain, which binds Isu1. Afterwards the N-terminal domain of Hsc20 interacts with Hsp70 protein that results in ATP to ADP hydrolysis, a change of conformation of Hsp70 occurs, and the Fe-S cluster is released from the Isu protein and delivered to the apo protein. *Escherichia coli* system has also a dedicated protein machinery consisting of IscU (molecular scaffold), IscS (cysteine desulfurase), CyaY (frataxin), Fdx (ferredoxin) and ferredoxin reductase Fpr. In the second step *E.coli* chaperone system: HscA and HscB is involved. Main difference between these two systems is an involvement of the Mge1 protein (nucleotide exchange factor) in a yeast system, needed during the release of hydrolyzed ATP, whether in the case of *E.coli* system such component is not necessary.

The aim of this work was to compare bacterial and eukaryotic Hsp70 systems functioning in Fe-S cluster biogenesis. Phylogenetic analysis conducted in our laboratory showed that HscA and Ssq1 are distantly related and that the orthologs of HscA protein are not present in eukaryotic proteomes. This result suggested that the gene coding for HscA protein was lost during evolution of mitochondria, and the mitochondrial multifunctional Hsp70, which is the descendent of bacterial DnaK, took its role. Moreover, the results of our phylogenetic analysis suggest that the gene encoding the specialized Ssq1 protein is the product of a duplication of the gene encoding the mtHsp70 protein that occurred in *S. cerevisiae*. On the other hand, the main question was how biochemically similar or different both Hsp70 involved in Fe-S cluster biogenesis are.

To answer that question I needed to purify proteins from *S.cerevisiae*, *E.coli* and Isu1 from *Chaetomium thermophilum*, because of the high concentration of Isu protein required for biochemical analysis. To check if the purified proteins are active I performed the ATPase activity assay to determine the activity of Hsp70 proteins in the absence and in the presence of J- proteins and substrates to determine if they properly stimulate Hsp70 system.
In order to check whether the purified proteins interact with each other according to the ATPase cycle, I performed pull-down experiments of protein complexes. My comparative analysis showed incredible similarities in biochemical properties of both systems.

Further research focused on the role of chaperones in the biogenesis process. For this purpose, I recreated the physiological environment for the reaction using *E.coli* proteins. Firstly, I developed protocols for efficient purification of *E.coli* proteins (IscS, CyaY, Fdx, Fpr) which are necessary for effective Fe-S cluster synthesis on IscU protein. To monitor whether proper Fe-S cluster is formed on IscU protein I used Circular Dichroism spectroscopy (CD). For transfer studies I used two different acceptor proteins ferredoxin (Fdx) and glutaredoxin D (GrxD), both in apo form without Fe-S clusters. During the experiment, I could distinguish if the transfer occurred to the Fdx or to GrxD because of the differences in CD spectra of those proteins.

Finally, the last step of my PhD thesis was to verify the influence of the Hsp70 chaperone system on a transfer of Fe-S cluster from the molecular scaffold - IscU to the acceptor proteins - Fdx or GrxD. This analysis allowed me to show the significant effect of bacterial chaperones in the presence of ATP on the increase in the rate of Fe-S cluster transfer from IscU to GrxD. Obtained results are fully consistent with the *in vivo* data published so far. The *in vitro* research model I have developed opens up new possibilities for studying the impact of mutations that disrupt specific protein: protein interactions, on the efficiency of the synthesis and transfer of Fe-S clusters.