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# „Biochemical reconstitution of protein complexes involved in mitochondrial iron-sulfur clusters biogenesis”

Iron-sulfur clusters FeS are prosthetic groups critical for the activity of many proteins that are essential for the survival of the cell. In all Eukaryotes FeS cluster biogenesis takes place in mitochondria, where they are synthetized by dynamic complex of interacting proteins. Until today over 20 different proteins were identified to be important in this process, but the very core of FeS cluster assembly complex is formed by molecular scaffold protein Isu1, cysteine desulfurase Nfs1(Isd11) and frataxin Yfh1. Isu1 serves as a place of cluster biosynthesis, Nfs1(Isd11) is a sulfur donor and Yfh1 is a Nfs1 activity regulator and/or iron donor. Although it’s well understood that these proteins have to interact with each other, little is known about the molecular mechanism of this phenomena.

Using available structural and biochemical data I predicted surface exposed residues critical for individual protein:protein interactions within tripartite Yfh1:Isu1:Nfs1(Isd11) iron-sulfur assembly complex of yeast *Saccharomyces cerevisiae*. I verified my predictions biochemically using a semi-quantitative *in vitro* pull-down assay utilizing GST-Yfh1 and Isu1-GST fusion proteins. First I observed that the GST-Yfh1 protein binds only to preformed Isu1:Nfs1 complex and does not interact with either of those proteins individually. Next, using mutated proteins $Yfh1^{D86K/E89K}$ and $Nfs1^{R313E/R316E/R318E}$ (Isd11), I showed that these residues are responsible for Yfh1:Nfs1(Isd11) interaction within FeS assembly complex. In similar manner I showed the importance of Yfh1 W131 residue and the P134, V135, K136 (PVK) motif of Isu1 in their direct interaction of those proteins within FeS assembly complex. To verify the functional meaning of my findings, I analyzed those mutations in context of regulation of desulfurase enzymatic activity. When I used wild type proteins, Nfs1(Isd11) activity was inhibited by Isu1 alone, but stimulated upon simultaneous addition of Yfh1, which did not influence Nfs1(Isd11) activity individually. According to pull-down results, mutated proteins lost the ability to interact with each other and therefore were not able to regulate

Nfs1(Isd11) activity. Disruption of these protein:protein interactions resulted in yeast growth impairment, although mutated proteins were produced on level equal to wild type proteins.

These results indicate that frataxin Yfh1 binds to FeS cluster assembly complex by its D86 E89 residues, that interact with Nfs1 R313, R316, R318 residues, and by W131 residue that interacts with PVK motif of Isu1. What's more, all those residues were highly evolutionary conserved among bacteria and eukaryotes, which strongly suggests that identified binding interfaces could be responsible for general molecular mechanism of "FeS cluster synthesis complex" formation.