Julia Magdalena Majewska

Streszczenie rozprawy doktorskiej w języku angielskim: **Structure-function bases for the interaction of cysteine desulfurase Nfs1(Isd11), with other components of FeS cluster biogenesis.**

In mitochondria FeS clusters, prosthetic groups critical for the activity of many proteins, are assembled on Isu1, a 14kDa scaffold protein, and then transferred to recipient apoproteins. The assembly process involves interaction of Isu1 with Nfs1(Isd11), the cysteine desulfurase serving as a sulfur donor, and the yeast frataxin homologue Yfh1, a regulator of cysteine desulfurase activity. Then the transfer of FeS cluster from Isu1 to acceptor protein requires co-chaperone Jac1 and Hsp70 protein Ssq1.

The results of molecular modelling of Nfs1-Isu1 complex, which was guided by the available x-ray structure for bacterial homologs, predicted the importance of the hydrophobic patch required for the interaction of Nfs1 with Isu1. Within Nfs1 these were L479 and M482 residues, which aligned against L63, V72 and F94 Isu1 residues. Using the Nfs1 and Isu1 variants having alterations in those residues, the importance of predicted residues for Nfs1(Isd11)-Isu1 complex formation was validated by *in vitro* binding assays. *In vivo* experiments have shown that Nfs1(Isd11)-Isu1 complex plays indispensible role in the essential process of FeS cluster biogenesis. Next, guided by model of Nfs1-Isu1-Yfh1 assembly complex, I predicted electrostatic interactions between R313, R316 and R318 residues of Nfs1 and D86, E89 residues of Yfh1. *In vivo* data suggested that predicted residues of Nfs1 are not only involved in Yfh1 binding, but also serve as a binding site for ferrodoxin, a protein whose activity in needed for the reduction of sulfur during FeS cluster assembly.

In addition, Nfs1 was found to require the same hydrophobic L63, V72 and F94 residues of Isu1 for binding, as does Jac1, suggesting that Jac1 and Nfs1 binding is mutually exclusive. In support of this conclusion, Jac1 and Nfs1 compete for binding to Isu. Evolutionary analysis revealed that residues involved in these interactions are conserved and critical for the biogenesis of FeS cluster protein *in vivo*.

These results suggest, that exclusivity of Isu1 binding by Nfs1/Jac1 has functional consequences for the transition from the FeS cluster assembly process on Isu1 to the transfer of FeS cluster from Isu1 to recipient protein, and thus regulation of the biogenesis of FeS cluster proteins.