Molecular mechanism of the interaction between J-domain of Jac1 protein and its Hsp70 partner in the process of iron-sulfur cluster biogenesis.

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Iron-sulphur clusters (FeS) are inorganic cofactors that are indispensable for protein function in numerous key metabolic processes. FeS cluster biogenesis is performed in mitochondria. FeS clusters are first synthesised on the Isu protein. Next, they are transferred to recipient proteins by the Hsp70 molecular chaperone system. In *Saccharomyces cerevisiae*, this system is composed of a specialized Hsp70, Ssq1, a J-domain protein (JDP), Jac1, and a nucleotide exchange factor, Mge1. Through its J-domain, Jac1 stimulates the ATPase activity of Ssq1 which leads to Ssq1:Isu complex formation followed by the release of the FeS cluster to a recipient protein. The molecular mechanisms of Jac1:Isu and Ssq1:Isu interactions are known, while the nature of the Jac1:Ssq1 interaction is not understood. The aim of my project was to identify the amino acid residues responsible for this interaction.

I began my study by comparing the *S. cerevisiae* chaperone system, in which a specialized Hsp70 Ssq1 is responsible for FeS transfer, with the *Schizosaccharomyces pombe* system, in which the same process is facilitated by a multifunctional mtHsp70 protein. I have found that $Jac1_{Sc}$ from *S. cerevisiae* contains a larger number of positively charged residues in its J-domain in comparison to its orthologue, $Jac1_{Sp}$, from *S. pombe*. I replaced the charged residues in $Jac1_{Sc}$ with uncharged residues present in $Jac1_{Sp}$ and investigated the effects of that exchange. I have shown, that such an exchange decreases Ssq1 ATPase stimulation and inhibits yeast growth *in vivo*. These results confirm that charged residues participate in the functional interaction between $Jac1_{Sc}$ J-domain and Ssq1.

Based on the results of this comparative study and on the structural model of $Jac1_{Sc}$:Ssq1 complex, our team has identified residues that might facilitate the interaction between $Jac1_{Sc}$ J-domain and Ssq1. These residues are R37, K38, R41, K70 in $Jac1_{Sc}$ and D246, E248, D249, E253 in Ssq1. To verify these predictions, I investigated the effects of alanine substitutions of these residues on Ssq1 ATPase stimulation by $Jac1_{Sc}$ and on the stimulation of Ssq1:Isu complex formation. I have shown that substituting any of the predicted residues affects $Jac1_{Sc}$:Ssq1 interaction in both experimental assays. $Jac1_{Sc}$ R37A and R41A variants showed the strongest effects, as well as the RR/AA double variant. What is more, $Jac1_{Sc}$ RR/AA mutant inhibited yeast growth *in vivo*. I observed the strongest effects for Ssq1 D246A and E253A single variants as well as the double variant. Next, by using site-specific chemical cross-linking, I have shown that the physical $Jac1_{Sc}$:Ssq1 interaction is possible only when the electrostatic interactions are intact.

My results demonstrate that the J-domain of $Jac1_{Sc}$ interacts with Ssq1 through a network of electrostatic interactions, involving positively charged residues of the J-domain and negatively charged residues of Ssq1. These results may aid studies of other JDP:Hsp70 interactions, because Hsp70 systems are evolutionarily conserved.