Review

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# When less is more: novel mechanisms of iron conservation

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**Disorders of iron homeostasis are very common, yet the molecular mechanisms of iron regulation remain understudied. Over 20 years have passed since the first characterization of iron-regulatory proteins (IRP) as mediators of cellular iron-deficiency response in mammals through iron acquisition. However, little is known about other mechanisms necessary for adaptation to low-iron states. In this review, we present recent evidence that establishes the existence of a new iron-regulatory pathway aimed at iron conservation and optimization of iron use through suppression of nonessential iron-consuming processes. Moreover, we discuss the possible links between iron homeostasis and energy metabolism uncovered by studies of iron-deficiency response.**

## Iron and the importance of sufficient iron stores

Iron deficiency is the most common nutritional disorder in the world affecting over 2 billion people worldwide, and is especially prevalent in vulnerable groups such as pregnant women and young children [1]. The best-studied manifestation of low systemic iron stores is anemia; however, iron deficiency without a reduction in hemoglobin levels (referred to as iron depletion) also exists, and is considered pathologic. In developed countries iron depletion without anemia appears to be more prevalent than the anemia of iron deficiency [2–4]. A number of studies in humans and animals have revealed a link between reduced systemic iron and a vast array of gross physiologic abnormalities, including delayed development, reduced physical endurance and mental cognition, immune dysfunction, and impaired thermoregulation [5–7].

In addition to its well-recognized role in oxygen and carbon dioxide transport as a part of the hemoglobin molecule, iron serves as an essential cofactor for a number of critical enzymes that regulate virtually all aspects of cellular and whole-body physiology, including mitochondrial respiration and energy production [8], DNA replication and repair [9,10], protein and lipid biosynthesis [11,12], antioxidation and detoxification of foreign compounds [13,14], and more. Insufficient iron stores can potentially disrupt these processes, particularly in iron-rich tissues such as the brain, heart, and hematopoietic system. However, the molecular details of iron regulation on a cellular level are still not fully characterized. In this review, we will discuss recent breakthroughs in our understanding of cellular iron regulation and iron-deficiency response in yeast and mammalian cells, highlighting potential links between iron and a variety of cellular regulatory pathways, including metabolic processes.

## Iron-regulatory proteins: the first responders to iron deficiency

Maintenance of iron homeostasis on the level of a whole organism is primarily mediated by the hepatic hormone hepcidin, which inhibits iron recycling in the blood and dietary iron uptake in the duodenum, thus preventing systemic iron overload [15]. Moreover, hypoxia-inducible factor (HIF)1α and 2α were shown to increase intestinal iron absorption [16,17], iron uptake into erythroid progenitors [18], heme synthesis [19], and to suppress hepcidin

### Glossary

**Aft1/2p**: yeast transcription factors that translocate into nucleus in response to iron deficiency and induce expression of a subset of genes known as 'iron regulon' that are involved in iron acquisition and release from cellular storage vacuoles.

**Bio2/3/4p**: iron-dependent enzymes functioning in biotin synthesis in yeast. Transcription and activity of these proteins is suppressed in low-iron states to conserve cellular iron.

**Cth1/2p**: yeast tandem zinc finger proteins upregulated by Aft1/2p under iron deficiency. Cth1/2p bind to AU-rich elements (AREs) in the 3′ untranslated regions (UTRs) of target mRNA resulting in their degradation. Many of Cth1/2p targets encode iron-requiring proteins or participate in iron-consuming biologic pathways. Inhibition of these pathways reduces cellular iron utilization in nonessential processes and makes iron available for maintenance of viability.

**Hmx1p**: heme oxygenase enzyme responsible for breakdown of heme. *HMX1* expression is induced by iron deficiency, resulting in liberation of heme-bound iron and generation of biliverdin, an antioxidant molecule.

**Iron acquisition**: cellular adaptation to iron deficiency aimed at increasing cellular iron content via increased import from extracellular space, mobilization of cellular iron stores, and inhibition of cellular iron export. Iron-acquisition mechanism is mediated by iron-regulatory proteins 1 and 2 (IRP1/2) in mammals and Aft1/2p in yeast.

**Iron conservation**: mechanism by which cells optimize iron utilization under low-iron states by preferentially shutting down nonessential iron-consuming pathways and directing all available iron towards the functions vital for survival.

**Iron-regulatory proteins 1 and 2 (IRP1/2)**: mammalian proteins that are activated by iron deficiency and function to increase cellular iron levels through post-transcriptional regulation of key proteins involved in iron uptake, storage, and export in mammalian cells.

**Tristetraprolin (TTP)**: a mammalian homolog of yeast *CTH1/2* that is induced by iron deficiency and is required for cellular survival in low-iron states, likely by suppressing nonessential iron-requiring pathways and mediating the iron-conservation pathway.

**Vht1p**: an iron-independent yeast biotin importer; expression is upregulated by Aft1p in iron deficiency.

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production [20], thus ensuring adequate supply of iron to support erythropoiesis. Systemic iron regulation is reviewed in [21,22].

The mechanisms for maintaining iron homeostasis on a level of a single cell are distinct from systemic iron regulation. To ensure adequate iron content, mammalian cells regulate iron levels through uptake and export [23]. Iron enters the cell from the bloodstream in a complex with transferrin, which binds to transferrin receptor 1 (TfR1) on the plasma membrane, followed by receptor-mediated endocytosis, endosomal vesicle acidification, reduction of iron into its soluble form by STEAP3 metalloreductase, and release of iron into the cytosol by divalent metal transporter 1 (DMT1). In the cytoplasm, iron is incorporated into iron-containing enzymes, imported into the mitochondria for iron–sulfur (Fe/S) cluster and heme biosynthesis, or stored in a complex with ferritin multimers. Finally, iron can exit the mammalian cell through a membrane transporter ferroportin 1 (Fpn1) and its associated metalloreductase ceruloplasmin. For a detailed review of mammalian iron transport, see [23]. Tight regulation of iron import, export, and storage is critical to prevent iron deficiency and impairment of vital cellular functions or iron overload, which leads to generation of toxic radicals via an iron-catalyzed Fenton reaction.

Mammalian iron-regulatory proteins 1/2 (IRP1/2) function as central regulators of iron-deficiency response in the cell via modulation of mRNA stability and translation of key iron regulatory proteins (IRP), for a detailed review of IRP1/2 see [24]. In iron-replete states, IRP1 contains an Fe/S center and functions as a cytosolic aconitase (Figure 1A). IRP2 has no aconitase activity or Fe/S center, but instead is degraded by an iron-sensing ubiquitin ligase FBXL5. IRP1/2 activity is suppressed in cells with adequate iron stores. However, a drop in cellular iron activates IRP1 through its loss of Fe/S center, and stabilizes IRP2 protein via degradation of its negative regulator, FBXL5, which also loses its di-iron center and becomes a target of the proteosome [25]. Upon activation, IRP1/2 interact with iron-response elements (IRE) in the 3′ and 5′ untranslated regions (UTRs) of various mRNA molecules and alter their stability or translation. Specifically, activation of IRP1/2 response enhances iron uptake into the cell by binding to and stabilizing TfR1 mRNA, which harbors multiple IREs in its 3′UTR. By contrast, binding of IRPs to the 5′UTR suppresses translation of several mRNAs, including Fpn1 and ferritin, thus inhibiting iron export from the cell and promoting release of stored iron, respectively [24] (Figure 1A). A conceptually similar regulatory pathway exists in yeast *Saccharomyces cerevisiae*, where iron deprivation induces translocation of transcription factors Aft1/2p (see Glossary) from cytosol into the nucleus, which in turn upregulate transcription of the so-called iron regulon that includes genes involved in reductive and siderophore-mediated iron import [26] (Figure 1B). Overall, the mammalian IRP1/2 and yeast Aft1/2p mechanisms function to restore iron equilibrium by increasing iron import and release of iron from cellular stores, although in mammals this process is regulated on a

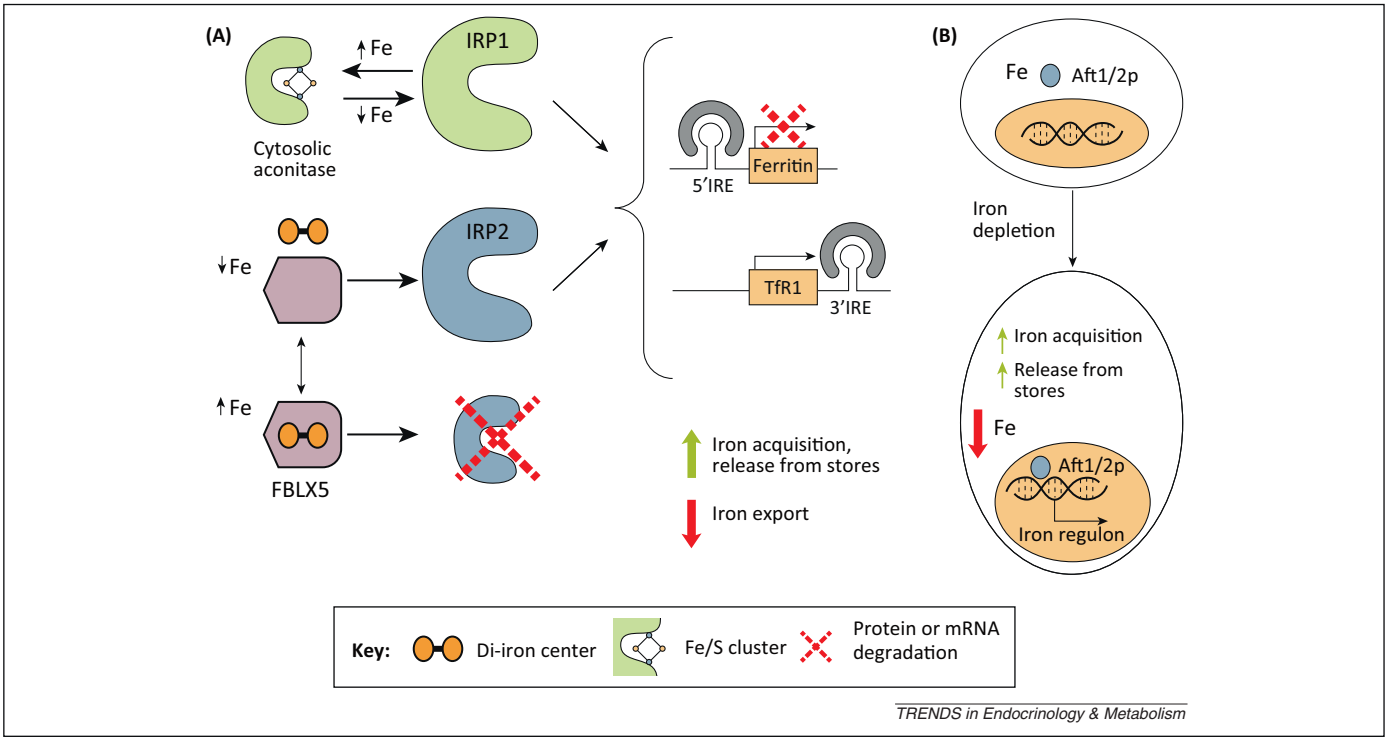


**Figure 1**. Canonical iron-acquisition pathway in mammals and yeast. **(A)** In mammalian cells iron-regulatory proteins 1 and 2 (IRP1/2) are activated in response to iron deficiency. Under iron-replete conditions, IRP1 contains an iron–sulfur cluster and functions as a cytosolic aconitase, and IRP2 is degraded by the proteosome. In low iron conditions IRP1 is activated by losing its iron–sulfur cluster and IRP2 protein is stabilized. IRP1/2 then bind to the iron response elements (IREs) in the 3′ and 5′ untranslated regions (UTRs) of target mRNA. Binding to the 3′UTR stabilizes target mRNA of transferrin receptor 1 (TfR1) responsible for iron uptake. Binding to the 5′UTR halts translation of the target mRNA, suppressing proteins involved in iron export [ferroportin 1 (Fpn1)] and storage (ferritin). Overall, IRP1/2 restore cellular iron balance by increasing iron import, mobilizing stored iron, and reducing iron export. **(B)** In iron-deficient yeast, Aft1/2 transcription factors translocate from cytosol into the nucleus and activate transcription of the iron regulon, which encodes genes required for iron uptake and release from intracellular stores, thus effectively increasing intracellular iron content. Abbreviations: Aft1/2, activator of ferrous transport 1/2; FBLX5, F-box/LRR-repeat protein 5.