Iron Homeostasis: Recently Identified Proteins Provide Insight into Novel Control Mechanisms

An-Sheng Zhang and Caroline A. Enns

Iron is an essential nutrient required for a variety of biochemical processes. It is a vital component of the heme in hemoglobin, myoglobin, and cytochromes and is also an essential cofactor for non-heme enzymes such as ribonucleotide reductase, the limiting enzyme for DNA synthesis. When in excess, iron is toxic because it generates superoxide anions and hydroxyl radicals that react readily with biological molecules, including proteins, lipids, and DNA. As a result, humans possess elegant control mechanisms to maintain iron homeostasis by coordinately regulating iron absorption, iron recycling, and mobilization of stored iron. Disruption of these processes causes either iron-deficient anemia or iron overload disorders. In this minireview, we focus on the roles of recently identified proteins in the regulation of iron homeostasis.

Iron Absorption and Recycling

Iron Absorption—Adults absorb ~1–2 mg of iron/day from the diet to compensate for daily iron loss due to the sloughing of epithelial cells, blood loss, and sweat. Most diets contain two different forms of iron, inorganic non-heme iron in vegetables and grains and heme iron (ferrous iron protoporphyrin IX) in red meat. Iron traverses both the apical and basolateral membranes of absorptive epithelial cells to reach the blood, where it is incorporated into Tf, the major iron transport protein. At least two different sets of transporters are used in this process. Non-heme iron is transported by DMT1 (divalent metal transporter 1), the intestinal iron importer. Dietary non-heme iron exists mainly as Fe^{2+} and has to be reduced prior to transport. DcytB, a reductase whose expression is induced by iron deficiency, is localized in the apical membrane of intestinal enterocytes and is a major but most likely not the only reductase. The transporter responsible for heme uptake remains controversial (1–3).

Cytoplasmic iron in intestinal enterocytes can be either stored in the cytosolic iron storage molecule, ferritin, or exported into plasma by the basolateral iron exporter, FPN. FPN is most likely the only cellular iron exporter in the duodenal mucosa as well as in macrophages, hepatocytes, and the syncytial trophoblasts of the placenta because targeted deletion of both FPN alleles in mice is embryonic lethal. The export of iron by FPN depends on the multicopper oxidase, Cpo, in the circulation and hephaestin on the basolateral membrane of enterocytes, which convert Fe^{2+} to Fe^{3+} for incorporation of iron into Tf. Tf-bound iron is the major iron source for most tissues.

Iron Distribution in the Body—Adults have a total of 3–5 g of iron. Approximately 65–75% is found in the hemoglobin of erythrocytes in the form of heme. The liver stores 10–20% in the form of ferritin, which can be mobilized easily when needed. About 3–4% of the body’s iron is in heme-bound myoglobin in striated muscle. The rest is distributed in other tissues. Under physiological conditions, ~25 mg of iron/day is consumed by immature erythrocytes in bone marrow for heme biosynthesis.

Iron Recycling within the Body—Macrophages in the liver and spleen are responsible for the recycling of heme iron from senescent erythrocytes. The hemoglobin-derived heme is catalyzed by the cytosolic heme oxygenase-1 to release iron, and the iron is subsequently exported into the circulation by FPN. In addition, heme can also be exported directly into the circulation via the heme exporter, FLVCR (feline leukemia virus subgroup C receptor), on macrophage plasma membranes. A recent study demonstrated that FLVCR also plays a critical role in the export of excess heme from immature erythrocytes and hepatocytes (4). Plasma heme is scavenged and transported by hemopexin to hepatocytes for degradation. Iron recycling from senescent erythrocytes in macrophages constitutes the major iron supply for hemo- globin synthesis.

Cellular Iron Sensing and Regulation

The majority of cells obtain their iron requirements by Tf-mediated iron uptake via Tfr1. Tfr1 is internalized into endosomes that are acidified, facilitating the release of iron from Tf (5, 6). The iron is reduced by a recently identified ferrireductase, Steap3, and transported across the vesicle membrane for utilization within the cell and/or storage (7). DMT1 is the transporter in immature red blood cells (8). Iron uptake is roughly proportional to the number of Tfrs on the cell surface. Regulation of Tfr1 is achieved via IRPs and mRNA stem-loop structures, IREs, which have been reviewed extensively (9, 10). The IREs in mRNA of Tfr1 negatively regulate the stability of Tfr1 mRNA when cytosolic iron levels are high. Under low iron conditions, the IRPs bind to the IREs, where they stabilize TFR1 mRNA. The double knock-out of IRP1 and IRP2 is embryonic lethal. The double knock-out of these genes in the intestine results in the death of intestinal epithelial cells, presumably by iron depletion (11), underscoring the importance of these proteins.
Liver as the Central Iron Regulatory Organ

Hepcidin, a peptide synthesized by the liver hepatocytes, plays a major role in regulating iron homeostasis in the body (9, 10). The mature form is 25 amino acids with four intersubunit disulfide bonds. The massive iron overload found in hepcidin knock-out mice suggests that hepcidin is an iron stores regulator involved in communication of body iron status to the intestine (12). In contrast, mice engineered to overproduce hepcidin are severely anemic (13). The discovery that a hepatic adenoma overexpressing hepcidin results in anemia and that the anemia is resolved upon removal of the tumor confirms the relationship between hepcidin expression and inhibition of iron uptake by the intestine (14). Studies have demonstrated that hepcidin binds FPN, which results in the internalization and degradation of FPN (15). Hepcidin therefore functions to decrease serum iron levels by blocking iron absorption from the intestine, iron recycling from macrophages, and mobilization of stored iron from liver hepatocytes.

The liver plays a major role in iron homeostasis in the body in addition to secreting hepcidin. Liver macrophages take up senescent red blood cells and hemoglobin through the hemoglobin-haptoglobin receptor (CD163), salvage the iron released from hemoglobin, and secrete the iron as Fe^{2+} via FPN. Hepatocytes synthesize both Tf and Cp. Cp facilitates the efflux of iron from cells as well as the loading of iron into Tf (16, 17). Hepatocytes take up Tf through TfR1 and the more recently identified TfR2 (18). They also take up other forms of non-Tf-bound iron, including heme via the heme hemopexin receptor (19), and are capable of storing large quantities of iron in ferritin and hemosiderin, a breakdown product of ferritin. Thus, the liver and, in particular, the hepatocyte are thought to sense and reflect body iron stores (20).

Iron Sensing and Regulation of Hepcidin Expression

Humans possess elegant mechanisms to maintain iron homeostasis by modulating the expression of hepatic hepcidin. HJV, BMPs, TfR2, HFE, and Tf are critical to this process. Hepcidin expression is also regulated by erythroid factors, hypoxia, and inflammation, regardless of body iron levels.

HJV and BMPs—HJV is a recently identified protein encoded by the gene HFE2. Both clinical and animal studies demonstrate that it plays a pivotal role in iron homeostasis. Homozygous or compound heterozygous mutations in HFE2 are responsible for the onset of the majority of juvenile hemochromatosis. Disruption of both Hfe2 alleles in mice (H_{jv}^{−/−}) results in a marked increase in iron deposition in the liver, pancreas, and heart (21–23). The severely suppressed expression of hepcidin, detected in juvenile hemochromatosis patients and in H_{jv}^{−/−} mice, indicates that HJV plays a central role in the regulation of hepatic hepcidin expression.

HJV is highly expressed in skeletal muscle and heart and at lower levels in liver and binds both BMPs and neogenin (24–26). Recent studies show that HJV is a co-receptor for BMP2, BMP4, BMP5, and BMP6 and that it increases hepatic hepcidin expression via enhancing BMP signaling (24, 27). BMPs are a subfamily of cytokines that belong to the TGF-β superfamily. The BMP subfamily signals through one set of receptor-acti-

FIGURE 1. Model of hepcidin regulation by iron. Central to this model is that hepcidin transcription is regulated by HJV, which acts as a co-receptor for BMP. Upon binding to the BMP receptor (BMPR), a signaling cascade is initiated resulting in the translocation of SMAD4 to the nucleus, where it stimulates hepcidin transcription. The binding of HJV to neogenin (neo) is necessary for the release of HJV from cells. The release is dependent on cleavage by the protease furin. Where in the cell this occurs remains to be determined. In addition, cleavage of HJV is inhibited by Tf. Soluble HJV inhibits BMP-mediated signaling. Mutations in TfR2, HFE, or Tf result in a decreased level of hepcidin mRNA. Tf stabilizes TfR2. The mechanism by which the Tf-TfR2-HFE complex affects hepcidin transcription is unknown.

vated SMADs (SMAD1, SMAD5, and SMAD8). The receptor-activated SMADs form heteromeric complexes with SMAD4, the central mediator in TGF-β/SMAD signaling, which translocates from the cytoplasm to the nucleus to regulate gene expression. The importance of BMP signaling in the regulation of hepcidin expression is further supported by studies in mice with liver-specific disruption of SMAD4, which show decreased hepcidin expression and severe iron accumulation in the liver as well as other organs (28).

HJV is a glycosylphosphatidylinositol-linked protein that undergoes regulated release from cells. Its release depends on its interaction with neogenin, a membrane protein widely expressed in different tissues, including liver and muscle, but not on BMP signaling (29). Release is negatively regulated by iron-loaded Tf and possibly non-Tf iron (29–31). Recent reports implicate the protease furin in the cleavage and secretion of HJV (30, 31), but the details of how it is controlled are controversial. In vitro and in vivo studies of soluble HJV suggest that the regulation of HJV release in response to body iron loading plays a key role in signaling (27). In this paradigm, HJV release from both skeletal muscle and liver is negatively regulated by body iron status to modulate the soluble HJV levels in serum (29). Soluble HJV competes with hepatocyte membrane HJV for the limited BMP local supply and negatively regulates hepatic hepcidin expression (Fig. 1).

TfR2—The recently identified TfR2 is a second, distinct TfR and is most likely responsible for the previously reported non-TfR1-mediated uptake of Tf into the liver. TfR2 is postulated to
be involved in the sensing of iron-loaded Tf levels in the blood. Mutations in TfR2 are associated with a recessive rare form of HH (32). The observation that TfR2 mice have similar iron overload as disease-causing mutations confirms that a loss of function in the TfR2 gene causes this form of HH (33). Like TfR1, TfR2 is a type II membrane glycoprotein with a large C-terminal ectodomain and small N-terminal cytoplasmic domain (18, 34). TfR2 shares 45% amino acid sequence identity with TfR1 in the extracellular region. Clear differences exist between the two TfRs. The affinity of TfR2 for iron-loaded Tf is ~30-fold lower than that of TfR1 (18, 35). Although both receptors have internalization motifs, there are no sequence similarities in their cytoplasmic domains. TfR2 is much less stable than TfR1, allowing changes in TfR2 over a shorter time period (36, 37). TfR2 is regulated at the level of protein degradation by a novel mechanism. It is stabilized by dier Rf in vitro and in vivo (36, 37). TfR2 increases in a time- and dose-dependent manner after addition of dieric Tf to the culture medium. The response to dieric Tf appears to be hepatocyte-specific. Non-hepatic cell lines that either endogenously express TfR2 such as K562 cells or are transfected with a plasmid encoding TfR2 do not respond to Tf (36, 37). Real-time quantitative reverse transcription-PCR analysis shows that TfR2 mRNA levels do not change in cells treated with dieric Tf (36). Rather, the Tf-mediated up-regulation of TfR2 is due to an increase in the half-life of the protein (36). The binding of Tf to TfR2 appears to be responsible for these effects. Unlike wild-type TfR2, the level of a mutant form of TfR2 that does not dierectly bind Tf does not increase in response to dieric Tf (38). These results support a role for TfR2 in monitoring iron levels by sensing changes in the concentration of iron-loaded Tf.

Animal studies are consistent with these observations. Rats fed an iron-decient diet have lower TfR2 levels and Tf saturations than rats fed a high iron diet (37). TfR2 and Tf saturation are higher in HFE knock-out mice compared with normal littermates (37). TfR2 is also lower in the hypotransferrinemic mouse, supporting the role of dieric Tf in the stabilization of TfR2 (37). Because both tissue culture and animal studies show that TfR2 levels are sensitive to Tf over physiological ranges of Tf saturation, we and others hypothesize that TfR2 is the sensor for body iron levels. In keeping with this observation is a report showing that mice with a disease-causing mutation in TfR2 have decreased hepcidin mRNA levels (39).

HFE—The most prevalent form of HH is the autosomal recessive disease caused by a mutation in HFE (40). In the United States, the carrier frequency of this mutation is ~1 in 9 for individuals of Northern European heritage, making it the most common potentially lethal inherited disease in this population. The penetration of the gene is still debated, with estimates ranging from 1:400 to 1:10,000 individuals having the clinical disease (41). The penetration in men is much higher than in women. The mutation in 83% of HH is a single base G-to-A transition in nucleotide 845 that converts Cys to Tyr (42). The HFE mutant fails to associate with β2-microglobulin and is not transported to the plasma membrane (43). Both Hfe−/− mice and β2-microglobulin knock-out mice are simi-
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Iron homeostasis occurs largely through the regulation of hepatic hepcidin expression. The BMP/SMAD signaling pathway appears to play a pivotal role in this process. Functional disruption of the body iron-sensing proteins (HJV, Tfr2, and Hfe) constitutes the major cause of HH. How these proteins coordinately sense body iron levels, modulate BMP/SMAD signaling, and regulate hepcidin expression remain to be determined.

Acknowledgments—We thank Julia Maxson, Juxing Chen, and Maja Chloupkova for critical reading of the manuscript.

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