Frontiers in Nephrology

Molecular Control of Iron Transport

Tomas Ganz

Departments of Medicine and Pathology, David Geffen School of Medicine, University of California, Los Angeles, California

The iron-regulatory hormone hepcidin is a 25–amino acid peptide that is synthesized in hepatocytes. Hepcidin binds to the cellular iron export channel ferroportin and causes its internalization and degradation and thereby decreases iron efflux from iron exporting tissues into plasma. By this mechanism, hepcidin inhibits dietary iron absorption, the efflux of recycled iron from splenic and hepatic macrophages, and the release of iron from storage in hepatocytes. Hepcidin synthesis is stimulated by plasma iron and iron stores and is inhibited by erythropoietic activity, ensuring that extracellular plasma iron concentrations and iron stores remain stable and the erythropoietic demand for iron is met. During inflammation, increased hepcidin concentrations cause iron sequestration in macrophages, resulting in hypoferremia and eventually anemia of inflammation. Hepcidin deficiency plays a central role in most iron overload disorders. The role of hepcidin abnormalities in anemias that are associated with renal disease and in resistance to erythropoietic therapies remains to be elucidated.

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Iron Economy

Iron is an essential element that is required for oxidative energy metabolism in nearly all species. In humans, iron is an essential component of the oxygen carriers hemoglobin and myoglobin and of cytochromes and other enzymes that are involved in oxidation or reduction of biologic substrates. The average male adult contains approximately 4 g of iron, a little more than 2 g of which is in hemoglobin (each 1 ml of packed erythrocytes contains approximately 1 mg of iron), 1 g in body stores predominantly in the liver, and the rest in myoglobin and other iron-containing proteins. Approximately 1 to 2 mg of iron is lost each day by epithelial shedding in the gastrointestinal tract and the skin and through blood loss in menstruating women. There is no physiologic mechanism for excreting larger amounts of iron, even in severely iron-overloaded individuals. The normal losses are balanced by absorption of iron from the diet. Western diets contain a much greater amount of iron (10 to 20 mg) than what is absorbed daily under normal circumstances (1 to 2 mg). Iron absorption increases several-fold in iron deficiency and is suppressed partly when iron stores are excessive. Approximately 20 mg/d iron is recycled from senescent erythrocytes by macrophages. Erythrocytes have a life span of 120 d, so each day, approximately 1% of erythrocytes is removed by macrophages from the circulation and iron is extracted from hemoglobin. Recycled and absorbed iron is delivered to transferrin in blood, and most of it is destined for the nascent erythrocytes in the bone marrow, whereas a much smaller portion is distributed to other tissues. The plasma transferrin compartment is relatively small, containing only approximately 3 mg of iron, which therefore must turn over every few hours.

Iron plays an important role in host defense responses to infectious agents. Several host defense proteins, including lactoferrin, siderocalin (also called neutrophil gelatinase–associated lipocalin, lipocalin 2, or NGAL), and the macrophage divalent metal transporter natural resistance associated macrophage protein 2, seem to function in infected tissues to sequester iron from invading microorganisms. On a systemic level, hypoferremia develops early during infection and, if prolonged, eventually leads to the characteristic anemia of inflammation (anemia of chronic disease).

Iron absorption, recycling, and movement in and out of stores is subject to regulation by three major influences: Systemic iron status (referred to as the "stores" regulator), the iron requirements of erythropoiesis ("erythropoietic" regulator), and the pathologic effects of inflammation ("inflammatory" regulator). As is discussed in detail, it has become clear that all three of these influences converge on a single iron-regulatory hormone, hepcidin, whose biology is central to systemic iron homeostasis.

Iron Absorption

Dietary iron is presented to the duodenum either as ferric iron complexed with macromolecules such as ferritin or phytates or in the form of heme or heme-containing proteins. At the epithelial surface, ferric iron is reduced to ferrous iron with the assistance of one or more apical ferric reductases, possibly including the duodenal cytochrome dCytB, and crosses into the cytoplasm *via* the apical enterocyte transporter

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Address correspondence to: Dr. Tomas Ganz, Departments of Medicine and Pathology, David Geffen School of Medicine, 10833 Le Conte Avenue, CHS 37-055, University of California, Los Angeles, CA 90095-1690. Phone: 310-825-6112; Fax: 310-206-8766; E-mail: tganz@mednet.ucla.edu

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divalent metal transporter 1 (DMT-1), as reviewed recently (1). DMT-1–mediated iron transport requires an inward proton gradient. Dietary heme is taken up by apical heme transporters, one of which was characterized recently (2). In the cytoplasm, iron is stored in ferritin. On the basolateral surface of duodenal enterocytes, cellular iron is exported to plasma by ferroportin (also called iron regulated transporter 1, mental transport protein 1, or SLC40A1) (3–5). Ferroportin cooperates with the multicopper ferroxidase hephaestin (6), which converts ferrous to ferric iron for uptake by plasma transferrin. As shown in mice with homozygous disruption of ferroportin genes, little or no iron transfer from enterocytes to plasma takes place without ferroportin, despite massive iron accumulation in enterocytes (7). Ferroportin therefore is a natural control point for intestinal iron absorption. The short lifespan of intestinal enterocytes (approximately 2 d) ensures that iron that is not transferred to plasma is shed with the enterocytes into the fecal stream.

Iron Recycling and Storage

As indicated in a recent review, despite its fundamental importance, the biology of iron recycling by macrophages is "one of the least well understood areas of iron metabolism" (8). At the end of their 4-mo lifespan, human erythrocytes undergo surface alterations that mark them to be phagocytosed and digested by macrophages in the spleen and the liver. In macrophages, iron is recovered from heme by the action of heme oxygenase (predominantly the inducible heme oxygenase HO-1). It is not certain at what subcellular location HO-1 performs its activity. Both DMT-1 and the closely related divalent metal transporter natural resistance associated macrophage protein 2 are expressed in macrophages and could transport iron across the phagosomal membrane to the cytoplasm, but it is not clear that either is required for this function. Macrophages, like other cells, store iron in ferritin. Iron eventually is exported to plasma transferrin by ferroportin with the aid of the multicopper ferroxidase ceruloplasmin (9), but it remains to be established whether ceruloplasmin interacts directly with ferroportin. Ceruloplasmin-deficient patients have a mild impairment of iron mobilization from macrophages as indicated by a mild anemia and gradual iron retention in macrophages, suggesting that the ferroxidase function of ceruloplasmin is partially redundant. Conversely, ferroportin is essential for iron recycling; ferroportin-deficient mice become severely anemic and rapidly accumulate iron in macrophages (7).

Hepatocytes are a major site of iron storage and express relatively low amounts of the iron exporter ferroportin on surfaces that face the sinusoids (5). In states of genetic or acquired iron overload, hepatocytes become a major site of iron deposition, presumably because their iron uptake exceeds the capacity for export. Hepatocytes display particularly high uptake rates for non–transferrin-bound iron, a form of iron that is present when iron load exceeds the iron-binding capacity of transferrin (10).

Hepcidin

The bioactive form of hepcidin is a 25–amino acid cationic peptide that contains four disulfide bonds (11–15). It is encoded as an 84–amino acid prepropeptide and is synthesized, processed, and secreted predominantly by hepatocytes. Injection of synthetic hepcidin into mice induces profound hypoferremia within 1 h (14). The essential role of hepcidin in iron homeostasis was established in transgenic mouse models and human diseases that result in hepcidin deficiency (16–18) or excessive production of the peptide (19,20). Complete deficiency of hepcidin causes juvenile hemochromatosis, a severe form of genetic iron overload in which dietary iron absorption is dysregulated so that iron is taken up at a high rate despite excessive iron stores. Excessive production of hepcidin causes iron deficiency anemia as a result of the individual's inability to absorb iron, despite normal or even iron-enriched diet. The severity of both conditions indicates that any mechanisms that override the effects of hepcidin are relatively ineffective.

Hepcidin Assays

The small size and evolutionary conservation of hepcidin has hampered the development of sensitive immunoassays. In humans, urinary hepcidin measurements detect the bioactive form of hepcidin, have provided physiologically meaningful information (21,22), and have correlated well with hepcidin mRNA concentrations in liver biopsies from the same patients (23). A human serum prohepcidin assay (24) has been less reflective of iron or inflammatory physiology probably because prohepcidin is a processing intermediate without a direct biologic role in iron metabolism. In mice, hepcidin-1 mRNA is used as a proxy for the measurement of the bioactive hepcidin form. Although further studies are necessary to clarify this issue, it seems that hepcidin is regulated predominantly by the concentrations of its mRNA so that the conclusions from mRNA-based mouse studies and peptide-based human studies are very similar. More recently, semiquantitative assays that were based on mass spectrometry were developed for human urinary (25) and serum (26) hepcidin, and these hold promise especially for measurements of elevated hepcidin concentrations.

Regulation of Hepcidin Synthesis

Hepcidin is induced by iron loading ("stores" signal in the older literature) and by inflammation and is suppressed by erythropoietic activity (12,21,22,27,28). The effects of acute inflammation are best understood and are mediated at least in part by IL-6 (21,29) through the induction and binding of STAT-3 to the hepcidin promoter (30). Although the acute hypoferremic response and hepcidin induction both are impaired in IL-6–deficient mice (21), chronic inflammatory stimulation elicits hepcidin even in IL-6–deficient mice (31). Potential candidates for chronic stimulatory factors include bone morphogenic proteins and TGF-β and other ligands of the receptors of the TGF-β family (32–34). It is not yet known how iron and erythropoiesis regulate hepcidin. Iron ingestion or parenteral iron administration results in the induction of hepcidin (12,21), which in turn blocks iron absorption until the normal plasma iron levels are restored. Hepcidin deficiency as a result of dysregulation of its synthesis would be expected to lead to iron accumulation and hemochromatosis. Indeed, recent

studies indicate that hepcidin is deficient in hereditary hemochromatosis as a result of mutations in transferrin receptor 2, HFE, or hemojuvelin (35–38). These genes therefore must encode regulators of hepcidin synthesis. Recent studies showed that these molecules form a hepcidin-regulating complex, possibly also involving bone morphogenic proteins and their receptor (32). However, how iron is sensed by these molecules and which signaling pathways they activate to regulate hepcidin in response to iron remain to be elucidated.

Hepcidin also is regulated by anemia (27), but it seems that the effects of anemia largely are mediated by an unknown factor that is produced during erythropoietic activity (28,39). Hepcidin is suppressed appropriately in mice that are made anemic by phlebotomy or hemolysis, but this suppression is reversed when reactive erythropoiesis is inhibited by cytotoxic agents, radiation, or erythropoietin-neutralizing antibodies (28,39). The hepcidin-suppressive effect of erythropoietin also is inhibited by cytotoxic agents and therefore is substantially dependent on erythropoietic activity (39). Although hepcidin suppression during anemia and reactive erythropoiesis could be due in part to increased iron use and systemic iron depletion, hepcidin suppression also is seen in mouse models and human diseases in which severe systemic iron overload coexists with anemia and reactive erythropoiesis. Urinary hepcidin concentrations are very low in untransfused anemic patients with thalassemia intermedia and systemic iron overload (22), and hepcidin mRNA concentrations are low in anemic mice with hypotransferrinemia and severe iron overload (20). The hepcidin-regulatory factor that is generated during erythropoiesis and acts on hepatocytes remains to be identified.

Ferroportin

All cells require iron for energy metabolism and other metabolic processes. Depending on the cell type, multiple mechanisms exist for iron uptake, whether in the form of transferrin-bound iron, nontransferrin iron, heme, hemoglobin, or entire red blood cells. In contrast, the ability to export iron is limited to tissues that are engaged in iron transport, including small intestinal (mainly duodenal) epithelium, macrophages, hepatocytes, and embryonic or placental cells that interface to the maternal circulation. As shown in ferroportin knockout mice, ferroportin is the sole or predominant efflux channel for iron in all of these tissues (7). Autosomal dominant ferroportin mutations that lead to mislocalization and degradation of ferroportin cause accumulation of iron in macrophages (ferroportin disease), confirming the critical role of ferroportin in iron recycling by macrophages (40–42). Ferroportin is a multipass membrane protein whose topology has not yet been established with certainty. Moreover, little is known about how it transports iron.

Hepcidin Regulates Ferroportin

Hepcidin regulates iron efflux by binding to ferroportin and inducing its internalization and lysosomal degradation (43,44) (Figure 1). In cells that express ferroportin, hepcidin has a concentration-dependent inhibitory effect on iron export that parallels the hepcidin-induced loss of ferroportin from the plasma membrane (15,43). Therefore, ferroportin is both a hepcidin-regulated iron efflux channel and the hepcidin receptor. Several autosomal dominant mutations of ferroportin interfere with its ability to bind and internalize hepcidin (40–42,45,46) and cause systemic iron overload similar to that from hepcidin deficiency (45,47). The molecular details of hepcidin binding by ferroportin and of the ferroportin internalization pathway remain to be elucidated. On the hepcidin molecule, the five N-terminal amino acids seem to be required for the interaction with ferroportin (15), and hepcidin-20, a naturally occurring form that lacks these amino acids, essentially is inactive.

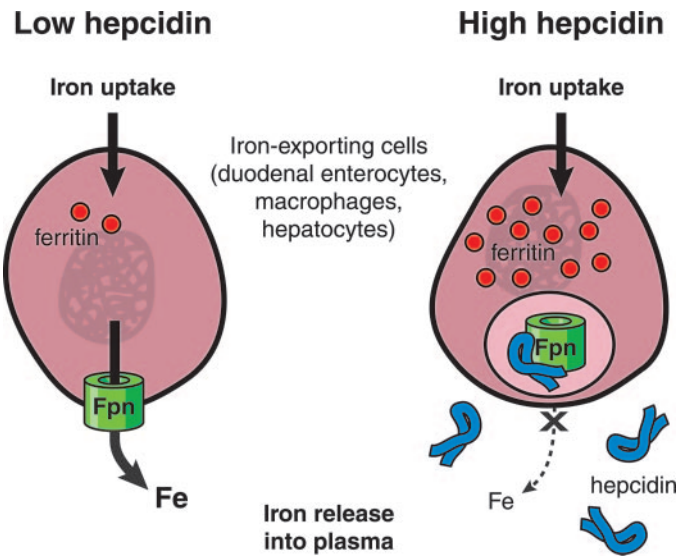

Figure 1. Hepcidin regulates cellular iron export into plasma. When hepcidin concentrations are low, ferroportin (Fpn) molecules are displayed on the plasma membrane and export iron. When hepcidin concentrations increase, hepcidin binds to ferroportin molecules and induces their internalization and degradation, and iron release is decreased progressively. Illustration by Josh Gramling—Gramling Medical Illustration.

Hepcidin Metabolism

Hepcidin is detectable in urine predominantly as the bioactive 25–amino acid form and, to a lesser extent, as N-terminally truncated 20– and 22–amino acid forms (11) that biologically are much less active (15) and probably represent degradation products. Renal clearance of hepcidin may be metabolically important, as suggested by its accumulation in patients with renal failure (26). Presumably because of its very small size, hepcidin is cleared efficiently by hemodialysis (26,48). When injected into mice, radiolabeled hepcidin is excreted rapidly in urine but also accumulates in ferroportin-rich tissues (14), including the proximal duodenum, the spleen, and the liver, where its uptake and degradation also could contribute to its removal from circulation.

Systemic Iron Homeostasis

Despite large fluxes of iron through the plasma compartment, human plasma iron concentrations normally are maintained in a relatively narrow range of 10 to 30 μM. This suggests that a homeostatic mechanism must exist to regulate

extracellular iron concentrations. Such a mechanism would be expected to contain an iron sensor, transduction machinery, and messenger molecules that regulate iron efflux from various cellular reservoirs into plasma. Recent studies suggest that the hepcidin-ferroportin interaction is at the core of this regulatory system (Figure 2). Intraperitoneal injection of 50 μg of hepcidin into mice causes profound hypoferremia as soon as 1 h after the injection (14). Serum iron does not return to normal until more than 48 h later, consistent with resynthesis of ferroportin that has been degraded in lysosomes under the influence of hepcidin. The key role of the hepcidin–ferroportin interaction in systemic iron homeostasis is supported by the phenotypic similarity of hemochromatosis as a result of hepcidin deficiency or mutations in ferroportin that interfere with hepcidin binding or ferroportin internalization (45,47).

Anemia of Inflammation

Anemia of inflammation (also called anemia of chronic disease) is defined by hypoferremia despite normal iron stores. Operationally, normal iron stores used to be established by the presence of stainable iron in bone marrow macrophages, but, more recently, adequate iron stores are assessed by normal or elevated serum ferritin. The anemia usually is mild to moderate and normocytic/normochromic. The pathophysiology of anemia of inflammation centers on iron-limited erythropoiesis that is exacerbated to a varying extent by shortened erythrocyte lifespan. The iron limitation is evidenced by hypoferremia, which in turn reflects the sequestration of iron in macrophages, intestinal enterocytes, and possibly hepatocytes. Other correlates of iron-limited erythropoiesis include elevation of zinc protoporphyrin as a result of substitution of zinc for iron during the formation of heme. It has been assumed that the hypoferremia of inflammation has a role in host defense against microbial infection, together with a number of other mechanisms that limit iron availability to invading microorganisms (49).

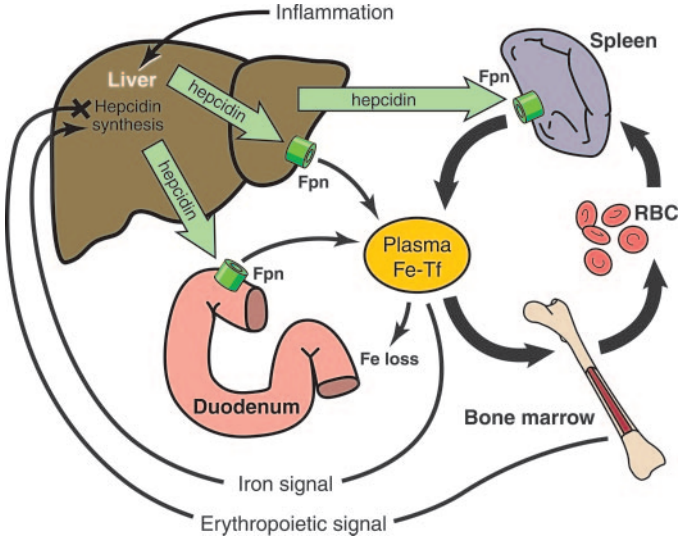

Figure 2. Systemic iron homeostasis. By regulating ferroportin, hepcidin controls the entry of iron into plasma. The major iron flows that are regulated by hepcidin–ferroportin interactions include the release of iron from macrophages that recycle iron in the spleen and other organs, dietary iron absorption in the duodenum, and the release of iron from storage in hepatocytes. The feedback stimulation of hepcidin by plasma iron saturation and iron stores ensures that extracellular iron concentration and iron stores stay within normal limits. Hepcidin synthesis is suppressed by erythropoietic activity, ensuring a sufficient supply of iron to the bone marrow when demand for erythrocytes is high. During inflammation, hepcidin production is stimulated and iron entry into plasma is inhibited, causing the hypoferremia and anemia of inflammation. Illustration by Josh Gramling—Gramling Medical Illustration.

Role of Hepcidin in Anemia of Inflammation

The inducibility of hepcidin by inflammatory stimuli (12,27,29,50) suggested that hepcidin, by limiting iron export from macrophages, could have a key role in anemia of inflammation. Excess hepcidin production in transgenic mice causes iron-restricted erythropoiesis (19), and anemia with hypoferremia also is seen with tumors that overproduce hepcidin in humans or mice (20,51). Patients with anemia of inflammation had elevated urinary hepcidin concentrations (50) that correlated with serum ferritin, a marker of inflammation. These studies strongly suggest that the final common pathway for anemia of inflammation involves cytokine stimulation of hepcidin synthesis, iron sequestration as a result of hepcidin-induced loss of ferroportin from macrophages, and iron-limited erythropoiesis. Inflammatory cytokines (including IL-1, TNF-α, IL-6, and IFN-γ) also may affect erythropoiesis through hepcidin-independent effects on erythroid development in the bone marrow (52) and by suppressing the production of erythropoietin (53). Moreover, ferroportin mRNA levels seem to be regulated by cytokines and toll-like receptor–dependent pathways by hepcidin-independent mechanisms (54). The relative impact of these mechanisms on erythropoiesis and iron metabolism *in vivo* remains to be determined.

Role of Hepcidin in Iron Overload Disorders

Acute or chronic dietary supplementation or iron injections increased hepcidin mRNA in normal mice (12,21), and the administration of 65 mg of oral ferrous iron to a group of human volunteers increased urinary hepcidin excretion more than five-fold within 24 h. Therefore, increased production of hepcidin represents the normal response to iron loading. The rapid response of urinary hepcidin to iron load in human volunteers (as little as 6 h; T. Ganz and E. Nemeth, unpublished observations) suggests that the system senses the iron saturation of transferrin, but both the form of iron and the specific molecular sensors that detect it remain to be identified. The expected effect of increased hepcidin after iron challenge would be homeostatic: To decrease further absorption of iron from the diet and inhibit its release from macrophages. Pathologic iron overload occurs in hereditary hemochromatoses, in anemias with massively increased erythropoiesis, or after repeated erythrocyte transfusions. In these settings, the normal response to iron loading is modified by genetic lesions that decrease or

ablate hepcidin synthesis (hereditary hemochromatoses) or by the suppressive effects of anemia and erythropoietic activity on hepcidin production. Hereditary hemochromatoses (55) are a group of diseases that are caused by autosomal recessive mutations in genes that encode the molecules HFE, human transferrin receptor 2, hemojuvelin, and hepcidin itself and certain autosomal dominant mutations in the hepcidin target ferroportin. Decreased production or absence of hepcidin or, in the case of ferroportin mutations, insensitivity to hepcidin seems to be the common feature of the disease, regardless of the specific genetic lesion involved. Hepcidin deficiency or resistance to hepcidin therefore is the fundamental cause of hemochromatosis.

In further support of the central involvement of hepcidin in the pathogenesis of hemochromatosis, transgenic correction of hepcidin deficiency in a mouse model of HFE hemochromatosis prevents the development of iron overload pathology (56). If hepcidin synthesis is switched on after hemochromatosis already developed, then increased hepcidin causes redistribution of iron from parenchymal cells to macrophages, where iron is relatively nontoxic (57). In iron-loading anemias (β-thalassemia and congenital dyserythropoietic anemia), the suppressive effect of erythropoiesis on hepcidin production (22,58) and the resulting increase in dietary iron absorption is sufficient to cause systemic iron overload and iron-mediated damage to the liver and myocardium even without blood transfusions. It remains to be seen whether exogenous hepcidin can correct the iron pathology in iron-loading anemias as well.

Role of Hepcidin in Anemia of Renal Disease

Anemia of renal disease has a multifactorial pathogenesis, including erythropoietin deficiency, inflammatory effects of the primary disease, inflammatory effects of its complications and of its treatments, and the potential consequences of decreased renal clearance of hepcidin. Both inflammation and the decreased clearance of hepcidin could raise blood hepcidin concentrations and lead to iron-restricted erythropoiesis. In milder cases, iron restriction could become manifest only when erythropoietic activity and iron demand increase as a result of treatment with recombinant erythropoietin. In these settings, iron restriction may lead to erythropoietin resistance with partial reversibility by parenteral iron therapy (59). Fundamental understanding of these processes is desirable but awaits further studies with improved serum hepcidin assays.

Conclusion

Hepcidin is the iron-regulatory hormone responsible for systemic iron homeostasis. It regulates intestinal iron absorption and the release of iron from macrophages and hepatic stores. Hepcidin acts by binding to the sole cellular iron exporter ferroportin and causing its internalization and degradation. Pathologic alterations of hepcidin regulation are central to disorders of iron metabolism, including hereditary hemochromatosis, iron-loading anemias, and anemia of inflammation. The role of hepcidin in anemias that are associated with renal diseases remains to be explored.

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Disclosures

None.

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