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Iron toxicity in yeast: transcriptional regulation of the vacuolar iron importer Ccc1

Liangtao Li[1] and **Diane M. Ward**[1]

[1]Division of Microbiology and Immunology, Department of Pathology, School of Medicine, University of Utah, Salt Lake City, UT 84132-2501, USA

Abstract

All eukaryotes require the transition metal, iron, a redox active element that is an essential cofactor in many metabolic pathways, as well as an oxygen carrier. Iron can also react to generate oxygen radicals such as hydroxyl radicals and superoxide anions, which are highly toxic to cells. Therefore, organisms have developed intricate mechanisms to acquire iron as well as to protect themselves from the toxic effects of excess iron. In fungi and plants, iron is stored in the vacuole as a protective mechanism against iron toxicity. Iron storage in the vacuole is mediated predominantly by the vacuolar metal importer Ccc1 in yeast and the homologous transporter VIT1 in plants. Transcription of yeast *CCC1* expression is tightly controlled primarily by the transcription factor Yap5, which sits on the *CCC1* promoter and activates transcription through the binding of Fe–S clusters. A second mechanism that regulates *CCC1* transcription is through the Snf1 signaling pathway involved in low-glucose sensing. Snf1 activates stress transcription factors Msn2 and Msn4 to mediate *CCC1* transcription. Transcriptional regulation by Yap5 and Snf1 are completely independent and provide for a graded response in Ccc1 expression. The identification of multiple independent transcriptional pathways that regulate the levels of Ccc1 under high iron conditions accentuates the importance of protecting cells from the toxic effects of high iron.

Keywords

Aft1; Ccc1; Fe; S clusters; Iron homeostasis; Snf1; Vacuolar transporter and Yap5

Introduction

Iron is an essential nutrient for all eukaryotes because it plays a role in a wide variety of cellular oxidation–reduction reactions such as cellular respiration, DNA replication and repair, lipid biosynthesis and oxygen transport, however, although iron is essential, it can also be toxic due to its ability to generate reactive oxygen species, which can damage nucleic acids, lipids and proteins. Both the necessity and toxicity of iron underscores the importance of acquiring iron and localizing it to subcellular compartments where it is

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utilized or stored. As there is no mechanism for regulated iron excretion, intracellular iron levels are regulated by acquisition and storage. Alterations in these parameters can result in iron-limited growth or iron toxicity and death. *Saccharomyces cerevisiae* has evolved complex mechanisms to obtain iron and to protect itself from the toxic effects of excess cellular iron [for review see (Martinez-Pastor et al. 2017; Outten and Albetel 2013; Philpott et al. 2012)]. Fungi and plants store iron in the vacuole to protect themselves from toxicity as they do not express the cytosolic iron-binding protein ferritin (Bode et al. 1995; Gollhofer et al. 2014; Kim et al. 2006; Li et al. 2001; Portnoy et al. 2000; Singh et al. 2007; Szczypka et al. 1997; Urbanowski and Piper 1999). Iron storage in the vacuole also assists in maintaining cytosolic iron levels. When cytosolic iron levels decrease, iron can be efficiently exported from the vacuole by specific iron transporters. In *S. cerevisiae*, those transporters include Smf3 and the high affinity iron transport system Fet5/Fth1 (Portnoy et al. 2000; Singh et al. 2007; Urbanowski and Piper 1999). When cytosolic iron levels are replete, iron is imported into the vacuole to protect from iron toxicity as well as to store iron for use under iron-limited growth conditions. Iron import into the vacuole occurs primarily through the membrane transporter Ccc1 or its homologue VIT1 in plants (Chen and Kaplan 2000; Gollhofer et al. 2014; Kim et al. 2006; Lapinskas et al. 1996; Li et al. 2001). The absence of Ccc1 results in increased sensitivity to iron toxicity and cell death. In yeast, Ccc1 levels are tightly regulated by transcription (Li et al. 2008, 2011, 2012, 2017; Rietzschel et al. 2015) and by mRNA degradation (Puig et al. 2005, 2008). In this review, we will focus on the transcriptional regulation of iron sensing in *S. cerevisiae* and how Ccc1 levels are controlled by the high iron-sensing transcription factor Yap5 and Snf1 kinase signaling to the stress transcription factors Msn2 and Msn4.

Iron sensing in *S. cerevisiae*

Under iron-limiting conditions, the low iron transcription factors Aft1 and Aft2 regulate the expression of a large group of genes (> 25 genes) collectively termed the “low iron regulon”. These genes encode for proteins involved in plasma membrane iron acquisition, vacuolar iron export, mitochondrial iron import and iron–sulfur cluster synthesis along with proteins involved in iron metabolism [for review see (Martinez-Pastor et al. 2017; Outten and Albetel 2013)]. These transcription factors, Aft1 and Aft2, predominantly respond to the levels of mitochondrial iron-sulfur (Fe–S) cluster biogenesis and not to cytosolic iron levels. When mitochondrial Fe–S cluster synthesis is diminished, cytosolic Aft1 and Aft2 are translocated to the nucleus and bind to target DNA resulting in transcription of the “low iron regulon”. While Aft1 and Aft2 are the major low-iron sensing transcription factors, mathematical modeling of yeast iron acquisition suggests that there may be other transcription factors that respond to changes in cytosolic iron levels (Cockrell et al. 2014).

Transcriptional regulation of CCC1

Notably, under low iron conditions there is little transcription of the vacuolar iron importer Ccc1. The lack of *CCC1* transcription ensures that cytosolic and mitochondrial proteins have maximal access to iron. Further, two Aft1-target genes, *CTH1* and *CTH2*, are responsible for the low iron induced degradation of *CCC1* mRNA (Puig et al. 2005, 2008). Under iron-replete conditions, the transcription factor Yap5 acts to increase *CCC1* transcription resulting in increased iron import into the vacuole (Li et al. 2008; Pimentel et al. 2012).

Yap5 is a member of the basic leucine zipper transcription regulators of which there are eight in fungi (Fernandes et al. 1997; Reinke et al. 2013). Yap5 is localized to the nucleus and occupies a consensus Yap binding site in the *CCC1* promoter regardless of cellular iron levels (Li et al. 2008). Yap5-mediated *CCC1* transcription is activated under iron excess and two different studies suggest that activation of transcription is mediated through sensing Fe–S clusters (Li et al. 2012; Rietzschel et al. 2015). Rietzschel et al. specifically determined that Yap5-dependent *CCC1* transcriptional activation is mediated through the binding of two 2Fe–2S clusters to cysteine-rich domains in the activation domain of Yap5, inducing a conformational change that is suggested to activate *CCC1* transcription.

The absence of Yap5 greatly reduces *CCC1* transcription, however, iron-responsive *CCC1* transcription still occurs. Cells deleted for *YAP5* have higher iron tolerance than cells deleted for *CCC1*. These results suggested that there were other transcriptional mechanisms that regulate *CCC1* expression in iron-replete conditions. Yeast have developed several stress-activated signaling networks to deal with deprivation or excess nutrients conditions (Ho and Gasch 2015; Nishida-Aoki et al. 2015; Yadav et al. 2016). Recently, a role for the low-glucose sensor Snf1 kinase complex [for review see (Conrad et al. 2014)] in regulating *CCC1* transcription has been identified (Li et al. 2017). Under iron-replete conditions, the absence of Yap5 and Snf1 has an additive effect on *CCC1* transcription with the concomitant decrease in Ccc1 protein levels, increased sensitivity to iron toxicity and poor growth. This additive effect suggests that Yap5 and the Snf1 complex act independently to activate *CCC1* transcription (Fig. 1). Indeed, Snf1 activation is independent of Fe–S cluster synthesis and does not affect the transcription of *TYW1*, another target of the Yap5-high iron regulon (Li et al. 2011). Iron-replete conditions can be sensed as a stress on cells and in fact, the general stress transcription factor Msn4 is upregulated in response to high iron (Du et al. 2012). Li et al. show that the Snf1-mediated activation of *CCC1* transcription works partially through Msn2/Msn4, but the Snf1 complex also activates further *CCC1* transcription through an unknown mechanism (Li et al. 2017).

Conclusions

There is a clear balance that must be maintained between cytosolic iron and mitochondrial iron levels depending upon iron utilization. Dysregulation of this balance results in iron toxicity and cell death. Studies have shown that removing cytosolic iron either by sequestration in the vacuole or in the mitochondria can act as a protective mechanism against iron toxicity (Chen and Kaplan 2000; Li et al. 2001; Lin et al. 2011). Ccc1 plays a key role in this balance by importing iron into the vacuole. The levels of Ccc1 are determined by an equilibrium between regulating *CCC1* transcription under iron-replete conditions and *CCC1* mRNA degradation under iron-limited conditions. Transcriptionally, *CCC1* expression is regulated through two known mechanisms including the high iron sensing transcription factor Yap5 and the Snf1-kinase stress pathway signal to transcription factors Msn2 and Msn4, however, additional studies are needed to determine other “signals” and transcription factors that contribute to increased *CCC1* transcription under high iron conditions.

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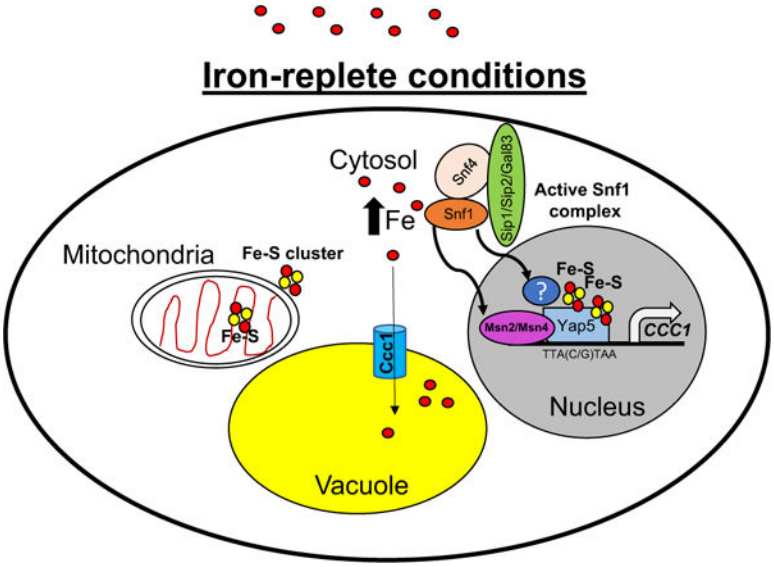

Fig. 1.

Model of transcriptional regulation of *CCC1* expression. Under iron-replete conditions, sufficient Fe–S clusters are synthesized in the mitochondria and nuclear localized Yap5 binds 2 2Fe–2S clusters to activate transcription. The high iron also acts by some unknown mechanism to activate the Snf1-complex (Snf1/Snf4/Sip1/Sip2/Gal83), which acts through the transcription factors Msn2/Msn4 and another mechanism (blue question mark circle) to induce *CCC1* transcription