Iron-sulfur cluster biosynthesis and trafficking - Impact on human disease conditions

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Abstract

Iron-sulfur clusters (Fe-S) are one of the most ancient, ubiquitous and versatile classes of metal cofactors found in nature. Proteins that contain Fe-S clusters constitute one of the largest families of proteins, with varied functions that include electron transport, regulation of gene expression, substrate binding and activation, radical generation, and, more recently discovered, DNA repair. Research during the past two decades has shown that mitochondria are central to the biogenesis of Fe-S clusters in eukaryotic cells via a conserved cluster assembly machinery (ISC assembly machinery) that also controls the synthesis of Fe-S clusters of cytosolic and nuclear proteins. Several key steps for synthesis and trafficking have been determined for mitochondrial Fe-S clusters, as well as the cytosol (CIA – cytosolic iron-sulfur protein assembly), but detailed mechanisms of cluster biosynthesis, transport, and exchange are not well established. Genetic mutations and the instability of certain steps in the biosynthesis and maturation of mitochondrial, cytosolic and nuclear Fe-S cluster proteins affects overall cellular iron homeostasis and can lead to severe metabolic, systemic, neurological and hematological diseases, often resulting in fatality. In this review we briefly summarize the current molecular understanding of both, mitochondrial ISC and CIA assembly machineries, and present a comprehensive overview of various associated inborn human disease states.
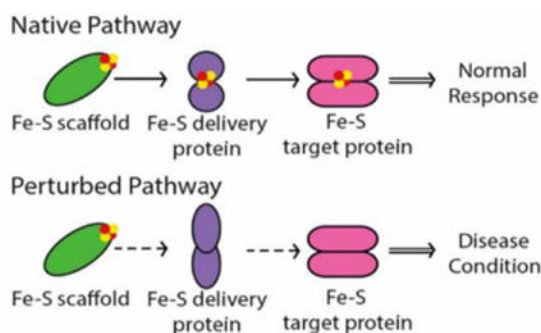
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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here].

Conflicts of interest

The authors declare no conflicts of interest.

Native Pathway

Fe-S scaffold → Fe-S delivery protein → Fe-S target protein ⇒ Normal Response

Perturbed Pathway

Fe-S scaffold ⇢ Fe-S delivery protein ⇢ Fe-S target protein ⇒ Disease Condition

Iron-sulfur cluster biogenesis and trafficking enables the function of a number of cellular proteins. As such, mutations or perturbations in these pathways lead to disease states.

Introduction

Iron-sulfur (Fe-S) clusters are small inorganic cofactors, composed of iron and sulfur. Protein-bound Fe-S clusters are among the most structurally and functionally versatile cofactors across all kingdoms of life.[1] These cofactors are evolutionarily conserved and ubiquitous in nature,[2] with the ability to be formed spontaneously in solution from Fe^{2+}/Fe^{3+}, RS^-, and inorganic S^{2-}, or from Fe^{2+}, R-SH and sulfur under reducing conditions. However, Fe-S clusters are susceptible to oxidation in the presence of oxygen, presenting challenges for cluster synthesis and binding to proteins, due to atmospheric oxygen. In addition, the iron-mediated Fenton reaction poses a severe threat to the genome due to cytotoxicity via DNA damage.[2a, 3] As such, highly conserved and controlled mechanisms are in place for cluster production and trafficking to target proteins to allow for their wide range of function as protein cofactors.

Over the last decade, several new roles associated with Fe-S clusters have emerged, both as a result of progress in identifying and purifying proteins with intact clusters, and also due to the higher level of sophistication of the biophysical techniques used in their characterization. Yet the presence of these clusters remains puzzling, given the challenges present with oxygen and the potential for cellular toxicity. This raises the fundamental question of why Fe-S clusters have been retained as a vital component of essential macromolecules ranging from proteins to nucleic acids, and highlights the need for mechanistic and biochemical studies to promote understanding of the role of these versatile cofactors. With recent advances, a variety of disease conditions have been associated with Fe-S cluster proteins that impact overall cluster trafficking. Herein, we briefly review the cluster biogenesis process and describe the associated inborn human disease phenotypes.

Mitochondrial Fe-S Cluster Biogenesis

Elucidation of the molecular and cellular basis of Fe-S cluster biogenesis and transfer to putative Fe-S cluster proteins has been an area of intense investigation since it was discovered that biogenesis is not spontaneous, but rather catalyzed.[4] Three distinct pathways have been identified for bacterial Fe-S cluster biosynthesis.[5] The ISC system is considered

the major Fe-S cluster assembly system in humans,[1a, 2b] required not only for the maturation of mitochondrial but also cytosolic and nuclear Fe/S proteins. This machinery primarily mediates three steps of Fe-S cluster biosynthesis; namely, (1) cluster assembly on the scaffold protein, (2) chaperone-assisted release of the Fe-S cluster and a currently unclear mechanism of mitochondrial export, if necessary, and (3) subsequent transfer to putative target apo-proteins.[1b, c] Much of the research concerning mitochondrial Fe-S cluster biosynthesis has been carried out in yeast cells, human cells, and with human, yeast, or bacterial proteins *in vitro*. The overview presented here summarizes a compilation of this research, with the goal of primarily describing the current model for the human system.

1. Cluster assembly on the scaffold protein

The first step in Fe-S cluster biogenesis is the assembly of iron and sulfur on the scaffold protein IscU. Sulfur is provided by the eukaryotic cysteine desulfurase complex of NFS1-LYRM4 (human ortholog of the Nfs1-Isd11 complex), which converts cysteine to alanine through a persulfide intermediate (Fig. 1; Table 1)).[6] The precise details of the assembly complex of Iscu-Isd11-Nfs are somewhat unclear, as larger complexes have been observed in yeast and bacteria, but the physiological significance of these oligomers is unknown.[7] Redox chemistry ensues, although the details are poorly understood, possibly with the assistance of a redox-active ferredoxin.[7d, 8] Mitochondria import ferrous iron in a membrane potential-dependent manner via the mammalian carrier proteins mitoferrin 1 and 2.[9] This iron is then transferred to IscU through an iron donor protein called frataxin (Fxn),[10] for complete assembly of a [2Fe-2S] cluster. Frataxin can also function as a regulatory partner in the Nfs1-Isd11-IscU complex to control not only sulfur production, but also iron entry during Fe-S cluster assembly, to accelerate the rate of Fe-S cluster biogenesis[11,12]

2. Chaperone assisted release of the Fe-S cluster and mitochondrial export, if necessary

Cluster release from IscU to target proteins is mediated by a dedicated chaperone system comprising the Hsp70-type protein HSPA9 (homolog of yeast Ssq1 and bacterial HscA) and the co-chaperone Hsc20 (homolog of yeast Jac1 and bacterial HscB) (Fig. 1; Table 1).[13] The chaperone complex binds and induces a conformational change in IscU, which enables release of the IscU-bound Fe-S cluster either to selected target proteins or to specific Fe-S cluster carrier proteins, with the proposed major target being the monothiol glutaredoxin, Grx5, a homodimeric protein, that binds a [2Fe-2S] cluster with two exogenous glutathione ligands for downstream cluster delivery and trafficking (Fig. 1).[14]

From Grx5, the [2Fe-2S] cluster can also be targeted to other downstream proteins within the mitochondria, or for export from the mitochondria to the cytosol via the ABC transporter ABCB7 (yeast Atm1) (Fig.1).[15] The crystal structures of yeast Atm1 and a related bacterial ABC transporter that confers heavy metal tolerance[16] indicate that the transporter exports a sulfur containing molecule from the mitochondria to the cytosol, which is critical for the CIA machinery.[17] A role for glutathione in the ISC export process has been established in yeast cells, where a depletion of glutathione results in a phenotype similar to that of Atm1 deficiency that produces iron overload in mitochondria.[18] Hence, glutathione is required for extra-mitochondrial Fe-S cluster biogenesis and therefore impacts cellular iron homeostasis. [19] Prior reports have provided evidence for a tetrameric GSH-coordinated [2Fe-2S] cluster

as a possible substrate for the exporter protein.[17a] In fact, mixing GSH-coordinated [2Fe-2S] complex with purified Atm1 stimulated its ATPase activity up to 1.8-fold.[17b] Structures were solved with potential glutathione and oxidized glutathione (GSSG) binding sites about 5 A° apart, which could readily accommodate a [2Fe-2S] cluster and provide support for a glutathione complexed Fe-S cluster.[16] *In vivo* determination of these glutathione complexed [2Fe-2S] clusters can further strengthen the physiological existence of these small molecules in the cell. Another study has suggested the molecule GSSSG, i.e., the oxidized form of GSSH (glutathione persulfide) and GSH, as the Atm1 substrate and potential sulfur carrier.[20] Nevertheless, an as yet ill-defined species X must be exported from the mitochondria in order to support proper Fe-S cluster trafficking in other cellular compartments.

3. Transfer to putative target apo-proteins

Recent studies have shown that a number of other proteins are further involved in Fe-S cluster biogenesis. Following release of the Fe-S cluster from the scaffold protein, it can either be directly delivered to [2Fe-2S] target proteins or converted to a [4Fe-4S] cluster.[1b, c] A current model suggests that [4Fe-4S] cluster maturation requires additional ISC proteins: IscA1 and IscA2, and the folate binding protein Iba57,[21] in a process that requires sequential delivery of a [2Fe-2S], postulated to be from Grx5, with reductive coupling to form a [4Fe-4S] cluster (Fig. 1).[22] However, knockdown of these IscA proteins does not eliminate production of [4Fe-4S] clusters, so other proteins and complexes may utilize a similar reductive coupling mechanism on target Fe-S cluster proteins.[21b, 21d] Other more specialized targeting factors have evolved to assist the maturation of dedicated subsets of [4Fe-4S] proteins and also for transfer of [2Fe-2S] clusters downstream of the scaffold assembly to final target proteins, such as Nfu, BolA3, Ind1, and SDHAF (Fig. 1; Table 1).[1a] However, the precise mapping of these pathways and all of the potential functions of these proteins remain unclear. For example, Nfu may act as an alternative scaffold protein to IscU, because Nfu can accommodate both a [2Fe-2S] cluster as well as a labile [4Fe–4S] cluster in vitro and pass these on to apo-proteins[23]

Cytosolic and Nuclear Fe-S Cluster Protein Biogenesis

The biogenesis of cytosolic and nuclear Fe-S proteins is heavily dependent on the conserved mitochondrial Fe-S cluster (ISC) assembly machinery in addition to the ABC transporter protein and cytosolic Fe-S cluster (CIA) assembly machinery. To date, nine components of the CIA machinery have been identified that play a role in mobilization of Fe-S cluster and its insertion into extra-mitochondrial apo-proteins. The assembly process has been primarily studied in yeast and comprises two mains steps:[1a, 4c] (1) [4Fe-4S] cluster assembly on the Cfd1-Nbp35 scaffold complex and (2) transfer of Fe-S clusters to target apo-proteins from the CIA scaffold complex.[1b, c] Yeast nomenclature has been used throughout this section and describes the proposed models based on the work in yeast Similar pathways are likely functional in humans too.

1. [4Fe-4S] cluster assembly on the CFD1-NBP35 scaffold complex

An initial step in cytosolic Fe–S protein biogenesis is the assembly and binding of a transient [4Fe–4S] cluster on the cytosolic scaffold protein complex, a heterotetramer

comprised of the two P-loop NTPases Cfd1 and Nbp35, which bind two different kinds of [4Fe-4S] clusters (Fig. 1).[4c, 24] A loosely bound [4Fe-4S] cluster at the C-terminus of both proteins bridges the two complex subunits together, whereas the other [4Fe-4S] cluster is tightly coordinated at the N-terminal domain of the Nbp35 protein. The lability of the C-terminus cluster is considered to be important for the second step, involving transfer of the cluster to target apo-proteins. Generation of the functionally essential N-terminal Fe-S cluster (N) of Nbp35 depends on the flavoprotein Tah18 (yeast homolog of NDOR1) and the Fe-S protein Dre2 (yeast homolog of CIAPIN1), which serve as an NADPH-dependent electron (e⁻) transfer chain (Fig. 1).[25] Cytosolic-nuclear Fe-S protein biogenesis additionally requires the cytosolic multidomain glutaredoxin, Grx3, yet its precise function and site of action in the pathway remains to be determined,[26] although studies from the yeast system suggest roles in iron regulation and maturation of other proteins such as ribonucleotide reductase.[26b, 27] Accordingly, it is speculated that these cytosolic monothiol glutaredoxins play a general role in iron trafficking.

2. Transfer of Fe-S clusters to target apo-proteins from the CIA scaffold complex

The bridging Fe-S clusters are released from Cfd1–Nbp35 and transferred to apo-proteins in a reaction mediated by the Fe-S protein Nar1 and the CIA targeting complex Cia1–Cia2–Mms19 (Fig. 1).[1a, 4c] Nar1 coordinates two [4Fe–4S] cofactors that are similar to those in hydrogenases and bridges the early and late stage CIA machinery.[61, 62] Cia1, Cia2 and Mms19 form dimeric and tetrameric complexes in both yeast and mammalian cells, and interact with target apo-proteins and assure specific Fe-S cluster insertion,[28] including the crucial function of maturation of nuclear Fe-S proteins (Fig. 1). Several of the proteins involved in mitochondrial Fe-S cluster biogenesis have also been reported to be localized in the cytosol such as IscU, Nfu, Nfs1 and Isd11.[29] However, their functional relevance is yet to be established.

Diseases Associated with Fe-S Cluster Biogenesis Proteins

A number of inborn human diseases have been identified with mutations associated either with the gene or the protein involved in Fe-S cluster biogenesis. In recent years, additional diseases have been identified with the increasing availability of information concerning the function and properties of Fe-S cluster proteins.[1b, c] Several of these disease states are discussed below (Table 1). Additional disease states related to Fe-S cluster maturation have been discussed in terms of infectious agents, but those will only be described briefly in this work.

Diseases Associated with Mitochondrial Fe-S Cluster Biogenesis

1. Friedreich's ataxia (FRDA)—Friedreich's ataxia (FRDA) is a neurodegenerative disease that is by far the most common disease linked to Fe-S cluster biosynthesis.[30] FRDA patients carry a GAA-triplet expansion mutation in the first intron of the frataxin gene (Fxn). This expansion leads to transcriptional silencing of frataxin thereby resulting in severely decreased levels of the frataxin protein. Nonsense and missense mutations or deletions also result in the same symptoms for patients.[30c, 31] No therapy for FRDA exists today, despite

much effort to increase frataxin levels or mitochondrial function, or alleviating secondary symptoms by antioxidants or iron chelator strategies.[32]

2. IscU myopathy—IscU myopathy is characterized by severe exercise intolerance, tachycardia, fatigue and pain in active muscles.[1a, 2b] Patients have decreased mitochondrial Fe-S cluster enzyme activities, as well as iron deposits.[33] Most affected patients are homozygous for a splice mutation in intron 4 of the IscU gene resulting in decreased IscU protein levels.[33–34] A therapeutic approach consisting of antisense oligonucleotides, in order to induce skipping of the splice site responsible for the condition and restore normal mRNA splicing in fibroblasts, is currently being tested,[35] as are treatment options that control miRNA levels to deal with the vascular symptoms and conditions.[36] IscU has also recently been found as a target for regulation by the tumor suppressor protein p53 with implications for modulating iron homeostasis and iron levels in cancer.[37]

3. Fdx2 myopathy—In addition to IscU myopathy, a second novel mitochondrial myopathy has been found in a patient due to a disruption on the FDX1L gene, which encodes the mitochondrial protein ferredoxin 2 (Fdx2).[38] The myopathy is due to a mutation (c. 1A>T) that disrupts the ATG initiation codon, resulting in low levels of Fdx2 in muscle and fibroblasts, and decreased activities of respiratory complexes I-III and mitochondrial aconitase.[38] Symptoms resemble those of IscU myopathy but are not as severe, possibly indicating a functional rescue by the homolog Fdx1 or a bypass mechanism compared to the role of IscU in Fe-S biogenesis.[38]

4. ISD11-Nfs1—Recently, the importance of ISD11 (human LYRM4) and Nfs1 in cluster biogenesis as part of the *de novo* cluster assembly complex was highlighted as a result of their roles in disease states. For Nfs1, patients were found with a missense mutation (p. Arg72Glu) that results in infantile mitochondrial complex II/III deficiency.[39] Levels of Nfs1 transcripts and protein levels were decreased in patient fibroblasts, as were the levels of respiratory complexes II and III, which could be indicative of an overall disruption in Fe-S cluster biogenesis and is also consistent with the absence of ISD11 association in co-immunoprecipitation assays.[39] However, treatment with mitochondrial cofactor therapy was successful for one of the three patients in preventing lethality during infancy, suggesting a potential therapeutic option.[39]

The requirement for ISD11 in stabilizing Nfs1 has been confirmed in patients with mutations on ISD11, who demonstrated a similar phenotype in the form of respiratory complex deficiency, termed combined oxidative phosphorylation deficiency 19 (COXPD19). [40] Interestingly, these patients also exhibited further downstream complications with deficiencies in complexes I, II and III, in addition to other mitochondrial and cytosolic Fe-S proteins.[40a] Both patients were homozygous for a missense mutation (p. Arg68Leu),[40a] which caused the levels of ISD11 to be undetectable and a decrease in respiratory chain complexes to below 40%. This mutation also impacted the interaction with Nfs1 by promoting aggregation and inhibiting desulfurase activity.[40a] Furthermore, IscU levels were also observed to decrease when the R68L mutation was present, suggesting that the complex is also essential for promoting the stability of IscU for overall Fe-S cluster biogenesis and trafficking.[40b]

5. Multiple Mitochondrial Dysfunction Syndrome (MMDS)—Multiple
mitochondrial dysfunction syndrome (MMDS) describes a class of fatal mitochondrial
diseases caused by mutations in proteins involved in Fe-S cluster biogenesis and trafficking.
To date, four types of MMDS have been identified: namely, MMDS1 due to mutations on
Nfu,[41] MMDS2 for mutations with BOLA3,[41b, 42] MMDS3 with mutations on Iba57,[43] and
MMDS4, with mutations on IscA2.[44] Recently, an IscA1 variant has been described that
results in MMDS-like symptoms, which may expand the class of disorders.[45] All MMDS
patients exhibit similar symptoms that include metabolic acidosis with hyperglycemia, and
deficiency of respiratory complexes and lipoic acid bound enzymes, such as pyruvate
dehydrogenase (PDH) and α-ketogluterate dehydrogenase (KGDH).[41f, 46] Lipoate
biogenesis relies on the mitochondrial [4Fe-4S] enzyme lipoate synthase (LIAS).

For MMDS1, five patients have been described with a homozygous mutation in Nfu
(p.Arg182Gln), where the mutation is close to a splice site and results in skipping of exon 6
and no detectable protein levels.[46] Most other patients were described with a homozygous
mutation (p.Gly208Cys) that affects an amino acid close to a highly conserved Fe-S cluster
binding domain of the Nfu protein. This mutation does not affect protein levels but does
impair Fe-S cluster binding and transfer, most likely due to a perturbed monomer-dimer
equilibrium that limits cellular reconstitution of Nfu.[41f, 47] Most recently, a study of patients
with the heterozygous gene for Nfu, one with the altered splice site or with the Gly208Cys
missense mutation and the native allele, has revealed that low levels of the wild type Nfu
mRNA were produced, which yielded sufficient functional LIAS to avoid lipoic acid
deficiency, but the wild type transcripts were insufficient to support functional respiratory
complex II.[41d]

In the case of MMDS2, 6 different patients have been identified with mutations on
BOLA3.[41b, 42] The first patient demonstrated a single base pair duplication that produced a
frameshift with a premature stop codon and therefore loss of the full length protein.[41b] Two
other patients have been characterized as having a missense mutation at a highly conserved
residue (p.Ile67Asn)[42b] that could affect the overall protein fold based on the solution NMR
structure of human BOLA3.[48] The remaining three patients all demonstrated a truncating
mutation of eight amino acids upstream of the first truncating mutation.[42a] To date, the
molecular role of BOLA3 remains to be elucidated; however the availability of an NMR
structure[48–49] with additional biochemical characterization will provide increasing
understanding of its role in MMDS.

Similar to BOLA3 in MMDS2, a wide variety of mutations on Iba57 have been identified for
MMDS3. Initial reports described different patients with a range of phenotypes. The first
case resulted in the most severe condition caused by a missense mutation (p. Gln314Pro)
that appeared to result in protein misfolding with subsequent degradation to concentrations
below critical levels.[43a] The mildest phenotype was observed in a patient with an Iba57
mutation that resulted in reduced mRNA levels because of aberrant splicing and a frameshift
mutation that yielded a truncated form of Iba57.[43c] The intermediate phenotype of MMDS3
was due to a mutation on Iba57 that drastically reduced its ability to function in Fe-S
biogenesis with diminished levels of [4Fe-4S] clusters.[43b] Most recently, four additional
mutations on Iba57 have been observed with generally the same symptoms and phenotypes.

[43d] The prevalence of such diverse mutations on Iba57 that all result in MMDS3 makes prediction of the disease difficult, based on genetic information, and strengthens the need for further biochemical characterization of Iba57 to understand why so many possible causes of the disease exist.

MMDS4 was the most recent class to be discovered, with six patients exhibiting a missense mutation (p.Gly77Ser) on IscA2, which is located in a highly conserved cluster binding domain. However, the exact effect of the mutation is unclear, since mRNA levels were not significantly impacted.[44] Interestingly, patient fibroblasts also showed lower levels of IscA1 and Iba57 than control fibroblasts.[44] How this mutation results in lower protein levels of these partner proteins remains unclear. Similarly, an IscA1 mutation (p.Glu87Lys) has been implicated in causing MMDS-like symptoms, which may result in a new class of the disorder, as one patient has been found with this mutation that is expected to lead to protein instability due to loss of a conserved salt-bridge.[45]

The class of MMDS disorders describes a newly discovered disease condition and further details on how mutations in key Fe-S biogenesis proteins influence cellular function, and their roles that result in disease, need to be determined in order to fully aid patients.

6. Respiratory complex 1—Fe-S clusters play essential roles in respiration and metabolism as cofactors in respiratory chain complexes. The manner in which Fe-S clusters are integrated into the respiratory complexes is currently unclear; however, respiratory chain complex maturation factor proteins have recently been identified that are required for correct formation of the complexes, either by promoting subunit association or cofactor integration.[50]

In particular, complex I of the respiratory complex, also known as NADH-CoQ oxidoreductase, is composed of 44 subunits, utilizes at least 10 assembly factors for correct complex formation, and accounts for one-third to one-quarter of mitochondrial disease, which occurs in about 1 out of every 5,000 births.[51] One such assembly factor, Ind1 or NUBPL, has been implicated as an Fe-S cluster delivery protein that is specifically required for complex I assembly and activity in yeast,[51] and mutations or perturbations of the Ind1 gene have resulted in a specific deficiency of complex I, while other respiratory complexes remain intact.[52] Genetic sequencing has found two mutations in patients: namely, a p. Gly56Arg missense mutation and a c. 815-27T>C branch site mutation. Of the two, pathogenicity has been more attributed to the branch site mutation, since it results in aberrant splicing, very low levels of the NUBPL protein, and reduced levels of fully assembled complex I, as little as 20%.[52–53] However, there remains the possibility that the missense mutation contributes to the pathogenicity of the branch site mutation. Furthermore, recent work in *Arabidopsis thaliana* suggests that Ind1 may also play a role in translational control,[54] which may contribute to phenotypes observed in the pathogenic state. However, no work has been done in human cell lines to either evaluate this additional functional role or examine the possible role of Ind1 in Fe-S cluster delivery.

7. Respiratory complex II—Respiratory complex II (or succinate dehydrogenase, SDH) is composed of four subunits (A-D). Subunits C and D anchor the complex to the membrane,

while subunits A and B exhibit catalytic activity.[55] All of the subunits have been implicated in disease states, because SDH plays vital cellular roles as a result of its involvement in both the Krebs cycle and oxidative phosphorylation. However, only subunit B (SDHB) contains iron-sulfur clusters.[55a] SDHB is the most commonly mutated subunit of the four,[55a] and mutations result primarily in either neurodegeneration, generally in the form of infantile leukodystrophy,[56] or cancer, particularly renal cell carcinoma, paraganglioma, pheochromocytoma, or WT gastrointestinal stromal tumors.[55, 57]

In terms of the neurological impact, one patient has been identified with a missense mutation at an evolutionarily conserved residue (p.Asp48Val) near an iron-sulfur cluster binding site, which is hypothesized to be crucial for efficient electron transport through the respiratory chain and resulted in an almost complete loss of SDHB and decreased levels of SDHA.[56] As with many mitochondrial disorders, there is no cure for SDH deficiency; however, the patient in this study was supplied with an oral treatment of coenzyme Q10 to yield a subjective improvement in strength.[56]

The remainder of patients with mutations on SDHB have been linked to some form of cancer. A recent analysis of the Leiden Open Variation Database showed that 37% of all the reported cancer cases linked to SDHB are due to mutations to the cluster cysteinyl ligands that prevented cluster binding, or in the L(I)YR motif, which blocks chaperone recognition by Hsc20 and the HSPA9 chaperone to prevent binding interactions with either of these proteins.[58]

As is the case for respiratory complex I, maturation factors for SDH have also been implicated in causing disease. In the case of SDHB, two assembly factors exist: SDHAF1 and SDHAF3.[55b, 59] Both factors are believed to help protect SDHB from oxidative damage during the assembly of the complete SDH complex, by shielding the nascent SDHB Fe-S clusters from damage by reactive oxygen species.[55b, 59] SDHAF1 also functions in concert with Fe-S cluster chaperones in recruiting the chaperone complex to SDHB via the L(I)YR motif,[60] and was found to co-immunoprecipitate with Hsc20.[58b] As such, mutations on SDHAF1 that disrupt the binding interface for either SDHB or Hsc20[60] have also been implicated in causing leukoencephalopathy,[61] where accumulation of succinate in the white matter of the brain is caused by a loss of SDH activity. The accumulation of succinate was due to degradation of SDHB, when non-functional SDHAF1 was present; however, succinate levels could be reduced following treatment with riboflavin, which serves to increase the flavinylation of SDHA and allows it to convert succinate to fumarate to restore the activity of the Krebs cycle.[60]

8. MitoNEET—Outside of the traditional mitochondrial Fe-S cluster components and targets, the outer mitochondrial membrane protein, mitoNEET has been implicated in numerous diseases.[62] MitoNEET was first discovered bound to thiazolidinedione, a drug used for the treatment of type 2 diabetes. Since then, its functional role has been under active investigation. Given its association with a diabetes drug, one of the physiological roles of mitoNEET is linked to diabetes, via control of lipid and glucose metabolism,[63] most likely by modulation of lipid accumulation,[64] which is related to insulin resistance. MitoNEET is also involved in controlling overall mitochondrial bioenergetics by regulating homeostasis

and the ratio of energy stored to the energy spent,[64] which connects with its role in causing obesity and potential regulation of mitophagy.[63] In conjunction with mitochondrial homeostasis, overexpression of mitoNEET results in diminished levels of mitochondrial iron, while knockdown leads to iron overload and reduced activity of the electron transport chain, resulting in overall lower cellular energy.[64] Accordingly, mitoNEET plays a key role in cellular and whole organism iron regulation and metabolism, and in maintenance of reactive oxygen species,[62, 65] potentially by binding a [2Fe-2S] cluster for delivery and repair of cytosolic aconitase (IRP1),[66] in a process that is under redox control.[67]

Given its broad role in metabolism, mitoNEET has also been associated with cystic fibrosis due to a decrease in mitoNEET mRNA,[68] Parkinson's disease due to an interaction with *PARKIN*,[69] and cancer and tumorogenesis,[62, 70] where mitoNEET was found to be upregulated in breast cancer cells.[71] Despite the association of mitoNEET with broad cellular processes and diseases, many questions remain unanswered concerning its specific cellular mechanism and the connection between its role in Fe-S cluster biosynthesis and overall mitochondrial regulation. Furthermore, mitoNEET is still under consideration as a target for drug development for the treatment of cancer and diabetes based on overall mitochondrial dysfunction,[62–63] with additional possibilities considering its likely roles in Parkinson's and cystic fibrosis. As more is learned concerning mitoNEET and its physiological roles, better therapeutics may be developed to compensate for disruptions in its function.

Diseases Associated with Cytosolic Fe-S Cluster Proteins

Although a number of proteins have been identified as components of the cytosolic Fe-S cluster machinery,[4b, 15, 72] many have only been discovered or characterized in depth over the last 5 years. For this reason, many of these proteins have not yet been linked to disease conditions. However, given their required roles in the biogenesis of essential Fe-S clusters and maintenance of iron homeostasis, there is the possibility of disease association as these proteins become better understood and disease states are examined in greater depth. For example, examination of proteins in the NAR family in *Arabidopsis thaliana*[73] and yeast[74] have indicated conserved roles in maintaining a response to oxidative stress, which may have downstream implications for genome maintenance,[72] due to the connection to DNA damage repair pathways and corresponding involvement in aging disorders.

Likewise, the cytosolic Fe-S protein MMS19, has not specifically been associated with any diseases. However, this protein has been characterized as required for maturation of many nuclear Fe-S cluster containing proteins that are particularly involved in DNA replication and repair, such as FANCJ, XPD, RTEL, and POLD (discussed below).[75] The essential nature of MMS19 in maturation of key nuclear proteins may link it to cancer and neurodegenerative phenotypes in the future.[75b]

Diseases Associated with Nuclear Fe-S Cluster Containing Proteins

With oxygen sensitivity and possible toxicity associated with Fe-S clusters, it was assumed that most nucleic acid enzymes did not contain Fe-S clusters. However, this view changed with the discovery of an Fe-S cluster in the DNA helicase XPD and associated protein

members that are involved in DNA repair,[76] and the possibility that Fe-S cluster containing enzymes find DNA damage by probing DNA integrity electronically.[77] Today, all DNA polymerases involved in replication and also certain helicases that participate in Okazaki fragment processing contain some form of Fe-S clusters.[2a, 78]

9. Glycosylases—The glycosylase family provided the first protein example to be characterized with an Fe-S cluster by way of endonuclease III in *E. coli*;[2a] however, mammalian forms are now being characterized. Members of this family conduct Base Excision Repair (BER) to detect, remove, and replace damaged DNA bases. In particular, the human protein MUTYH serves to repair mismatches associated with 8-oxoguanine.[79] A number of disease variants of MUTYH have been identified that result in cancer in the form of colorectal polyposis, termed MUTYH-associated polyposis (MAP).[80] To date, one of the mutations (P281L) was found at a highly conserved proline in the Fe-S cluster binding domain, which impairs the ability to bind substrate DNA.[80–81,82] A detailed understanding as to why variants, such as these, result in MAP will provide assistance to clinicians in patient-screening for cancer risk and subsequent treatment options.[82]

10. Helicases—Helicases constitute a second family of nuclear proteins that contain Fe-S clusters and function in DNA processing. Within the helicase family, the Fe-S cluster subset belongs to the SF2 grouping with shared homology of the helicase domain.[2a, 83] A number of these proteins have disease implications and are therefore discussed below.

FANCJ, in conjunction with 15 other genes, is implicated in causing Fanconi anemia; a condition characterized by congenital defects, progressive bone marrow failure, susceptibility to cancer with chromosome breaks, and sensitivity to DNA damaging agents that result in intra-strand cross-links.[84] Furthermore, FANCJ plays a role in genome stability by unwinding G-quadruplexes[85] and by conducting double-strand break repair.[86] Of the numerous mutations identified in the gene for *FANCJ* that are linked to various disease states, two of them (M299I and A349P) occur in the iron-sulfur cluster binding domain, where the M299I mutation has been found in breast cancer patients,[87,83a, 88] and the A349P mutation causes Fanconi anemia.[89] These mutational analyses have demonstrated the importance of the Fe-S cluster in the function of FANCJ and its role in downstream DNA processing activities in disease.[83]

A second Fe-S cluster-containing helicase is XPD, which serves key roles in nucleotide excision repair to remove photo-damaged DNA bases, and in transcription as a member of the TFIIH complex.[83] Mutations in XPD have been linked to xeroderma pigmentosum (XP), Cockayne's syndrome (CS), Trichothiodystrophy (TTD), and Cerebro-oculo-facial-skeletal (COFS) syndrome.[83] To date, only one of these disorders has been linked to a mutation in the Fe-S cluster binding domain. In patients with TTD, a R112H mutation is present at a highly conserved residue that removes a hydrogen bond to a cluster-ligating cysteine,[90] resulting in impaired interactions with proteins in the TFIIH complex and involvement in helicase inactivation.[83a, 90a]

The last two Fe-S cluster helicases that we will describe have been implicated in disease much more recently. The helicase ChlR1, or DDX11, is less understood than those already

discussed. Its specific role in DNA processing and genome integrity is unclear; however, it may serve primarily to unwind intermediate DNA structures present during replication and recombination,[91] with additional potential roles in sister chromatid cohesion, heterochromatin organization, viral genome maintenance, DNA repair, and G-quadruplex unwinding, via its helicase and ATPase activities[92]. Although its precise function is unknown, the first human patient with a defect in ChlR1 was identified in 2010 in a condition termed Warsaw breakage syndrome, due to either a splice site mutation[93] or p. Arg263Gln mutation in ChlR1, which perturbs a conserved arginine residue in the Fe-S cluster binding domain.[94]

The last Fe-S cluster helicase that we will discuss is the Regulator of Telomere Length 1 (RTEL1), which functions to maintain telomere integrity and unwinds various DNA structures that are assembled in the process of replication, recombination, and repair.[95] Thus far, a number of RTEL1 variants have been found that exhibit single nucleotide polymorphisms (SNPs) and increased susceptibility for brain tumors or hepatocellular carcinomas, suggesting a key role for the protein in tumorigenesis.[95] Mutations of RTEL1 in coding regions have instead been implicated in premature aging disorders[95], such as dyskeratosis congenita (DC)[96] and Hoyeraal-Hreidarsson syndrome (HHS),[97] which is considered a more severe form of DC. Both diseases are characterized by multi-system bone marrow failure, due to impaired telomere maintenance.[95] One mutation (p. Glu275Lys) has been found in the Fe-S cluster domain,[96b] but the biochemical characterization and impact of this residue on the cluster has not yet been examined.

Further biochemical characterization is required to understand the Fe-S cluster helicase family, particularly to determine the role of the cluster in relation to disease phenotypes. Efforts in examining the molecular mechanisms will aid current potential therapies that use chemical rescue by small molecules to restore function to mutated helicases and treat disease conditions.[2a]

11. Polymerases—The last class of Fe-S cluster containing DNA processing enzymes that we will briefly discuss are polymerases. All replicative DNA polymerases, such as delta (δ) and epsilon (ε), contain essential [4Fe-4S] clusters[2a, 98] that are matured via the CIA machinery.[99] These two polymerases have implications in cancer;[100] however, the precise functional role of the Fe-S cluster is unknown,[2a] and therefore its link to disease is unclear.

Conditions Resulting in Mitochondrial Iron Overload

Although the disorders below are linked primarily to the misregulation of iron, they occur specifically in Fe-S cluster proteins. Whether this link is due to a simple abundance of iron or to a more direct connection between Fe-S clusters and iron regulation, perhaps via the iron regulatory protein (IRP), remains uncertain and requires further research.

12. X-linked sideroblastic anemia (XLSA)—X-linked sideroblastic anemia is caused by mutations in the mitochondrial transporter protein ABCB7.[101] The condition is characterized by early-onset ataxia, sideroblastic anemia and iron overload in affected tissues, primarily in the mitochondria, which suggests that the transporter is unable to function properly for trafficking of clusters in the cytosol and nucleus. Expanding on the

previous disease symptoms, new phenotypes are being associated with ABCB7 mutations that give rise to cerebellar ataxia[102] and ringed sideroblasts.[103]

13. Sideroblastic-like anemia and iron overload—A homozygous mutation in the exon 1 of the *GRX5* gene causes problems with RNA splicing, resulting in sideroblastic-like anemia and iron overload condition.[104] This condition causes moderate anemia, hepatosplenomegaly, and iron overload, and leads to a decreased level of mRNA for Grx5 but not a complete loss of the Grx5 protein function.[14b, 104a] The decreased protein levels result in impaired cluster transfer to downstream Fe-S proteins, such as IRP1 and ferrochelatase (FECH).[104c] An additional sideroblastic anemia patient has been found with a mutation in *GRX5*, which is a compound heterozygous mutation p. Lys101Gln and p. Leu148Ser.[105] *In vitro* analysis of these mutations in Grx5 revealed that the L148S mutation results in similar effects as observed in cases of patients with diminished protein levels, where cluster transfer to downstream targets was impaired.[106] The K101Q mutation yielded different effects, in that this variant was unable to bind a Fe-S cluster,[106] and correlates with the fact that this residue participates in GSH binding,[107] which is required as a cluster ligand. Aside from implications in sideroblastic anemia, patients have also been found to display variant non-ketotic hyperglycinemia, with symptoms similar to those observed for patients with MMDS2 due to mutations in BOLA3.[42a] In these three patients, Grx5 was found with a p. Lys51del mutation that impaired transfer to downstream targets and resulted in low levels of lipoate synthase activity,[42a] indicating that this mutation has functional consequences similar to the mRNA splicing defect and L148S variants[106]. Although Grx5 appears to function primarily in the early stage of Fe-S cluster trafficking,[14a, b, 104a, 108] additional multifunctional cellular roles may exist given the variety of phenotypes observed in disease conditions related to Grx5.[42a, 104c, 105–106]

Most recently, a phenotype similar to that observed for sideroblastic anemia has been linked to mutations in the mitochondrial Fe-S cluster biosynthesis chaperone, HSPA9, which has also been implicated in key roles in erythroid differentiation.[109] Mutations to HSPA9 result in lower mRNA expression levels with incomplete loss of function leading to the anemia,[110] a phenotype which may be under a process of complex regulation.

Fe-S Cluster Proteins Linked to Neurological Disorders

Similar to the diseases related to iron overload, neurological disorders are often related to iron misregulation; however, it remains to be seen if there is a more direct connection between Fe-S clusters and these conditions. Recent work has indicated a link between mitochondrial dysfunction and neurodegenerative disorders, such as Parkinson's disease, Alzheimer's disease, and Huntington's disease.[111] These neurodegenerative conditions are thought to exist as a feedback loop, where mitochondrial dysfunction causes increased production of reactive oxygen species, which decreases Fe-S cluster and heme biosynthesis and leads to IRP activation and iron overload in mitochondria.[111–112] This accumulated iron in turn generates additional reactive oxygen species via Fenton chemistry. In the case of Alzheimer's disease, these factors promote oligomerization of amyloid-β to form plaques[113] and the formation of Fe^{3+}-paired helical filament aggregates[114] to further drive iron accumulation and decrease the levels of the soluble Tau protein.[115] Similarly, in

Huntington's disease the Huntington protein (Htt) is believed to play a role in maintaining iron homeostasis via the transferrin receptor;[111b, 116] when mutated in the disease state, Htt likely perturbs iron uptake and contributes to mitochondrial dysfunction.[111b] In the class of the neurodegenerative disorders, Parkinson's disease has most been associated with iron-sulfur cluster proteins.

14. Parkinson's Disease—Parkinson's disease is a neurodegenerative disease affecting roughly 1:800 individuals with a survival rate of about 15 years after diagnosis. The degenerative process affects dopaminergic neurons leading to motor function deficits, such as resting tremor, rigidity, and postural instability.[111–112] Age is considered to be the main risk factor for Parkinson's-however, environmental toxins or genetic susceptibility are also implicated.[117] Data suggests that patients with Parkinson's also have a defect in ISC synthesis, partial inhibition of Complex I of the respiratory chain, and increased iron levels in dopaminergic neurons (in the substantia nigra),[112, 118] again due to erroneous regulation of iron homeostasis by IRP.[111–112] In addition to this, the pathway which delivers transferrin-bound iron to mitochondria and to complex I of the respiratory chain is found in the substantia nigra dopaminergic neurons, but also consists of transferrin receptor 2.[119] In Parkinson's, there is accumulation of oxidized transferrin inside mitochondria, which results in the release of ferrous iron from transferrin and the generation of hydroxyl radicals via Fenton chemistry.[119a, 120] Furthermore, glutaredoxin 2 (Grx2) has been implicated in promoting the iron accumulation feedback loop in Parkinson's disease based on its ability to regulate cellular redox status by acting on its substrate glutathione (GSH) to impact overall iron homeostasis.[112, 121] As our understanding of these neurodegenerative disorders is expanded, therapeutic approaches are being explored as well. Thus far, some success has been observed in patients with Parkinson's and other diseases in this area through the use of iron-chelation therapy.[111b] However, only one of the chelators has been successful in clinical trials,[122] since many of them cause adverse side effects due to lack of targeting to specific tissues, resulting in systemic iron depletion.[111b, 112] New compounds or approaches that include targeting with fewer broad effects could provide a successful treatment option.

15. Erythropoietic Protoporphyria—Proper Fe-S cluster biosynthesis is also crucial for the pathway of heme biosynthesis, since the last step in the process is catalyzed by ferrochelatase (FECH), which binds a [2Fe-2S] cluster.[123] Mutations in FECH account for 98% of erythropoietic protoporphyria (EPP) with patients affected worldwide[124] and prevalence as high as 45% in some countries.[125] Symptoms of EPP include heightened and painful photosensitivity, and in some severe cases, liver damage occurs;[124] all of which are due to the accumulation of the heme precursor, protoporphyrin IX from a decrease in FECH activity to less than 30%.[126] The majority of patients present with the IVS3-48T>C low expression allele in conjunction with a missense mutation that causes a loss of FECH function, although other combinations have been observed, where patients can simply exhibit multiple loss of function mutations.[124, 127] As of 2015, at least 130 different mutations on FECH have been implicated in EPP, with new mutations or sites being identified, as sequencing is extended and population studies are undertaken.[127–128] Therefore, specific genotype-phenotype correlations and understanding of the specific molecular mechanism are challenging. Furthermore, patients with EPP were also found to

display abnormal mRNA levels of mitoferrin-1,[129] which has been found to complex with FECH and ABCB10 for heme biosynthesis[130] and may also contribute to the phenotype of EPP. As familial screening becomes more common, doctors can be more aware of carriers to provide better treatment plans for patients,[128] which typically involve avoiding sunlight and tools to reduce excess protophorphyrin for the less severe cases, and solutions, such as bone marrow transplants for long term solutions in the case of drastic phenotypes.[124]

Fe-S Cluster Protein Maturation in human infectious agents

Iron-sulfur clusters also play a vital role in prokaryotic metabolism, as catalysts, redox sensors or electron trafficking agents in a variety of important proteins. For example, across different strains of *E. coli* there are over 150 proteins that contain Fe-S clusters and over 50 different proteins in *Mycobacterium tuberculosis*.[131] Fe-S clusters have consequently been exploited for bactericidal toxicity, where antibiotics promote cell respiration, forming endogenous superoxide and peroxide species, which degrade Fe-S clusters that serve important roles in bacterial cell cycles, and DNA and protein biogenesis.[132] Fe-S clusters have also been targeted in terms of human pathogens in *Pseudomonas aeruginosa*. Deletion of the transcriptional regulator iscR leads to increased susceptibility to peroxides and severely decreased virulence in mouse and Drosophila models.[133] Much work has been done in studying Fe-S clusters in *Mycobacterium tuberculosis*, a facultative pathogen that can live inside macrophages and lead to tuberculosis. These bacteria contain a family of WhiB-like 4Fe-4S cluster-containing transcriptional regulators that could be exploited as an attractive potential antibacterial target, since these regulators play a vital role in Mycobacterial cell division, lipid metabolism, oxidative stress, and antibiotic resistance.[134]

Summary and Perspectives

Since Fe-S clusters are required in a number of essential proteins or as necessary cofactors for a multitude of enzymatic reactions and physiological processes, conditions that perturb the process of cluster biogenesis and trafficking often lead to disease states. Additionally, a number of these perturbations lead to lethal consequences, making them challenging to investigate, which highlights the importance of understanding the molecular details of Fe-S cluster biosynthesis. Furthermore, recent work in bacteria, utilizing various double mutants and media supplementation, has demonstrated that these genetically engineered forms can grow, albeit slowly, without Fe-S clusters.[135] The implications of this work in relation to human Fe-S cluster-based diseases are currently unclear, but may provide alternative treatment strategies in the future.

To date, the majority of inborn human diseases related to biogenesis or Fe-S cluster binding proteins are caused by mitochondrial proteins.[1a, 2b] No specific diseases have been associated with the cytosolic system, even though they have demonstrated roles in yeast viability (Table 1).[1a, 4c, 15, 75, 98, 136] Furthermore, diseases have been linked to nuclear proteins that bind Fe-S clusters to function in DNA replication and repair; [2a, 80, 82–84, 86, 90a, 93, 95, 100c, d] however, the presence of the cluster and some of the specific cellular functions remain unclear, presenting challenges when trying to understand the role of the cluster in disease conditions. Aside from diseases associated with specific Fe-S cluster

proteins, a subset of neurological disorders, such as Alzheimer's and Parkinson's, have been linked to the Fe-S cluster biogenesis pathway based on the presence of free iron and overall iron dysregulation in mitochondria.[111] In addition, Fe-S cluster proteins from all of the cellular compartments have been implicated in a variety of cancers.[3] The essential nature and necessary function of these metal cofactors dictates the need for a better understanding of their biogenesis and trafficking mechanisms to clarify the molecular basis of disease states and lead to potential therapeutic approaches.

Acknowledgments

Biographies

Christine Wachnowsky first studied biochemistry at Allegheny College in the lab of Dr. Margaret Nelson, focusing on cell differentiation in *Dictyostelium discoideum*. She earned her Ph.D. at the Ohio State University as part of the Ohio State Biochemistry Program. Christine conducted research in the laboratory of Dr. James A. Cowan on the topic of iron-sulfur cluster biosynthesis and trafficking in relation to disease states.

Insiya Fidai completed her Bachelor's and Master' in Biotechnology from the University of Mumbai, India. During her Masters, she worked on biocompatible synthesis of noble metal nanoparticles using various reducing sugars under the guidance of Dr. Pankaj Poddar at the National Chemical Laboratory, Pune, India. She then earned her Ph.D. at The Ohio State University as part of the Ohio State Biophysics Graduate Program. In Dr. Cowan's laboratory, Insiya worked on ATCUN complexes as potential catalytic metallodrugs and role of iron-sulfur clusters in biogenesis, trafficking and storage, and implications for disease states.

James A. Cowan graduated with first class honours in Chemistry from the University of Glasgow, before moving to Cambridge University to pursue research in synthetic organic chemistry and chemical physics. Subsequently he took up a postdoctoral appointment at Caltech to pursue studies in bioinorganic chemistry before taking up a faculty position at The Ohio State University. Dr. Cowan's research interests cover metal catalysis, metals in medicine, and the cellular chemistry of iron and metal cofactors

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Significance to metallomics

Herein, we summarize current models for iron-sulfur cluster biogenesis, which reflects the complex and intricate series of cellular exchange pathways required for proper iron homeostasis and metal cofactor trafficking. Given the complexity of iron-sulfur cluster assembly and delivery, which begins in the mitochondria and includes a mechanism for nuclear delivery, malfuctions in these trafficking processes can lead to a number of disease conditions. In this review, we report on Fe-S cluster-based diseases that have been attributed to defects in cluster trafficking proteins and pathways and can lead to lethal consequences.
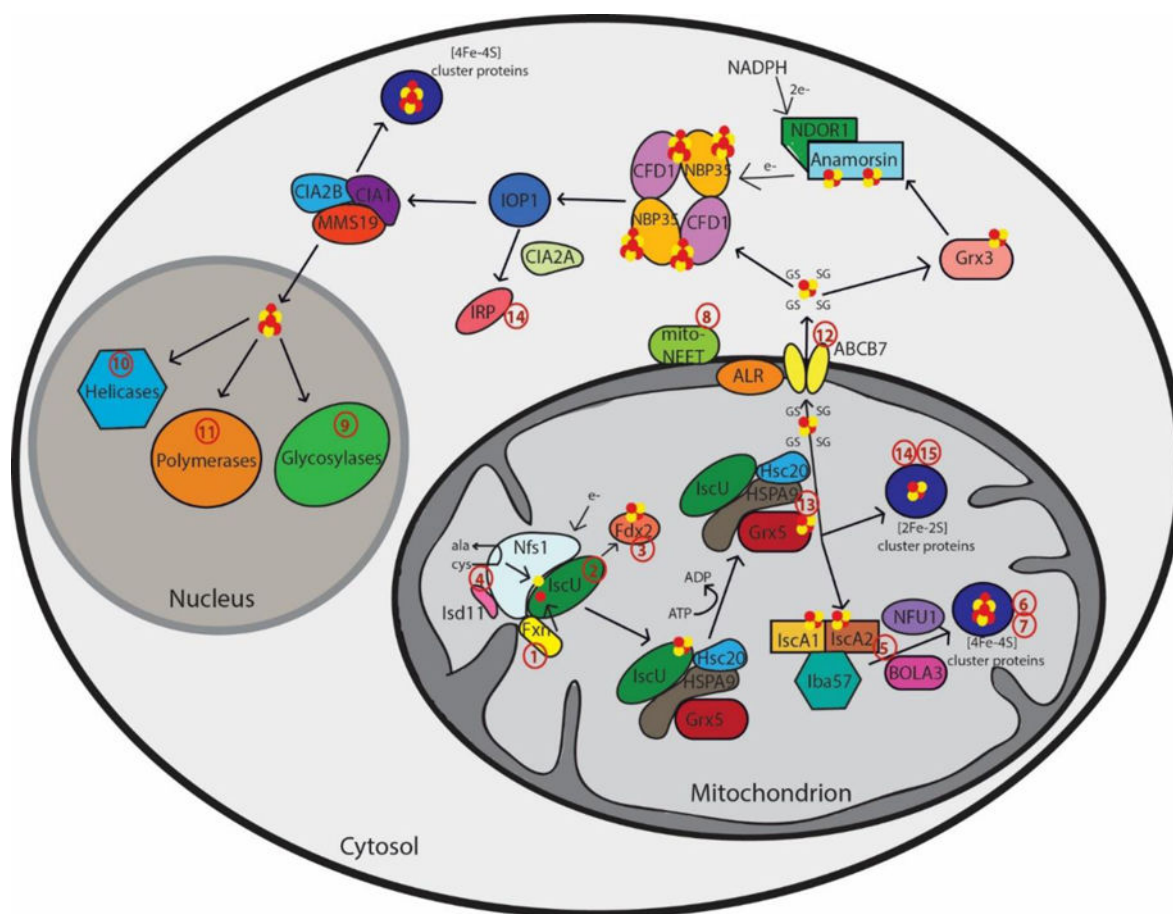
Figure 1.
Schematic representation summarizing current models of mammalian Fe-S cluster
biogenesis based on disease conditions. Nfs1, the cysteine desulfurase, with its cofactor
protein Isd11 donates sulfur from L-cysteine for nascent Fe-S cluster assembly. Together,
they bind to the primary scaffold protein IscU, the regulator protein frataxin (Fxn), which
controls cysteine desulfurase activity and iron delivery, and a source of electrons. The newly
formed IscU-bound [2Fe-2S] is subsequently transferred via a dedicated chaperone-co-
chaperone system (Hsc20 – HSPA9) to Grx5. From Grx5, the Fe-S clusters are either
directly inserted into mitochondrial [2Fe-2S] proteins or transferred via specific carrier
systems such as the one composed of IscA1, IscA2 and Iba57 to mitochondrial [4Fe-4S]
proteins. To date, the presence of another cluster type (such as glutathione complexed
[2Fe-2S] cluster) under physiological conditions cannot not be excluded. For extra-
mitochondrial Fe-S cluster, an uncharacterized compound (X) is exported for cytosolic and
nuclear cluster trafficking via the Fe-S cluster export machinery consisting of ALR,
glutathione and ABCB7. Species X has been proposed to be a glutathione-complexed
[2Fe-2S] cluster and is depicted as the [2Fe-2S](GS)$_4$ complex.[17] Cytosolic cluster
trafficking has been studied primarily in yeast. Fe-S clusters can be trafficked through Grx3
to anamorsin, which provides electrons to the cytosolic scaffold CFD1-NBP35. From there,
[4Fe-4S] clusters are transferred via IOP1 to the CIA proteins and final target proteins, such
as IRP. The CIA proteins can complex with MMS19 for cluster delivery to nuclear targets,

like polymerases, helicases, and glycosylases. The red numbers in the figure correspond to the disease numbers in the review and in Table 1.

Table 1

Iron-sulfur cluster proteins in relation to disease states. The number in the first column corresponds with the number in the review and figure 1. All of the proteins listed are shown in the general schematic of cluster biosynthesis, except Grx2 and FECH.

Number	Protein	Function	Disease	Most Common Cause
1	Frataxin	Iron donor; allosteric effector of NFS1	Friedreich's ataxia (FRDA)	GAA-triplet expansion causing transcriptional silencing
2	IscU	Fe-S cluster scaffold	IscU myopathy	Splice mutation in intron 4 resulting in decreased protein levels
3	Fdx2	Electron transport	Fdx2 myopathy	c. 1A>T mutation disrupting the initiation codon
4	Nfs1	Sulfur donor for cluster assembly	Infantile mitochondrial complex II/III	p. R72Q decreasing transcripts and protein levels
4	ISD11	Stability factor	Combined oxidative phosphorylation deficiency 19 (COXPD19)	p. R68L resulting in undetectable ISD11 protein levels
5	Nfu	Late acting [Fe-S] targeting factor	MMDS1	p. G208C resulting in insufficient downstream holo targets
5	BOLA3	Late acting [Fe-S] targeting factor	MMDS2	Truncation mutation resulting in insufficient downstream holo targets
5	Iba57	[4Fe-4S] cluster assembly factor	MMDS3	p. Q314P causing protein misfolding and degradation
5	IscA1/2	[4Fe-4S] cluster assembly	MMDS4	p. G77S resulting in insufficient downstream holo targets
6	Ind1	Assembly factor for complex I	Complex I deficiency	p. G56R and a c. 815-27T>C branch site mutation leading to aberrant splicing
6	SDHB	Respiratory complex II	Neurodegeneration and cancer	Mutations at Fe-S cluster ligating cysteines preventing cluster binding
7	SDHAF1	Assembly factor for complex II	leukoencephalopathy	Mutations that disrupt binding interface for either Hsc20 or SDHB prevent cluster maturation on SDHB
8	MitoNEET	Maintenance of iron homeostasis	Association with cystic fibrosis, Parkinson's disease, and cancer	Related to decrease in mRNA levels
9	MUTYH	Base excision repair of 8-oxoguanine:adenine mismatches	Colorectal polyposis	p. P281L resulting in low protein that cannot bind DNA
10	FANCJ	Genome maintenance and double-strand break repair	Fanconi anemia and breast cancer	p. M299I upregulates helicase activity or p.A349P that impairs DNA-protein interactions
10	XPD	Nucleotide excision repair and transcription	Xeroderma pigmentosum (XP), Cockayne's syndrome (CS), Trichothiodystrophy (TTD), and Cerebro-oculo-facial-skeletal (COFS) syndrome	p. R112H that impairs transcriptional function and inactivates helicase activity
10	ChlR1	Genome maintenance	Warsaw breakage syndrome	p.R263Q inhibits DNA binding and DNA-dependent hydrolysis
10	RTEL1	Maintenance of telomeres	Cancer, dyskeratosis congenita (DC), and Hoyeraal-Hreidarsson syndrome (HHS)	Protein mutations that prevent telomere maintenance
11	POL δ and ε	DNA polymerases	Cancer	Unclear connection
12	ABCB7	Fe-S cluster export from the mitochondria	X-linked sideroblastic anemia (XLSA)	Protein mutations that result in mitochondrial iron overload

Number	Protein	Function	Disease	Most Common Cause
13	Grx5	[2Fe-2S] cluster carrier	Sideroblastic-like anemia	Aberrant splicing leading to decreased Fe-S cluster transfer and impaired iron homeostasis
14	IRP	Iron homeostasis	Parkinson's disease	Erroneous regulation generating reactive oxygen species
14	Grx2	Cluster carrier and redox regulation	Parkinson's disease	Erroneous regulation of GSH levels
15	FECH	Heme biosynthesis	Erythropoietic protoporphyria	Over 130 different mutations that impair function