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On the mechanism of iron sensing by IRP2: new players, new paradigms

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Abstract

Two iron regulatory proteins (IRP1 and IRP2) regulate translation and/or stability of mRNAs encoding proteins required for iron storage, acquisition and utilization. Rather than IRP2 directly sensing iron concentrations, iron has been shown to regulate the level of the SKP1-CUL1-FBXL5 E3 ubiquitin ligase protein complex, which is responsible for IRP2 degradation.

Iron is an essential element but can be toxic at high concentrations. Consequently, cells strictly regulate iron acquisition and storage. Vertebrates regulate translation and/or stability of mRNAs encoding proteins required for iron storage, acquisition and utilization through the binding of specific proteins termed iron regulatory proteins (IRPs). Determining how the IRPs sense and respond to iron is critical for understanding mammalian iron homeostasis. Two recent papers^{1,2} have identified a new form of regulation for the IRP in which iron does not affect the IRP directly but rather affects the enzyme that induces IRP degradation.

Two different IRPs, IRP1 and IRP2, can bind to specific sequences of nucleotides, termed iron responsive elements (IREs), in the untranslated regions of mRNA (for review see ref. 3). Binding of either IRP to the IRE prevents translation if the IRE is in the 5' untranslated region or increases message stability if the IRE is in the 3' untranslated region. In the presence of iron, the IRPs are removed from the IREs, thus permitting translation of messages responsible for iron storage and utilization and decreasing the stability of messages involved in iron acquisition. IRP1 and IRP2 respond to iron in very different ways. When cytosolic iron is high, the mRNA binding site of IRP1 becomes occupied by an iron-sulfur cluster, thereby converting the RNA binding site into a catalytic site for aconitase⁴. IRP2 is homologous to IRP1, but it does not form an iron-sulfur cluster in the presence of iron⁵. In contrast, iron induces the degradation of IRP2 by the ubiquitin proteasome system. It was assumed that iron bound to IRP2 or that iron catalyzed the oxidation of amino acids within IRP2, permitting its recognition by a ubiquitin ligase and resulting in its degradation by the proteasome. Studies attempting to identify iron-binding activity or iron-induced protein modifications of IRP2, however, have been unsuccessful.

Vashisht *et al.*¹ and Salahudeen *et al.*² have identified the mechanism of iron sensing and degradation for IRP2. They showed that IRP2 does not sense iron, but rather iron regulates the level of the ubiquitin ligase that is responsible for IRP2 degradation. They identified

SKP1-CUL1-FBXL5 as the E3 ubiquitin ligase protein complex responsible for degradation of IRP2. FBXL5 is a member of the F-box family of proteins, which determine the substrate specificity of the SCF (SKP1-CUL1-Fbox) ubiquitin ligase. Salahudeen *et al.*² used RNAi against a panel of ubiquitin ligases to identify the specific enzyme that affected the degradation of IRP2. Vashisht *et al.*¹ generated a modified FBXL5 protein that could bind substrate but would not mediate degradation, thus allowing the modified protein to accumulate with its bound substrate. They then identified IRP2 by mass spectrometry as a protein that bound to this catalytically inactive “bait.” Both groups showed an iron-dependent physical interaction between FBXL5 and IRP2; they also both showed that IRP2 was ubiquitinated and degraded in a FBXL5-dependent manner. IRP2 accumulated when FBXL5 was silenced using RNAi, and IRP2 decreased when FBXL5 was overexpressed. The IRP2 that accumulated in FBXL5-silenced cells was functional, indicating that iron does not directly affect IRP2 activity.

Both authors showed that FBXL5 is the iron sensor, as the stability of FBXL5 is regulated by iron (Fig. 1a). FBXL5 protein levels are low in iron-limiting conditions and are increased when iron is replete. FBXL5 has a hemerythrin-like domain in its N terminus that directly binds to iron. Hemerythrins are oxygen-binding proteins that bind oxygen through a diiron metal center. Mutation of the putative iron-binding amino acid residues reduces the stability of the hemerythrin-like domain of FBXL5. Salahudeen *et al.*² showed that the hemerythrin-like domain of FBXL5 binds oxygen, and both groups showed that the stability of FBXL5 is regulated by oxygen. Similar to its regulation by iron, FBXL5 protein levels are low in oxygen-limiting conditions. In iron- or oxygen-limiting conditions, FBXL5 is destabilized, and that inhibits the assembly of the SKP1-CUL1-FBXL5 ubiquitin ligase protein complex, thus leading to accumulation of IRP2. The regulation of FBXL5 by oxygen explains the fact that IRP2 is stabilized during hypoxia.

The current studies provide a mechanism for iron and oxygen sensing for IRP2-mediated post-transcriptional regulation of iron metabolism; IRP2 does not directly sense iron, but rather the iron/oxygen sensor is the enzyme that effects IRP2 degradation. These studies form an interesting complement to the mechanism by which oxygen and iron regulate transcription (Fig. 1b). The HIF family of transcription factors regulates the transcriptional activation of many genes involved in oxygen and iron metabolism (for review see ref. 6). The concentration of HIF is regulated by oxygen and iron, but HIF itself does not bind oxygen or iron; rather, oxygen and iron are used by an enzyme, prolyl hydroxylase, that modifies HIF. The modified HIF is recognized by a ubiquitin ligase and degraded. In the absence of oxygen or iron, the prolyl hydroxylase is inactive and HIF levels accumulate, thereby permitting transcription. Thus, iron and oxygen sensing is not done by proteins involved in transcriptional and post-transcriptional regulation, but rather is mediated by enzymes that modulate the levels of the regulatory proteins. It would be of great interest to determine whether there are other substrates for SKP1-CUL1-FBXL5 ubiquitin ligase and whether this general iron/oxygen regulatory mechanism can be extended to other metabolic pathways.

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Figure 1.

Iron- and oxygen-mediated modification of proteins required for regulation of iron metabolism. **(a)** IRP2 is ubiquitinated by the SKP1-CUL1-FBXL5 E3 ubiquitin ligase complex in an iron-dependent manner and degraded. Under low iron conditions, IRP2 binds to specific sequences in the untranslated regions of mRNA that encode proteins involved in iron acquisition and storage. Binding of IRP to a 5'-IRE inhibits mRNA translation, and binding to a 3'-IRE increases mRNA stability. In the presence of iron, there is an increase in the stability and thus concentration of the FBXL5-containing E3 SCF ubiquitin ligase. This E3 ligase ubiquitinates IRP2, leading to its degradation by the proteasome. The loss of IRP2 permits translation of 5'-IRE-containing mRNA and degradation of 3'-IRE-containing mRNA. Domains in the FBXL5 subunit of the SCF E3 ligase sense iron and oxygen. **(b)** Prolines within HIF-2α are hydroxylated by a cytosolic iron-containing prolyl hydroxylase (PHD) in a reaction that requires oxygen as a substrate. Hydroxylated HIF-2α is recognized by the von Hippel-Lindau tumor suppressor (pVHL), which is a component of an E3 ubiquitin ligase complex, which ubiquitinates hydroxylated HIF-2α, leading to its degradation by the proteasome. In the absence of oxygen or iron, HIF-2α is not hydroxylated and not degraded. Increased amounts of HIF-2α permit it to bind to a second

cytosolic protein, HIF-1 β (also known as ARNT). The heterodimeric complex translocates into the nucleus where it activates transcription of the ferrireductase encoded by *CYBRD1* and the iron transporter encoded by *DMT1*, genes required for iron acquisition.

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