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Mini Review

Compartmentalization of iron between mitochondria and the cytosol and its regulation

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ABSTRACT

Iron is essential for life. Its coordinated distribution between intracellular compartments and the adaptation of iron uptake to intracellular demands are central for a balanced iron homeostasis. Mitochondria take center stage in cellular iron metabolism as they harbor the two major iron-utilizing pathways, the synthesis of heme and the biogenesis of iron–sulfur (Fe/S) proteins. Consistent with this central role, mitochondria are also critical regulators of cellular iron homeostasis. They directly influence cellular iron uptake and the status of iron-utilizing metabolic processes through iron-dependent co-factors or by control of gene expression. For all these aspects of cellular iron metabolism, the uptake of iron into mitochondria is critical. During the last decade, considerable progress has been made with respect to the functional characterization of mitochondrial iron acquisition and the identification of transporters involved. The model organism *Saccharomyces cerevisiae* has been especially useful for the elucidation of this process. Here, we summarize the recent advances in the mechanism of mitochondrial iron transport and the impact of mitochondria on the regulation of cellular iron homeostasis.

1. Introduction

Iron is a key trace element for virtually all organisms. It functions as an essential co-factor in central cellular processes such as respiration, metabolite biosynthesis, DNA synthesis and repair, ribosome biogenesis, and oxygen transport in vertebrates. Although iron is highly abundant, its bioavailability is low due to its poor solubility under ambient conditions. Therefore all cells have developed efficient iron uptake systems to meet cellular iron demands (for recent reviews see Anderson and Vulpe, 2009; Blaiseau et al., 2011; Dlouhy and Outten, 2013; Haas, 2014; Kaplan and Kaplan, 2009; Labbe et al., 2013; Lane et al., 2015; Lawen and Lane, 2013; Muckenthaler et al., 2008). For microbial pathogens, acquisition of iron from infected cells or tissues is frequently crucial for virulence (Cassat and Skaar, 2013; Haas, 2012; Nairz et al., 2014; Parrow et al., 2013). Accordingly, mammals react to microbial infections by inducing iron withholding defence systems, in order to deprive the environment of pathogens from essential iron ions (Nairz et al., 2014; Parrow et al., 2013; Weinberg, 2009).

Cellular iron levels must be delicately balanced, as intracellular iron is both a source and an amplifier of reactive oxygen species and thus toxic at higher concentrations (Lyons and Eide, 2007). To maintain appropriate cellular iron levels and to avoid iron-loading, cells have developed sophisticated systems for assuring a balanced cellular iron homeostasis (Dlouhy and Outten, 2013; Dunn et al., 2007; Hentze et al., 2010; Kaplan and Kaplan, 2009; Labbe et al., 2013; Muckenthaler et al., 2008; Outten, 2014; Richardson et al., 2010). At the cellular level, this balance is achieved through a strict coupling of cellular iron uptake at the plasma membrane to intracellular iron demands, and a balanced intracellular distribution of iron between the cellular compartments involved in iron-utilization and storage. A tightly regulated iron metabolism is essential, and disruption or over-expression of iron-related molecules can have significant health consequences. Defects in mammalian proteins involved in iron transport, regulation, or utilization in mitochondria are frequently associated with recessive chronic degenerative disorders with either chronic anemia or systemic iron overload (Beilschmidt and Puccio, 2014; Heeney and Andrews, 2004; Hentze et al., 2010; Kaplan et al., 2011; Lane et al., 2015; Muckenthaler et al., 2008; Papanikolaou et al., 2005; Stehling et al., 2014). In the latter case, the cytotoxic effects of elevated intracellular iron levels result in

Abbreviations: ISC, iron sulfur cluster assembly; CIA, cytosolic sulfur cluster assembly; Grx, glutaredoxin; MCF, mitochondrial solute carrier family.

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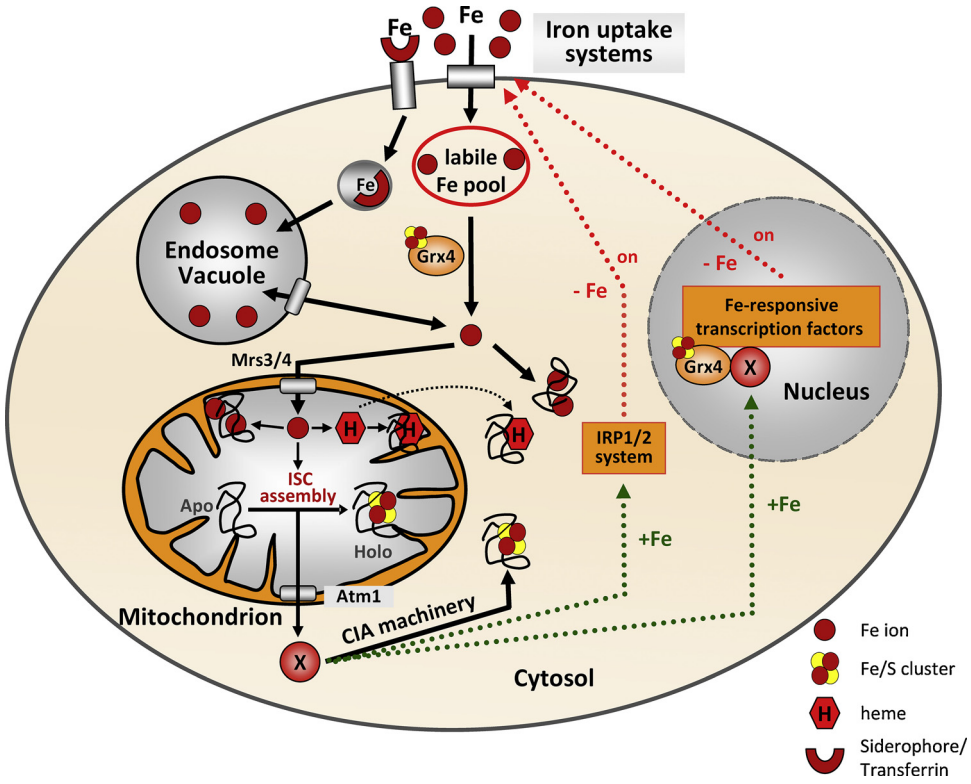

Fig. 1. Intracellular iron trafficking and the regulation of cellular iron uptake in eukaryotes. Iron ions acquired at the plasma membrane by high- and low-affinity iron uptake systems enter the cytosol, where they likely bind to diverse low molecular weight biological ligands, forming the labile iron pool. In parallel, fungi internalize iron siderophore complexes and vertebrates internalize iron bound to transferrin by receptor mediated endocytosis. Internalized iron is released in the endosomal/vacuolar compartment from where it is exported into the cytosol by the high affinity iron transporter Fth1/Fet5 and Smf3 (vertebrate DMT1) (Philpott, 2006). Cytosolic iron is transported into the mitochondrial matrix by the mitochondrial carrier family proteins Mrs3/4 (vertebrate MFRN1 and 2) where it is used for heme synthesis and the *de novo* synthesis of Fe/S clusters which is catalyzed by the mitochondrial ISC assembly system (Lill et al., 2012). In fungi, excess cytosolic iron is exported into the vacuole by the vacuolar divalent metal transporter Ccc1. In vertebrates, iron is stored in ferritin in the cytosol. The essential cytosolic monothiol glutaredoxin Grx4 (mammalian PICOT) plays a central role in cytosolic iron trafficking. Grx4 accepts iron from the cytosolic labile iron pool in form of an Fe/S cluster and is crucially involved in the donation of iron to cytosolic iron-dependent enzymes and the cytosolic iron sulfur protein assembly (CIA) system (Paul and Lill, 2015). The latter further requires an unknown sulfur-containing low-molecular weight solute (X) that is produced by the mitochondrial ISC system and exported into the cytosol by the mitochondrial inner membrane ABC transporter Atm1 (vertebrate ABCB7) (Lill et al., 2014a). Cellular iron acquisition is tightly regulated. In vertebrates, the cytosolic iron regulatory proteins IRP1 and IRP2 play key roles in the posttranscriptional regulation of iron metabolism. Both bind to iron-responsive elements (IREs) of iron-regulated mRNAs. Under high iron conditions, the CIA system assembles a [4Fe-4S] cluster on IRP1 which transforms IRP1 into a cytosolic aconitase that no longer binds to IREs (Fig. 3). In fungi, genes involved in iron uptake at the plasma membrane are controlled by iron-responsive transcriptional regulators that respond to two iron-dependent intracellular signals: (1) A Grx4 bound Fe/S-cluster that functions as sensor for the status of the cytosolic iron pool. (2) A key regulatory molecule (X) that signals the iron status of the mitochondrial ISC systems (Fig. 5).

chronic progressive tissue damage and ultimately failure of the organs involved (Heeney and Andrews, 2004; Munoz et al., 2011; Papanikolaou et al., 2005).

In eukaryotes, mitochondria are the major site of iron-utilization. These well recognized “power-houses” of the cell are major consumers of iron as they harbor several abundant iron-dependent proteins that play essential roles in the respiratory chain (complexes I–IV), the citric acid cycle (aconitase) and the biosynthesis of amino acids and vitamins (*e.g.*, lipoate synthase). More importantly, they are also central sites for the synthesis of iron-containing co-factors (Figs. 1 and 2). The initial step in the biosynthesis of heme, and the final step, the insertion of ferrous iron into protoporphyrin IX, are located in mitochondria. Proteins with heme co-factors are involved in a variety of essential metabolic pathways, and heme deficiency is lethal, unless cells are supplied with an external source of heme (Schultz et al., 2010; Sinclair and Hamza, 2015; Yuan et al., 2013). Moreover, mitochondria harbor the mitochondrial ISC (iron–sulfur cluster assembly) and ISC export systems that are essential for the maturation of all cellular proteins with iron–sulfur (Fe/S) co-factors, whether located in mitochondria, the cytosol or the nucleus (Lill et al., 2014b; Pain and Dancis, 2014; Rouault, 2012; Lill et al., 2015). The maturation of extra-mitochondrial proteins requires the export of a small solute that is produced by the mitochondrial ISC system and exported *via* the mitochondrial ABC transporter Atm1 into the cytosol where it is used by the cytosolic Fe/S protein assembly (CIA) system for the formation of cytosolic and nuclear Fe/S proteins (Lill et al., 2014a; Netz et al., 2014; Paul and Lill, 2015; Sharma et al., 2010). Several cytosolic and nuclear Fe/S proteins play essential roles in protein translation (*e.g.*, the ABC protein Rli1) (Hopfner, 2012), DNA synthesis and repair (*e.g.*, DNA polymerases and helicases) (Fuss et al., 2015; White, 2009), and other aspects of genome stability (Gari et al., 2012; Lill et al., 2014b; Stehling et al., 2012; Wu and Brosh, 2012). Hence, many of the 17 known members of the mitochondrial ISC systems are essential for cell viability, and mutations in genes encoding ISC members are associated with recessive diseases with complex neurodegenerative, hematological and metabolic phenotypes (Beilschmidt and Puccio, 2014; Rouault, 2012; Stehling and Lill, 2013).

Consistent with the crucial role of mitochondria in cellular metabolism, perturbations of mitochondrial functions activate a variety of downstream signaling pathways. Mitochondrial dysfunctions, especially those caused by damage of the citric acid cycle or respiration, induce the mitochondrial retrograde signaling pathway that results in transcriptional and metabolic reconfigurations, such as carbohydrate and nitrogen metabolism, in order to accommodate cells to defects in mitochondria (Liu and Butow, 2006). Accumulation of unfolded proteins within mitochondria triggers