Iron & Disease - Section 1

Mechanisms of FeS protein biogenesis and related diseases

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Introduction
Iron-sulfur (FeS) clusters are versatile protein cofactors involved in electron transfer processes, catalytic reactions, and the sensing of environmental conditions. They are part of mitochondrial, cytosolic and nuclear proteins involved in, e.g., respiration, metabolic reactions, RNA modification, antiviral defense, DNA replication and repair as well as ribosome assembly and recycling (Figure 1).1,2 Synthesis, trafficking and polypeptide insertion of [2Fe-2S] and [4Fe-4S] clusters are complex processes involving more than 30 known proteins (Figure 2).3-5 These evolutionarily conserved biogenesis factors belong to either the mitochondrial iron-sulfur cluster assembly (ISC) machinery, the mitochondrial ABCB7 export system, or the cytosolic iron-sulfur protein assembly (CIA) machinery. Both the ISC and CIA machineries follow similar biosynthetic mechanisms with two major steps: i) the de novo assembly of a FeS cluster on a scaffold protein, and ii) the trafficking and insertion of the newly assembled cofactor into target apoproteins. Genetic defects of almost any of the ISC components and of ABCB7 are associated with severe rare diseases displaying neurological, metabolic and hematological phenotypes (Figure 2A).6,7 Some cytosolic FeS proteins required for tRNA modification have been connected to neuropahties,8 and several nuclear FeS proteins involved in genome maintenance (e.g., DNA polymerases and helicases, Fanconi anemia protein FANCJ, Xeroderma pigmentosum group D protein XPD) are linked to various forms of cancer and aging (Figure 1).9

Mitochondrial FeS protein biogenesis
Maturation of all cellular FeS proteins is initiated in the mitochondrial matrix by the core ISC complex that de novo assembles a [2Fe-2S] cluster on the scaffold protein ISCU (Figure 2A).9 Sulfide is generated by the cytosine desulfurase complex NFS1-ISD11-ACP1 from cysteinyl sulfur which is reduced by the NADPH - ferredoxin reductase - ferredoxin (FDX2) electron transfer chain during cluster synthesis.10-12 Frataxin may act as an iron donor and/or may stimulate sulfur transfer from NFS1 to the ISCU scaffold.13 The newly assembled [2Fe-2S] cluster is then released from ISCU by a dedicated Hsp70 (HSPA9) and Hsp40 (HSC20) chaperone system,14 and transferred to a dimer of the monothiol glutaredoxin GLRX5.15,16 The GLRX5-bound cofactor can then be directly inserted into [2Fe-2S] target proteins or become converted to a [4Fe-4S] cluster with the help of the late-acting ISC factors ISC A1, ISC A2, and IB A57 (Figure 2A).17,18 Cluster insertion into the dedicated polypeptide chains is finally achieved by the specific targeting factors BO L A3, N FU1, and IN D1.19,20 The latter two proteins transiently bind the moving [4Fe-4S] cluster. The core ISC components and GLRX5 are additionally involved in the generation of a sulfur-containing compound (X-S in Figure 2B) that is exported by the mitochondrial ABC transporter ABCB7 to the cytosol for use by the CIA machinery.

Cytosolic-nuclear FeS protein biogenesis
The first step in the CIA pathway is the generation of a [4Fe-4S] cluster on the scaffold complex CFD1-NBP35 (Figure 2B). This reaction requires reduction by the electron transfer chain NADPH-NDOR1-CAPIN1 (as found in yeast). The [4Fe-4S] cluster is then released in an ATP-dependent fashion from the scaffold, and transferred via IOP1 and the CIA targeting complex (CIAO1, CIA2B, and MMS19) to dedicated recipient apoproteins.21 Some target FeS proteins require dedicated assembly factors. For instance, iron regulatory protein 1 (IRP1) depends on CIA2A, a homolog of CIA2B, for FeS cluster insertion.21 The FeS cluster-containing ABC protein ABCE1 involved in ribosome recycling is specifically recruited to the CIA targeting complex by a dedicated adapter pair ORAOV1-YAE1D1 (as found in yeast) for cofactor insertion (Figure 2B).22

Take Home Messages
- In eukaryotes, FeS cluster synthesis, trafficking and insertion into apoproteins is catalyzed by the mitochondrial ISC machinery and the cytosolic CIA system.
- Almost all of the ISC genes is associated with a disease displaying diverse neurological, hematological, or metabolic phenotypes with frequent lethality in early childhood.
- Progress in the molecular understanding of mitochondrial FeS protein biogenesis has improved the interpretation of the biochemical phenotypes of the diseased states, but not the prediction of the overall clinical phenotype.
Diseases in FeS protein biogenesis

Genetic defects in almost any of the ISC factors are causative for the so-called ‘FeS diseases’,
which can be dissected into two major groups: The first group comprises functional deficiencies in early-acting ISC factors including GLRX5 (Figure 2A). These defects affect FeS protein biogenesis in both mitochondria and the cytosol-nucleus, and may show a concomitant impact on cellular iron homeostasis with mitochondrial iron accumulation, as seen in some forms of sideroblastic anemia, e.g., in individuals with mutations in GLRX5 or ABCB7.16,23 This is explained, at least in part, by the role of the core mitochondrial ISC system and of ABCB7 in the maturation of IRP1, which switches its iron regulatory function by FeS cluster assembly or disassembly.4,24 Defective IRP1 maturation leads to increased cellular iron uptake and trafficking to mitochondria, which import the metal via mitoferrin (MFRN1/2) (Figure 2A). Abnormal MFRN1 expression has been linked to erythropoietic protoporphyria due to the low mitochondrial iron. The mitochondrial iron accumulation seen in mutations in core ISC genes can also contribute to a diminished heme content, possibly by creating an oxidative stress condition that decreases synthesis and increases degradation of heme. Other phenotypes of mutations in early-acting ISC genes are respiratory deficiencies and lactic acidosis (NF51 and ISD11),25 myopathies (ISCU, FXD2),26 and neurological symptoms (Friedreich’s ataxia (frataxin), FXDR).27 In contrast, the second group of FeS diseases caused by genetic defects in the late-acting ISC factors (ISCA1, ISCA2, IBA57, NFU1, BOLA3, and IND1) is not associated with conspicuous alterations in cellular iron homeostasis, because these ISC factors do not play a role in cytosolic-nuclear FeS protein assembly (Figure 2A). Affected patients present with rather diverse phenotypes summarized as ‘multiple mitochondrial dysfunction syndromes’ (MDDS).6,7 Frequently this includes severe neurological symptoms, e.g., encephalopathy or leukodystrophy. At the biochemical level, assembly defects of dedicated mitochondrial [4Fe-4S] but not of [2Fe-2S] proteins are observed (Figure 2A) providing insights into the target specificity of these ISC factors. The most obvious biochemical deficiencies are in the respiratory chain complexes I and II, and in the [4Fe-4S] protein lipoate synthase, leading to respiratory defects and impaired protein lipoylation.28,29 The latter defect can be used as a simple and sensitive diagnostic tool to help diagnose these types of FeS diseases.

Future perspectives

The substantial advances in the identification and mechanistic characterization of the machinery assisting cellular FeS protein biogenesis in the last two decades have improved the interpretation of the biochemical phenotypes associated with mitochondrial FeS diseases. Depending on the defective assembly step either all cellular FeS proteins (in case of mutations in core ISC genes), preferentially cytosolic FeS proteins (in case of mutations in ABCB7), or only mitochondrial [4Fe-4S] proteins (in case of mutations in late-acting ISC genes) are functionally impaired. In the former two cases, also a disturbance of the cellular iron...
Figure 2. A current model of cellular FeS protein biogenesis and disease-linked proteins. A) The early and late steps of the mitochondrial ISC pathway, and the role of the core ISC factors and the ABC transporter ABCB7 for the CIA system and cellular iron regulation. The ISC proteins highlighted in yellow are linked to various forms of human disease. The major ‘FeS disease’ phenotypes are presented in the boxes. B) The early and late steps of the CIA pathway and its dependence on mitochondrial ISC function. Recipient FeS proteins are shown in light blue. The models are based on studies conducted mainly in yeast, mouse, Trypanosomes, and human cells. For further details see text. MMDS, multiple mitochondrial dysfunction syndromes; GSH, glutathione; pmf, proton motive force; red, reduced; ox, oxidized; e-, electron; X-S, sulfur-containing compound exported by ABCB7.
homeostasis is observed, due to insufficient maturation of IRP1. In contrast, the explanation of the overall clinical phenotypes of the various FeS diseases, whether neurological, metabolic or hematological, remains to be a future challenge, which is similar to other mitochondrial diseases. In all likelihood, genetic diseases linked to the CIA machinery will be discovered. It will be interesting to define the biochemical and clinical consequences of such disorders. Given the essential character of all known CIA proteins, even subtle functional impairments of these factors may be fatal. The considerable insights into the molecular mechanisms of cellular FeS protein biogenesis and its linked diseases has now opened avenues for developing dedicated treatment strategies.

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