Published in final edited form as:

Curr Opin Genet Dev. 2016 June ; 38: 31–37. doi:10.1016/j.gde.2016.02.004.

Mitochondrial iron overload: causes and consequences

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Abstract

Pathological overload of iron in the mitochondrial matrix has been observed in numerous diseases, including sideroblastic anemias, which have many causes, and in genetic diseases that affect iron-sulfur cluster biogenesis, heme synthesis, and mitochondrial protein translation and its products. Although high expression of the mitochondrial iron importer, mitoferrin, appears to be an underlying common feature, it is unclear what drives high mitoferrin expression and what other proteins are involved in trapping excess toxic iron in the mitochondrial matrix. Numerous examples of human diseases and model systems suggest that mitochondrial iron homeostasis is coordinated through transcriptional remodeling. A cytosolic/nuclear molecule may affect a transcriptional factor to coordinate the events that lead to iron accumulation, but no candidates for this role have yet been identified.

Iron is critical for function of the mitochondrial respiratory chain

Mitochondria of eukaryotes represent discrete compartments that are separated from the cytosol by their outer and inner membranes and the inter-membrane space. Using complexes I–V of the respiratory chain, mitochondria capture energy from organic food-stuffs in the form of ATP and NADH, and their highly efficient capture of energy was likely an important factor that enabled eukaryotic cells to develop large and complex nuclear genomes [1]. Mitochondria also possess their own much smaller genomes, which support synthesis of several highly hydrophobic subunits of the respiratory chain that are too hydrophobic to be synthesized and imported from the cytosolic protein synthesis machinery using nuclear transcripts as templates [2]. Twelve iron sulfur (Fe-S) clusters essential to function of respiratory chain complexes are distributed among three complexes: Complex I (NADH dehydrogenase) contains eight Fe-S clusters, Complex II (Succinate dehydrogenase) contains three, and complex III (cytochrome bc₁ complex) contains one. Electrons released by NADH (to Complex I) and succinate (to Complex II) ascend through respiratory complexes I and II by tunneling between Fe-S clusters that generate a ladder-like path along which electrons can readily travel [3]. Energy released when electrons move to higher oxidation potentials is then used by complexes I and III to export protons from the mitochondrial matrix into the inter-membrane space by incompletely understood means,

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which creates a proton gradient across the inner mitochondrial membrane. As protons flow down the concentration gradient back into the mitochondrial matrix, ATP is produced [4].

Thus, correct synthesis and positioning of the Fe-S clusters in mitochondria is key to optimal eukaryotic cellular function. Fe-S clusters are synthesized by a group of dedicated proteins, which include in mammals a cysteine desulfurase (NFS1) that generates sulfur, and a scaffold protein upon which nascent Fe-S clusters are assembled (ISCU). The machinery dedicated to Fe-S synthesis has been highly conserved in bacteria, and eukaryotes, including plants and animals (reviewed in [5], [6]). In mammals, the initial Fe-S synthesis complex consists of NFS1, a cysteine desulfurase that requires a partner, ISD11, for desulfurase function [7], and ISCU, a scaffold protein. The cysteine desulfurases form a dimer, but the ISCU partner proteins are situated at opposite ends of the multimeric complex [8] [9]. Ferredoxins likely provide electrons to generate the intact Fe-S cluster [10] [11], and frataxin likely has a role in allosteric regulation of Fe-S generating activity [12] and ISCU binds Fe-S clusters that contain two iron atoms and two inorganic sulfurs [13]. Holo-ISCU with its [2Fe-2S] complex then forms a complex with a chaperone and co-chaperone pair, known as HSPA9 and HSC20 in humans, similar to bacteria [14]. HSPA9 is a member of the HSP70 ATPase family, and HSC20 activates ATPase activity and also binds to Fe-S recipient proteins by binding iterations of the tripeptide motif, LYR in their primary sequence. Forming a complex with recipient proteins likely facilitates transfer and binding of intact Fe-S clusters to the correct ligands in recipient proteins. Examples of recipient proteins include SDHB [15] and SDHAF1, an accessory protein that aids SDHB assembly (Maio et al., Cell Metabolism, in press).

The mystery of mitochondrial iron overload diseases- how is mitochondrial iron homeostasis regulated?

Despite the fact that much is now known about how mitochondrial Fe-S clusters are assembled and transferred, several major mysteries remain about how mitochondria regulate iron homeostasis in the mitochondrial matrix. In the cytosolic compartment of cells, it is well known that cytosolic iron levels are highly regulated by iron regulatory proteins (IRP1 and IRP2) (reviewed in [16]). These proteins sense cytosolic iron levels and bind to mRNA transcripts of important iron metabolism proteins, including ferritin, an iron storage heteropolymer, and transferrin receptor 1 (TFRC), an important iron uptake protein. When cells are iron-depleted, ferritin translation is repressed by binding of IRPs to an RNA stem-loop structure in the 5'UTR of ferritin H and L transcripts known as an iron-responsive element (IRE), whereas TFRC mRNA is stabilized by IRP binding to IREs in the 3'UTR of the mRNA. Iron is an important cofactor for proteins involved in many cellular processes, partly because its flexible chemistry enables it to accept or donate single electrons as needed to complete metabolic transformations.

Mitochondria express iron uptake proteins known as mitoferrins (reviewed in [17]), which are members of the mitochondrial carrier family (MCF), also known as solute carrier protein family 25 (SLC25). These proteins share topologic features and import many crucial metabolites into the mitochondrial matrix, including amino acids, nucleotides, carboxylates,

and other substrates such as phosphorous and iron [18] [19]. The two mitoferrins in mammals account for most iron uptake in erythroid cells (MFRN1 or SLC25A37) [19] and in non-erythroid cells (MFRN2 or SLC25A28). Erythroid expression of MFRN1 is largely driven by the transcription factors, GATA1 and GATA2 [20]. It seems likely that the iron transported by mitoferrins is derived from the cytosolic iron pool, but it is unclear whether the iron is delivered to mitoferrins in association with a carrier or chaperone-like protein [17].

Less is known about how iron exits the mitochondrial matrix. The ABC transporter ABCB7 was once thought to export iron in the form of Fe-S clusters (based on the yeast orthologue, Atm1) [21], but the exported substrate remains uncharacterized, even though two structures of this exporter were recently reported, and glutathione was considered as a possible substrate [22] [23], though there was evidence that it transported silver or mercury to protect from toxicity [22]. Other mechanisms by which iron could exit the mitochondrial matrix include export of the heme molecule, which may be facilitated by one or more specific transporters [24].

To understand overall mitochondrial iron homeostasis, more proteins involved in mitochondrial iron trafficking need to be identified. A growing list of rare human diseases manifest severe mitochondrial iron overload in the course of disease, and insights into pathophysiology of these diseases may help to identify the proteins involved in mitochondrial iron homeostasis [25]. These diseases interfere with effective Fe-S biogenesis (Table 1), heme synthesis (Table 2), and mitochondrial protein synthesis in the case of MLASA syndromes (Table 3). They cause sideroblastic anemia, in which the mitochondrial matrix accumulates iron that is detected as blue deposits in the Prussian blue stain in areas surrounding the nucleus of red cell precursors (Figure 1a). Early in the disease, small black electron dense iron deposits may be detected (Figure 1b) and late in the disease, the mitochondrial matrix may become loaded with black deposits that outline the cristae because of iron accumulation (Figure 1c).

The fact that mitochondria become iron overloaded in diseases with specific genetic impairments is compatible with the notion that a product of Fe-S or heme biosynthesis is involved in overall regulation of mitochondrial iron homeostasis. The regulatory entity could be a peptide or an iron or heme containing protein that activates a particular transcription factor that coordinates mitochondrial iron acquisition and retention, perhaps by increasing expression of mitoferrin, or decreasing expression of putative iron or heme exporters. Interestingly, in two diseases in which Fe-S assembly is compromised, ISCU myopathy [26] and Friedreich ataxia [27], array analyses showed that expression of mitoferrin was significantly increased. In ISCU myopathy, expression of ALAS1 was also increased [28]. In ISCU myopathy, affected cells would be expected to be heme deficient because ferrochelatase requires a Fe-S cluster for stability and function in the final step of heme biosynthesis [26].

It is interesting that in the MLASA syndromes (Mitochondrial **M**yopathy, **l**actic **a**cidosis and **s**ideroblastic **a**nemia), each of the identified genetic causes affects general mitochondrial protein synthesis or function of ATP6, the putative transporter of protons back into the

mitochondrial matrix that ultimately drives ATP synthesis. It is theoretically possible that MLASA is caused by ATP deficiency, which would adversely affect skeletal muscles, cause lactic acidosis, and perhaps detection of insufficient energy production leads to remodeling of nuclear transcription to attempt to enhance energy production, perhaps by provision of the arguably most critical element- iron.

In Friedreich ataxia, the failure of Fe-S biogenesis appears to precede mitochondrial iron overload [29], but the mitochondrial iron overload that follows may be very destructive on its own. Indeed, down regulation of increased mitoferrin expression reversed mitochondrial iron accumulation and ameliorated nervous system degeneration in a *Drosophila* model of Friedreich's ataxia [30]. In general, the mitochondrial iron deposits in these syndromes contain oxidized iron [31], though iron is sometimes found in mitochondrial ferritin, an iron storage molecule [32] [33] [34]. Many myelodysplastic syndromes with sideroblastic anemia are attributable to mutations in the splicing factor *SF3B1*.

Conclusions- what is the pathophysiology of mitochondrial iron overload?

With the discovery of more genetic defects that cause mitochondrial iron overload, it is possible that a unifying understanding of regulation of mitochondrial iron homeostasis will emerge. Perhaps coexpression profiles and array studies will reveal new proteins involved in iron trafficking through the mitochondria. Also, the molecular causes of some acquired diseases of mitochondrial iron overload may be identified. For instance, a form of sideroblastic anemia that could be cured by administration of pyridoxine was recognized many decades before any mechanism could be proposed. Now it is accepted that the pyridoxine deficiency disables ALAS2, which is a pyridoxal phosphate dependent enzyme involved in the initial step of heme biosynthesis. Another candidate gene that could be responsive to pyridoxine would be NFS1, which causes rare infantile leukoencephalopathies when mutated.

The challenge ahead is to identify the full roster of transporters involved in mitochondrial iron homeostasis and to try to identify a regulatory "overlord" that regulates a nuclear transcriptional response to perceived iron deficiency. Since mitoferrin expression clearly increases in several models of Friedrich's ataxia [35] [30] and ISCU myopathy [28], nuclear remodeling of transcription of critical mitochondrial iron homeostasis genes seems to be the mechanism for coordinating the increase in mitochondrial iron concentrations. Greater understanding could have important implications for numerous human diseases that are not readily treatable at this point. Other candidates for involvement in mitochondrial homeostasis have emerged in model system studies. For instance, in *S. cerevisiae*, all of the proteins involved in early Fe-S biogenesis cause mitochondrial iron overload when disabled [36]. In addition, mutations of the yeast homologues of the putative scaffold proteins, ISA1 and ISA2 cause mitochondrial iron overload. Yeast counterparts of ABCB7, GFER (Erv1 in yeast), and GLCLC (Gamma-Glutamylcysteine Synthetase- the human homologue of GSH1, a glutathione synthase in yeast, cause mitochondrial iron overload. Intriguingly, defective MTM1, and member of the SLC25 superfamily, causes iron overload, but its substrate is not yet known. Also SLC25A15 mutations in yeast Ggc1 mutations, a GTP-GDP exchanger, also cause mitochondrial iron overload. Other candidates that should be carefully examined

with respect to potential roles in mitochondrial iron homeostasis include ABCB6 and ABCB8, two members of the ATP binding cassette (ABC) transporters that are found in mitochondria, with ABCB6 intriguingly occupying a position on the mitochondrial outer membrane [37]. It is possible that mitochondrial iron overload is missed when phenotyping is performed on animal models, because detection of iron requires either special stains with Prussian blue, or correct interpretation of black iron deposits in electron micrographs.

The goal of understanding mitochondrial iron homeostasis is important, and enough different examples are accumulating that insights will likely be forthcoming in the years ahead. Since insights into fundamental pathophysiology are incomplete, regimens to deplete mitochondrial iron overload regionally have been used with some success [38]. However, it would be much better to intervene to prevent mitochondrial iron overload at an earlier stage in the development of disease, but such interventions will require understanding what drives mitochondrial iron overload in an increasingly wide range of diseases.

Acknowledgments

The author thanks the intramural program of NICHD for support, and members of the Rouault lab for their help with this mini-review.

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Figure 1.

- a. A bone marrow smear stained with Prussian blue for iron detection and counterstained with nuclear fast red, which stains erythrocytes and nuclei in immature red cells. The meshwork of blue stained dots surrounding the nuclei represents the mitochondrial network of erythroblasts. When a high percentage of erythroblasts contain high iron, the cells are referred to as “sideroblasts” and the frequent association of this phenotype with anemia is the basis for diagnosing sideroblastic anemia.
- b. An electron micrograph of early sideroblastic anemia, in which several black spots visible within the mitochondrial matrix represent early accumulations of insoluble iron.
- c. An electron micrograph of a sideroblast with advance mitochondrial iron overload. Note that black iron deposits fill the matrix, but the morphology of mitochondria can still be discerned because cristae are not iron-loaded.

Table 1

Defective Fe-S biogenesis

Gene/mutation	Initial Year of identification	Disease gene function	References	Special features
Frataxin	1996	Likely an allosteric regulator of initial Fe-S cluster synthesis	[1] [2] [3]	Mitochondria of Dorsal root ganglia, cardiomyocytes and deep cerebellar nuclei are adversely affected, but other tissues are relatively spared
Glutared oxin 5 (GLRX5)	2005 (in zebrafish)	Likely involved in late stage Fe-S biogenesis, exact function unknown	[4] [5] [6]	Deficiency causes cytosolic iron deficiency, which activates IRE binding activity of IRP1 and represses ALAS2, the first step of heme synthesis, causes sideroblastic anemia
ISCU	Syndrome described in 1964-gene identified in 2008	Scaffold protein upon which nascent clusters are initially assembled	[7] [8] [9] [10] [11]	Abnormal retention of intron in spliced transcript causes loss of function of ISCU as primary scaffold for Fe-S formation
HSPA9 (Hsp 70 homologue)	2015	Enables transfer of nascent Fe-S clusters to recipient proteins	[12] [13] for data on function	Congenital sideroblastic anemia with pseudodominant inheritance pattern
NFS1	2014	Cysteine desulfurase, pyridoxyl phosphate dependent, that requires formation of complex with ISD11 for function	[14]	infantile mitochondrial complex II/III deficiency, a novel autosomal recessive mitochondrial disease characterized by lactic acidemia, hypotonia, respiratory chain complex II and III deficiency, multisystem organ failure and abnormal mitochondria. Functional loss in yeast causes mitochondrial iron overload [15]
ISD11/LY RM4	2013	Enables cysteine desulfurase, NFS1, to function by forming tight	[16]	Neonatal OXPHOS deficiency. Mitochondrial iron overload not

Gene/mutation	Initial Year of identification	Disease gene function	References	Special features
		functional complex.		reported in patients, but would be expected. However, functional loss in <i>S. cerevisiae</i> causes mitochondrial iron overload [17] [18]
ABCB7	2009	An ABC cassette mitochondrial that presumably exports a small molecule to cytosol. Candidates include glutathione, metals, sulfur compounds and peptides [19] [20]	[21]	X-linked sideroblastic anemia and ataxia (XLSA/A) -a recessive disorder characterized by an infantile to early childhood onset of non-progressive cerebellar ataxia and mild anemia

Table 2

Defective heme synthesis

Gene/ mutation	Initial Year of identification	Disease gene function	References	Special features
ALAS2	1995	Enzyme that condenses succinyl CoA with glycine to form amino-levulinic acid	[22] [23]	X-linked sideroblastic anemia- ALAS2 is a pyridoxine dependent enzyme, and provision of pyridoxine ameliorates anemia [24]
FECH	2008	Inserts iron into protoporphyrin IX in final step of heme biosynthesis	[25]	Case report of child with congenital sideroblastic anemia related to mutations in FECH promoter
SLC25A38	2009	Putative importer of glycine used in synthesis of amino-levulinic acid	[26] [27]	Multiple different mutations cause congenital sideroblastic anemia
ABCB10	2014	Forms complex with mitoferrin and ferrochelatase -may export ALAS from mitochondrial matrix [28]Res, 113, 279–87}	[29]	Mitochondrial iron overload found in knockout mouse model

Table 3

Causes of mammalian mitochondrial iron overload in the MLASA (mitochondrial myopathy, lactic acidosis and sideroblastic anemia syndromes)

Gene/mutation	Initial Year of identification	Disease gene function	References	Special features
Pseudouridylate Synthase 1 (PUS1)	2004	Performs post-transcriptional trans-glycosylation of ribosomal, transfer RNAs and mRNAs-encodes nuclear and mitochondrial isoforms-function unknown	[30] [31] [32] [33]	May function as structural “glue” in ribosomal RNAs and tRNAs. Modifications may occur co-transcriptionally
mitochondrial tyrosyl-tRNA synthetase (YARS2)	2010	Mitochondrial protein that charges tRNAs with tyrosine	[34] [35] [36]	May impair mitochondrial protein synthesis and ribosome recycling
ATP6 (Component of Complex V)	2014	Generates ATP using energy from mitochondrial proton gradient	[37]	Decrease in oligomycin-sensitive respiration, a finding which is consistent with a complex V defect-may play a direct role in translocation of protons across membrane.