Structure of the Human Cysteine Desulfurase Complex

Iron-sulfur (Fe-S) clusters are small, inorganic cofactors that are essential for all living organisms by participating in a variety of functions such as substrate activation, DNA replication and repair, and respiration. Iron-sulfur (Fe-S) clusters are synthesized and distributed in eukaryotic mitochondria by an evolutionarily conserved set of proteins in the iron-sulfur cluster (ISC) assembly pathway. In order to assemble a cluster, eukaryotes utilize NFS1, a cysteine desulfurase, to catalyzes the conversion of cysteine to alanine and persulfide sulfur. This persulfide sulfur is ultimately transferred to the scaffold protein ISCU2 where [2Fe-2S] cluster assembly occurs with the addition of electrons from ferredoxin (FDX2). Interestingly, NFS1 requires a eukaryotic specific adaptor protein ISD11 and acyl-carrier protein (ACP) in order to function properly. In addition, the most notable protein in this pathway is frataxin (FXN), associated with Freidreich's ataxia, and has been shown to activate NFS1 and increase the rate persulfide transfer to ISCU2 and ultimately [2Fe-2S] cluster assembly.[1]

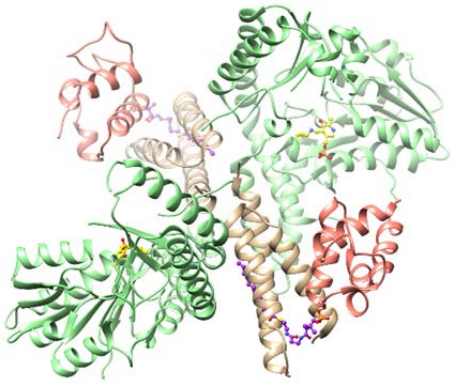

Figure 1. X-ray crystal structure of human NFS1-ISD11 in complex with *E. coli* ACP. NFS1 (light green) is a type I cysteine desulfurase (PLP, yellow) that forms a tight complex with the essential human protein ISD11 (tan). The three-helix bundle fold of ISD11 is stabilized by both electrostatic interactions with *E. coli* ACP (salmon) and hydrophobic interactions provided by ACP's bound lipid (purple). The NFS1-ISD11-ACP_{ec} complex forms a two-fold symmetrical $\alpha_2\beta_2\gamma_2$ complex.

When compared to the prokaryotic version of the ISC pathway, there are distinct mechanistic differences which remain poorly understood. First, the prokaryotic cysteine desulfurase, IscS, is fully active in the absence of any adaptor proteins.[2] Second, the prokaryotic homologue of FXN, CyaY, acts as an inhibitor of Fe-S cluster biosynthesis in *in vitro* assays.[2-3] Although these mechanistic differences have been discovered, little structural information has shed light on these differences and provided insights into FXN based activation of the eukaryotic Fe-S cluster biosynthetic complex.

This study, led by researchers at Texas A&M University, University of Utah, and Massachusetts Institute of Technology, shows, for the first time, the x-ray crystal structure of a eukaryotic cysteine desulfurase (NFS1) in complex with two essential adaptor proteins (ISD11 and ACP_{ec}).[4] Using molecular replacement – single wavelength anomalous dispersion (MR-SAD), the structure of human NFS1-ISD11 in complex with *E. coli* ACP_{ec} was determined to 3.09 Å. The NFS1-ISD11-ACP complex is the central sub-complex involved in providing sulfur for cluster synthesis. The data was collected at SSRL BL 7-1 and published in *PNAS* (June 2017). The structure revealed that NFS1 forms an unexpected cysteine desulfurase dimer architecture that is stabilized by the 3-helix adaptor protein ISD11. Furthermore, ISD11 is stabilized by interactions with ACP through both an electrostatic interface and a hydrophobic interface facilitated by the lipid bound to ACP's 4'phosphopantetheine cofactor (Figure 1). Negative stain electron microscopy was also used to evaluate the new architecture and yielded a 15 Å resolution 3D reconstruction that fit well with the x-ray crystal structure. Furthermore *in vitro* and *in vivo* assays were used to evaluate the biosynthetic complex and probe various protein-protein interfaces. This first x-ray

structure provides a framework for further mechanistic studies of human Fe-S cluster biosynthesis which will aid the understanding of diseases associated with this assembly pathway.

References

1. J. J. Braymer and R. Lill, "Iron-Sulfur Cluster Biogenesis and Trafficking in Mitochondria", *J Biol Chem.* **292**, 12754 (2017) doi: 10.1074/jbc.R117.787101.
2. J. Bridwell-Rabb, C. Iannuzzi, A. Pastore and D. P. Barondeau, "Effector Role Reversal during Evolution: The Case of Frataxin in Fe–S Cluster Biosynthesis", *Biochemistry* **51**, 2506 (2012) doi: 10.1021/bi201628j.
3. S. Adinolfi, C. Iannuzzi, F. Prischi, C. Pastore, S. Iametti, S. R. Martin, F. Bonomi and A. Pastore, "Bacterial Frataxin CyaY is the Gatekeeper of Iron-Sulfur Cluster Formation Catalyzed by IscS", *Nat. Struct. Mol. Biol.* **16**, 390 (2009) doi: 10.1038/nsmb.1579.
4. S. A. Cory, J. G. Van Vranken, E. J. Brignole, S. Patra, D. R. Winge, C. L. Drennan, J. Rutter and D. P. Barondeau, "Structure of Human Fe-S Assembly Subcomplex Reveals Unexpected Cysteine Desulfurase Architecture and Acyl-ACP-ISD11 Interactions", *Proc. Natl. Acad. Sci. USA* **114**, E5325 (2017) doi: 10.1073/pnas.1702849114.

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