Cell Metabolism
Minireview

Regulation of Intestinal Iron Absorption: The Mucosa Takes Control?

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DOI 10.1016/j.cmet.2009.06.009

Two studies (Shah et al., 2009; Mastrogiannaki et al., 2009) show that the hypoxia inducible factor HIF-2α is a major player in regulating iron absorption by directly controlling the transcription of iron transporters in the intestine in response to changes in mucosal iron or oxygen levels. The HIF-2α mechanism has major effects on iron metabolism which can override the well-known hepcidin-ferroportin regulatory axis.

Prior to the discovery of hepcidin, the duodenal mucosa itself was believed to be an important site for regulating the entry of iron into the body, and it was thought by many in the field that cells of the crypts were involved in sensing body iron levels. This was called the "crypt cell sensing hypothesis" (initially proposed by Conrad and Crosby (1963) (for recent review, see Roy and Enns, 2000), which stated that increased body iron requirements led to early changes in the crypt enterocyte iron content, then subsequently to changes in iron transporter expression in mature enterocytes. Thus, there was a delay between changes in body iron requirements and changes in iron absorption, since it takes 1–2 days for a crypt cell to mature and migrate to the villus tip. Tantalizing evidence had supported this idea without providing a truly robust confirmation (Schumann et al., 1999; Trinder et al., 2002) as molecular mechanisms for iron regulation of iron absorption gene transcription had not yet been completely described. Posttranscriptional control of iron metabolism genes has, however, been well studied (Muckenthaler et al., 2008). Iron regulatory proteins (IRP1 and 2) bind to iron-responsive elements in mRNA's for key iron metabolism proteins when iron levels are low and either block translation (e.g., in the case of the iron storage protein ferritin) or increase mRNA stability (in the case of proteins such as transferrin receptor 1 that are involved in cellular iron uptake). The IRP/IRE translational/mRNA degradation mechanism has been shown to operate on ferroportin (Fpn, the iron efflux protein that transports iron from intestinal cells [enterocytes] to blood) in the duodenum (Galy et al., 2008). This control mechanism was thought to link systemic regulation of iron absorption (see below) to local control by changes in enterocyte iron level (Chen et al., 2003). The ubiquitous IRP system, however, may be viewed as primarily operating to control cellular levels of iron for housekeeping purposes. The effect of IRP's on ferroportin mRNA would mean that low enterocyte iron levels would result in a block in ferroportin mRNA translation. This is contradicted by the observation that iron absorption is enhanced in iron deficiency. A recent study by Zhang et al. (2009) has provided a mechanism that can resolve this apparent contradiction between the enterocyte's own housekeeping iron needs and body iron requirements. Zhang et al. showed that alternate splicing leads to a minor fraction of ferroportin mRNA that lacks the IRE (Fpn1B), thereby meaning that ferroportin translation is only partially controlled by enterocyte iron levels. This finding helps explain why IRP2 knockout (KO) has remarkably little effect on iron absorption rates (Galy et al., 2005). The mRNA for the highly iron-regulated apical ferric reductase (Dcytb) has no IRE, and therefore its regulation has never been adequately explained by the IRP system. A more rapid local intestinal regulation was deduced from the observation that a bolus of oral iron taken before iron absorption measurements resulted in downregulation of absorption of subsequent doses of iron (referred to as the mucosal block) (Hahn et al., 1943). This was thought to be due to "local" control of iron transporter expression by iron. Molecular investigation of the latter has shown downregulation of the major brush border membrane iron transporter DMT1 and Dcytb at the protein level (Yeh et al., 2000; Frazer et al., 2003) and mRNA level (Frazer et al., 2003).

In recent years, focus has shifted away from the intestine to the liver as the major site for control of iron metabolism, as this is where hepcidin is produced. The discovery of hepcidin as a liver-expressed peptide that regulated iron absorption (reviewed by Ganz, 2008) was a major breakthrough and prompted Frazer, Anderson, and coworkers to carefully analyze the time dependence of iron absorption responses and changes in gene expression in both the intestine and liver in response to physiological challenges to iron metabolism (Frazer et al., 2002; 2003). Their data suggested much that had been attributed to local sensing and regulation of absorption by the intestine, could be explained by liver sensing changes in body iron requirements with hepcidin acting as a hormone to negatively regulate iron absorption. The demonstration that hepcidin binds to the iron transporter ferroportin resulting in its degradation (Nemeth et al., 2004) seemed to offer the key that finally unlocked the "local" iron absorption regulation riddle. Hepcidin binding to ferroportin would lead to blocking of iron efflux to the plasma, which in turn might result in increases in enterocyte iron that could downregulate the intestinal iron uptake proteins DMT1 and Dcytb (Frazer et al., 2003)—but the mechanism for this downregulation remained obscure. Mouse KO studies strongly support the suggestion that hepcidin is the most potent regulator of iron absorption (Lesbordes-Brion et al., 2006); however, some indirect evidence (Schumann et al., 1999; Raja et al., 2005) left open the possibility that other regulatory mechanisms operate.

Hypoxia inducible factor (HIF) was initially discovered as the major oxygen regulated transcription factor that controls

erythropoietin expression (Semenza and Wang, 1992) and it thereby links tissue oxygen supply to red cell production. HIF levels are regulated by a protein degradation pathway mediated by the E3 ubiquitin ligase von Hippel-Lindau tumor suppressor (VHL) (Maxwell et al., 1999), and an oxygen-sensing mechanism is provided by the action of prolyl hydroxylases (PHD) enzymes that modify the α subunit of HIF, thus labeling it for degradation by VHL (Epstein et al., 2001) (Figure 1). Other proteins are essential for the HIF/PHD mechanism, including VHL, which is essential for the hydroxylation reaction and Arnt (HIF-1β), which forms the HIF transcription factor complex. The HIF system shows tissue specificity through variable expression of the several HIF alpha subunit isoforms (HIF-1α, HIF-2α, HIF-3α) and PHD isoforms (PHD1, PHD2, PHD3). A growing list of proteins involved in iron metabolism have been identified as HIF-responsive targets, including Transferrin receptor 1, Ceruloplasmin, Heme Oxygenase 1, and hepcidin (Peyssonnaux et al., 2008). Prolyl hydroxylases require not only oxygen, but also iron and ascorbate in order to hydroxylate HIF; thus, PHDs can potentially act as iron sensors (Berra et al., 2006) and HIF could operate as an iron-sensitive transcription factor.

Two recent papers have now turned iron regulation on its head again by providing compelling data that the duodenum is both an important sensor and regulator of iron absorption through regulation of iron transport proteins by the transcription factor HIF-2α, with PHDs emerging as potential iron sensors in the mucosa. DMT1 and particularly Dcytb are among the genes most highly upregulated by both iron deficiency and hypoxia in the duodenum. While DMT1 contains a 3′IRE, Dcytb does not have a recognizable IRE, and hence its strong regulation by iron and hypoxia have always been unexplained. In the first study, Shah et al. (2009) showed that liver-specific KO of either HIF1α alone or both HIF-1α and HIF-2α had virtually no effect on response to iron deficiency, indicating this response does not require liver HIFs. The authors then focused on the role of HIFs in the intestine. First they showed that HIF-2α but not HIF-1α mRNA was induced in the duodenum by iron deficiency. They then found an intestinal-specific VHL KO (which blocks HIF degradation) produced a large induction of HIF-2α but not HIF-1α expression and made the interesting finding that both Dcytb and DMT1 were among the genes most highly activated in duodenum of these mice, suggesting that both Dcytb and DMT1 were transcriptionally regulated by HIF-2α. Furthermore, both proteins and, in the case of Dcytb, functional ferric reductase activity were increased in the intestine of VHL KO mice. Moreover, VHL KO mice became iron overloaded despite an intact hepcidin response. Using an intestine specific double VHL/HIF-1α KO mouse model, they still found robust upregulation of Dcytb and DMT1, thus implying that the effects were due to HIF-2α rather than HIF-1α. In contrast to VHL KO mice, in double VHL/Arnt KO mice (Arnt is required for the formation of functional HIF-1α and HIF-2α transcription complexes) the effects on iron metabolism seen in VHL KO mice (increased DMT1 and Dcytb mRNA and protein; increased functional ferric reductase activity and iron loading) were all reversed. The authors then went on to characterize a number of hypoxia response elements (HREs) within the promoter regions of the Dcytb and DMT1 genes, demonstrating that HIF-2α but not HIF-1α activated transcription.

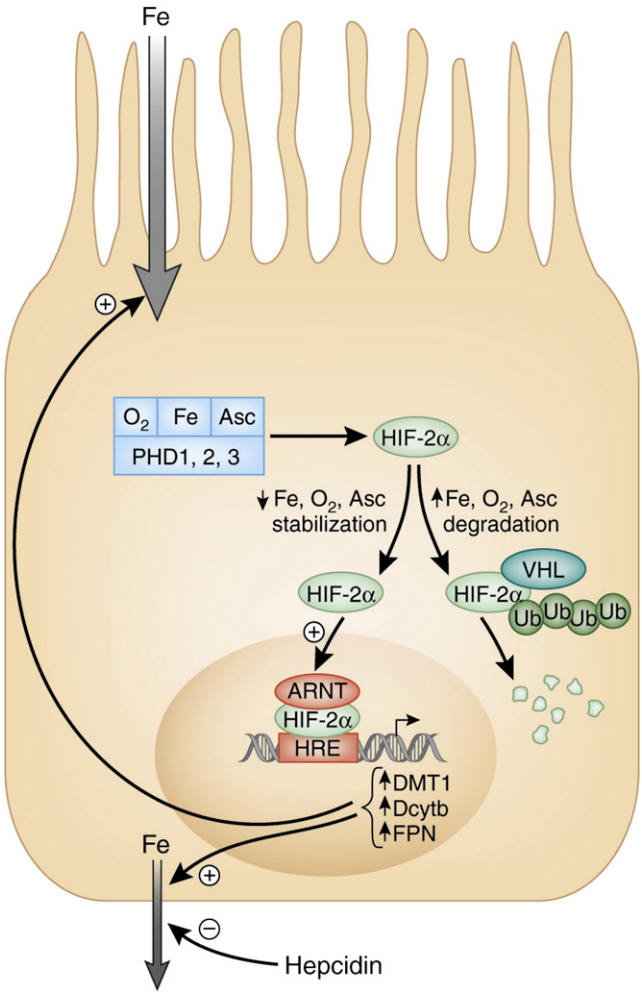

Figure 1. Overview of Transcriptional Control of Intestinal Iron Transport Genes by HIF-2α

HIF-2α but not HIF-1α is a major iron-inducible transcription factor controlling transcription of iron transporters within enterocytes. Increases in intracellular iron (Fe), oxygen (O_2), or ascorbate (Asc) levels increase prolyl hydroxylase (PHD) activity, leading to degradation of HIF-2α via von Hippel-Lindau (VHL) -mediated ubiquitilation (Ub), reduced DMT1, Dcytb, and FPN transcription and reduced dietary iron absorption. Conversely, decreases in intracellular iron (e.g., in dietary iron deficiency), oxygen, or ascorbate lead to reduced enterocyte PHD activity, increased HIF-2α levels, increased transcription of iron transporters, and increased iron uptake. HIF-2α-induced changes in iron transport can override the effects of the hepcidin-ferroportin regulatory pathway.

Interestingly, the authors found that the intestinal Arnt KO mice in fact became anemic and had lower serum iron when fed an iron-deficient diet due to the lack of a transcriptional response of Dcytb and DMT1 genes (lowered hepcidin levels were not able to compensate for this effect), thus providing strong if indirect physiological evidence for the role of HIF-2α.

Whereas Shah et al. used VHL KO and double VHL/HIF-1α KO to indirectly show effects of HIF-2α, in a second paper, Mastrogiannaki et al. (2009) directly and selectively deleted HIF-1α and HIF-2α in the intestine. Whereas intestinal deletion of HIF-1α had no effect on iron metabolism, intestinal HIF-2α KO had a similar phenotype to intestinal Arnt KO mice reported

by Shah et al. with markedly reduced mRNA levels for DMT1, Dcytb, Fpn, and hepcidin-reduced iron stores, serum iron, and hemoglobin. The only difference was that Shah et al. only saw effects on iron metabolism of Arnt KO on an iron-deficient diet, whereas Mastrogiannaki et al. observed effects of HIF-2α deletion even on a regular diet. One point of conflict between the Shah et al. and Mastrogiannaki et al. studies was that the former implied that loss of the intestinal HIF mechanism led to loss of iron sensing in the intestine. Mastrogiannaki et al. found that intestinal HIF-2α KO mice retained a significant responsiveness of Dcytb and DMT1 mRNA levels to dietary iron deficiency, suggesting an additional iron sensing mechanism is present.

HIFs are of course more widely associated with oxygen, rather than iron sensing. It has long been known that hypoxia induces a very early increase in iron absorption (Hathorn, 1971) at 6–8 hr after onset of hypoxia. This increase precedes alterations in serum or plasma iron, erythropoiesis (Hathorn, 1971; Raja et al., 1988), or hepcidin (G.O. Latunde-Dada and R.J. Simpson, unpublished data) and is due to increases in the mucosal uptake of iron (Raja et al., 1988). HIFs acting as local sensors of enterocyte oxygen provide an explanation for this early response. The additional requirement of PHDs for ascorbate adds an extra degree of complexity to the regulation of HIFs. Iron, oxygen, and ascorbate are closely linked, not only metabolically, but also, the three can react together chemically, and duodenal levels of ascorbate tend to change in the opposite direction to changes in iron, increasing in iron deficiency (Atanasova et al., 2005). Further study is required to figure out how these factors coordinately regulate iron absorption via regulation of PHDs.

It also appears that the HIF-2α regulation of DMT1 explains some puzzling aspects of DMT1 regulation by iron that were not explained by the DMT1 IRE. Galy et al. (2008) showed in an intestine-specific IRP1 plus IRP2 KO that DMT1 non-IRE mRNA was upregulated and DMT1-IRE was only mildly decreased. This, taken with more drastic effects of the IRP KO on other IRE-regulated proteins, had implied some transcriptional upregulation of DMT1 gene. The enterocytes of the IRP double intestinal KO mice were likely to be severely functionally iron deficient due to upregulation of Fpn and ferritin protein, and the HIF mechanism now provides a molecular mechanism for a compensatory increase in DMT1 gene transcription.

Both the Shah et al. and Mastrogiannaki et al. studies showed that modulation of HIF-2α in the intestine altered serum iron and iron stores, with HIF-2α intestinal KO and Arnt intestinal KO causing anemia and VHL intestinal KO causing iron overload. Their data suggested that the hepcidin mechanism and liver iron sensing were functioning correctly but was unable to counteract the altered expression of iron transporters in the intestine. Interestingly, there are suggestions that HIF-2α may exert more control on the apical iron uptake pathway (Dcytb and DMT1) than the basolateral iron transport (FPN and Hephaestin) (Mastrogiannaki et al. show a significant effect on Fpn; however, the effect is not significant in the Shah et al. studies). If confirmed, such a mechanism could explain earlier data, which showed that iron ingestion rapidly changes duodenal DMT1 and Dcytb mRNA levels but leaves FPN and Hp unchanged (Frazer et al., 2003).

The papers together are an important advance not only because they shine a light on how Dcytb, DMT1, and ferroportin are transcriptionally regulated by iron and hypoxia, but also because they provide a major molecular mechanism to explain how local changes in enterocyte iron (as in the mucosal block phenomenon) or oxygen alter duodenal transporter expression and dietary iron absorption. They may therefore suggest new gene candidates for inherited defects in iron absorption and show that alterations in HIFs at the intestinal level can override systemic regulation via hepcidin. This may therefore provide alternative therapeutic strategies for interventions in control of iron absorption in iron-overload conditions. Such alternatives could focus on local control of iron absorption at the intestinal level.

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