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Post-transcriptional regulation of gene expression in response to iron deficiency: co-ordinated metabolic reprogramming by yeast mRNA-binding proteins

Sandra V. Vergara and Dennis J. Thiele[1]
Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC 27710, U.S.A.

Abstract

Saccharomyces cerevisiae (baker's yeast) is an excellent model for understanding fundamental biological mechanisms that are conserved in Nature and that have an impact on human disease. The metal iron is a redox-active cofactor that plays critical biochemical roles in a broad range of functions, including oxygen transport, mitochondrial oxidative phosphorylation, chromatin remodelling, intermediary metabolism and signalling. Although iron deficiency is the most common nutritional disorder on the planet, little is known about the metabolic adjustments that cells undergo in response to iron deficit and the regulatory mechanisms that allow these adaptive responses. In the present article, we summarize recent work on genome-wide metabolic reprogramming in response to iron deficiency, mediated by specific mRNA degradation mechanisms that allow *S. cerevisiae* cells to adapt to iron deficiency.

Introduction

Virtually all aerobic organisms require iron as a catalytic cofactor for essential biochemical processes. Given the ability of iron to exist in two states, Fe^{2+} and Fe^{3+}, its powerful redox chemistry drives a number of biochemical reactions. These include oxygen transport via haemoglobin, mitochondrial oxidative phosphorylation mediated by cytochrome oxidase, changes in chromatin structure due to the action of iron-dependent histone demethylases and a plethora of metabolic activities by iron-dependent enzymes such as those found in the tricarboxylic acid cycle [1,2]. While the redox activity of iron is useful for enzyme catalysis, free iron can also generate reactive oxygen species such as hydroxyl radical, which damages proteins, lipids and nucleic acids [3].

Although iron is essential for normal growth and development, iron deficiency is the leading nutritional disorder on earth, thought to affect over 2 billion people. Moreover, iron deficiency has a disproportionately large impact on pregnant women and children. The consequences of severe human iron deficiency include impaired physical and cognitive development, decreased oxidative metabolism, altered fuel homoeostasis, fatigue and anaemia [4–6]. However, the detailed biochemical, metabolic and regulatory changes that occur in response to a modest or severe iron deficiency are not well understood.

Saccharomyces cerevisiae has proved to be an outstanding model for the identification of genes involved in iron homoeostasis including those encoding proteins that function in iron uptake, incorporation into haem and Fe–S (iron–sulfur) clusters, compartmentalization to and mobilization from intracellular stores and other functions in iron balance. In response to iron excess, *S. cerevisiae* cells utilize the Yap5 transcription factor to induce expression of *CCC1*, encoding a vacuolar iron storage transporter that protects cells from iron overload [7,8]. Under conditions of iron deficiency, the partially redundant Aft1 and Aft2 iron-responsive transcription factors bind to iron-response elements in the promoters of many genes that encode proteins involved in iron homoeostasis and a wide array of other cellular processes [9–15]. As shown in Figure 1, these include the *FRE1* and *FRE2* genes encoding proteins that carry out Fe^{3+} reduction, *FTR1* and *FET3*, encoding an iron permease–oxidase complex that transports iron across the plasma membrane, *FTH1* and *FET5*, which mobilize vacuolar iron stores, iron–siderophore transporters as an alternative iron-uptake pathway, as well as genes involved in lipid and sterol metabolism and other processes. Although we know the identity and function of many of these Aft1/Aft2 targets under conditions of iron deficiency, the role of many of these gene products under these conditions remains to be elucidated.

Discussion

One member of the iron regulon whose role has recently been identified is *CTH2*, a gene whose mRNA levels are elevated nearly 200-fold in response to iron deficiency. *CTH2* encodes a member of the CCCH zinc finger mRNA-binding protein family, typified by the mammalian TTP (tristetraprolin) protein that is conserved from fungi to plants and animals. This family of RNA-binding proteins has been demonstrated

Key words: AU-rich element (ARE), iron deficiency, metabolic reprogramming, mRNA-binding protein, mRNA degradation, yeast.

Abbreviations used: ARE, AU-rich element; GCN4, general control non-derepressible 4; TTP, tristetraprolin; UTR, untranslated region.

[1]To whom correspondence should be addressed (email dennis.thiele@duke.edu).

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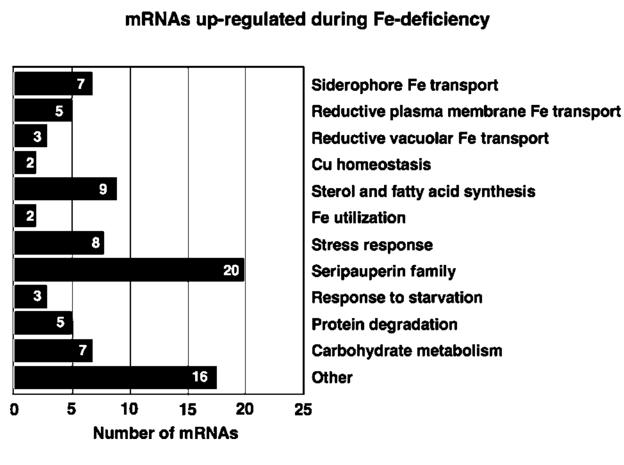
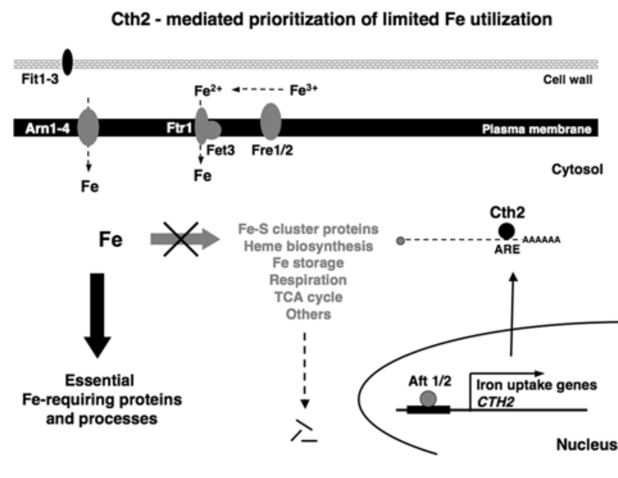
Figure 1 | The *S. cerevisiae* iron regulon
Iron deficiency results in the activation of the Aft1 and Aft2 iron-responsive transcription factors. The iron regulon mRNAs encode proteins with a number of functions, shown here, with the number of specific mRNAs encoding proteins in each functional group.



to bind specifically to consensus RNA sequences that are AU-rich and thus are termed AREs (AU-rich elements). Mammalian TTP binds to ARE elements within the 3′-UTR (untranslated region) of the TNFα (tumour necrosis factor α) mRNA, strongly stimulating its turnover via recruitment of components of the RNA decay machinery [16,17]. Interestingly, genome-wide transcriptomic analysis using DNA microarrays demonstrated that under conditions of iron deficiency, *cth2Δ* cells hyper-accumulate over ~80 distinct mRNAs, compared with isogenic wild-type cells grown under the same iron-deficient conditions [18]. These mRNAs encode proteins with a wide range of cellular functions that include: haem biosynthesis, Fe–S cluster biogenesis, cytochrome oxidase subunits involved in mitochondrial oxidative phosphorylation, enzymes in the tricarboxylic acid cycle and other enzymes of intermediary metabolism, proteins involved in iron storage in the yeast vacuole and a host of additional known and unknown activities. In addition to their up-regulation in a *cth2Δ* strain, these mRNAs are down-regulated in a wild-type strain under conditions of iron deficiency and all harbour one or more consensus AREs in the 3′-UTR.

Indeed, experiments using the three-hybrid technique, a surrogate genetic approach to investigate protein–RNA interactions [19], indicate that wild-type Cth2 protein binds to AREs within the 3′-UTR of several of these mRNAs and that this binding is dependent on both a functional ARE and the functional integrity of the Cth2 zinc finger RNA-binding domain [18,20]. Moreover, the 3′-UTR of Cth2 target mRNAs, such as that derived from *SDH4*, functions as a portable unit. When the *SDH4* 3′-UTR is fused to the open reading frame of the *GCN4* (general control non-derepressible 4) gene, it is able to confer reduced stability on the *GCN4–SDH4* hybrid transcript under conditions of iron deficiency and in a manner that is dependent on the integrity of the *SDH4* ARE elements and the Cth2 mRNA-binding domain.

Figure 2 | Iron prioritization by the Cth2 mRNA-binding protein
Iron (Fe) is transported into yeast cells via the action of cell-wall and plasma-membrane proteins that include Fit1–Fit3 in the cell wall, the Arn1–Arn4 siderophore–iron uptake transporters, the Fre1 and Fre2 Fe^{3+} metalloreductases and the Fet3–Ftr1 iron permease–oxidase transporters. Available intracellular iron pools are incorporated into iron-dependent enzymes involved in Fe–S cluster biogenesis, haem biosynthesis, mitochondrial oxidative phosphorylation and other metabolic and regulatory activities. In response to iron deficiency, expression of the iron regulon is activated by the Aft1 and Aft2 iron-responsive transcription factors, resulting in enhanced iron uptake and the expression of dozens of additional genes. One member of the iron regulon, *CTH2*, encodes an mRNA-binding protein that binds to AREs in the 3′-UTR of many mRNAs. Cth2 binding stimulates the rate of target mRNA decay, presumably via the recruitment of mRNA de-capping and de-adenylation activities and RNases. The selective targeting of mRNAs by Cth2 results in the inactivation of iron-storage mechanisms and reductions in the levels of mRNAs encoding dispensable iron-requiring enzymes, providing a mechanism for iron prioritization under conditions of iron deficiency.



Taken together, these experiments suggest a model by which cells cope with iron deficiency both through the regulation of transcription and through post-transcriptional mechanisms. Under iron-replete conditions, a low-level expression of the components involved in cell surface iron uptake ensures a continued supply of iron, and expression of the vacuolar iron storage importer, *CCC1*, facilitates the storage of excess iron and prevents toxicity. When cells encounter conditions where iron becomes limiting, the Aft1 and Aft2 iron-responsive transcription factors activate the expression of the iron regulon (Figure 1), resulting in enhanced expression of the iron-uptake machinery, increased mobilization of vacuolar iron stores, enhanced sterol and fatty acid synthesis and other activities that are required for adjustment to either increasing iron uptake or growth under iron-deficient conditions. When little extracellular iron is available, the action of Cth2, a member of the iron regulon, is required for metabolic adaptation of iron deficiency, via the targeted degradation of ~80 distinct mRNAs (Figure 2). This robust mRNA degradation results in the rheostatic

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down-regulation of dispensable iron-dependent processes, perhaps sparing residual iron for more essential processes.

Many avenues for understanding metabolic reprogramming in response to iron deficiency remain for further investigation. What is the function of the plethora of genes identified as members of the iron regulon and what are their roles in response to iron deficiency? Similarly, what are the roles of the proteins encoded by Cth2 target mRNAs and how do they facilitate cellular responses to iron deficiency? Interestingly, the *S. cerevisiae* genome encodes a protein homologous with Cth2 called Cth1. While a *cth1Δ* mutation exacerbates the iron deficiency growth phenotype of a *cth2Δ* strain, the precise role of Cth1 in this process and its relationship to Cth2 have not yet been clarified. Understanding the complete picture of how *S. cerevisiae*, as a model eukaryotic cell, responds to conditions of iron deficiency will likely have great predictive power for identifying key mechanisms that lead to adaptation or health consequences in mammalian iron deficiency.

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