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Current Opinion in Genetics & Development

Mitochondrial iron overload: causes and consequences

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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.

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Current Opinion in Genetics & Development 2016, **38**:31–37

This review comes from a themed issue on **Molecular and genetic bases of disease**

Edited by **Jason Bielas** and **Carolyn Suzuki**

For a complete overview see the Issue and the Editorial

Available online 25th March 2016

http://dx.doi.org/10.1016/j.gde.2016.02.004

0959-437X/Published by Elsevier Ltd.

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, 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 (39).

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

Table 1

Defective Fe-S biogenesis

Gene/mutation	Initial year of identification	Disease gene function	Refs	Special features
Frataxin	1996	Likely an allosteric regulator of initial Fe-S cluster synthesis	[1–3]	Mitochondria of dorsal root ganglia, cardiomyocytes and deep cerebellar nuclei are adversely affected, but other tissues are relatively spared
Glutaredoxin 5 (GLRX5)	2005 (in zebrafish)	Likely involved in late stage Fe-S biogenesis, exact function unknown	[4–6]	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	[7–11]	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 functional complex	[16]	Neonatal OXPHOS deficiency. Mitochondrial iron overload not reported in patients, but would be expected. However, functional loss in *S. cerevisiae* 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

All references mentioned in this table refer to the section ''References for tables'' in the second part of the Reference list.