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When Less is More: Novel Mechanisms of Iron Conservation

Marina Bayeva, Hsiang-Chun Chang, Rongxue Wu, and Hossein Ardehali*

Feinberg Cardiovascular Research Institute, Northwestern University School of Medicine, Tarry
14-733, 303 E Chicago Ave, Chicago, IL

Abstract

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 existence of a new iron regulatory pathway aimed at iron conservation and optimization of iron use through suppression of non-essential iron-consuming processes. Moreover, we discuss the possible links between iron homeostasis and energy metabolism uncovered by studies of iron deficiency response.

Keywords

iron deficiency; metabolism; diabetes; tristetraprolin; Cth1/2

Iron and the importance of sufficient iron stores

Iron deficiency is the most common nutritional disorder in the world that affects 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 pathological. In the 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 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],

*Corresponding Author: Hossein Ardehali (h-ardehali@northwestern.edu).

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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 factors (HIF) 1 α and 2 α were shown to increase intestinal iron absorption [16–17], iron uptake into erythroid progenitors [18], heme synthesis [19], and to suppress hepcidin 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 the systemic iron regulation. To ensure adequate iron content, mammalian cells regulate iron levels through both 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 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 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 (for a detailed review of IRP1/2 see [24]). In iron-replete states, IRP1 contains an iron-sulfur (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 proteasome [25]. Upon activation, IRP1/2 interact with iron response elements (IRE) in the 3' and 5' untranslated regions (UTR) 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. On the other hand, 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 *S. cerevisiae*, where iron deprivation induces translocation of transcription factors Aft1/2p 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 post-transcriptional level, while the yeast mechanism is regulated through modulation of gene transcription.

Although the IRP1/2 system had remained the only iron regulatory pathway known in mammalian cells, the mechanism by which iron-starved cell survived under iron deficient conditions could not be fully explained by the IRP1/2 actions, suggesting the existence of another iron regulatory pathway.

Iron acquisition and iron conservation in yeast

A reduction in cellular iron levels due to increased demand, such as during rapid division, or as a result of a small decrease in extracellular iron content, results in activation of Aft1/2p- or IRP1/2-dependent uptake mechanisms, in yeast and mammalian cells, respectively. Induction of this so-called *iron acquisition* pathway restores cellular iron content by bringing exogenous iron into the cell and mobilizing intracellular iron stores. Surprisingly, an in-depth analysis of Aft1/2 downstream targets revealed a subset of genes whose function was unrelated to any of the iron uptake/mobilization pathways, but which, nevertheless, were critical for cellular adaptation to iron deficiency, as shown through genetic deletion studies [27–28]. Moreover, the iron acquisition model failed to explain the mechanism for cellular adaptation to severe or prolonged iron deficiency, when Aft1/2-mediated iron uptake alone was insufficient to normalize cellular iron. If restoration of cellular iron balance depended solely on iron uptake and redistribution, iron-starved cells would not be able to survive or proliferate under conditions of very low extracellular iron. Yet, yeast subjected to strong iron chelation displayed over 20-fold reduction in cellular iron levels, but only about 20% reduction in growth rate [12], demonstrating that cells were able to survive and proliferate despite failure of the Aft1/2 mechanism to correct cellular iron deficiency and the resultant lower steady-state intracellular iron content.

A number of recent studies supported the existence of a previously unrecognized iron-regulatory pathway aimed at optimizing the use of iron inside the cell through suppression of non-essential iron-consuming pathways. This so-called *iron conservation pathway* acts in parallel to the IRP1/2 or Aft1/2-mediated iron acquisition response. Specifically, iron-deficient cells activate iron uptake mechanisms first, which may be sufficient to restore cellular iron levels. However, if intracellular iron remains low despite induction of iron import/redistribution, the conservation pathway is activated and preferentially delivers iron to the most vital cellular processes by shuttling it away from non-essential pathways [29].

Conceptually, identification of the iron conservation pathway is the first major breakthrough since the discovery of IRP1/2 mechanism more than 20 years ago in our understanding of iron regulatory mechanisms on a cellular level. Studies in yeast not only confirmed the importance of iron conservation pathway in cellular adaptation to iron deficiency, but also uncovered a novel link between iron homeostasis and cellular energy metabolism. As discussed below, both transcriptional and post-transcriptional mechanisms of iron sparing have been described to date.

Iron Conservation through Transcriptional Regulation

The transcriptional mechanism of iron conservation is characterized by a switch from an iron-requiring pathway to an iron-independent one, diverting iron away from non-essential processes. One example of such regulation is biotin homeostasis in yeast. Endogenous synthesis of biotin requires the activity of iron-containing Bio2/3/4p enzymes that are actively transcribed under iron-replete conditions [30]. In addition, yeast cells are capable of importing biotin from the extracellular environment via a high-affinity transporter Vht1p [31], which does not require iron cofactor and is a downstream target of Aft1p [27]. Iron deficiency leads to increased transcription of the *VHT1* gene, while simultaneously suppressing transcription of *BIO2/3/4*, thus shutting down an iron-requiring synthetic pathway in favor of the iron-independent biotin import (Figure 2A). Importantly, while *VHT1*^{+/+} yeast cells were able to grow normally on both iron-poor and iron-rich medium, in the presence and absence of the synthetic biotin precursor KAPA, *VHT1*^{-/-} cells only grew on iron-rich medium supplemented with KAPA, consistent with inhibition of biotin uptake and complete reliance on the endogenous synthetic pathway. However, when grown in iron-poor medium, *VHT1*^{-/-} yeast failed to proliferate both in the presence and absence of KAPA, highlighting the critical importance of the Aft1p-Vht1p biotin transport mechanism in adaptation to iron deficiency [27].

Another example of the transcriptional adaptation to iron deficiency is the glutamate biosynthetic pathway, although the role of Aft1/2p transcription factors in this pathway is less clear. Yeast can synthesize glutamate using an iron-dependent Glt1p enzyme or an iron-independent Gdh3p enzyme. In low-iron states, *GDH3* transcription is upregulated and *GLT1* transcription is suppressed, diverting iron away from the iron-consuming pathway [27].

Finally, the heme biosynthesis pathway is a major consumer of cellular iron, and suppression of heme synthesis significantly increases iron availability for other cellular processes. In iron deficient yeast, reduction in heme levels is achieved through an Aft1-dependent transcriptional activation of heme oxygenase (*HMX1*) that is responsible for heme degradation, and transcriptional suppression of heme biosynthetic enzymes [32]. These two processes together increase the amount of “usable” iron in the cell. Cells with a deletion of *HMX1* contain higher levels of intracellular heme when grown in low iron, and display reduced growth rate in iron-poor medium supplemented with hemin, compared to cells with normal expression of this enzyme [32].

In summary, iron-deficient yeast cells use transcriptional regulatory mechanisms to steer away from iron-requiring pathways and towards iron-independent ones, conserving limited

iron supply while maintaining normal levels of essential molecules. It is important to note that, unlike *S. cerevisiae*, other species of fungi do not rely on Aft1/2 for iron regulation, but instead utilize iron-sensing transcriptional repressors [29]. Moreover, it remains to be determined whether similar transcriptional mechanisms also operate in iron-starved mammalian cells and which transcription factors are involved in regulating iron deficiency responses.

Iron Conservation through Post-transcriptional Mechanisms

Post-transcriptional regulation of iron-consuming pathways by Cth1 and Cth2 proteins represents another important adaptive response to iron deficiency in yeast [28]. Cth1/2p are tandem zinc finger (TZF) proteins with homology to the mammalian tristetraprolin (TTP), a protein induced by iron deficiency and required for the survival of mammalian cells in low-iron states [33]. Cth1/2p bind to AU-rich elements (ARE) in the 3'UTR of their target mRNA, followed by recruitment of DExD/H-box helicase 1 (Dhh1) RNA helicase, mRNA de-capping and degradation [34]. *CTH1/2* transcription is strongly induced in iron deficiency through the direct regulation by Aft1p [28], yet there is no known role for Cth1/2p in the majority of the classical Aft1/2-dependent responses. Instead, Puig *et al.* showed that Cth1/2p function in cellular iron conservation by regulating the stability of cellular proteins involved in multiple pathways that consume iron. Deletion of *CTH1/2* genes in yeast revealed 94 ARE-containing transcripts that were upregulated, suggesting that Cth1/2 proteins target these mRNAs for degradation [35]. Among the Cth1/2p targets was a large subset of genes coding for proteins that require iron for their structural assembly or enzymatic activity, such as Fe/S cluster-containing lipoic acid synthase (LIP5), isopropylmalate isomerase (*LEU1*), RNase L inhibitor (RLI1), and Rieske protein of the ubiquinol cytochrome *c* reductase complex. Thus, by directly suppressing production of iron-consuming proteins, Cth1/2p may liberate iron for use in other pathways.

Cth1/2p also suppress a number of other targets that do not require iron for their structure and function, but are involved in cellular pathways that depend on iron availability, including iron-independent components of tricarboxylic acid (TCA) cycle, fatty acid and ergosterol biosynthesis, as well as heme and Fe/S cluster biosynthetic pathways [35]. Thus, *CTH1/2* activation by Aft1/2p results in a global reprogramming of cellular iron utilization by suppression of multiple iron-consuming pathways at distinct regulatory points and sparing limited iron supply for essential functions (Figure 2B). However, as discussed below, the consequences of Cth1/2p activation go beyond the simple optimization of iron use within a cell, and may potentially lead to widespread changes in carbohydrate, lipid and protein metabolism.

Mitochondrial Energy Metabolism—A number of mitochondrial proteins functioning in the TCA cycle have been identified as Cth1/2p targets, including citrate synthase (*CIT1*), mitochondrial aconitase (*ACO1*), alpha-ketoglutarate dehydrogenase (α -*KGD*), succinate dehydrogenase 2/4 (*SDH2/4*) subunits and mitochondrial fumarase (*FUM1*). Moreover, critical components of ETC subunits, including cytochrome *c* oxidase and ubiquinol cytochrome *c* reductase are downregulated by Cth1/2p [35] (Figure 3). The combined effect of Cth1/2p on mitochondrial energetic function results in a shift from mitochondria-

dependent oxidative phosphorylation to cytosolic glucose utilization, including adaptive upregulation of high-affinity glucose transporters, glycolytic enzymes and enzymes involved in glycogen breakdown [35]. These changes in glucose metabolism during iron deficiency resemble those seen in glucose-deprived cells and are consistent with results from metabolomics analysis showing a depletion of intracellular glucose and glycolytic intermediates in iron-deficient yeast [12]. These results suggest that in addition to functioning as “iron rheostats”, Cth1/2p may play an important role in regulating glucose homeostasis, potentially linking iron regulation to cellular energy metabolism (Figure 3).

Lipid Metabolism—Inhibition of mitochondrial function by Cth1/2p is expected to halt beta-oxidation of fatty acids (FAs) and force a cell to utilize glucose as its preferred substrate, although the effects of Cth1/2p on beta-oxidation have not been assayed directly. The effects of Cth1/2p on FA synthesis in iron deficiency appear to be complex. Several FA synthetic enzymes, including alpha and beta subunits of FA synthase enzyme (*FAS1/2*), contain 3'AREs and are targeted by Cth1/2p for degradation [35]. However, an iron-requiring δ -9 fatty acid desaturase (*OLE1*) essential for synthesis of monounsaturated FAs is not targeted by Cth1/2p and its expression is significantly upregulated by iron deficiency. In fact, metabolomics analysis of iron deficient yeast shows an 8-fold upregulation of *OLE1* mRNA, and maintenance of Ole1 protein content and activity, with preservation of normal levels of monounsaturated FAs in the cell [12]. Thus, Cth1/2p might function in the “fine-tuning” of lipid homeostasis [29], although this hypothesis remains to be tested.

Heme and Fe/S Cluster Biosynthesis—As discussed earlier, the heme biosynthetic pathway consumes significant amount of iron, and yeast with deletion of *HMX1* are unable to grow in iron-limiting conditions. In addition, accumulation of protoporphyrin IX (PPIX), an unmetallated precursor of heme, induces significant oxidative damage to the cell [36]. Thus, suppression of heme synthesis will concurrently conserve iron and reduce oxidative stress in iron-starved yeast. To effectively inhibit heme synthesis, yeast employ both transcriptional (discussed above) and post-transcriptional regulatory mechanisms. In particular, Cth1/2p target several critical steps of heme synthetic pathway, including the rate limiting enzyme 5-aminolevulinate synthase (*HEM1*), uroporphyrinogen III synthase (*HEM4*), and ferrochelatase (*HEM15*) which inserts iron into PPIX [35]. The physiological consequences of heme deficiency in iron-limited cells have not yet been reported, although reduction in heme levels is expected to affect multiple heme-dependent cellular processes including energy production, ROS and xenobiotic breakdown, oxygen sensing, and more.

While there is a drastic suppression of heme synthesis in iron deficiency, the effects of low iron and Cth1/2p on Fe/S cluster biosynthesis are less clear. Three enzymes from the Fe/S biosynthetic pathway were identified as Cth1/2p targets: Isa1p (an enzyme required for mitochondrial Fe/S cluster assembly), Nfu1p (a protein needed for maturation of mitochondrial Fe/S cluster containing enzymes), and Yah1p (ferredoxin required for the formation of cellular Fe/S cluster proteins and heme A synthesis) [35]. However, as none of the enzymes essential for Fe/S cluster biosynthesis are regulated by Cth1/2p, the functional significance of this finding needs to be determined. Moreover, some of the critical Fe/S cluster consuming pathways, such as synthesis of essential amino acids, are minimally

affected by iron deficiency, although this may also be due to recycling of amino acids by autophagy and upregulation of transcription of many proteins involved in amino acid biosynthesis [29].

To summarize, Cth1/2p-dependent regulation of mRNA stability is an important mechanism for adaptation to iron deficiency. Through suppression of iron-requiring pathways, Cth1/2p divert iron away from non-essential processes to be used for maintenance of cell viability in states of prolonged or severe iron deficiency.

Iron Conservation Pathway in Mammalian Cells

As mentioned above, the importance of sufficient iron stores in humans has been established, and iron deficiency has been linked to several physiologic abnormalities. Looking at the effects of iron deficiency on the central nervous system, a recent study identified distinct abnormalities in the ratio of glycolytic metabolites in the cerebrospinal fluid (CSF) of iron-deficient infant primates [37], making a potential link between low iron, metabolism and brain development. Another study found that iron deficiency induced long lasting auditory and visual dysfunctions in human infants [38], a finding consistent with the notion that iron is required for proper myelination of neurons [39]. However, despite breakthrough studies elucidating iron conservation pathways in yeast, surprisingly little is known about the existence and function of similar pathways in higher eukaryotes.

Mammalian TTP

Similar to yeast Cth1/2p, mammalian TTP is a zinc-finger-containing protein that binds to AU-rich elements of target mRNAs such as cytokines and growth factors, and induces their degradation. It was recently shown that protein TTP appears to be a functional homolog of Cth1/2p in iron regulation [33], as overexpression of human TTP in *CTH1* /*CTH2* yeast restored iron-dependent suppression of the two known Cth1/2p targets, *ACO1* and *SDH2*. Moreover, TTP was strongly induced by iron deficiency in mouse embryonic fibroblasts (MEFs) and H9c2 cardiac myoblasts, although the mechanism appeared to be independent of IRP1/2 proteins. Additionally, TTP was found to negatively regulate mRNA levels of mammalian lipoic acid synthase (Lias) and ABCE1 (RNase L inhibitor) gene products, both of which harbor putative AREs in their 3'UTR and code for Fe/S-containing proteins. Treatment of wild-type (WT) MEFs with the iron chelator deferroxamine (DFO) resulted in ~50% reduction in ABCE1 and Lias mRNA, mirroring earlier observations in iron-deficient yeast [28]. However, instead of a decrease, iron-starved MEFs null for TTP exhibited about a 5-fold increase in ABCE1 and Lias mRNA, suggesting that TTP is necessary for the downregulation of at least some of the iron-containing proteins in low-iron states.

In addition to regulating specific iron-containing proteins, TTP may target multiple iron-consuming pathways by degrading mRNAs that code for iron-dependent and -independent enzymes in mammalian cells. Interestingly, computational analysis of the 3'UTR AREs using an online Universitat of Wien research platform algorithm (<http://rna.tbi.univie.ac.at/cgi-bin/AREsite.cgi?>) which specifically predicts TTP binding sites, revealed a number of evolutionary-conserved putative AREs in the key proteins functioning in three major iron-dependent pathways in mammals: TCA cycle, mitochondrial ETC and heme synthesis

(Table 1), although the precise role for TTP in regulating these pathways in normal and iron-deficient cells remains to be determined.

Similar to Cth1/2p in yeast, TTP plays an essential role in cellular response to iron starvation. Treatment with two mechanistically-distinct iron chelators (2,2-bipyridyl and DFO), resulted in ~2-fold increase in cell death in WT MEFs, but over 6-fold increase in cell death in TTP null MEFs. Taken together, these findings support an important function of mammalian TTP in cellular iron deficiency response, likely by optimizing cellular iron utilization (Figure 4).

In addition to establishing TTP as a mammalian homolog of Cth1/2p, TTP is negatively regulated by the mammalian target of rapamycin (mTOR) pathway [33, 40–41], suggesting a molecular link between cellular iron homeostasis and energy metabolism. While TTP has been primarily studied in suppression of immune responses, several studies link TTP to metabolic derangements. First, TTP expression is highly and transiently responsive to insulin in cultured mouse adipocytes [42–43]. Two independent studies showed that stimulation of NIH 3T3 adipocytes with insulin resulted in a dramatic accumulation of TTP mRNA within 10 minutes, and peaking at 30–45 minutes of insulin treatment [42, 44]. The degree of TTP upregulation by insulin positively correlated with the number of insulin receptors (IR) expressed on the cell surface [44], suggesting a function for TTP in insulin-responsive tissues.

Studies of human patients with insulin resistance further support the proposed connection between TTP and metabolism. First, a genetic linkage analysis identified TTP as a candidate gene for metabolic syndrome with a strong correlation between two TTP polymorphisms and increased weight/body mass index (BMI), diastolic blood pressure and LDL-cholesterol levels in a cohort of 709 severely obese men and women [45]. Second, low TTP mRNA levels were observed in visceral adipose tissue of obese men with metabolic syndrome compared to healthy obese men, suggesting that TTP may protect against the development of obesity-related metabolic complications [45]. In a follow-up study, TTP expression in the omental adipose tissue of obese women with metabolic syndrome was about two-fold lower than in obese women without metabolic syndrome, and correlated strongly with diabetic phenotype, including high fasting insulin levels, insulin resistance index and low adiponectin content [46].

The molecular mechanism for the protective effects of TTP against metabolic derangements is likely to be complex due to this protein's ability to modulate many critical cellular functions. Given that metabolic syndrome is associated with increased inflammation, several groups suggested that TTP may counteract insulin resistance by suppressing TNF α and other cytokine production and thus reducing systemic inflammation. However, no correlation was observed between TTP expression in women with metabolic syndrome and their levels of circulating TNF α , indicating that the anti-inflammatory function of TTP may not be the primary benefit [46]. Interestingly, several large epidemiological studies reported higher iron levels in diabetic patients [47–49], which would be expected to suppress TTP expression. On the other hand, TTP is significantly upregulated by low iron [33], and short-term iron depletion was shown to promote insulin sensitivity in mice and humans [50–51].

This suggests that cellular iron may play an important role in modulating energy metabolism. The link between iron, TTP and glucose is yet to be characterized in higher eukaryotes, but studies in yeast offer an intriguing insight into the insulin-sensitizing effects of iron depletion. As discussed earlier, iron starvation in yeast induces transcription of Cth1/2p, which shut down mitochondrial oxidative phosphorylation to conserve iron. As a result, yeast cells are no longer able to rely on mitochondria for energy production, and thus shift their metabolism toward glucose utilization by increasing expression of proteins functioning in glucose transport, glycolysis, and reserve carbohydrate metabolism [35]. A similar transition to glucose utilization in iron-depleted mammalian cells through activation of TTP and inhibition of mitochondrial oxidative phosphorylation is expected to facilitate glucose removal from the bloodstream and thus promote insulin sensitivity. Taken together, these findings raise an intriguing possibility of a molecular interplay between iron regulation and glucose homeostasis, opening up new avenues for research.

Concluding remarks and future perspectives

While identification of IRP1/2 as critical players in iron deficiency response was a major advance in our understanding of iron regulation on a cellular level, little progress has been made to uncover additional iron-regulatory pathways. The last five years have witnessed revival of our interest in the field of iron biology, which led to the discovery of iron conservation program in yeast and mammalian cells. Unlike the classic pathway of iron acquisition, iron conservation response is aimed at optimizing the use of limited iron that is already available in the cell by shuttling it towards pathways essential for survival. However, a number of important questions remain to be answered (Text Box 2), including the mechanism for TTP induction by iron deficiency, characterization of the downstream pathways affected by TTP, the role of TTP in regulation of whole-body iron deficiency response, and further exploration of the link between iron and metabolism.

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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 (UTR) of target mRNA resulting in their degradation. Many of Cth1/2p targets encode iron-requiring proteins

	or participate in iron-consuming biological pathways. Inhibition of these pathways reduces cellular iron utilization in non-essential processes and makes iron available for maintenance of viability.
Hmx1p	heme oxygenase enzyme responsible for breakdown of heme. <i>HMX1</i> 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 non-essential 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 <i>CTH1/2</i> which is induced by iron deficiency and is required for cellular survival in low-iron states, likely by suppressing non-essential iron-requiring pathways and mediating iron conservation pathway.
Vht1p	an iron-independent yeast biotin importer, whose expression is upregulated by Aft1p in iron deficiency.

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Highlights

- Cells respond to iron deficiency through iron acquisition and iron conservation
- To conserve iron, cells shut down many iron-consuming processes
- Iron-regulatory pathways may influence metabolism and insulin sensitivity

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 proteasome. In low iron, 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 (UTR) of target mRNA. Binding to the 3'UTR stabilizes target mRNA of transferrin receptor (TfR1) responsible for iron uptake. Binding to the 5'UTR halts translation of the target mRNA, suppressing proteins involved in iron export (ferroportin, 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.

Figure 2. Transcriptional and post-transcriptional mechanisms of iron conservation in yeast

(A) Regulation of biotin homeostasis is an example of transcriptional iron conservation response. In iron-replete cells, biotin is actively synthesized from its precursors by Bio2/3/4p enzymes, which require iron for their function, although biotin can also be imported from extracellular space by Vht1p transporter. In iron deficiency, yeast cells upregulate transcription of iron-independent *VHT1* and inhibit transcription of iron-dependent *BIO2/3/4* synthetic enzymes. As a result, iron-depleted cells preferentially acquire biotin through an iron-independent import pathway which reduces utilization of cellular iron.

(B) Cth1/2p proteins mediate post-transcriptional pathway of iron conservation. In low-iron states, Aft1/2p activate transcription of *CTH1/2*, which bind to the AU-rich elements (AREs) in the 3'UTR of mRNA and target them for degradation. Some of the Cth1/2p targets include proteins that directly incorporate one or several iron atoms into their structure. In addition, a subset of Cth1/2p targets does not code for iron-containing proteins but instead functions in iron-requiring metabolic pathways. Through these two mechanisms Cth1/2p optimize cellular iron utilization by shuttling iron away from non-essential pathways and conserving it for use in maintenance of cell viability. Dashed line indicates degraded mRNA molecules.

Figure 3. Cth1/2p regulation of metabolic pathways

Activation of Cth1/2p by iron deficiency results in significant changes to cellular metabolic pathways. Cth1/2p target multiple components of the citric acid cycle and electron transport chain (marked in yellow). This results in suppression of mitochondrial oxidative phosphorylation, which secondarily increases cellular dependence on glucose oxidation by glycolysis.

Figure 4. Iron acquisition and iron conservation pathways in mammalian cells

Under iron deficiency, mammalian cells first activate the IRP1/2 pathway which brings iron into the cell from extracellular space, releases iron from intracellular stores, and reduces iron export. If iron deficiency is mild or short in duration, IRP1/2 are sufficient to restore iron balance. However, if after activation of IRP1/2, cellular iron levels are still low, iron conservation response is initiated through activation of TTP. TTP binds to the ARE in 3'UTR of target molecules and reduces cellular iron utilization through selective suppression of non-essential iron-consuming pathways. As a result, all iron that is available in the cell is used to support only those functions that are essential for survival.

Table 1*In silico* analysis of AU-rich elements in major mammalian iron-consuming pathways^a

Pathway	Gene	Total AREs	Conserved AREs
TCA cycle			
	Citrate Synthase	6	2
	Aconitase	1	0
	Isocitrate dehydrogenase	3	3
	Fumarase	4	1
ETC			
	NADH-ubiquinone oxidoreductase (Complex I)	7	2
	Succinate dehydrogenase cytochrome b560 subunit (Complex II)	5	2
	Succinate Dehydrogenase complex, subunit D (Complex II)	4	2
	Cytochrome b-c1 complex subunit Rieske (Complex III)	1	1
	Cytochrome b-c1 complex subunit 7 (Complex III)	9	2
	Cytochrome c oxidase polypeptide 7A2 (Complex IV)	3	3
	Cytochrome c oxidase assembly protein COX11 (Complex IV)	10	6
Heme Synthesis			
	ALAS2	1	1
	Ferrochelatase	7	4

^aThe number of the 3'UTR AREs shown here was computed using an online Universitat of Wien research platform algorithm (<http://ma.tbi.univie.ac.at/cgi-bin/AREsite.cgi?>) which specifically predicts TTP binding sites. Abbreviation: ARE; AU-rich elements