Iron is an essential element that is toxic when it accumulates in excess. Intricate regulatory mechanisms have evolved to maintain iron homeostasis within cells and between different tissues of complex organisms. This review discusses the proteins involved in iron transport and storage and their regulation in health and disease.

Iron is an essential metal, required as a co-factor in proteins that transfer electrons and manage oxygen. Until recently, there was little molecular understanding of how mammalian tissues meet the challenge of acquiring adequate amounts of iron without risking the toxic effects of iron excess. Our current knowledge of mammalian iron physiology and homeostasis is painted in broad brushstrokes, with many details remaining to be discovered. This review summarizes recent progress in identifying key protein transporters and the molecules that regulate their activities.

Although all cells need a small amount of iron, erythropoietic cells require substantial amounts to produce hemoglobin. Accordingly, anemia is a prominent manifestation of iron deficiency. Other cell types also have specialized roles that are important to consider in assembling a systemic model of iron homeostasis. Intestinal epithelial cells (enterocytes), extraembryonic visceral endoderm cells, and placental syncytiotrophoblasts serve in the acquisition of iron from the external environment. Of these, iron handling by enterocytes is best understood. Liver hepatocytes serve a depot function, removing excess iron from circulating plasma and safely storing it until it is needed. Tissue macrophages recognize and phagocytose old and damaged erythrocytes, recovering their iron for reuse and storage. Molecular signals must coordinate the operations of each of these cell types. To date, no efficient, regulated excretion mechanism for iron has been described, underscoring the importance of meticulous regulation of iron acquisition and distribution.

**Intestinal Iron Absorption**

Mammals absorb dietary iron through the duodenal epithelium of the small intestine (29, 66), which is organized in villous structures to maximize its absorptive surface area. Enterocyte precursors are present in crypts at the bases of villi, migrating up the villous axis as they differentiate. Membrane extensions at the apical surface of enterocytes form a brush border that further increases the surface area available for absorption. Mature enterocytes live for only 1–2 days. Iron that accumulates within them is lost from the body when senescent enterocytes are shed into the gut lumen.

Proteins involved in the uptake of heme and inorganic iron reside on the brush-border membrane (Figure 1). Most non-heme iron in the diet is present as the Fe$^{3+}$ form. The major transporter involved in cellular non-heme iron uptake (import) is divalent metal transporter 1 (DMT1; also known as Nramp2, DCT1, and SLC11A2), which has 12 predicted transmembrane segments (26, 33, 35). As its name implies, DMT1 also transports other divalent metals including Mn$^{2+}$, Co$^{2+}$, Cu$^{2+}$, and Zn$^{2+}$ (35). DMT1 is active in a low-pH environment, as found in the duodenum, because it requires proton cotransport (35). A requirement for DMT1 in intestinal iron absorption is clearly supported by animal models with spontaneous and induced mutations (20, 25, 26, 34). DMT1 exclusively transports divalent metals, necessitating luminal conversion of Fe$^{3+}$ to Fe$^{2+}$. McKie et al. (60) identified a candidate intestinal iron reductase, duodenal cytochrome b (Dcytb; also known as Cybrd1). Expression of this putative transmembrane, di-heme protein is induced in the intestinal mucosa of mice with increased intestinal iron absorption due to anemia, iron deficiency, or hypoxia (60). However, it was recently reported that disruption of the murine Dcytb gene did not significantly impair intestinal iron absorption under normal conditions (36), suggesting that other intestinal reductases may substitute or that mice have an efficient mechanism for non-enzymatic iron reduction.

The intestine also absorbs heme iron from the diet. Cell-culture studies of the intestinal cell line Caco-2 suggested that heme absorption is a saturable, carrier-mediated process (103). Other studies have described a heme receptor on duodenal brush-border membranes and erythroleukemia cells (28, 32). Recently, an enterocyte heme importer was described that likely mediates dietary heme uptake (92). This molecule, termed heme carrier protein 1 (HCP1), resembles bacterial proteins that transport metal-tetracycline complexes. It has no close mammalian homologs. Once dietary heme has entered the intestinal epithelial cell, it is likely cleaved by intracellular heme oxygenase 1 to release iron (85). Subsequently, it probably joins the same intracellular pool as non-heme iron. Two other proteins, FLVCR and Bcrp, both function as cellular heme exporters (55, 84), but neither has been shown to be involved in intestinal heme transport.
across the basolateral membrane. A spontaneous mouse mutant, sex-linked anemia (sla), has impaired iron absorption and accumulation of iron in duodenal enterocytes attributable to a mutation in the gene encoding hephaestin (5, 82, 98). This phenotype is similar to, but less severe than, the phenotype seen in mice with intestine-specific inactivation of ferroportin (22). Hephaestin is a membrane-bound homolog of the serum multi-copper oxidase ceruloplasmin (40). The current model suggests that hephaestin oxidizes Fe^2+ released by ferroportin, facilitating its incorporation into transferrin, the major serum iron carrier protein. The anemia of sla mice resolves after the neonatal period, suggesting that hephaestin is primarily needed for iron transfer during the accumulation of initial stores and that serum ceruloplasmin may substitute later.

**FIGURE 1. Intestinal iron absorption**
An individual enterocyte is depicted. Dietary non-heme iron (Fe^{3+}) must be reduced for transport across the apical brush border. DMT1 mediates proton-dependent Fe^{2+} import. Dietary heme iron is transported by HCP1. Once inside the cell, heme oxygenase releases iron from protoporphyrin, presumably allowing it to enter the same pool as non-heme iron. Some iron is used or stored within the enterocyte in ferritin. This iron is later lost from the body when the intestinal mucosa is sloughed. Some iron is exported across the basolateral membrane by ferroportin and oxidized to Fe^{3+} for incorporation into serum transferrin.

Intercellular enterocyte iron can either be stored in the multimeric protein ferritin (95) or transported across the basolateral membrane of the enterocyte into the circulation. Ferroportin (also known as IREG1, MTP1, and SLC40A1), a distinct transporter with 10–12 transmembrane segments, was identified as a compelling candidate for the basolateral iron exporter (1, 21, 61). Intestinal expression of ferroportin mRNA and protein increases in response to iron deficiency and hypoxia (61). Recently, selective inactivation of the murine ferroportin gene in intestinal cells established that ferroportin is the major, if not only, intestinal iron exporter (22). Ferroportin is probably also selective for Fe^{2+}. Multicopper oxidases that oxidize Fe^{2+} to Fe^{3+} also play a role in the transport of iron across the basolateral membrane. Dietary non-heme iron (Fe^{3+}) must be reduced for transport across the apical brush border. DMT1 mediates proton-dependent Fe^{2+} import. Dietary heme iron is transported by HCP1. Once inside the cell, heme oxygenase releases iron from protoporphyrin, allowing it to enter the same pool as non-heme iron. Some iron is used or stored within the enterocyte in ferritin, and is later lost when the intestinal mucosa is sloughed. Some iron is exported across the basolateral membrane by ferroportin and oxidized to Fe^{3+} for incorporation into serum transferrin.
alternative splicing (56). The TF cycle is completed when the endosome returns to and fuses with the plasma membrane, returning apoTF to the circulation and TFR1 to the plasma membrane and allowing both molecules to start the cycle again.

Cellular iron in excess of immediate needs is stored as an iron oxide solid within the central cavity of ferritin, a polymeric protein composed of varying ratios of heavy (H) and light (L) ferritin polypeptides (94). To ensure that very little iron is free within cells, iron regulatory proteins (IRP1 and IRP2) control the posttranscriptional expression of genes modulating cellular iron uptake and storage. Under low iron conditions, both proteins bind to conserved, hairpin-like iron regulatory elements (IREs) present in the untranslated regions (UTRs) of target mRNAs. Classical studies defined the roles of IRPs and IREs in regulating mRNAs encoding TFR1 and ferritin subunits. The TFR1 mRNA contains five IRE elements in its 3'-UTR. IRP binding protects the mRNA from endonucleolytic degradation (42). Thus, when the cell is iron depleted, more TFR1 protein is made, resulting in increased iron acquisition. In contrast, binding of IRPs to the single IREs in the 5'-UTRs of ferritin mRNAs does not affect mRNA stability but rather inhibits translation of the mRNA into protein (42). Accordingly, less ferritin protein is made when it is not needed for iron storage. IRP1 and IRP2 activities are regulated by iron, but through distinct mechanisms. IRP1 is inactivated by the incorporation of a 4Fe•4S cluster, which converts the iron transporter to an efficient calcium transporter (38). IRP2 is inactivated by iron-dependent degradation (37, 47). In addition to interpreting cellular iron status, IRP1 and IRP2 both respond to nitric oxide levels, IRP1 responds to H2O2, and IRP2 is regulated by hypoxia (39, 42, 77).

Several other iron-related mRNAs also contain IRE elements. A single IRE is present in the 3'-UTR of one splice isoform of DMT1 mRNA (45). 5'-IREs are present in mRNAs encoding the erythroid form of the heme biosynthetic aminolevulinic acid synthase (eALAS; Refs. 14, 17) and the iron transporter ferroportin (1, 21, 61). The 5'-IRE in the eALAS mRNA likely serves to coordinate initiation of heme biosynthesis with iron availability. The presence of IREs in DMT1 and ferroportin mRNAs suggests that their expression may be controlled, at least in part, by cellular iron content. Although this is not yet understood in detail, evidence for in vivo activity of the IRE in the ferroportin mRNA comes from the spontaneous mouse mutant polycythemia (pcm), which has a genomic deletion that inactivates the ferroportin IRE, resulting in a complex phenotype with transient polycythemia in heterozygotes (pcm/+). IRP2 knockdown in homozygotes (pcm/pcm) (63).

Data from multiple animal models establish that the TF cycle is critical for iron uptake into erythroid cells (6, 20, 25, 26, 34, 58, 102). In addition, human mutations associated with anemia have been identified in both the TF and DMT1 genes (7, 46, 62). However, studies of non-erythroid cells indicate that there must be distinct mechanisms for non-TF-bound iron (NTBI) uptake. Although the proteins normally involved in this process have not been identified (9), two possible mechanisms for mammalian NTBI uptake have been described.

Purified lipocalin 2 (also known as Ngal and 24p3) (30, 106) contains iron complexed in bacterial siderophores. It is not yet clear whether this form has a physiological role in iron transport. Nonetheless, the complex is taken up by cells through an as yet unidentified receptor-mediated process. To date, studies implicate the iron-binding activity of lipocalin 2 in early renal development and in innate immunity, but further study will be necessary to determine whether it plays a general role in NTBI uptake (27, 106).

L-type calcium channels have also been implicated in NTBI uptake. Mammals express four highly homologous L-type calcium channels (Ca1.1-1.4). L-type calcium channels have been shown to mediate cellular iron uptake in vitro (96) and to be involved in iron deposition in the murine heart in the setting of iron overload in vivo (76). Pharmacological calcium channel blockers interfere with this activity. Interestingly, the spontaneous mk mutation in murine DMT1 effectively converts the iron transporter to an efficient calcium channel, consistent with the idea that similar transmembrane pores conduct Fe2+ and Ca2+ (104). L-type calcium channels are widely expressed and thus could be involved in NTBI uptake in other tissues.

Iron Utilization and Recovery

Hemoglobin production in erythroid precursors is a complex process requiring meticulous coordination of iron acquisition, protoporphyrin biosynthesis, and globin protein production. Perturbation of this balanced process invariably results in disease. Disorders of hemoglobin synthesis give insight into iron homeostasis.

The protoporphyrin precursor of heme is constructed through a series of enzymatic reactions that take place alternately in the mitochondrion and the cytoplasm. Mutations in the enzymes involved in heme biosynthesis lead to a spectrum of diseases, several of which have iron phenotypes. Mutations in the first enzyme, ALAS2, result in sideroblastic anemia (15), characterized by accumulation of unused iron in the mitochondria of erythroid precursors, likely in the form of mitochondrial ferritin (11). The final enzyme of heme biosynthesis, ferrochelatase, introduces iron into protoporphyrin IX to produce heme (16). Mutations in this protein result in erythropoietic protoporphyria, a rare disease resulting from accumulation of protoporphyrin lacking iron (12, 89, 91, 97). These patients can also have sideroblasts, similar to
patients with ALAS2 mutations. Sideroblastic anemia can also result from mutations in proteins with less direct roles in heme biosynthesis (24). For example, X-linked sideroblastic anemia with spinocerebellar ataxia results from mutations in the mitochondrial transporter ABC7 (4), which is required for the maturation of cytosolic iron sulfur (Fe•S) clusters.

Recycling of iron from senescent erythrocytes provides most of the iron utilized by developing red blood cells. Specialized macrophages in the spleen, bone marrow, and liver remove effete red cells from circulation for recovery of their iron. Neither the signal on the effete erythrocyte nor the macrophage receptor has been clearly identified to date, but several promising candidates have been described. Accumulation of phosphatidylserine in the outer leaflet of the erythrocyte membrane seems to be a primary event (13), but Ca\(^{2+}\) flux (57), removal of sialic acids on the cell surface, and opsonization of red blood cells by autoantibodies are all possible signals for turnover (8). The scavenger receptor CD36 (53, 93) is a plausible candidate for the macrophage-specific receptor.

After the red cell has been internalized into an acidic phagosome, heme oxygenase liberates iron from heme (83). The iron is likely transferred out of the phagosome by DMT1 (48) for cellular storage or return to the serum. The factors that influence whether the macrophage retains or releases iron are not known, but intracellular iron content (99), cytokine activity (86, 101), and reactive oxygen species such as nitric oxide (31) may be involved. Iron egress from macrophages is partly, if not entirely, mediated by ferroportin at the cell surface to initiate ferroportin internalization and degradation (69). In the duodenal enteroctye, hepcidin-dependent regulation of ferroportin reduces dietary iron absorption. Loss of hepcidin protein results in severe iron overload in mice (70) and human patients (78, 88), underscoring the central role of hepcidin in regulating iron balance.

Consistent with its role as a negative regulator of iron absorption, hepcidin expression is decreased in response to anemia and hypoxia (71) (FIGURE 2). This is advantageous, because it makes more iron available for erythropoiesis. In contrast, hepcidin expression is increased in response to inflammation (67) and nongenetic iron overload (81). This response minimizes the availability of iron, inhibiting the growth of pathogens and limiting the iron burden. Neither the factor that induces hepcidin in response to iron loading nor the factor that inhibits hepcidin in response to anemia or hypoxia has been identified.

The unidentified erythroid suppressor of hepcidin expression appears to overcome the putative sensor for iron loading, however, because hepcidin expression is suppressed in anemias complicated by iron overload such as hypotransferrinemia (100) and thalassemia (2). Although we have a general knowledge of the conditions that regulate hepcidin expression, little is known about the molecular mechanisms of hepcidin regulation. The discovery of such factors will probably result from studies of hepatocytes, where several membrane proteins (HFE, TFR2, and HJV) have already been

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**FIGURE 2. Regulation of hepcidin expression**

Hepatocellular production of the peptide hormone hepcidin is influenced by iron needs and stores. Anemia and hypoxia result in decreased hepcidin synthesis; inflammation, the inflammatory cytokine interleukin-6 (IL-6), and increased iron stores (possibly through ferric-TF) result in increased hepcidin synthesis. The molecular pathways involved in signal transduction in response to these conditions have not yet been elucidated. The protein products of hemochromatosis disease genes (HFE, TFR2, and HJV) are presumed to act as regulators of hepcidin expression because mutations in each result in inadequate hepcidin production. A soluble cleavage product of HJV (sHJV) also has been shown to inhibit hepcidin production.
implicated in the modulation of hepcidin expression in vivo. Homozygous or compound heterozygous mutations in any of these three proteins result in genetic hemochromatosis, a common iron-overload disorder in human populations.

HFE is an atypical major histocompatibility class I protein that is mutated in the majority of patients with hemochromatosis (23). Mice (3, 65) and humans (10) deficient in HFE have reduced hepatic expression of hepcidin despite iron overload. HFE is highly expressed in hepatocytes and is also expressed in Kupffer cells (107). The physiologically significant site of HFE expression remains controversial. The fact that HFE forms a protein complex with TFR has led to the attractive hypothesis that a soluble factor such as diferric Tf (which competes with HFE for TFR binding) might modulate HFE activity and regulate a potential pathway signaling to the hepcidin (HAMP) promoter. To date, direct evidence supporting this hypothesis is lacking.

Transferrin receptor 2 (TFR2; a homolog of TFR) is mutated in a small subset of patients with genetic hemochromatosis. Its normal function is unclear. However, like HFE, deficiency of TFR2 attenuates hep-

FIGURE 3. The hepcidin-ferroportin axis
In hemochromatosis, hepcidin is deficient or absent, resulting in increased ferroportin on the cell surface and accelerated iron release. Consequently, intestinal iron absorption is increased and serum iron levels rise.
Hepcidin expression in mice (50) and humans (68) and results in moderate iron overload. TFR2 is highly expressed in hepatocytes (52) but is also expressed in erythroblasts (51). No erythropoietic abnormalities have been observed in mice or humans with TFR2 deficiency, suggesting that the TFR2 requirement for hepcidin regulation is intrinsic to the hepatocyte and not the result of feedback through the anemia/hypoxia regulatory pathway. Diferric TF is also an attractive candidate for communicating iron status to regulate hepcidin expression via TFR2 as TF induces TFR2 expression in hepatocytes (49, 87).

Hemojuvelin (HJV) is a homolog of proteins involved in neural patterning. It was recently described as a hemochromatosis disease gene (78). HJV deficiency in mice (43, 72) and humans (44, 78) reduces hepcidin levels further than either HFE or TFR2 deficiency and, accordingly, results in more severe iron loading. HJV is expressed in a number of tissues including liver, heart, and skeletal muscle, and found in plasma in a soluble form. Recent data suggest that cell-associated HJV is required for normal expression of hepcidin in hepatocytes and that soluble HJV negatively regulates hepcidin expression in hepatocytes (59). The cellular source of soluble HJV and the conditions that regulate its production are currently unknown, but high expression of HJV in skeletal and cardiac muscle suggests that these iron-rich tissues may communicate their iron needs through soluble HJV.

Induction of hepcidin in response to inflammation is mediated, at least in part, by interleukin-6 (IL-6) (67). The relationship between IL-6 induction of hepcidin expression and regulation by HFE, TFR2, and HJV remains uncertain. Hepcidin induction causes hypoferremia through cellular iron-withholding, a response that contributes to innate immunity (67). However, there is a co-existing deleterious effect. Increased hepcidin levels also restrict the availability of iron for erythropoiesis, resulting in the anemia of inflammation, a common disorder observed in patients with inflammation, infection, organ failure, or recent trauma.

Pathogenesis of Iron-Overload Disorders

Studies focused on the regulation of iron homeostasis have converged on hepcidin as a common effector molecule, acting through its modulation of ferroportin activity (FIGURE 2). All known heritable disorders of iron overload involve mutations affecting hepcidin itself, regulators of hepcidin (HFE, TFR2, HJV), or the hepcidin target ferroportin.

Patients with mutations in HFE and TFR2, presumed regulators of hepcidin expression, generally present in midlife with elevated transferrin saturation, parenchymal iron loading, and proportionally less iron in tissue macrophages (80). Each of these features can be explained by modest hepcidin deficiency in these individuals. Without hepcidin, there is inadequate regulation of ferroportin at the basolateral surface of enterocytes, contributing to increased dietary iron uptake. Likewise, ferroportin activity remains elevated in macrophages, explaining their relative iron deficiency (FIGURE 3).

Patients with mutations in hepcidin and HJV present in the second or third decade of life with elevated transferrin saturation and severe iron loading. The clinical picture is dominated by cardiomyopathy and endocrinopathies. If untreated, iron-related organ damage is lethal by the fourth decade of life. Their hepcidin levels are lower than those in patients with HFE or TFR2-related hemochromatosis, consistent with the accelerated iron loading in these individuals.

Mutations in ferroportin result in either of two distinct iron overload diseases, each inherited in an autosomal-dominant pattern (19, 64, 74, 90). Similar to other forms of hemochromatosis, some patients present with parenchymal iron overload and elevated transferrin saturation. These patients have mutant ferroportin that is expressed at the cell surface and is capable of iron egress but resistant to regulation by hepcidin. In contrast, other ferroportin mutations cause mislocalization and/or loss of transporter function. These patients have little or no parenchymal iron loading and low to normal serum iron levels, but tissue macrophages are iron-laden.

Future Directions

Now that most of the key proteins involved in iron homeostasis have been identified, future investigations must focus on understanding their molecular functions. To date, there have been few structural studies of DMT1, ferroportin, or HCP1, and it is not yet known how they carry out transmembrane iron transport. Although the stimuli that modulate hepcidin expression are well described as phenomena, we do not yet understand how physiological signals are transduced to regulate hepcidin production. The potent erythroid suppressor of hepcidin synthesis has not yet been identified. The detailed molecular activities of hemochromatosis disease proteins HFE, TFR2, and HJV have not yet been reported. Undoubtedly, the next decade should yield a more comprehensive understanding of iron metabolism and likely inform other studies of the whole organism.

References


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