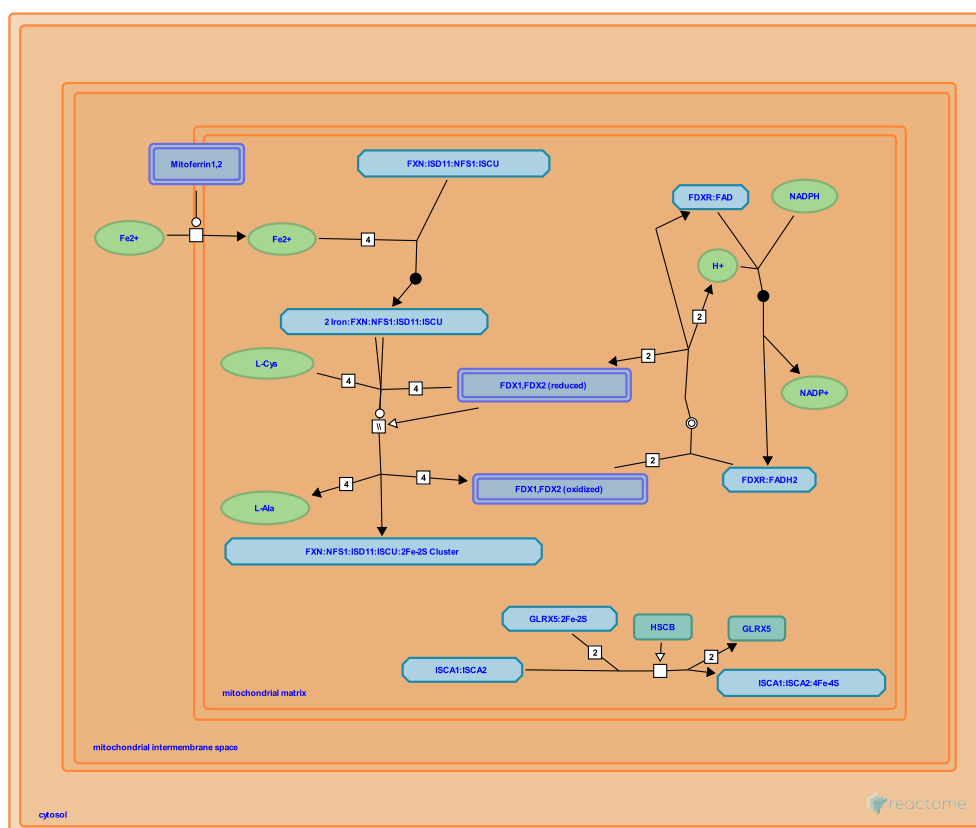


Mitochondrial iron-sulfur cluster biogenesis



Banci, L., Lill, R., May, B., Rouault, TA., Tong, WH.

European Bioinformatics Institute, New York University Langone Medical Center, Ontario Institute for Cancer Research, Oregon Health and Science University.

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This is just an excerpt of a full-length report for this pathway. To access the complete report, please download it at the [Reactome Textbook](https://reactome.org/page/about-us).

03/05/2024

Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

Literature references

Fabregat, A., Sidiropoulos, K., Viteri, G., Forner, O., Marin-Garcia, P., Arnau, V. et al. (2017). Reactome pathway analysis: a high-performance in-memory approach. *BMC bioinformatics*, 18, 142. [↗](#)

Sidiropoulos, K., Viteri, G., Sevilla, C., Jupe, S., Webber, M., Orlic-Milacic, M. et al. (2017). Reactome enhanced pathway visualization. *Bioinformatics*, 33, 3461-3467. [↗](#)

Fabregat, A., Jupe, S., Matthews, L., Sidiropoulos, K., Gillespie, M., Garapati, P. et al. (2018). The Reactome Pathway Knowledgebase. *Nucleic Acids Res*, 46, D649-D655. [↗](#)

Fabregat, A., Korninger, F., Viteri, G., Sidiropoulos, K., Marin-Garcia, P., Ping, P. et al. (2018). Reactome graph database: Efficient access to complex pathway data. *PLoS computational biology*, 14, e1005968. [↗](#)

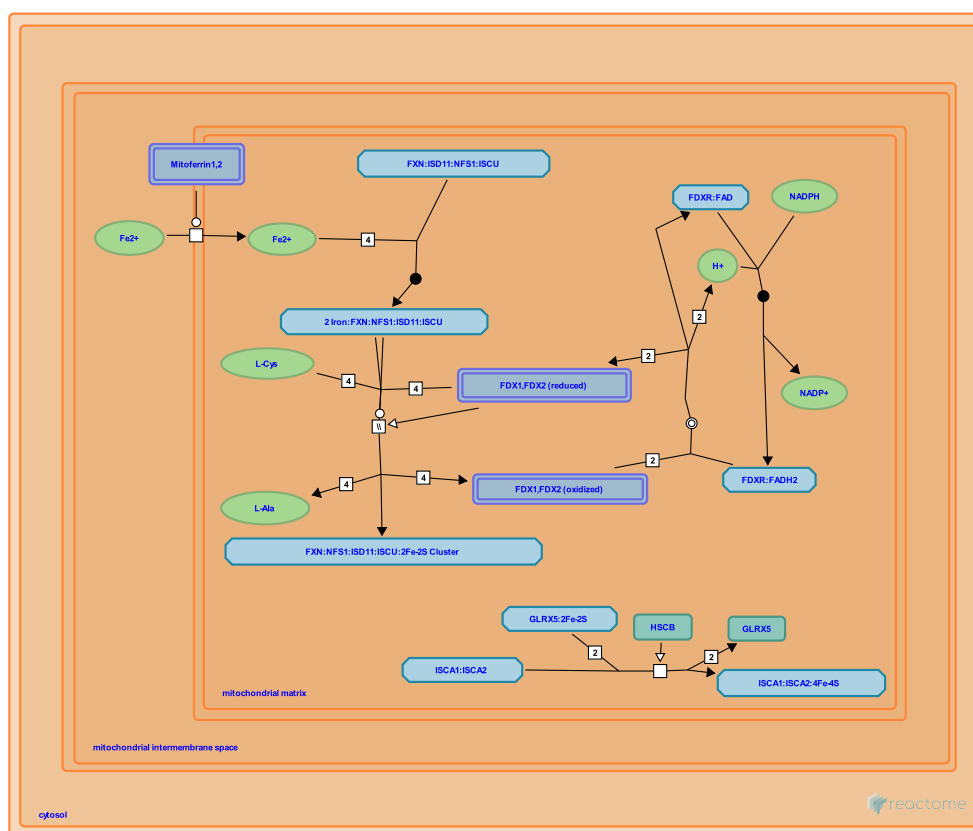
Reactome database release: 88

This document contains 2 pathways and 4 reactions ([see Table of Contents](#))

Mitochondrial iron-sulfur cluster biogenesis [↗](#)

Stable identifier: R-HSA-1362409

Compartments: mitochondrial inner membrane, mitochondrial matrix, mitochondrial intermembrane space



Iron-sulfur (Fe-S) proteins are localized in the cytosol, nucleus, and mitochondria of mammalian cells (reviewed in Stemmler et al. 2010, Rouault 2012, Bandyopadhyay et al. 2008, Lill 2009, Lill et al. 2012). Fe-S protein biogenesis in the mitochondrial matrix involves the iron-sulfur cluster (ISC) assembly machinery. Ferrous iron is transported across the inner mitochondrial membrane into the mitochondrial matrix by Mitoferrin-1 (SLC25A37) and Mitoferrin-2 (SLC25A28). (Mitoferrin-1 is enriched in erythroid cells while Mitoferrin-2 is ubiquitous.) Frataxin binds ferrous iron in the mitochondrial matrix. The cysteine desulfurase NFS1 in a subcomplex with ISD11 provides the sulfur by converting cysteine into alanine and forming a persulfide which is used for cluster formation on ISCU, the scaffold protein. Interaction between NFS1 and ISD11 is necessary for desulfurase activity. Frataxin binds to a complex containing NFS1, ISD11, and ISCU and is proposed to function as an iron donor to ISCU or as an allosteric switch that activates sulfur transfer and Fe-S cluster assembly (Tsai and Barondeau 2010). Cluster formation also involves the electron transfer chain ferredoxin reductase and ferredoxin. ISCU initially forms clusters containing 2 iron atoms and 2 sulfur atoms ([2Fe-2S] clusters). They are released by the function of HSP70-HSC20 chaperones and the monothiol glutaredoxin GLRX5 and used for assembly of [2Fe-2S] proteins. Assembly of larger clusters such as [4Fe-4S] clusters may involve the function of ISCA1, ISCA2, and IBA57. The clusters are transferred to apo-enzymes such as the respiratory complexes, aconitase, and lipoate synthase through dedicated targeting factors such as IND1, NFU1, and BOLA3.

Literature references

- Lill, R., Stehling, O. (2013). The role of mitochondria in cellular iron-sulfur protein biogenesis: mechanisms, connected processes, and diseases. *Cold Spring Harb Perspect Biol*, 5, a011312. [↗](#)
- Rouault, TA. (2012). Biogenesis of iron-sulfur clusters in mammalian cells: new insights and relevance to human disease. *Dis Model Mech*, 5, 155-64. [↗](#)
- Tsai, CL., Barondeau, DP. (2010). Human frataxin is an allosteric switch that activates the Fe-S cluster biosynthetic complex. *Biochemistry*, 49, 9132-9. [↗](#)

Webert, H., Pierik, AJ., Lill, R., Hoffmann, B., Stehling, O., Uzarska, MA. et al. (2012). The role of mitochondria in cellular iron-sulfur protein biogenesis and iron metabolism. *Biochim. Biophys. Acta*, 1823, 1491-508. [↗](#)

Lill, R. (2009). Function and biogenesis of iron-sulphur proteins. *Nature*, 460, 831-8. [↗](#)

Editions

2011-06-04	Authored, Edited	May, B.
2012-09-24	Authored	Lill, R.
2012-10-12	Reviewed	Rouault, TA., Tong, WH.

Mitoferrin translocates iron from the mitochondrial intermembrane space to the mitochondrial matrix ↗

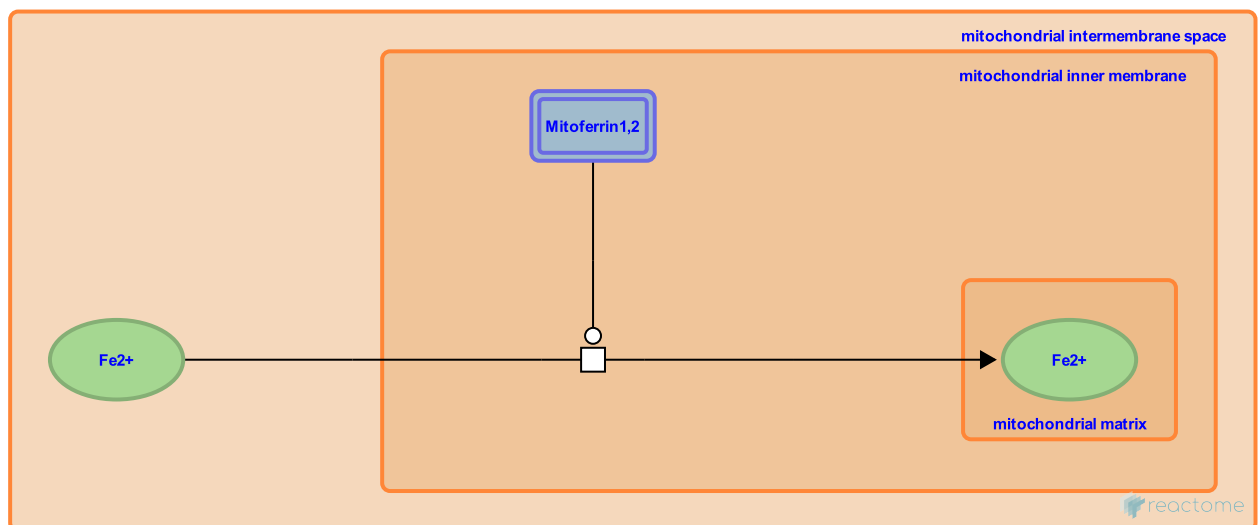
Location: [Mitochondrial iron-sulfur cluster biogenesis](#)

Stable identifier: R-HSA-1362417

Type: transition

Compartments: mitochondrial inner membrane, mitochondrial intermembrane space, mitochondrial matrix

Inferred from: [MRS3,4 Transports Iron Across the Mitochondrial Inner Membrane \(Saccharomyces cerevisiae\)](#), [Mitoferrin Transports Iron Across the Mitochondrial Inner Membrane \(Mus musculus\)](#)



As inferred from biochemical studies in yeast and phenotypic studies in mouse, Mitoferrin-1 (SLC25A37) and Mitoferrin-2 (SLC25A28) transport ferrous iron across the inner mitochondrial membrane. Mitoferrin-1 is essential for maintaining mitochondrial iron uptake in developing erythroid cells; mitoferrin-2 is ubiquitously expressed. Defects in Mitoferrin-1 and Mitoferrin-2 cause a reduction in mitochondrial iron acquisition and biogenesis of iron-sulfur clusters and heme.

Followed by: [Fratxin binds iron](#)

Editions

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2012-10-12	Reviewed	Rouault, TA., Tong, WH.

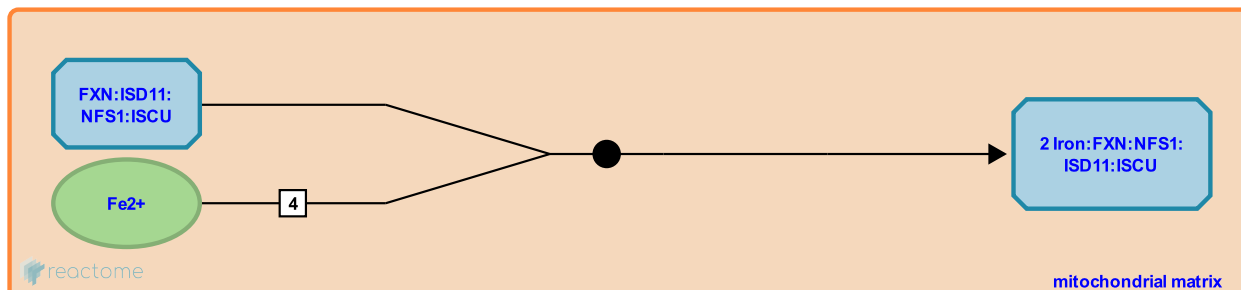
Frataxin binds iron ↗

Location: [Mitochondrial iron-sulfur cluster biogenesis](#)

Stable identifier: R-HSA-1362416

Type: binding

Compartments: mitochondrial matrix



Frataxin (FXN) specifically binds 2 atoms of ferrous iron per monomer (reviewed in Stemmler et al. 2010). Iron bound to Frataxin may (Yoon and Cowan 2003, Gerber et al. 2003) or may not (Schmucker et al. 2011) enhance the interaction of Frataxin with NFS1, ICSU, and ISD11. Frataxin was shown to stimulate the cysteine desulfurase activity of NFS1 and was proposed to be a regulator of sulfur production (Tsai et al. 2010). The formation of sulfide by NFS1 is most efficiently observed when NFS1 is in complex with ISD11, ISCU, and FXN in the presence of cysteine and iron. This means that only the complete system of NFS1, ISD11, ISCU, FXN, cysteine, and iron is fully active as a desulfurase. FXN therefore seems to be a regulator of the cysteine desulfurase permitting sulfide production only when all components needed for Fe-S cluster synthesis are present and the ISCU-bound Fe-S cluster can be formed.

Preceded by: [Mitoferrin translocates iron from the mitochondrial intermembrane space to the mitochondrial matrix](#)

Followed by: [FXN:NFS1:ISD11:ISCU assembles 2Fe-2S iron-sulfur cluster](#)

Literature references

- Cowan, JA., Yoon, T. (2003). Iron-sulfur cluster biosynthesis. Characterization of frataxin as an iron donor for assembly of [2Fe-2S] clusters in ISU-type proteins. *J Am Chem Soc*, 125, 6078-84. ↗
- Winn, AM., Barondeau, DP., Bridwell-Rabb, J. (2011). Structure-function analysis of Friedreich's ataxia mutants reveals determinants of frataxin binding and activation of the Fe-S assembly complex. *Biochemistry*, 50, 7265-74. ↗
- Reutenauer, L., Puccio, H., Colin, F., Schmucker, S., Wattenhofer-Donzé, M., Martelli, A. et al. (2011). Mammalian frataxin: an essential function for cellular viability through an interaction with a preformed ISCU/NFS1/ISD11 iron-sulfur assembly complex. *PLoS One*, 6, e16199. ↗
- O'Neill, HA., Cavadini, P., Benada, O., Isaya, G. (2002). Assembly and iron-binding properties of human frataxin, the protein deficient in Friedreich ataxia. *Hum Mol Genet*, 11, 217-27. ↗
- Tsai, CL., Barondeau, DP. (2010). Human frataxin is an allosteric switch that activates the Fe-S cluster biosynthetic complex. *Biochemistry*, 49, 9132-9. ↗

Editions

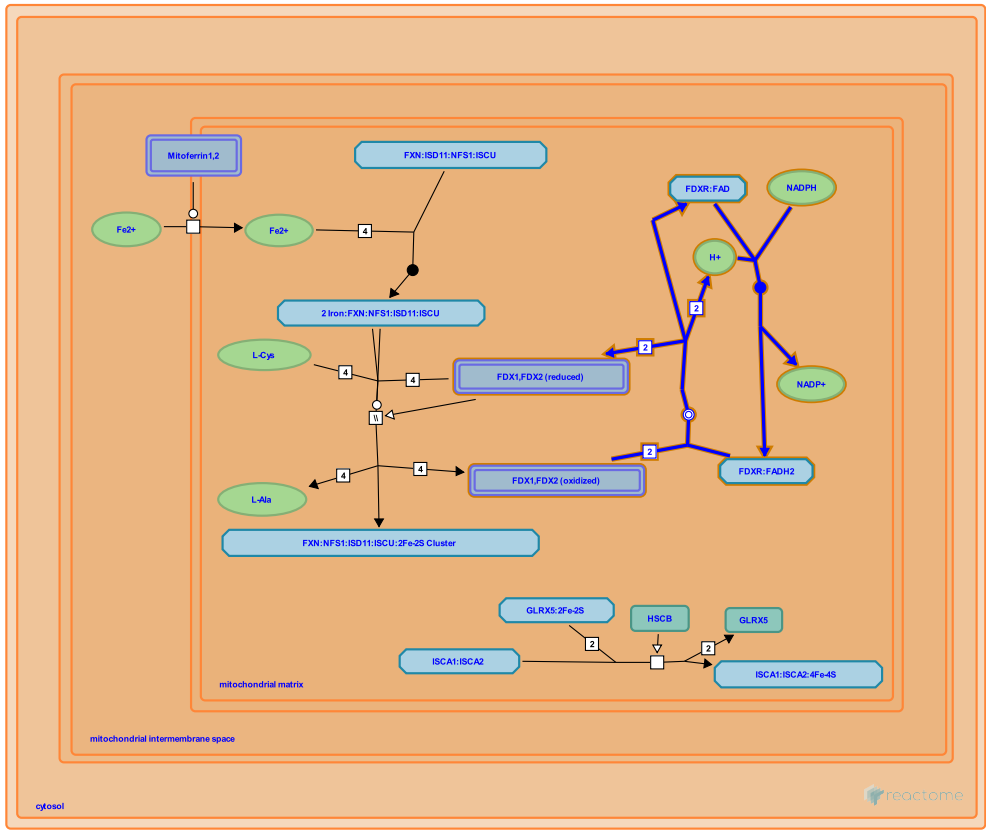
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Electron transport from NADPH to Ferredoxin ↗

Location: Mitochondrial iron-sulfur cluster biogenesis

Stable identifier: R-HSA-2395516

Compartments: mitochondrial matrix



NADPH, ferredoxin reductase (FDXR, Adrenodoxin reductase), and ferredoxins (FDX1, FDX1L) comprise a short electron transport chain that provides electrons for biosynthesis of iron-sulfur clusters and steroid hormones (Sheftel et al. 2010, Shi et al. 2012, reviewed in Grinberg et al. 2000, Lambeth et al. 1982).

Literature references

Bernhardt, R., Heinemann, U., Schiffler, B., Grinberg, AV., Müller, J., Hannemann, F. (2000). Adrenodoxin: structure, stability, and electron transfer properties. *Proteins*, 40, 590-612. ↗

Hobler, A., Webert, H., Pierik, AJ., Lill, R., Elsässer, HP., Bernhardt, R. et al. (2010). Humans possess two mitochondrial ferredoxins, Fdx1 and Fdx2, with distinct roles in steroidogenesis, heme, and Fe/S cluster biosynthesis. *Proc. Natl. Acad. Sci. U.S.A.*, 107, 11775-80. ↗

Kamin, H., Seybert, DW., Lancaster, JR., Lambeth, JD., Salerno, JC. (1982). Steroidogenic electron transport in adrenal cortex mitochondria. *Mol. Cell. Biochem.*, 45, 13-31. ↗

Shi, Y., Kovtunovych, G., Crooks, DR., Ghosh, M., Rouault, TA. (2012). Both human ferredoxins 1 and 2 and ferredoxin reductase are important for iron-sulfur cluster biogenesis. *Biochim. Biophys. Acta*, 1823, 484-92. ↗

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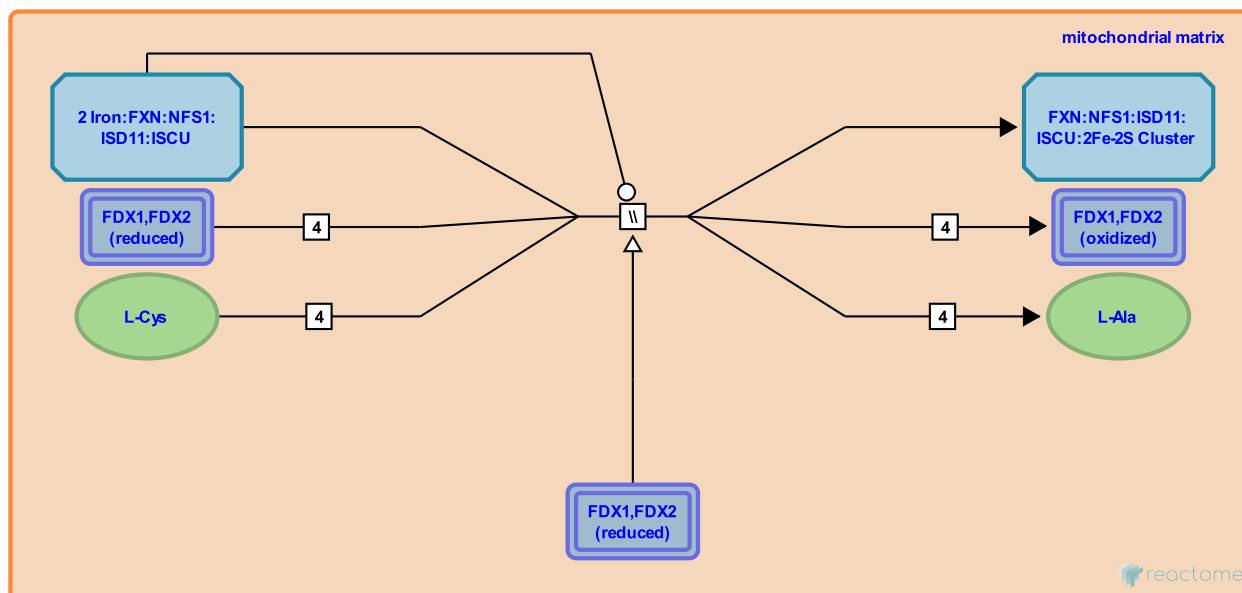
FXN:NFS1:ISD11:ISCU assembles 2Fe-2S iron-sulfur cluster ↗

Location: Mitochondrial iron-sulfur cluster biogenesis

Stable identifier: R-HSA-1362408

Type: omitted

Compartments: mitochondrial matrix



Iron-sulfur clusters are assembled on the scaffold, ISCU. Based on homology with bacterial IscU:IscS complexes (reviewed in Johnson et al. 2005), one molecule of ISCU is bound to each subunit of a NFS1 dimer (Marinoni et al. 2012). A single complex may thus be capable of assembling two 2Fe-2S clusters. Sulfide is provided by desulfuration of cysteine by NFS1:ISD11 (Biederbick et al. 2006, Shi et al. 2009, Tsai and Barondeau 2010). It has been proposed that ferrous iron is delivered by FXN (Gerber et al. 2003, Yoon and Cowan 2003, Schmucker et al. 2011) bound to ISCU (inferred from yeast, Wang and Craig 2008), although more recent studies suggested that FXN functions as an allosteric effector to stimulate sulfide transfer (Tsai et al. 2010). Holo-ISCU (ISCU bound to a newly synthesized 2Fe-2S cluster) transiently interacts with a dedicated HSP70 chaperone system including Mortalin (GRP75) and HSP20 and GLRX5 (GRX5). Electrons supplied by FDX2 (FDX1L) are required (Tong et al. 2003, Cai et al. 2017) and may reduce the sulfur from S⁰ to S²⁻ (sulfide). NFU1 binds an Fe-S cluster (Tong et al. 2003, inferred from bacteria Yuvaniyama et al. 2000) and, from biochemical studies of bacterial NFU1 homologues, is proposed to be an intermediate Fe-S cluster carrier (Bandyopadhy et al. 2008). Mutations in human NFU1 affect only a subset of Fe-S proteins (Navarro-Sastre et al. 2011).

Preceded by: Frataxin binds iron

Literature references

- Lill, R., Gerber, J., Mühlenhoff, U. (2003). An interaction between frataxin and Isu1/Nfs1 that is crucial for Fe/S cluster synthesis on Isu1. *EMBO Rep.*, 4, 906-11. ↗
- Johnson, MK., Dean, DR., Johnson, DC., Smith, AD. (2005). Structure, function, and formation of biological iron-sulfur clusters. *Annu. Rev. Biochem.*, 74, 247-81. ↗
- Chakrabarti, M., McCormick, SP., Fox, NG., Barondeau, DP., Lindahl, PA. (2015). The Human Iron-Sulfur Assembly Complex Catalyzes the Synthesis of [2Fe-2S] Clusters on ISCU2 That Can Be Transferred to Acceptor Molecules. *Biochemistry*, 54, 3871-9. ↗
- Iannuzzi, C., Pastore, A., Barondeau, DP., Bridwell-Rabb, J. (2012). Effector role reversal during evolution: the case of frataxin in Fe-S cluster biosynthesis. *Biochemistry*, 51, 2506-14. ↗
- Shi, Y., Kovtunovych, G., Crooks, DR., Ghosh, M., Rouault, TA. (2012). Both human ferredoxins 1 and 2 and ferredoxin reductase are important for iron-sulfur cluster biogenesis. *Biochim. Biophys. Acta*, 1823, 484-92. ↗

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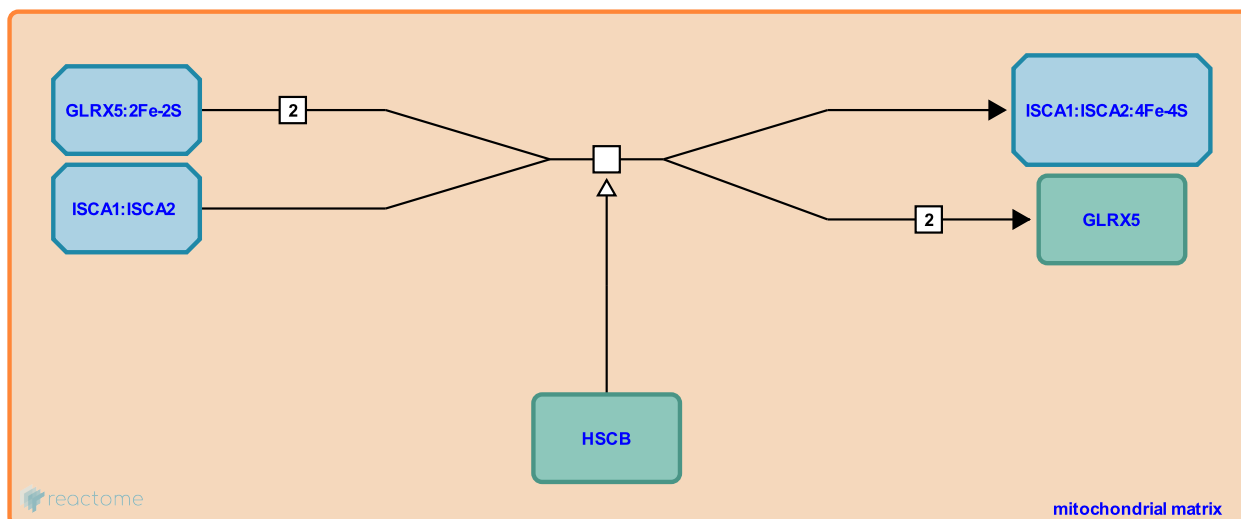
Formation of 4Fe-4S cluster on ISCA1:ISCA2 ↗

Location: Mitochondrial iron-sulfur cluster biogenesis

Stable identifier: R-HSA-8878815

Type: transition

Compartments: mitochondrial matrix



Iron-sulfur clusters containing 4Fe-4S are assembled from 2Fe-2S clusters on ISCA1:ISCA2 heterodimers (Banci et al. 2014, Brancaccio et al. 2014, inferred from *Saccharomyces cerevisiae* in Mühlenhoff et al. 2011). GLRX5:2Fe-2S can donate 2Fe-2S clusters to ISCA1:ISCA2 in vitro (Banci et al. 2014, Brancaccio et al. 2014). It is unclear if other proteins also donate 2Fe-2S clusters. Two conserved C-terminal cysteines of ISCA1:ISCA2 heterodimers extract [2Fe-2S] clusters from GLRX5, forming a ISCA1:ISCA2:GLRX5 intermediate containing two 2Fe-2S clusters (Brancaccio et al. 2017). The physiological electron donor required to convert the two 2Fe-2S clusters bound to the intermediate into a 4Fe-4S cluster is not yet characterized. ISCA1, ISCA2, and IBA57 are required for formation of holoenzymes such as aconitase that contain 4Fe-4S clusters (Sheftel et al. 2012). HSCB (HSC20), the homolog of yeast JAC1, interacts with HSPA9 and appears to facilitate the reaction (Uhrigshardt et al. 2010).







Literature references

- Pierik, A.J., Lill, R., Pines, O., Richter, N., Mühlenhoff, U. (2011). Specialized function of yeast Isa1 and Isa2 proteins in the maturation of mitochondrial [4Fe-4S] proteins. *J. Biol. Chem.*, 286, 41205-16. ↗
- Ciofi-Baffoni, S., Banci, L., Piccioli, M., Brancaccio, D., Gallo, A., Novellino, E. (2017). [4Fe-4S] Cluster Assembly in Mitochondria and Its Impairment by Copper. *J. Am. Chem. Soc.*, 139, 719-730. ↗
- Ciofi-Baffoni, S., Banci, L., Mikolajczyk, M., Piccioli, M., Zovo, K., Brancaccio, D. et al. (2014). Formation of [4Fe-4S] clusters in the mitochondrial iron-sulfur cluster assembly machinery. *J. Am. Chem. Soc.*, 136, 16240-50. ↗
- Kovtunovych, G., Ghosh, M., Singh, A., Rouault, T.A., Uhrigshardt, H. (2010). Characterization of the human HSC20, an unusual DnaJ type III protein, involved in iron-sulfur cluster biogenesis. *Hum. Mol. Genet.*, 19, 3816-34. ↗
- Niggemeyer, B., Lill, R., Elsässer, H.P., Stehling, O., Sheftel, A.D., Wilbrecht, C. et al. (2012). The human mitochondrial ISCA1, ISCA2, and IBA57 proteins are required for [4Fe-4S] protein maturation. *Mol. Biol. Cell*, 23, 1157-66. ↗

Editions

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