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SYSTEMIC IRON HOMEOSTASIS

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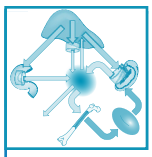

Ganz T. Systemic Iron Homeostasis. *Physiol Rev* 93: 1721–1741, 2013; doi: 10.1152/physrev.00008.2013.—The iron hormone hepcidin and its receptor and cellular iron exporter ferroportin control the major fluxes of iron into blood plasma: intestinal iron absorption, the delivery of recycled iron from macrophages, and the release of stored iron from hepatocytes. Because iron losses are comparatively very small, iron absorption and its regulation by hepcidin and ferroportin determine total body iron content. Hepcidin is in turn feedback-regulated by plasma iron concentration and iron stores, and negatively regulated by the activity of erythrocyte precursors, the dominant consumers of iron. Hepcidin and ferroportin also play a role in host defense and inflammation, and hepcidin synthesis is induced by inflammatory signals including interleukin-6 and activin B. This review summarizes and discusses recent progress in molecular characterization of systemic iron homeostasis and its disorders, and identifies areas for further investigation.

I. INTRODUCTION

A. Biological Roles of Iron

Iron is an essential trace element for nearly every living organism. Because it readily accepts or donates electrons, free iron is highly reactive and toxic. In biological organisms, its chemical reactivity is constrained and directed by its association with prosthetic groups and proteins. Proteins may contain iron in the prosthetic form of iron-sulfur clusters or heme, or the element is directly coordinated by amino acid side chains, frequently including histidine, glutamate, aspartate, and tyrosine. Iron-containing proteins carry or store oxygen (e.g., hemoglobin or myoglobin); catalyze metabolic, signaling-related, and antimicrobial redox reactions (e.g., cytochromes, ribonucleotide reductase, nitric oxide synthase, NADPH oxidase, myeloperoxidase); and transport or store iron (e.g., transferrin, lactoferrin, or ferritin) (90). Because they fulfill these important functions, iron-containing proteins are essential for energy metabolism and intermediary metabolism including nucleotide synthesis, and play a role in signaling pathways as well as host defense. This review is focused on systemic iron homeostasis, i.e., the mechanisms that regulate dietary iron absorption and the concentration of iron in plasma and the extracellular milieu. The complementary but largely separate subject of cellular iron regulation was recently reviewed elsewhere (109, 157). The goal of this review is to provide the reader with an overview of human iron homeostasis and its disorders, emphasizing the exciting new developments in this field during the last 15 years.

B. The Iron Economy

Although iron is one of the most abundant elements in the Earth's crust, iron scarcity is the prevalent condition for most organisms living in the currently oxygen-rich Earth's environments. This paradox has been attributed to the low solubility of naturally occurring oxidized forms of iron. For humans, iron is inefficiently absorbable from plant-based foods where it is complexed in insoluble forms, and this is reflected in the high prevalence of iron deficiency in human populations that consume predominantly vegetarian diets (275). Heme iron from meat, poultry, and fish is efficiently absorbed (65), but the ready availability of these foods is evolutionarily recent and societally and geographically limited. Humans and other mammals evolved in an environment where iron deficiency was common, and this is reflected in mechanisms for efficient conservation and internal recycling of iron. At the other extreme, faced with dietary iron surplus common in prosperous countries, most humans are able to limit dietary absorption of iron and avoid the toxicity of excessive iron accumulation.

The average adult human contains ~3–4 g iron, most of which is in erythrocyte hemoglobin (~2–3 g iron). Other iron-rich tissues include the liver and the spleen, the ma-

jor reserve organs for iron where iron is stored in macrophages and hepatocytes in a specialized cytoplasmic iron storage protein, ferritin. Muscle contains iron predominantly in myoglobin, an oxygen storage protein. All cells contain smaller concentrations in iron-containing proteins essential for energy production, synthetic metabolism, and other important functions. Iron is distributed to tissues through blood plasma which contains only 2–4 mg iron, bound to the iron-transport protein transferrin. Plasma iron turns over every few hours as ~20–25 mg iron a day move through this compartment. Of all cells, erythrocytes have the highest concentration of iron, ~1 mg/ml packed volume. Although smaller amounts of iron from other cell types are also recovered by macrophages, most plasma iron is derived from aged erythrocytes that are recycled by macrophages in the spleen and other organs. As the lifespan of human erythrocytes is ~120 days, ~0.8% (or ~15–25 mg) of all erythrocyte iron must be recycled every day. In turn, iron is extracted from the plasma compartment mostly for hemoglobin synthesis by erythrocyte precursors, regulated separately by erythropoietin in response to tissue oxygenation. Despite rapid turnover and changes in iron utilization, plasma iron concentrations are generally stable, indicating that the delivery of iron from recycling macrophages into plasma must be homeostatically controlled **(FIGURE 1)**.

Radioiron tracer studies in the 1950s and 1960s showed that iron losses from the body are only 1–2 mg/day mainly from desquamation of epithelial surfaces (100). Under normal circumstances, the losses are balanced by dietary iron absorption, mainly in the proximal duodenum. As a result of these relatively small losses, dietary iron absorption normally contributes little to the total iron flux in humans. The respective contributions of recycling and dietary absorption to the daily iron turnover differ in other animal species depending on the lifetime of their erythrocytes and daily iron losses. Because losses of iron from the body are not significantly modulated by systemic iron deficiency or excess, regulation of the iron content of the body is completely dependent on close control of dietary iron absorption.

Physiological mechanisms that control dietary iron absorption in humans must contend with differentials in iron bioavailability in different food sources, ranging from 5–12% in vegetarian diets to 14–18% in mixed diets (115). Despite these variations and changes in iron demand due to growth or occasional blood loss, iron stores are stable in most humans consuming an iron-adequate diet. From the 1930s to the 1980s, extensive experimental studies (reviewed in Ref. 72) provided detailed support for the existence of homeostatic mechanisms that control the total iron content of the body by regulating dietary iron absorption, and control plasma iron concentration predominantly by regulating the release of recycled iron from macrophages. Since then, the effort has focused on understanding the specific molecules and mechanisms involved.

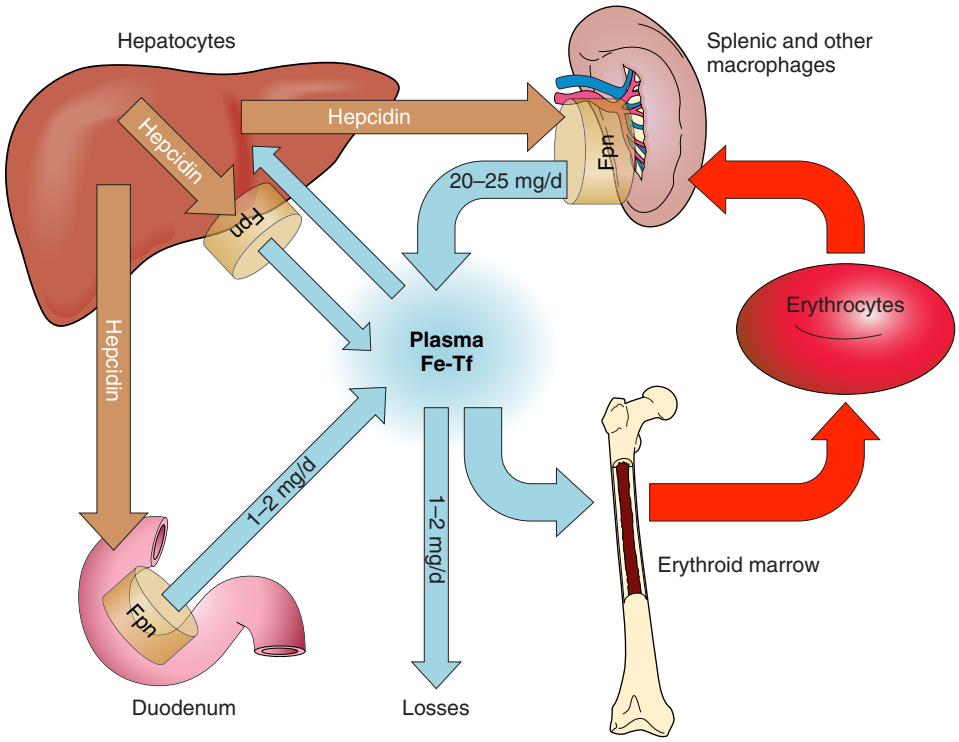

FIGURE 1. Major iron flows and their regulation by hepcidin and ferroportin. Iron in transferrin is indicated in blue, and iron in erythrocytes is in red. Hepcidin controls the iron flow into plasma by inducing the endocytosis and proteolysis of the iron exporter ferroportin (brown).

II. IRON HOMEOSTASIS: TISSUES AND TRANSPORTERS

A. Tissues, Cells, and Fluxes

The three key cell types involved in postnatal iron homeostasis are duodenal enterocytes that absorb dietary iron, macrophages which recycle iron from erythrocytes and other cells, and hepatocytes which store iron and can release it when needed. During fetal development, the placental syncytiotrophoblast transfers iron from the mother to the fetus. The key regulated step in all iron-transporting tissues is the transfer of iron from these cells to plasma. The iron regulatory hormone hepcidin is produced by hepatocytes (including fetal hepatocytes) and controls the transfer of iron to plasma from enterocytes, macrophages, hepatocytes, and syncytiotrophoblasts by mechanisms described in section III. Iron not transferred to plasma is retained in macrophages, hepatocytes, and perhaps in the placenta and functions as a storage compartment. Iron retained in enterocytes is rapidly lost from the body because these cells turn over every 2–5 days in humans (50), and are sloughed into the fecal stream, carrying off any iron that had not been transferred to plasma. Therefore, the partitioning of iron between duodenal enterocytes (the mucosa) and the plasma effectively determines the body iron content (60).

The predominant forms of iron in the human diet are heme, ferritin, and ferric iron, complexed with other macromolecules. The acid environment of the stomach and exposure to digestive enzymes cause a partial release of these iron forms from the digestate. Heme and non-heme iron appear to be absorbed by separate mechanisms (266), and there may be yet another pathway involved in ferritin absorption (241). Despite the importance of heme and ferritin as dietary sources of iron, and despite some promising leads (196, 222, 241), little is known about their transport and metabolism in the enterocyte. In contrast, the transport of inorganic iron has been studied in detail for several decades. Duodenal iron absorption requires that iron cross the apical membrane, followed by variable storage in cytoplasmic ferritin, then iron transport across the enterocyte and the transfer of iron across the basolateral membrane. Much evidence, especially the consequences of genetic disorders and mouse mutations that disable basolateral iron export, indicates that iron from ferritin or heme exits the enterocyte by the same route, i.e., that iron of heme and ferritin must be liberated in the absorptive endosome or in the cytoplasm (241, 266). Thus no matter how it is taken up by the enterocyte, iron in its ferric form is delivered to plasma transferrin near the basolateral surface. It has been suggested that ferric iron could also move across the enterocyte in a vacuole without crossing cell membranes (144), but the physiological contribution of this type of process has not been convincingly demonstrated.

An analogous sequence of events takes place in macrophage lysosomes when they phagocytose senescent erythrocytes. The hydrolytic environment of the phagolysosome digests the erythrocyte and its hemoglobin, releasing heme, which is then degraded by the inducible heme oxygenase-1 (HO-1), freeing iron for cytoplasmic storage or export to blood plasma. The subcellular location of the events that take place after heme is released in the phagolysosomes has not been determined with certainty. In one model, HO-1 acts within the phagolysosomes, and the released iron is transported across the phagolysosomal membrane (227–229) then stored in the cytoplasm or exported across the macrophage cell membrane to plasma. This model has been challenged on one hand by the lack of HO-1 in the phagolysosome or its membrane, and on the other by the presence of heme transporters (heme responsive gene-1, HRG-1) in the phagolysosomal membrane (57), leading to an alternative model in which heme is transported across the phagosomal membrane then degraded within the cell but outside the phagolysosome **(FIGURE 2)**. Although normally most iron is exported to plasma across the macrophage cell membrane, during hemolytic stress heme may be exported intact by heme transporters then bound to hemopexin, a plasma heme carrier.

Thus, in both enterocytes and in macrophages, two sets of transporters and the cytoplasmic iron storage protein ferritin participate in iron movement to blood plasma. The uptake transporters deliver iron to the cytoplasm, and a second set of transporters transfers iron from the cytoplasm to blood plasma.

B. Iron Import

In the enterocyte, the most important apical uptake transporter of inorganic iron is the divalent metal transporter DMT1 which imports ferrous iron as well as several other divalent metals (117) but not trivalent (ferric) iron (reviewed in Ref. 221). DMT1 is an integral membrane protein predicted to have 12 transmembrane domains with both termini in the cytoplasm (101, 221). DMT1 is expressed on the brush-border membrane of duodenal enterocytes (29) and also abundant in erythrocyte precursors where it colocalizes with transferrin in recycling endosomes (30). Extensive evidence shows that in erythrocyte precursors and other cell types dependent on transferrin for their iron supply, DMT1 fulfills a critically important and nonredundant role by transporting to the cytoplasm iron delivered by transferrin (73, 74). Furthermore, DMT1 and a related molecule, natural resistance associated macrophage protein-1 (Nramp1), are also involved in iron transport in macrophages (227–229), but their specific function in these cells remains uncertain. Thus, despite its name, the transporter seems to be essential for normal iron homeostasis but less important for that of the other divalent metals. Initial studies of DMT1 were greatly facilitated by the discovery of

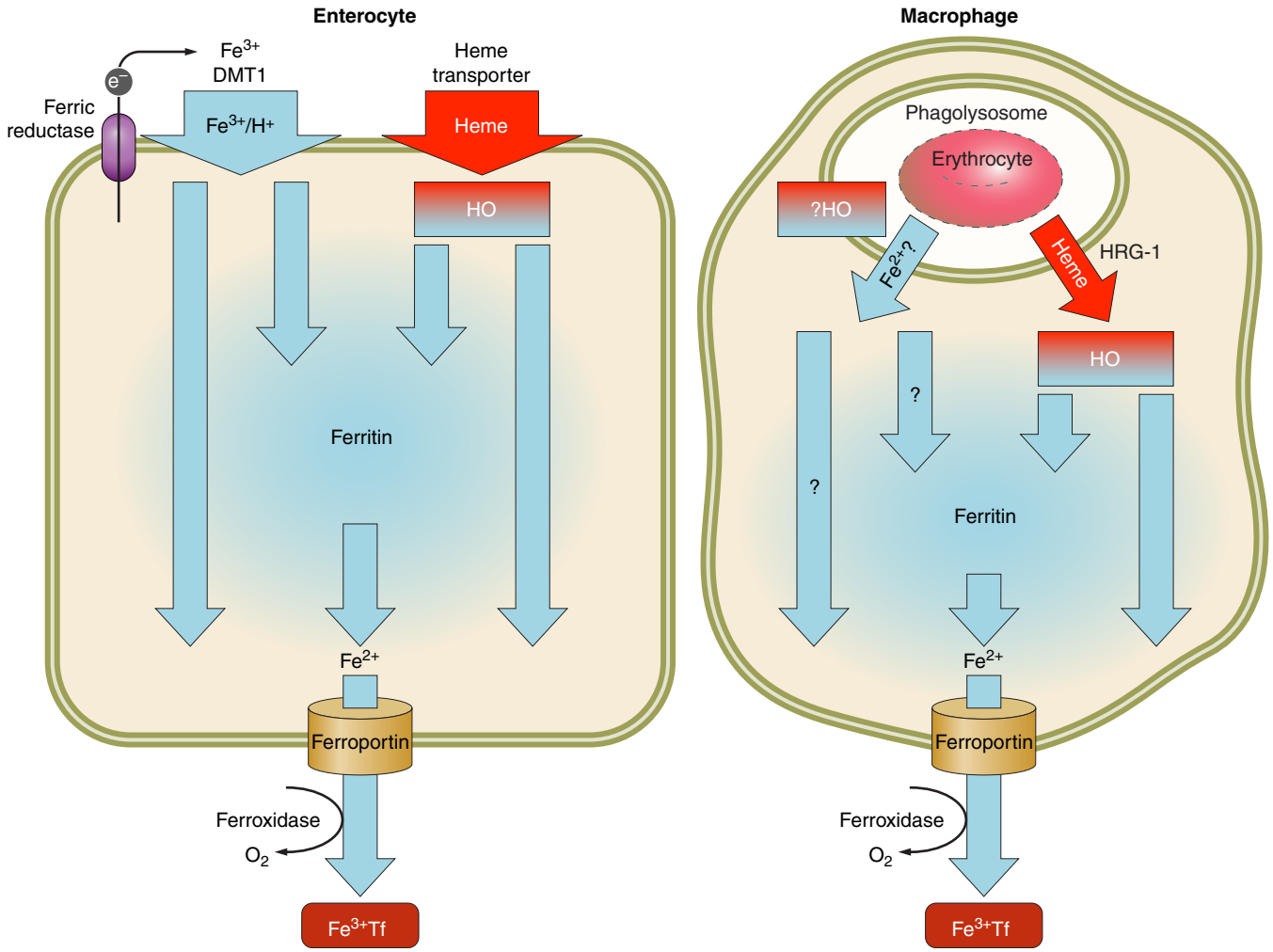

FIGURE 2. Transport of iron by enterocytes and macrophages. Iron uptake takes place at the apical brush border of enterocytes, mainly by DMT1 and an unknown heme transporter. Ferric reductases at the apical surface facilitate the conversion from the predominant dietary form Fe^{3+} to Fe^{2+}, which is then transported by DMT1 into the cytoplasm. The heme transporter must be coupled to a cytoplasmic heme oxygenase (HO) which extracts iron from heme. The pathways of cellular transport of iron to the basolateral membrane are not known. Intracellular iron is stored in ferritin. Basolateral iron (Fe^{2+}) export is solely mediated by ferroportin, but before it can be loaded onto the iron carrier transferrin (Tf), it is converted to Fe^{3+} by the oxygen-dependent ferroxidases hephaestin and ceruloplasmin. Iron-transporting macrophages take up iron predominantly in the form of senescent erythrocytes which undergo proteolysis in the phagolysosome, followed by heme and iron transport into the cytoplasm. The transport of iron within the cytoplasm is not well understood. As in the enterocyte, the export of iron to plasma is dependent on ferroportin and ceruloplasmin.

mice (microcytic anemia or Mk mouse) and rats (Belgrade rat) with hypomorphic mutations in DMT1(73, 87). More recently, human mutations giving rise to microcytic anemia have also been identified (reviewed in Ref. 118). These hypomorphic defects in mice, rats, and humans affect iron homeostasis in a complex manner, usually causing severe microcytic anemia due to decreased ability of erythrocyte precursors to utilize transferrin-bound iron, as well as causing demonstrable deficits in intestinal iron absorption, accompanied by paradoxical hyperabsorption of iron through residual iron transport activity in the intestine (19, 118, 244). Animal models with complete tissue-specific ablation of DMT1 helped clarify the role of DMT1 in intestinal iron transport. Conditional intestine-specific knockout mice generated by crossing floxed DMT1 and villin-Cre mice (102) develop postnatal anemia and systemic iron deficiency but can be rescued by parenteral iron administration bypassing the intestinal tract. This finding establishes the essential role of DMT1 in intestinal iron transport.

Xenopus oocytes overexpressing rat or human DMT1 transport Fe^{2+} inward, a process that is stimulated by low pH, and accompanied by proton influx. This and similar evidence from other preparations expressing DMT1 indicates that DMT1 is an Fe^{2+}/H^{+} cotransporter (101, 221). Human DMT1 is found in at least 4 isoforms which share a core of 531 amino acids but differ in the NH_2 or COOH termini as a result of differing transcription initiation sites

and exon splicing (114). Most importantly, the mRNAs for some of the isoforms contain a 3' iron-responsive element (3'IRE) that binds iron-regulatory proteins 1 and 2 to stabilize the mRNA and increase DMT1 synthesis when cellular iron concentrations are low. The 3'IRE+ forms are predominantly expressed in epithelial cells while DMT1 mRNAs in erythroid cells lack the 3'IRE. The variations in NH_2- and COOH-terminal protein sequence may help direct subcellular localization but do not affect the transport properties of DMT1 (221).

Effective iron transport by DMT1 depends on the concentration of ferrous iron and on the cotransport of protons. DMT1 function therefore requires the conversion of dietary ferric iron to ferrous iron prior to its transport and an acid microenvironment in the brush border of enterocytes. Duodenal cytochrome B (dcytB) contributes to the reduction of luminal ferric iron but is not required for DMT1 function (42, 102), perhaps because other ferric reductase activities contribute as well. The distinct role of dcytB is highlighted by its effect as a genetic modifier of iron overload in hereditary hemochromatosis (45). Ascorbate, a known potentiator of dietary iron absorption, increases the reductase activity of dcytB, likely by acting as its preferred intracellular electron donor (150). The intestinal Na^+/H^+ exchanger appears to be responsible for generating the proton concentrations necessary for DMT1 to function (145).

C. Heme Import

There are currently two candidates for heme transporters that move heme into the cytoplasm. Heme carrier protein 1 (HCP1) (222) was isolated by subtractive hybridization of duodenal versus ileal genes from hypotransferrinemic mice where iron-related transporters are expected to be highly induced. Although the molecule is clearly capable of transporting heme, it later turned out to transport folate and to be mutated in patients with genetic folate deficiency (195), indicating that its nonredundant function was folate uptake in the duodenum. Morever, mice with ablated HCP1 developed folate deficiency anemia and could be rescued by parenteral folate derivatives but not by parenteral iron or heme. The contribution of HCP1 to heme transport remains to be determined but is clearly not essential. The second mammalian heme uptake transporter, heme responsive gene-1, was cloned by homology to heme transporters identified in the heme auxotroph *Caenorhabditis elegans* (196). It localizes to the phagolysosomes of macrophages (57, 196), suggesting that it could be involved in the transport of heme recovered from senescent erythrocytes and its recycling for iron **(FIGURE 2)**.

D. Ferritin and Cytoplasmic Storage

Ferritin is a spherical heteropolymeric protein composed of 24 subunits of heavy (H) or light (L) type. Relevant to systemic iron homeostasis, cytoplasmic ferritin can store large amounts of iron in its interior. The H-ferritin subunits function as ferroxidases to facilitate the conversion of cytoplasmic Fe^{2+} to an oxidized mineral form for storage. Targeted deletion of the H-subunit in the intestine caused systemic iron dysregulation with increased intestinal iron absorption and mild systemic iron overload manifested by increased plasma and hepatic iron concentrations (252). The ability of the ferritin compartment to store iron in enterocytes may be required for controlled delivery of iron to the basolateral iron exporters.

Ferrous iron is delivered to ferritin by cytoplasmic chaperones, chiefly poly (rC)-binding protein 1 (PCBP1) (223). Exit of iron from ferritin may occur through gated pores or by autophagy and lysosomal degradation of ferritin (129, 240). How iron transits from ferritin to iron exporters is not known.

A soluble, relatively iron-poor form of ferritin is found in blood plasma. This form is a 24-subunit polymer containing mostly L-ferritin, and is derived primarily from macrophages (44). Serum concentrations of ferritin correlate with iron stores in most but not all physiological and pathological conditions (46, 120, 174), with exceptions reflecting pathological situations in which the macrophages are much less or much more iron-loaded than parenchymal tissue, or situations where ferritin synthesis is primarily driven by inflammation.

E. Iron Exporters

The sole known mammalian iron exporter is ferroportin [also called Scl40a1, iron-regulated gene 1 (IREG1), or metal transporter protein 1 (MTP1)] (1, 59, 151). It is expressed at all sites involved in iron transfer to plasma **(FIGURE 1)**, i.e., the basolateral membranes of duodenal enterocytes (28, 59), the membranes of macrophages (28), the sinusoidal surfaces of hepatocytes (197), and in the basal surface of the placental syncytiotrophoblast facing the fetal circulation (59). Like DMT1, ferroportin is thought to be a 12-transmembrane domain protein with both termini in the cytoplasm (142, 200), but the exact boundaries of the exposed segments of its extracellular and cytoplasmic faces are not certain (142, 200). Ferroportin exports Fe^{2+} and also Zn^{2+} but not divalent Mn, Cu, or Cd (155); the mechanisms of transport have not been reported. Ferroportin is encoded by two tissue-specific differentially spliced transcripts, FPN1A and FPN1B, that encode the same protein but differ in the presence (FPN1A) or absence (FPN1B) of a 5'IRE that functions to translationally repress ferroportin synthesis when cellular iron is scarce (274). FPN1B is highly expressed in the duodenum and in erythroid precursors, allowing perhaps for altruistic export of iron by these cells even when they sense iron deficiency (274). A mutation in the 5'IRE causes transient polycythemia in mice (156) by a mechanism that is not well understood.

Cellular iron export is dependent on members of a family of copper-containing ferroxidases (130), including ceruloplasmin, hephaestin and perhaps also Zyklopen (37, 38, 104, 258) that use molecular oxygen to oxidize ferrous to ferric iron **(FIGURE 2)**. Ceruloplasmin is a 130-kDa copper-containing protein highly expressed in the liver and the retina. Alternative splicing generates a membrane GPI-linked form and a soluble plasma form. Hephaestin and zyklopen are related 130- and 150-kDa transmembrane proteins expressed predominantly in enterocytes and the placenta, respectively. All three ferroxidases are found in the brain. Hephaestin-deficient mice (sex-linked anemia or *sla*) manifest iron deficiency anemia with accumulation of iron in enterocytes (258), indicating that the basolateral transfer of iron to plasma is defective. Ceruloplasmin deficiency impedes both intestinal iron absorption and the release of iron from macrophages (37, 38, 104, 208), and causes accumulation of iron in the brain and in hepatocytes (103, 104). Although detailed characterization of the respective tissue-specific roles of these ferroxidases remains to be done, it is likely that they facilitate ferroportin-mediated Fe^{2+} efflux by oxidizing iron to its ferric form Fe^{3+}, allowing its uptake by apotransferrin and thereby maintaining a low concentration of Fe^{2+} at the cell surface and Fe^{2+} gradient to the extracellular face of ferroportin that can drive iron transport. It remains to be established how the four known ferroxidase forms cooperate to provide ferroxidase function for enterocytes, macrophages, hepatocytes, and the placenta.

F. Heme Exporters

Feline leukemia virus, type C, receptor 1 (FLVCR1) is a 12-transmembrane domain 60-kDa protein and the sole known heme exporter whose ablation in mice causes a severe fetal anemia lethal in mid-gestation (128). Recent studies reveal that there are two functional isoforms of FLVCR1. FLVCR1b contains only the COOH-terminal half of FLVCR, is expressed in mitochondria, and may mediate heme export from mitochondria to the cytoplasm of erythrocyte precursors and other cells with active heme synthesis (40). FLVCR1b is required for erythroid development and differentiation, presumably because without it heme does not reach the cytoplasm and is not incorporated into hemoglobin. The full-length form of FLVCR1a is found in the plasma membrane and is not required for erythroid development. Selective disruption of FLVCR1a causes embryonic lethality by interfering with vascular and skeletal development and causing hemorrhages. Thus FLVCR1b is essential for heme export from mitochondria to the cytoplasm but does not appear to be involved in systemic iron homeostasis. The function of FLVCR1a in iron regulation is uncertain at this time.

G. Extracellular Iron Carriers

Under normal circumstances, ferric iron exported from cells becomes bound to the plasma iron carrier transferrin, a 75- to 80-kDa glycosylated protein that can carry up to two ferric ions, and deliver them to target tissues for uptake by the transferrin receptor-1 (TfR1). The essential and nonredundant role of transferrin in delivering iron for erythropoiesis is revealed by the severe anemia in genetic hypotransferrinemia or atransferrinemia in humans and in mice (reviewed in Refs. 12, 13, 95). Paradoxically, the disorder results in systemic iron overload, showing that other tissues can take up non-transferrin-bound iron (NTBI) in quantities that meet or exceed their requirements. In atransferrinemia, or if iron enters plasma in excess of the carrying capacity of transferrin, iron becomes complexed to citrate, acetate, and albumin, and these NTBI forms are taken up by tissues by alternative mechanisms reviewed elsewhere (12, 24, 49, 188). In addition to the NTBI, plasma ferritin may also deliver iron to some tissues (66, 154), but the relative physiological contribution of this process is not understood. In addition to carriers that bind inorganic iron, hemopexin and haptoglobin are plasma proteins that bind free heme and free hemoglobin, respectively, limiting their toxic effects and scavenging them for recycling into iron (116, 133, 245). Hemopexin and haptoglobin have an important homeostatic role during hemolytic stress and diseases (245).

III. HORMONAL CONTROL OF IRON HOMEOSTASIS BY HEPCIDIN AND ITS RECEPTOR FERROPORTIN

A. Hepcidin

Despite fluctuations in the iron content of human diets and occasional blood loss from trauma or child birth, most adult humans maintain plasma iron concentrations in the range of 10–30 μM and iron reserves of ~0.2–1 g (46). Moreover, iron absorption is increased in mice or humans during periods of iron deficiency, and absorption is decreased by parenteral iron overload (reviewed in Ref. 72). These observations have led to the expectation that one or more systemically acting hormones regulate the major flows of iron and are in turn regulated by iron (72). Surprisingly, the hormone and its function in iron homeostasis were only discovered during the last decade (history reviewed in Ref. 82).

The iron-regulatory hormone hepcidin is a 2.7-kDa (25 amino acid) peptide **(FIGURE 3)** containing four disulfide bonds (132, 179, 186). Hepcidin is synthesized and secreted by hepatocytes, circulates in blood plasma mostly free except for weak binding to albumin and α2-macroglobulin (119, 180), and is filtered by the kidneys (179). The NH_2-

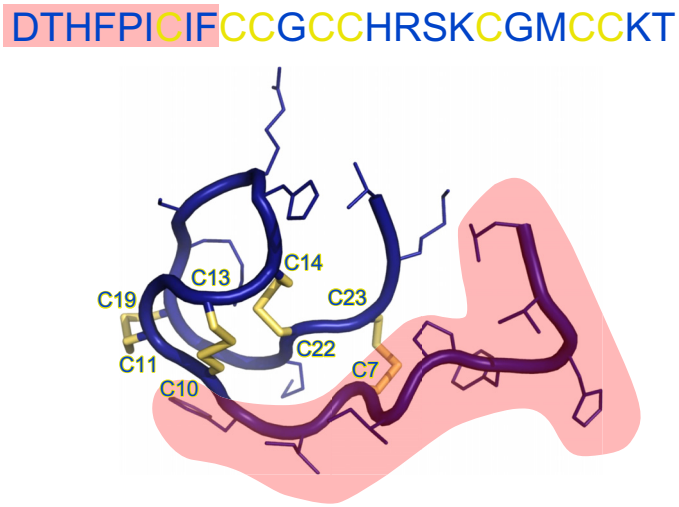

FIGURE 3. Hepcidin: the amino acid sequence and structure. The NH_2-terminal segment known to interact with ferroportin (193) is shaded in light red. The characteristic cysteines and their disulfide bonds are shown in yellow.

terminal hepcidin segment of six amino acids is highly conserved, unstructured, and essential for the iron-regulatory function of hepcidin and its interaction with its receptor ferroportin (161). The rest of the molecule is a bent β-hairpin crosslinked by highly conserved disulfide bonds (123), with the turn of the hairpin the most variable portion of the molecule. The mature 25-amino acid peptide is generated from an 84-amino acid prepropeptide containing a characteristic NH_2-terminal 24-amino acid signal sequence which is cleaved to yield the cellular intermediate prohepcidin (132, 179, 186, 250, 260). There is no evidence that this intermediate has any function other than as a precursor of mature hepcidin. The generation of the mature 25-amino acid form requires furin-like prohormone convertases that cleave prohepcidin at the COOH-terminal peptide bond after a characteristic polybasic sequence (250). In addition, NH_2-terminally truncated shorter forms (22 and 20 amino acids) are also found in human urine and the 20-amino acid form in human plasma, generally at much lower concentrations than the full-length 25-amino acid hepcidin (27, 179). Human hepcidin is encoded by a single three exon gene on chromosome 19 (132, 179, 186), and a similar three exon structure is conserved among other vertebrate hepcidin genes. In the mouse, there are two hepcidin genes, but only hepcidin-1 is involved in iron homeostasis (143). Among other vertebrate species, some fish also have two or more hepcidin genes (61, 224), some of which are not liver-specific and appear to be transcribed only during infections (61). These findings suggest that hepcidin may have evolved in early vertebrates, perhaps as an antimicrobial peptide that became secondarily involved in iron regulation.

B. Mechanism of Action of Hepcidin

Hepcidin acts by posttranslationally controlling the membrane concentration of its receptor, the sole known cellular iron exporter ferroportin (164) **(FIGURE 4)**. As ferroportin is the transporter that delivers dietary, stored, or recycled iron to blood plasma, the hepcidin-ferroportin interaction effectively controls the flux of iron into plasma and the iron supply available to all the iron-consuming tissues. Injection of 1–2 μg/g synthetic hepcidin into mice elicits a profound decrease in serum iron concentrations within 1 h, and the hypoferremic effect persists for many hours (202). Chronic transgenic overexpression of hepcidin causes iron deficiency anemia (167, 206), both by inhibiting iron absorption and restricting the release of stored iron. Hepcidin overexpression during fetal life can impair iron transfer to the fetus sufficiently to cause severe iron deficiency anemia at birth with most mice dying perinatally (167). At the other extreme, hepcidin deficiency in mice or humans causes hyperabsorption of iron and iron overload in parenchymal organs including the liver, pancreas, and the heart, coupled with the paradoxical loss of macrophage iron stores (137, 166, 204). These effects of hepcidin excess or deficiency are evidence of the fundamental role of hepcidin in the control of iron absorption and the release of recycled iron from macrophages. Importantly, the phenotype of hepcidin deficiency is mimicked by heterozygous human ferroportin mutations that interfere with hepcidin binding (218, 219), confirming the critical role of the hepcidin-ferroportin interaction in iron homeostasis, and suggesting that ferroportin may be the sole target of hepcidin.

C. Hepcidin-Independent Homeostatic Mechanisms

Although ferroportin is evolutionarily ancient with conserved sequences down to plants, worms, and other multicellular animals, its ligand hepcidin is found only in vertebrates, with the possible exception of birds (110). The absence of hepcidin in invertebrates suggests that alternative mechanisms for systemic regulation of ferroportin may exist in invertebrates and persist in vertebrates, although they may not be sufficiently effective to compensate for pathological situations in which hepcidin is deficient or excessive. The two factors that may affect iron homeostasis in a hepcidin-independent manner are hypoxia and cellular iron deficiency, when affecting cells and tissues involved in systemic iron transport. In the mouse, duodenal ferroportin mRNA is increased by hypoxia and iron deficiency (39, 147, 151, 274), and hypoxia and anemia increase ferroportin mRNA in macrophages but not hepatocytes (39). These tissue-specific effects may be mediated by hypoxia-inducible factors, especially HIF-2α (217, 239), regulating the transcription of ferroportin. The degradation of HIFs by prolyl hydroxylases is dependent on both iron and oxygen, so HIF-2α concentrations could be increased by cellular iron deficiency or hypoxia alone. It should be noted that a potential counterregulatory mechanism could decrease ferroportin during cellular iron deficiency. One isoform of ferroportin mRNA contains a 5'IRE and could undergo

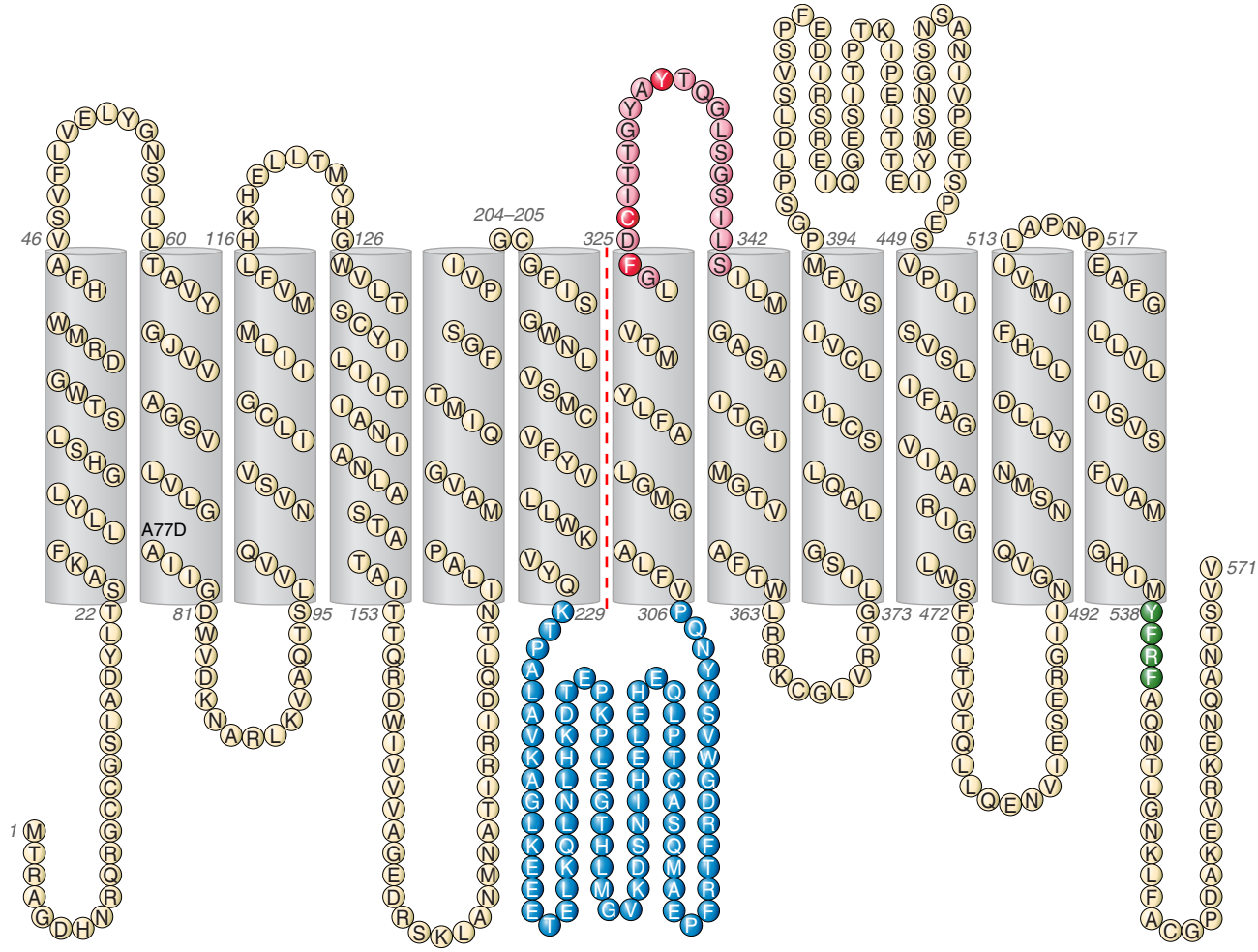

FIGURE 4. Human ferroportin topology and functional domains. Both termini are in the cytoplasm, and transmembrane helices (142) are shaded in gray. The extracellular hepcidin-binding loop is in red, with boundaries as determined by cysteine-scanning mutagenesis and cysteine-directed biotinylation (193). Key residues required for hepcidin binding are shown in dark red. The cytoplasmic loop that undergoes hepcidin-dependent ubiquitination (194) is shown in blue. The AP-2 adapter-binding motif for clathrin-dependent endocytosis is shown in green. An axis of approximate symmetry is shown by a dashed red line.

translational repression during iron deficiency, but the alternative splice form of ferroportin abundant in the duodenum and lacking the 5′IRE would evade this effect (274). Iron deficiency may also repress HIF-2α translation through the interaction of iron regulatory proteins (IRP1 and -2) with the iron-regulatory element (IRE) in the 5′ region of the HIF-2α mRNA (207). Other components of the iron transport machinery in the duodenum, including DMT1 and dcytB, are also targets of HIF-2α, and their mRNAs are increased during hypoxia and iron deficiency (217). It is possible that direct regulation of ferroportin by cellular hypoxia and cellular iron deficiency functions best in small animals where the oxygen tension and iron content of iron-transporting cells are representative of the organisms as a whole. Tissue differences in iron content and oxygen tension in larger animals may have favored a more centralized model of iron regulation with a systemically acting hormone, hepcidin, produced by the principal iron storage organ, the liver. It remains to be determined whether hypoxic regulation represents the evolutionarily more ancient form of iron homeostasis. If so, hepcidin may have initially evolved as a vertebrate host defense mediator (see sect. IV) that overrode the more primitive iron homeostatic responses during infection, and hepcidin may then have been secondarily adopted and evolved for iron homeostasis.

D. Regulation of Ferroportin by Hepcidin

Hepcidin binds directly to ferroportin, inducing the endocytosis of ferroportin and its proteolysis in lysosomes (164). The concentrations of hepcidin required for this effect are in the 10–100 nM range (164), and these correspond approximately to the upper range of hepcidin concentrations in human blood plasma under pathological conditions causing hypoferremia (84).

The identification of the binding interface between hepcidin and ferroportin was achieved by studying the effects of natural

and experimental mutations in both partners (43, 161, 193). For hepcidin, the NH_2-terminal five amino acids were necessary for bioactivity (161), and the NH_2-terminal nine amino acids **(FIGURE 3)** were sufficient for bioactivity (193) provided that the C7 cysteine involved in disulfide bonding was replaced by a thiol cysteine. On the ferroportin side, identification of the potential receptor site was greatly aided by the discovery of an informative family which manifested resistance to hepcidin as a result of an isosteric C326S substitution in ferroportin (63, 67, 218, 219), located in an extracellular loop **(FIGURE 4)** in both of the reasonably supported ferroportin models (142, 200). Alanine scanning of this ferroportin loop identified additional residues critical for hepcidin binding (193). In addition to the critical role of the ferroportin thiol C326 in binding hepcidin, several aromatic amino acid side chains (phenylalanine and tyrosine) in the interacting segments of hepcidin and ferroportin were experimentally identified as important for hepcidin-ferroportin interactions, and were seen to interact in a docking model of hepcidin to the hepcidin-binding loop of ferroportin (193).

The binding of hepcidin to ferroportin is followed within minutes by the ubiquitination of lysines in a cytoplasmic loop of ferroportin (194) **(FIGURE 4)** which appears to be required for the subsequent endocytosis of ferroportin (194, 205). An earlier report that phosphorylation of a pair of adjacent tyrosines preceded ubiquitination and was required for endocytosis (54) could not be verified despite extensive efforts (205). It is not yet certain whether ferroportin endocytosis is mediated by clathrin (54), one of the alternative endocytic pathways (9) or both, and there could be differences between the predominant endocytic pathways in different cell types.

IV. REGULATION OF HEPCIDIN BY IRON

A. Dual Regulation of Hepcidin by Extracellular Iron and Iron Stores

The relative stability of plasma iron concentrations despite rapid turnover of iron suggests feedback regulation of hepcidin by plasma iron **(FIGURE 5)**. Experimentally, regulation of hepcidin by plasma iron concentrations was detected in human volunteers (84) given small doses of iron sufficient to raise plasma iron concentrations transiently but too small to contribute significantly to iron stores. Serum hepcidin levels were observed to rise dramatically in response to transient increases in plasma iron with a delay of ~8 h (84). On the other hand, the relatively narrow distributions of estimated body iron stores in men and women on varied diets suggest that body stores could regulate hepcidin independently of the short-term effects of plasma iron concentrations (46). Surprisingly, experimental evidence of dual regulation of hepcidin by tissue iron stores and plasma iron concentrations was not obtained until recently (47, 66, 198, 270). In humans, observations that support hepcidin regulation by iron stores include the correlation between hepcidin mRNA and iron stores in human liver biopsies (7, 58, 88) and the strong correlation between serum ferritin, a recognized marker of iron stores, and serum hepcidin (84). Hepcidin regulation by plasma iron and by tissue iron stores appears to operate on different time scales (hours vs. days), and this could allow the two regulators to function in parallel.

B. Tissues Involved in Hepcidin Regulation

Hepatocytes are the predominant producers of hepcidin (132, 179, 186, 273). Unlike the distribution of ceruloplasmin and ferroportin which is predominantly periportal (197, 247), hepcidin mRNA appears to be evenly distributed among hepatocytes (247). Other cell types including macrophages and adipocytes (15, 273) contain much lower concentrations of hepcidin mRNA. Although non-hepatocyte sources could in principle exert autocrine or paracrine effects, these have not yet been documented.

C. Sensors and Pathways That Regulate Hepcidin

To date, the only known mode of hepcidin regulation is transcriptional. The molecular mechanisms that mediate

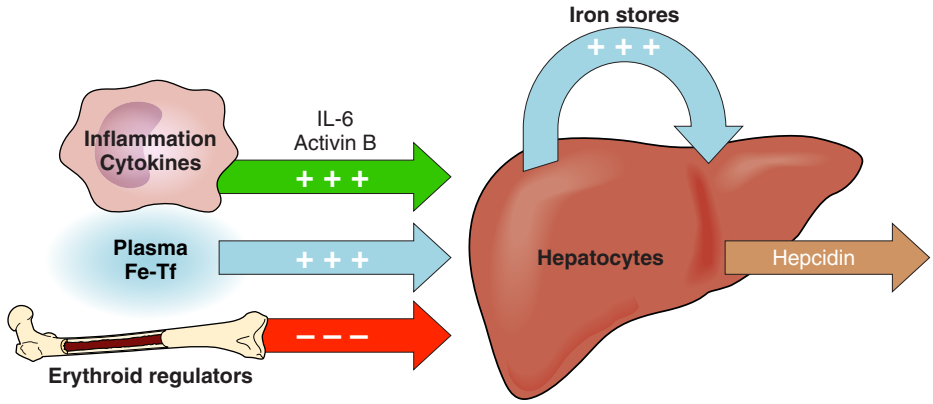

FIGURE 5. Regulation of hepcidin synthesis in hepatocytes. The major regulatory influences include iron-transferrin and iron stores (blue), inflammation (green), and erythroid activity (red).

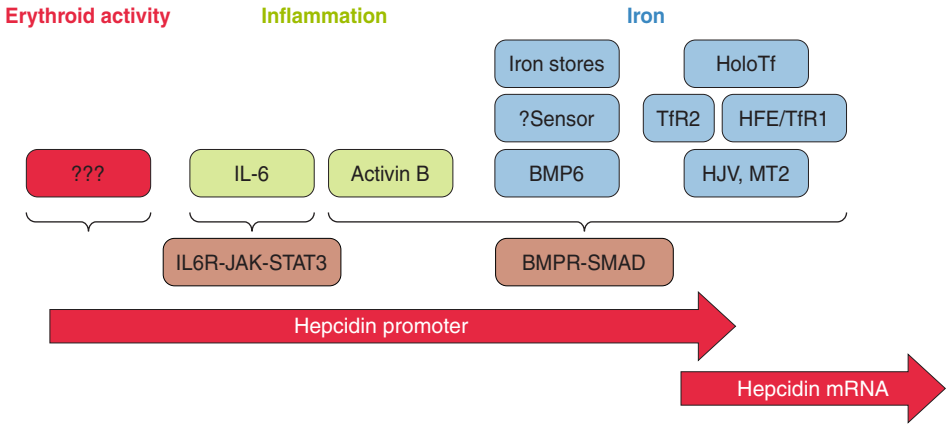

FIGURE 6. Molecular pathways regulating hepcidin transcription. JAK-STAT3 and BMPR-SMAD are the two key pathways that regulate hepcidin promoter activity. Iron-related mediators are shown in blue, and inflammatory mediators are in green. The erythroid regulator (red) and its transduction pathways are not known.

hepcidin regulation by iron appear to be surprisingly complicated **(FIGURE 6)**. Much of what we know about these mechanisms was learned through studies of human genetic diseases or mouse transgenic models in which hepcidin is dysregulated. **TABLE 1** lists genes implicated in hepcidin regulation by iron.

The BMP receptor and its canonical SMAD pathway are at the core of the hepcidin-regulating complex as indicated by the strong induction of hepcidin by multiple BMPs (10, 11), the presence of several functional BMP-response elements in the hepcidin promoter (31, 249, 254), and the profound effect of liver-specific SMAD4 ablation on hepcidin expression (262). The specific form of the BMP receptor used for iron-related hepcidin regulation in hepatocytes probably includes Alk2 or Alk3 as type I subunits (232) and predominantly ActRIIA as the type II subunit (267). BMP6 is the essential and specific BMP receptor ligand for iron-related signaling, at least in mice, as BMP6 knockout mice have very low hepcidin and develop severe iron overload, with no evidence of any other abnormalities (6, 153). The GPI-linked protein hemojuvelin is an essential co-receptor for activation of the BMP receptor for iron-related signaling. Its ablation leads to severe hepcidin deficiency and severe iron overload in humans (early onset, juvenile form of hereditary hemochromatosis) and in mice (113, 170, 177). The hepatic form of hemojuvelin appears to be essential for hepcidin regulation while the muscle form is dispensable (36, 95). The role of muscle-associated hemojuvelin remains to be elucidated. A soluble form of hemojuvelin is generated by proteolytic cleavage by furin (139, 140, 225) and can act as an antagonist of BMP signaling, but its physiological role is not known. Soluble hemojuvelin binds to BMPs, with highest affinity for BMP-2, -4, and

Table 1. *Genetic lesions in hepcidin regulators and their phenotypic consequences*

	Rate of Iron Accumulation		Hepcidin Relative to Iron Load		
Disrupted Gene	**Mouse**	**Human**	**Mouse**	**Human**	**Reference Nos.**
HFE	+	+	↓	↓ but variable	3, 22, 94, 198, 259
TfR2	++	++	↓ ↓	↓ ↓	93, 126, 198, 259
HFE + TfR2	+++	+++	↓ ↓ ↓	↓ ↓ ↓	183, 259
HJV	+++	+++	↓ ↓ ↓	↓ ↓ ↓	113, 170, 177, 198
BMP6	+++	NF	↓ ↓ ↓	NF	6, 153, 198
Neogenin	+++	NF	↓ ↓	NF	136
Transferrin	+++	+++	↓ ↓ ↓	↓ ↓ ↓	13, 246, 248
Liver SMAD4	+++	NF	↓ ↓ ↓	NF	262
BMPR (Alk2)	+	NF	↓	NF	232
BMPR (Alk3)	+++	NF	↓ ↓ ↓	NF	232
Hepcidin	+++	+++	0	0	137, 166, 204
MT2 (TMPRSS6)	− −	− −	↑ ↑	↑ ↑	64, 69

NF, not found; + to +++, increased compared with normal; − −, decreased compared with normal; 0, absent.

-6 (10, 265, 267), and this could explain its ability to antagonize BMP signaling.

The nature of the extracellular iron sensors and how they couple to the BMP pathway is less certain. Transferrin receptor 2 is a strong candidate as a sensor of extracellular holotransferrin concentration. TfR2 is stabilized by holotransferrin, and the disruption of TfR2 in mice or humans causes a loss of extracellular iron sensing (99, 121, 122, 198, 203). The hemochromatosis-related membrane protein HFE and holotransferrin compete for binding to TfR1 (135), so the TfR1/HFE complex could be another sensor for holotransferrin, perhaps independent of TfR2 (213, 259) or interacting with it and with hemojuvelin (52). In support of the independent roles of HFE and TfR2, overexpression of HFE stimulates hepcidin production (213, 214) whether or not Tfr2 is present. Neogenin promotes iron-related signaling as evidenced by decreased hepcidin despite severe iron overload in mice with neogenin-attenuating retrotransposon insertion (136), but the mechanism of this effect is uncertain (267, 272). Finally, a membrane serine protease matriptase-2 (also called TMPRSS6) functions as a negative regulator of hepcidin-related BMP signaling, acting by cleaving and inactivating the BMP agonist hemojuvelin (64, 69, 71, 76, 226). Genetic loss of matriptase-2 (transmembrane serine protease 6, TMPRSS6) activity in mice or humans causes iron-refractory iron deficiency anemia by stimulating excessive hepcidin synthesis that leads to sequestration of iron in macrophages and decreased dietary iron absorption.

Iron stores are clearly potent regulators of hepcidin, but less is known about how they regulate hepcidin transcription. It appears that the BMP receptor is also involved in this pathway. As iron accumulates in the liver, the expression of its ligand BMP6 is regulated by hepatic iron stores in about a fourfold range (47, 124, 198, 271). Ablation of BMP6 or hemojuvelin profoundly interferes with the hepcidin response to increased iron stores (198, 271), but neither alone completely abolishes the response. It is not clear which hepatic cell type produces BMP6 relevant to hepcidin regulation and how and where the iron stores are sensed. Sinusoidal endothelial cells can take up ferritin, contain higher BMP6 mRNA concentrations than other hepatic cell types (270), and could potentially serve as sensors of ferritin concentrations (66) which reflect iron stores.

V. REGULATION OF HEPCIDIN BY ERYTHROPOIESIS

Intestinal iron absorption is greatly increased in response to hemorrhage or erythropoietin (reviewed in Ref. 72) leading to the hypothesis that an "erythroid regulator" modulated intestinal iron absorption (72), assuring adequate supply of iron when needed for accelerated erythropoiesis (**FIGURES 5 AND 6**). Patients with ineffective erythropoiesis (e.g., in β-thalassemia), whose erythroid precursor populations are greatly expanded but fail to mature into functional erythrocytes, also have increased intestinal iron absorption despite often severe systemic iron overload (33, 190). Although blood transfusions given for severe anemia (e.g., in β-thalassemia major) contribute to the lethal iron overload in ineffective erythropoiesis, many patients with less severe anemia (exemplified by β-thalassemia intermedia) receive few or no transfusions but still become severely iron-overloaded (33, 190).

After the discovery of hepcidin, the erythroid regulator concept was modified from that of a direct regulator of iron absorption to a regulator of hepcidin. Hypoxia or erythropoietin were initially thought to regulate hepcidin directly (168, 181, 189), but the preponderance of data now supports a model in which the bone marrow produces a hepcidin suppressor, in response to erythropoietin (141, 148, 176, 257). A similar suppressive substance has been postulated in anemias with ineffective erythropoiesis where hepcidin is decreased despite iron overload and even in the absence of transfusions (86, 174, 178, 263). GDF-15, a BMP family member whose serum concentrations are greatly increased in iron-loading anemias (32, 125, 236, 237), has been proposed as a hepcidin suppressor in β-thalassemia and in congenital dyserythropoietic anemias, but its contribution to hepcidin suppression and iron overload in these conditions remains uncertain. Based on studies in blood donors, GDF-15 is unlikely to function as the physiological suppressor of hepcidin after blood loss (238). Identification of the physiological and pathological erythroid regulators of hepcidin is an important priority for future studies.

VI. HEPCIDIN IN INFLAMMATION, LIVER INJURY, AND HOST DEFENSE

A. Hepcidin Is Induced by Infections and Inflammation

The structural similarity of hepcidin to three- and four-disulfide antimicrobial peptides including mammalian, insect, and plant defensins and related molecules (34, 179) stimulated the hypothesis that hepcidin has an important role in innate immunity and may be regulated by inflammatory signals. Initial studies of hepcidin revealed its intrinsic antimicrobial activity which is less potent than that of defensins (132, 179). Subsequently, microbial molecules, the plant-derived inflammatory agent turpentine, and cytokines were shown to be potent inducers of hepcidin synthesis (162, 165, 168, 186). Interleukin (IL)-6 is a key hepcidin-inducing cytokine in vivo (162), but other cytokines, including IL-22 and activin B, may also contribute (8, 17). In multiple myeloma, a plasma cell malignancy which almost invariably causes anemia, serum hepcidin is greatly in-

creased, and both BMP4 and IL-6 were implicated as its pathogenic inducers (146, 220). The stimulatory effects of inflammation are mediated by the dual and in some cases synergistic (146, 184, 254, 255, 264) regulation of hepcidin transcription by SMAD and STAT3 transcription factors.

B. Modulation of Hepcidin During Liver Injury and Disease

Additional regulatory factors could influence hepcidin synthesis during liver injury and disease. Endoplasmic reticulum (ER) stress is a pathological signal that modulates hepcidin synthesis, via cAMP response element-binding protein H (CREBH), an ER stress-activated transcription factor and the stress-inducible transcription factor and through CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP) (173, 253). Hepatic oxidative stress may suppress hepcidin production after alcohol ingestion or in viral liver diseases and cause iron overload which exacerbates the liver injury (7, 77, 78, 92, 105–107, 131, 171, 172, 234). Finally, epidermal and hepatocyte growth factors (EGF and HGF), which contribute to liver regeneration after injury, also suppress hepcidin (97). How much each of these pathways contributes to physiological or disease-associated regulation of hepcidin remains to be seen.

C. Role of Hepcidin in Host Defense

It is now widely presumed that the induction of hepcidin by microbial and inflammatory factors serves a host defense function, but specific evidence for this hypothesis is surprisingly sparse. Patients with hereditary hemochromatosis, nearly all of whom have absolute or relative hepcidin deficiency, are known to be more susceptible to certain infections, e.g., *Vibrio vulnificus, Yersinia enterocolitica,* and *Listeria monocytogenes* (14, 16, 35, 91, 112), but it is not clear to what extent the individual factors of liver disease, tissue iron overload, high extracellular iron concentration, the lack of hypoferremic response to infections, and the lack of hepcidin contribute to increased susceptibility to infections. The involvement of hepcidin and iron in resistance to other microbial infections, including such global scourges as tuberculosis and malaria, is an active area of investigation (62, 81, 211, 231). There is evidence that induction of hepcidin during erythrocyte infection with malaria interferes with superinfection with another strain (192), by redistributing iron from hepatocytes to macrophages thus inhibiting the early hepatic phase of superinfecting malaria. However, such protective effects of hepcidin may not be applicable to all infections. Under some conditions, hepcidin-induced iron redistribution from extracellular spaces and hepatocytes to macrophage cytoplasm could even favor the growth of certain intracellular microbes (41), depending on the specific subcellular compartment they utilize and the effect of hepcidin on its local iron concentrations.

D. Role of Hepcidin in Regulating Inflammation

It has been hypothesized that not only does inflammation regulate hepcidin but that the reverse relationship, in which hepcidin levels modulate inflammatory signals, is also biologically important (55). Two studies have reported that systemic anti-inflammatory effects of hepcidin in mouse models (55, 175). Comparing hepcidin knockout (KO) to wild-type mice, one study found increased sensitivity of hepcidin KO mice to the inflammatory cytokine-inducing and lethal effects of lipopolysaccharide (LPS) (55). Resistance of hepcidin KO mice to LPS could be restored by injections of hepcidin. In another study, a similar proinflammatory effect of iron deficiency in a mouse model was documented (175) and attributed to the physiological suppression of hepcidin and the resulting derepression of inflammation. Specifically, the authors showed that iron deficiency potentiated the inflammatory cytokine-inducing effects of LPS injections. Although the study did not show that ferroportin pathway was directly involved in regulating inflammation, the inflammatory phenotype of the iron-deficient mouse was reversed by hepcidin treatment and was decreased in mice with high hepcidin expression due to ablation of TMPRSS6 genes.

The mechanism of the reported anti-inflammatory effect of hepcidin was proposed to involve hepcidin-induced ferroportin signaling (55). Specifically, it was suggested that hepcidin binding to ferroportin activated Jak2 binding to ferroportin, Jak2 phosphorylation of ferroportin and Stat3, followed by transcriptional activation of genes regulating inflammation, including IL-17, IL-17 receptor, and SOCS3 (suppressor of cytokine signaling-3) (55). With the use of siRNA to SOCS3, the increased expression of SOCS3 was shown responsible for the suppression of IL-6 and tumor necrosis factor (TNF)-α by hepcidin. This mechanism has since been contradicted by detailed experiments in which no activation of the Jak2-Stat3 pathway by hepcidin binding to ferroportin was observed in similarly prepared cells (205). Moreover, the study showed that Jak2 and the putative ferroportin phosphorylation sites were not required for hepcidin-induced endocytosis of ferroportin (205).

In view of the conflicting evidence to date, the mechanisms underlying the reported anti-inflammatory effects of hepcidin and proinflammatory effects of iron deficiency remain to be clarified.

VII. GENETIC DISORDERS OF THE HEPCIDIN-FERROPORTIN SYSTEM

Mutations in the genes encoding hepcidin, its various regulators, or its molecular target ferroportin may manifest as disorders of iron regulation (23, 26) **(TABLE 1)**. Hepcidin deficiency or ferroportin resistance to the endocytic effect of hepcidin results in hereditary hemochromatosis, a group of diseases

characterized by systemic iron overload due to hyperabsorption of dietary iron, with subsequent injury to iron-overloaded tissues (80, 185). Although the pathogenesis of iron-mediated toxicity is not well understood, in part because of the lack of suitable animal models that mimic human disease, the ability of iron to catalyze the production of reactive oxygen species is the main suspect in this process. Depending on the age of onset and the severity of the disease, the destructive process may affect the liver, causing cirrhosis and liver cancer; the heart, leading to heart failure; and endocrine glands where the effects are wide-ranging, including delayed growth and sexual development in the juvenile forms of the disease and diabetes mellitus in the juvenile and adult forms. The age of onset and the rate of disease progression correlate roughly with the severity of the hepcidin deficiency but are likely to be modulated by genes not yet identified as part of the iron-regulatory system, as well as alcohol use and abuse (5, 75, 172), dietary factors, and blood loss through menstruation. The epidemiology, diagnosis, and treatment of hereditary hemochromatosis is well covered in recent reviews (25, 80, 185).

At the opposite end of the spectrum of genetic iron disorders are conditions in which the production and blood concentrations of hepcidin are inappropriately high or the membrane concentration or iron-transporting capacity of ferroportin is decreased. Genetic lesions in the negative hepcidin regulator matriptase-2 (TMPRSS6) affecting both of its alleles cause hepcidin overproduction resulting in a syndrome of iron deficiency anemia due to decreased iron absorption and sequestration of iron in macrophages (64, 68, 69, 152). Treatment with parenteral iron can bypass the block of iron absorption but does not fully overcome the iron-restrictive effect of the block to macrophage iron export.

Heterozygous loss-of-function mutations in the ferroportin gene result in decreased membrane concentration of ferroportin or its diminished ability to transport iron. The disorder, named "ferroportin disease" (182), is manifested by trapping of iron in macrophages, high serum ferritin levels, and a tendency to anemia if therapeutic bleeding for iron overload is attempted. If iron loading is limited to macrophages, the disorder rarely causes clinically significant disease. A mouse model of classical ferroportin disease recapitulates this human condition (276). A nonclassical form of this disorder manifests parenchymal iron loading, attributable to partial or complete resistance to hepcidin, as reviewed recently (149). A puzzling feature of all forms of ferroportin disease is that it invariably involves heterozygous missense mutations acting in a dominant manner, so it cannot be attributed to simple haploinsufficiency. Although mistrafficking of ferroportin multimers containing both wild-type and mutant forms of ferroportin could explain the dominant negative effect, the existence and importance of ferroportin multimerization have been contested (53, 96, 187, 200, 212, 276).

VIII. TARGETING OF THE HEPCIDIN-FERROPORTIN AXIS FOR THE TREATMENT OF IRON DISORDERS

A. Hepcidin Agonists

Hepcidin production is inappropriately low in most forms of hereditary hemochromatosis (89, 113, 137, 163, 166, 169, 170, 177, 178, 204, 256, 261) and certain iron-loading anemias, including β-thalassemia (2, 21, 85, 160, 174, 178) and congenital dyserythropoietic anemias (32, 125, 236). Hepcidin deficiency in these diseases causes hyperabsorption of dietary iron and pathological iron overload with attendant tissue and organ damage. In iron-loading anemias, the contribution of dietary iron to total iron overload varies depending on whether and how often the patients are receiving erythrocyte transfusions (21, 33, 51, 127, 174, 190), but even patients who are never transfused are at risk for lethal iron overload (158). Unexpectedly, iron overload in mouse models of β-thalassemia has a deleterious effect on erythropoiesis that can be reversed by interventions that increase hepcidin production (85, 159). It remains to be seen whether hepcidin agonists can improve erythropoiesis in human iron-loading anemias.

The mainstay of current treatment for hereditary hemochromatosis (80) is blood removal by phlebotomy, during which each 1 ml of packed erythrocytes removes ~1 mg of iron. During the "deironing" phase, patients undergo treatments where 1 unit of blood (~450 ml, equivalent to 200–250 mg iron) is removed as often as once a week until serum ferritin levels indicate that iron stores are in the normal range. In the maintenance phase, the frequency of phlebotomy is decreased to maintain iron balance for each individual patient. This approach is inexpensive, safe, and effective in reversing many but not all complications of iron overload but is not well tolerated by a minority of patients. In iron-loading anemias, phlebotomy is not feasible because the patients become even more anemic. Here various iron chelators (134), parenteral (desferoxamine) or oral (defer-

Table 2. *Hepcidin agonists under development for the treatment of iron overload*

Class	Compounds	Stage (2012)	Reference Nos.
Hepcidin analogs	7–9 amino acid peptides	Preclinical	193, 199
BMP6 analogs	proteins	Conceptual	48
MT2 (TMPRSS6) antagonists	siRNAs, antisense oligonucleotides	Preclinical	20, 70, 159, 215

Table 3. *Hepcidin antagonists under academic or commercial development for the treatment of anemia of inflammation and anemia of renal diseases*

Class	Compounds	Stage (2012)	Reference Nos.
Hepcidin traps	Antibodies, lipocalin scaffold, spiegelmers	Preclinical to phase I studies	83, 111, 201, 209, 216, 251
BMP antagonists or traps	Heparin derivatives, BMP receptor kinase inhibitors, soluble hemojuvelin	Conceptual to preclinical	191, 235, 243
Inhibitors of TfR2 or hepcidin synthesis	siRNAs, antisense oligonucleotides	Preclinical	4, 43
Inhibitors of hepcidin binding to ferroportin	Antibodies, small molecules	Conceptual to healthy human volunteers	79, 138

iprone and deferasirox), are administered to induce the excretion of iron in urine and stool. These are life-saving but at the cost of significant patient burden and side effects. Like phlebotomy in hereditary hemochromatosis, iron chelators can be used for deironing, maintenance, or prevention of iron overload. There is a general agreement that a broader repertoire of therapeutics for all iron overload diseases is needed.

Hepcidin agonists include compounds that mimic the activity of hepcidin and agents that increase the production of hepcidin by targeting hepcidin-regulatory molecules **(TABLE 2)**. It should be noted that these potential therapeutics cannot substantially increase iron excretion and would have to be used in a preventive mode, before iron overload develops or after deironing is completed. The potential of these future drugs to improve erythropoiesis in β-thalassemia is suggested by their effects in mouse models of the disease.

B. Hepcidin Antagonists

Plasma concentrations of hepcidin are increased in iron-refractory iron deficiency anemia due to autosomal recessive mutations in TMPRSS6 (18, 64, 69, 152), in anemias associated with a variety of inflammatory disorders and malignancies (56, 84, 108, 146, 209, 210, 220, 230, 242, 243), and in chronic renal disease with or without inflammatory etiology (84, 268, 269). Although treatment of milder anemias in these settings may not warrant even the relatively rare risks and side effects involved, more severe anemias can impair the quality of life and are statistically associated with poor outcomes (as reviewed in Ref. 98). In situations where the underlying disease cannot be sufficiently mitigated to reverse a clinically important anemia, erythropoiesis-stimulating agents (erythropoietin and other drugs that mimic or induce its activity) are currently used with or without relatively large doses of parenteral iron, but the risks or potential risks of these agents have limited their indications (98).

In the hope that new approaches would increase the safety and efficacy of the treatment of these anemias, hepcidin antagonists have been developed targeting either hepcidin itself or its regulators **(TABLE 3)** and show substantial promise in preclinical animal models of anemia of inflammation and anemia of renal failure (209, 216, 233, 235, 243). As of 2012, several agents have entered early human trials.

IX. SUMMARY AND AREAS FOR FUTURE STUDY

In the past 15 years, iron researchers succeeded in identifying many of the key molecules involved in iron regulation. Areas where further studies are needed are summarized in **TABLE 4**.

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Table 4. *Areas for future research*

Areas For Future Research
Regulators of apical iron absorption in enterocytes
Heme transport and its regulation
Mechanism of iron transport by ferroportin and its structural basis
Subcellular iron transport in enterocytes and macrophages
Architecture and biochemistry of the pathway of hepcidin regulation by holotransferrin
Iron stores sensors and their pathways
Molecules and pathways that regulate hepcidin in response to erythroid demand for iron
Molecular etiology of hepcidin deficiency in β-thalassemia
Gender effects in iron homeostasis
Fetal iron homeostasis
Comparative iron homeostasis in invertebrates

DISCLOSURES

The author is a cofounder and Chief Medical Officer of Intrinsic LifeSciences, a company engaged in the development of iron-related diagnostics, and a cofounder and major stockholder in Merganser Biotech, a company engaged in the development of iron-related pharmaceuticals.

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