

Tangled Up In Red: Intertwining of the Heme and Iron-Sulfur Cluster Biogenesis Pathways

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A large-scale computational and genetic analysis study by Nilsson et al. (2009) has identified five genes that coexpress with heme biosynthetic enzymes and are required for normal heme synthesis. Several are implicated in iron-sulfur cluster biogenesis, and malfunction of these genes may repress heme synthesis by activating the IRE/IRP posttranscriptional regulatory system.

The eukaryotic heme biosynthetic pathway consists of eight enzymes that function in mitochondria for the first and last several steps or in the cytosol in the intervening steps. After the first enzymatic reaction and before the last, heme intermediates must cross mitochondrial membranes, and some of the transporters involved in the complicated heme biosynthetic pathway remain unidentified, including a mitochondrial heme exporter proposed to function in erythroid cells. Because heme intermediates must be transported between the mitochondrial and cytosolic compartments, it would make sense for required transport and regulatory proteins to be coordinately regulated. To identify such factors, Nilsson et al. used large-scale computational screening to identify mitochondrial transcripts that coexpress with the eight basic heme biosynthetic enzymes (Nilsson et al., 2009 [this issue of *Cell Metabolism*]). They identified five transcripts that warranted further study, and they established that morpholino knockdown of each of the five transcripts impaired heme synthesis in the zebrafish model system.

There are two major classes of cofactors that utilize iron in cells: heme groups and iron-sulfur (Fe-S) clusters. Heme molecules consist of a porphyrin ring with a single iron atom bound in the center, and Fe-S clusters are nonheme prosthetic groups in which Fe atoms are bound to inorganic sulfur (S) atoms and to S atoms of cysteines from associated proteins (Lill and Muhlenhoff, 2008; Rouault and Tong, 2008). The presence of iron often facilitates the ability of the prosthetic group-bearing protein to accept or donate a single electron, and fine-tuning of the redox potentials of 12 Fe-S clusters and

6 heme groups promotes electron transport through mitochondrial respiratory chain complexes I–IV.

Interestingly, there are two points in the heme biosynthetic pathway of mammalian and zebrafish erythroid cells at which defects in the Fe-S cluster biogenesis pathway can indirectly suppress heme synthesis. The first intersection occurs in the synthesis of the erythroid form of aminolevulinic acid synthase, ALAS2, which catalyzes the first step of heme biosynthesis. The ALAS2 transcript contains an iron-responsive element (IRE) in its 5'UTR and is thus subject to regulation by cytosolic iron regulatory proteins (IRPs). The relationship between Fe-S cluster biogenesis and heme synthesis was first identified in zebrafish that were deficient in glutaredoxin 5 (*GLRX5*), an Fe-S cluster biogenesis protein. Genetic experiments demonstrated that translation of ALAS2 was repressed by activation of iron regulatory protein 1 (IRP1) (Wingert et al., 2005), a cytosolic regulatory protein that binds to IREs when it lacks a cubane Fe-S cluster at the active site (Rouault, 2006; Muckenthaler et al., 2008). A second point of convergence between the heme and Fe-S cluster biogenesis pathways occurs in the final step of heme synthesis. Ferrochelatase of higher eukaryotes, but not yeast, contains a [2Fe-2S] cluster that is thought to contribute to structural stabilization of the enzyme (Wu et al., 2001). Thus, defects that interfere with Fe-S cluster biogenesis can potentially interrupt heme synthesis by repressing ALAS2 synthesis in erythroid cells or by inactivating ferrochelatase.

Notably, two of the five genes that were coexpressed with heme biosynthetic enzymes, *ISCA1* and *C1orf69*, have been

previously shown to be directly involved in Fe-S cluster biogenesis (Gelling et al., 2008), which is interesting because, even though the heme and Fe-S cluster biosynthetic pathways were previously known to intersect, this study revealed that they are also coregulated. The authors suggested that *ISCA* and *C1orf69* deficiency impaired heme synthesis in their zebrafish assay by activating (IRP1) and repressing ALA synthesis.

To pursue identification of a possible heme intermediate transporter or regulatory protein, they more intensively studied *SLC25A39*, a member of the solute carrier (SLC) family of mitochondrial membrane proteins that transport a diverse array of molecules, ranging from inorganic ions to organic molecules such as citrate and amino acid intermediates (Palmieri, 2008). They established that knockdown of *SLC25A39* reduced heme synthesis and iron incorporation into heme but did not cause accumulation of late intermediates of the heme biosynthetic pathway, thus eliminating ferrochelatase deficiency as a cause of the heme biosynthetic failure. They performed genetic complementation studies and established that human and mouse *SLC25A39* are the orthologs of yeast *MTM1*, a previously characterized yeast solute carrier protein. Although specific roles are known for many SLCs, the role of *MTM1*, originally believed to have a role in manganese transport, remains unknown. More recent phenotypic characterizations in yeast suggested that *MTM1* may affect the Fe-S cluster biogenesis pathway because *MTM1* deficiency resulted in misincorporation of Fe into manganese SOD, similar to a phenotype described for several other proteins defective in yeast Fe-S

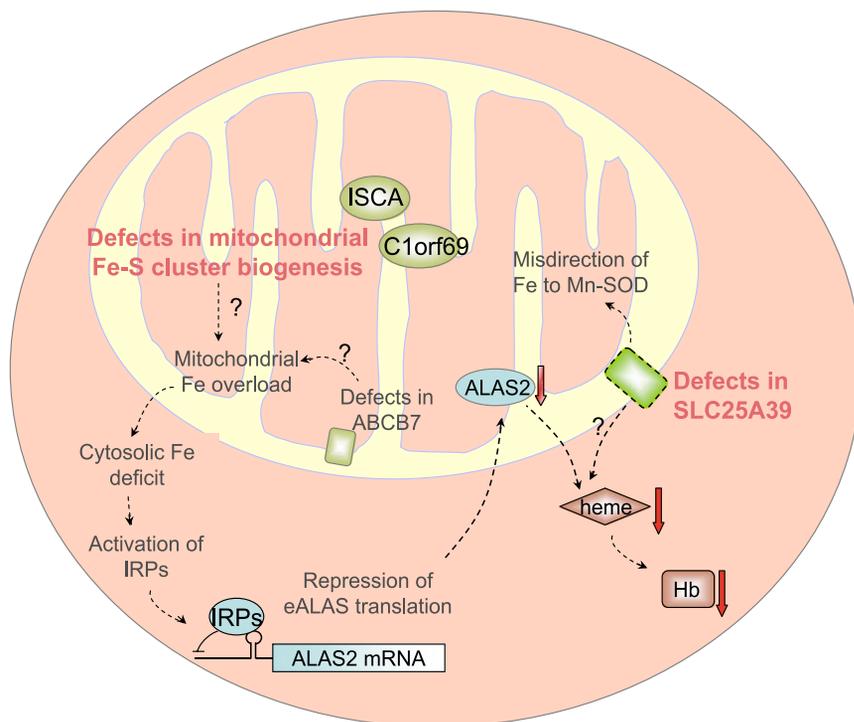


Figure 1. Intersections between the Fe-S Cluster and Heme Biosynthetic Pathways in Erythroid Cells and Proposed Regulation of Mitochondrial Iron Homeostasis

In erythroid cells of mammals and zebrafish, defects in iron-sulfur cluster biogenesis proteins have been observed to result in mitochondrial iron overload and cytosolic iron depletion. Loss of mitochondrial Fe-S cluster biogenesis and cytosolic iron depletion can result in activation of iron regulatory proteins 1 and 2 (IRPs) to bind to iron-responsive elements (IREs), which are RNA stem-loop elements that are found in transcripts of some iron metabolism genes. When IRPs bind to transcripts such as ALAS2 that contain an IRE in the 5' UTR (shown as a stem-loop in the ALAS2 mRNA), they are expected to repress translation and diminish ALAS2 protein levels. ALAS2 encodes the first heme biosynthetic enzyme, and the repressive role of the IRE in zebrafish heme biosynthesis was detected in genetic rescue experiments in animals deficient in the Fe-S cluster biogenesis protein, glutaredoxin 5 (data not shown). IRP-dependent repression of ALAS2 translation may account for the repression of heme synthesis in many experimental settings, including some of the morpholino studies described by Nilsson et al. (2009) in this issue of *Cell Metabolism*. SLC25A39 and ABCB7 are mitochondrial membrane transporters with unknown substrates that could participate in regulation of mitochondrial iron homeostasis or transport of heme intermediates. In yeast, malfunction of the SLC25A39 ortholog, MTM1, results in misdirection of iron into the active site of the mitochondrial manganese superoxide dismutase, MnSOD, and increased oxidative stress. Misincorporation of iron into the MnSOD has been observed in several yeast strains that have defective iron-sulfur cluster biogenesis, and the misincorporation may be caused by overload of a particular type of bioavailable iron in mitochondria.

cluster biogenesis. Loss-of-MTM1 activity appears to facilitate generation of a form of mitochondrial iron that can occupy the manganese-binding site of Mn SOD and exclude manganese binding, thereby inactivating mitochondrial superoxide dismutase (Naranuntarat et al., 2009).

Thus, SLC25A39/MTM1 joins the ranks of mitochondrial membrane proteins whose functions in iron metabolism are incompletely characterized, including ABCB7 and its yeast homolog, Atm1 (Lill and Muhlenhoff, 2008). A possible reason that the transported species of SLC25A39 and ABCB7 remain uncharacterized could be that these proteins transport regulatory molecules, rather

than basic components of metabolism. Numerous studies in yeast and mammalian cells point to the conclusion that interruption of Fe-S cluster biogenesis results in mitochondrial iron overload (Lill and Muhlenhoff, 2008; Rouault and Tong, 2008). In addition, human ALAS2 mutations result in sideroblastic anemia, a type of anemia that is characterized by mitochondrial iron overload in developing erythroid cells (Camaschella, 2008), suggesting that loss of heme or a heme intermediate can also drive mitochondrial iron overload under some circumstances. Moreover, recent data show that a state of cytosolic iron depletion can coexist with a state of mitochondrial iron overload in mammalian cells that

have disruptions of the Fe-S cluster machinery, suggesting that iron homeostasis is regulated by distinct processes in the cytosolic and mitochondrial compartments (Rouault and Tong, 2008).

Although much more needs to be discovered about how mitochondrial iron homeostasis is regulated, it is possible that export of a molecule that contains an intact Fe-S cluster and/or a heme intermediate from mitochondria might be used to convey the signal that mitochondria have sufficient iron. Then, if either heme synthesis or Fe-S cluster synthesis was disrupted, the cytosolic/nuclear compartment would perceive mitochondrial iron deficiency and might respond by significantly increasing mitochondrial iron stores. As is shown Figure 1, defects in Fe-S cluster biogenesis proteins, ABCB7, and SLC25A39 can decrease heme synthesis, and repression of ALAS2 translation may be the common underlying mechanism. Although the fundamental heme biosynthetic enzymes were identified long ago, this new work illustrates that much remains to be discovered about the relationships between the heme biosynthetic pathway, Fe-S cluster biogenesis, and mitochondrial iron homeostasis.

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