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Iron misregulation and neurodegenerative disease in mouse models that lack iron regulatory proteins

Manik C. Ghosh, De-Liang Zhang, Tracey A. Rouault *

Section on Human Iron Metabolism, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA

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ABSTRACT

Iron regulatory proteins 1 and 2 (IRP1 and IRP2) are two cytosolic proteins that maintain cellular iron homeostasis by binding to RNA stem loops known as iron responsive elements (IREs) that are found in the untranslated regions of target mRNAs that encode proteins involved in iron metabolism. IRPs modify the expression of iron metabolism genes, and global and tissue-specific knockout mice have been made to evaluate the physiological significance of these iron regulatory proteins (Irps). Here, we will discuss the results of the studies that have been performed with mice engineered to lack the expression of one or both Irps and made in different strains using different methodologies. Both *Irp1* and *Irp2* knockout mice are viable, but the double knockout ($Irp1^{-/-}Irp2^{-/-}$) mice die before birth, indicating that these Irps play a crucial role in maintaining iron homeostasis. $Irp1^{-/-}$ mice develop polycythemia and pulmonary hypertension, and when these mice are challenged with a low iron diet, they die early of abdominal hemorrhages, suggesting that Irp1 plays an essential role in erythropoiesis and in the pulmonary and cardiovascular systems. $Irp2^{-/-}$ mice develop microcytic anemia, erythropoietic protoporphyria and a progressive neurological disorder, indicating that Irp2 has important functions in the nervous system and erythropoietic homeostasis. Several excellent review articles have recently been published on *Irp* knockout mice that mainly focus on $Irp1^{-/-}$ mice (referenced in the introduction). In this review, we will briefly describe the phenotypes and physiological implications of $Irp1^{-/-}$ mice and discuss the phenotypes observed for $Irp2^{-/-}$ mice in detail with a particular emphasis on the neurological problems of these mice.

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* Corresponding author.
E-mail address: rouault@mail.nih.gov (T.A. Rouault).
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Introduction

In mammals and most organisms, iron is indispensable because of its essential role in DNA synthesis, mitochondrial respiration, hemoglobin synthesis and formation of iron–sulfur clusters. Iron is a transition metal with two stable oxidation states, +2 (Ferrous, Fe^{2+}) and +3 (Ferric, Fe^{3+}). Since the redox potential of aqueous Fe^{3+}/Fe^{2+} system (0.77 V) is neither too high nor too low, conversion from one oxidation state of iron to the other does not require much energy. Thus, iron has a unique ability to act both as an electron acceptor and an electron donor, making it indispensible for many biological reactions involving electron transfer and oxygen transport.

Maintenance of iron homeostasis is extremely vital. When cellular iron concentrations are low, the functions of numerous iron proteins are impaired, causing diseases such as anemia and cognitive deficits that affect millions of people worldwide. On the contrary, excess cellular iron catalyzes the formation of reactive oxygen species that are associated with diseases like hemochromatosis. In addition, excess iron in the brain is associated with diseases like Alzheimer's, Parkinson's and Friedreich's ataxia (Zecca et al., 2004). Thus, organisms and cells must tightly regulate iron metabolism to ensure that sufficient, but not excess, iron is supplied to heme, the iron–sulfur prosthetic groups of mitochondrial respiratory chain complexes, and other cellular iron proteins. This elegant regulation of iron is largely orchestrated at the cellular level by two iron regulatory proteins, IRP1 and IRP2, and at the systemic level by iron regulatory hormone, hepcidin (reviewed in Rouault, 2006, 2013; Hentze et al., 2010; Anderson et al., 2012; Ganz and Nemeth, 2012; Wilkinson and Pantopoulos, 2014; Zhang et al., 2014).

Cellular iron metabolism

Iron absorption in the enterocyte

Dietary ferric iron is first reduced in duodenum by the ferrireductase, duodenal cytochrome B (DcytB) (McKie et al., 2001; Choi et al., 2012). The divalent ferrous iron is then transported by a membrane iron transporter, divalent metal transporter 1 (DMT1) into the enterocyte (Gunshin et al., 1997). Internalized ferrous iron either remains accessible in a labile iron pool (LIP) by binding to hitherto unknown molecules, is stored in the iron storage protein ferritin or exits the enterocyte through the basolateral iron exporter, ferroportin 1 (FPN1) (Abboud and Haile, 2000; Donovan et al., 2000; McKie et al., 2000). Exported ferrous iron is then oxidized by hephaestin to ferric iron (Vulpe et al., 1999), which binds to transferrin (Tf), and the resulting diferric transferrin complex (diferric-Tf) circulates through the plasma to deliver iron to the tissues (Fig. 1).

Iron transport

In most tissues, the circulating pool of diferric-Tf serves as the major source of iron. This diferric-Tf binds to transferrin receptor 1 (TfR1) on the plasma membrane, and the resulting diferric-Tf-TfR1 complex internalizes into the endosome, where V-ATPase-mediated acidification causes conformational changes in Tf and TfR1, facilitating release of ferric iron from diferric-Tf. Ferric iron is reduced to ferrous iron by the endosomal ferrireductase known as six-transmembrane epithelial antigen of the prostate 3 (Steap3) (Ohgami et al., 2005). Ferrous iron is then transported by DMT1 into the cytosol (Fleming et al., 1998). The apo-Tf-TfR1 complex is unstable because apo-Tf has a very low affinity for TfR1. Thus, after the release of iron, both Tf and TfR1 recycle back to the cell surface. DMT1 can also directly transport non-transferrin-bound iron (NTBI) into cells, especially in conditions of hemochromatosis and hemolytic anemia when serum iron concentrations exceed the binding ability of Tf (Chua et al., 2004; Sarkar, 1970). ZIP14, a member of the SLC39A zinc transporter family, also transports NTBI in iron overload conditions (Liuzzi et al., 2006; Guo et al., 2008; Bishop et al., 2010; Pinilla-Tenas et al., 2011). In the cytosol, iron is incorporated into iron proteins or transported to cellular organelles, and excess iron is sequestered and stored in the iron storage protein, ferritin. The iron that is not used or stored, is exported by the iron exporter, FPN1, into plasma where it is oxidized to ferric iron by ceruloplasmin or hephaestin (Wolf et al., 2006; Vulpe et al., 1999). Tf binds ferric iron and circulates iron to other cells and tissues (Fig. 1).

Regulation of cellular iron metabolism by IRPs

Cells maintain optimum cytosolic iron levels by regulating the expression of the iron import protein TfR1, the iron transport protein DMT1, the iron storage protein ferritin and the iron export protein FPN1. The expression levels of TfR1, DMT1, ferritin and FPN1 are post-transcriptionally regulated by binding of IRP1 or IRP2 to IREs in the transcripts that encode these iron metabolism proteins.

IRP1 and IRP2 are homologous proteins with 56% sequence identity (Pantopoulos, 2004). IRP2 has an additional cysteine-rich 73 amino acid domain with unknown function (Bourdon et al., 2003; Wang et al., 2004). Both IRP1 and IRP2 are expressed ubiquitously. The expression of Irp1 is dominant in kidney, liver and brown fat, whereas the expression of Irp2 is dominant in the central nervous system (Meyron-Holtz et al., 2004a,b). The activities of these iron regulatory proteins are also regulated by iron, but through different mechanisms (Rouault, 2006).

IRP1 is a bifunctional protein that exists in an equilibrium between the [4Fe-4S] containing holo-form, which has cytosolic aconitase activity, and its apo-form, which binds to IREs. At high cellular iron concentrations, this equilibrium shifts toward cytosolic aconitase, and at low cellular iron levels, the equilibrium shifts toward the IRE binding form. Thus, iron changes the IRE binding activity of IRP1, but usually not the protein concentration. In contrast, at high iron concentrations, IRP2 undergoes proteasomal degradation by an E3 ubiquitin ligase complex that contains an F-box protein, FBXL5, which is activated when iron and oxygen bind to a hemerythrin domain in FBXL5 (Salahudeen et al., 2009; Vashisht et al., 2009; reviewed in Rouault, 2009). Therefore, both the activity and protein level of IRP2 decrease when the cells are iron replete (reviewed in Rouault, 2006).

Thus, at low intracellular iron concentrations, both IRP1 and IRP2 bind to IREs with high affinity. If an IRE is located in the 5′ UTR of target mRNAs, binding of iron regulatory proteins inhibits the translation of target mRNAs. Ferritin (both L and H chain transcripts) (Hentze et al., 1987; Theil, 1990), FPN1 (Abboud and Haile, 2000; Donovan et al., 2000; McKie et al., 2000), mitochondrial aconitase (ACO2) (Kim et al.,

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