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Review

Two to Tango: Regulation of Mammalian Iron Metabolism

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Disruptions in iron homeostasis from both iron deficiency and overload account for some of the most common human diseases. Iron metabolism is balanced by two regulatory systems, one that functions systemically and relies on the hormone hepcidin and the iron exporter ferroportin, and another that predominantly controls cellular iron metabolism through iron-regulatory proteins that bind iron-responsive elements in regulated messenger RNAs. We describe how the two distinct systems function and how they "tango" together in a coordinated manner. We also highlight some of the current questions in mammalian iron metabolism and discuss therapeutic opportunities arising from a better understanding of the underlying biological principles.

Plasma Iron: Key to Iron Overload and Iron Deficiency Anemias

Iron is essential for fundamental metabolic processes in cells and organisms. The key to systemic iron supply and homeostasis lies in the regulation of adequate plasma iron levels. Iron circulates in plasma bound to the glycoprotein transferrin, which has two high-affinity binding sites for Fe(III). Transferrin binding maintains iron in a soluble form, serves as a major vehicle for iron delivery into cells (via the transferrin receptor, TfR1), and limits the generation of toxic radicals. In humans, plasma transferrin is normally about 30% saturated with iron. A transferrin saturation <16% indicates iron deficiency, whereas >45% saturation is a sign of iron overload. When the saturation exceeds 60%, non-transferrin-bound iron begins to accumulate in the circulation and to damage parenchymal cells.

The homeostatic system thus has to maintain transferrin saturation at physiological levels, responding to signals from pathways that consume iron (such as erythropoiesis) and sending signals to the cells that supply iron to the blood stream (Figure 1). Iron is released into the circulation from duodenal enterocytes, which absorb 1–2 mg of dietary iron per day, and from macrophages, which internally recycle 20–25 mg of iron from senescent erythrocytes. Hepatocytes play a dual role in systemic iron metabolism: they are the major site of iron storage and they secrete the regulatory hormone hepcidin (Hamp, LEAP1). Hepcidin orchestrates systemic iron fluxes and controls plasma iron levels by binding to the iron exporter ferroportin (SLC40A1, Solute carrier family 40, member 1) on the surface of iron-releasing cells (Figure 1), triggering its degradation and hence reducing iron transfer to transferrin (Nemeth et al., 2004). Inherited and acquired disorders that perturb hepcidin production consequently cause iron deficiency (high hepcidin levels) or iron overload (hepcidin deficiency).

Assessing the concentration of serum ferritin is a clinically useful measure of iron storage. Low serum ferritin levels indicate depleted stores, whereas increased levels may indicate iron overload. Inflammatory conditions (or infections, cancer, and liver disorders) can also increase serum ferritin. Given its clinical utility, it is surprising that the physiological function(s) of serum ferritin and its source (that is, whether it is derived from damaged cells or actively secreted by a regulated mechanism) still remain to be defined. Serum ferritin is predominantly composed of L chain subunits, partially glycosylated, and iron poor.

Iron Absorption

Inorganic dietary iron is absorbed at the brush border of duodenal enterocytes via the divalent metal transporter 1 (DMT1/SLC11A2, solute carrier family 11, member 2) (Gunshin et al.,1997). Given that iron largely adopts the oxidized state, it must first be reduced by the membrane-associated ferrireductase DcytB (Cybrd1). DcytB may not be the only ferrireductase of the apical membrane of enterocytes, as knockout mice appear to have normal iron metabolism (see review by McKie, 2008). Heme iron is absorbed independently by mechanisms that remain uncertain, because the proposed transporter SLC46A1 appears to carry mostly folate (Qiu et al., 2006). Heme iron is released intracellularly by hemoxygenase, mainly by the inducible hemoxygenase 1 (HOX1) (Ferris et al., 1999). Cytosolic iron can then be exported into the circulation by the basolateral iron exporter ferroportin (McKie et al., 2000; Donovan et al., 2000). Enterocytic iron export through ferroportin requires hephaestin, a multicopper oxidase homologous to ceruloplasmin, which oxidases Fe(II) to Fe(III) for loading onto transferrin. Consistent with this function, hephaestin-deficient mice display iron deficiency anemia with mucosal iron retention.

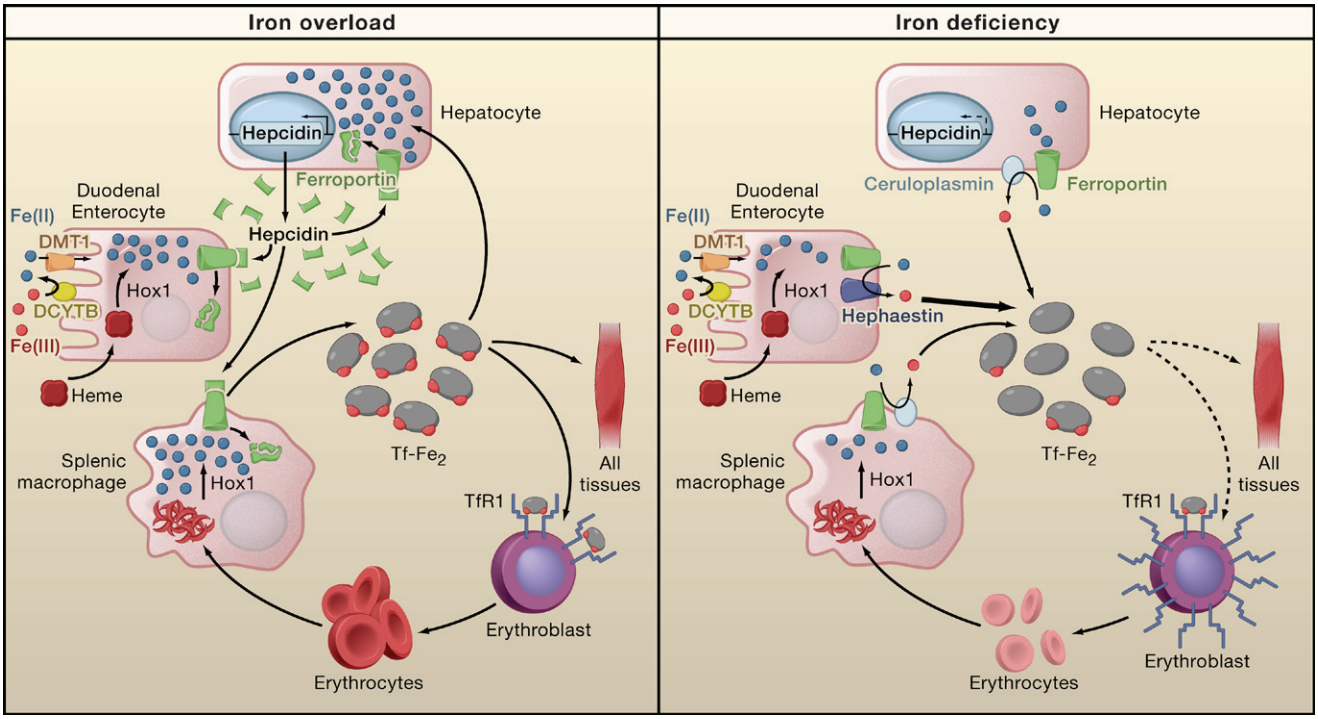

Figure 1. Regulation of Systemic Iron Homeostasis

Cells involved in systemic iron regulation are shown. Divalent metal transporter 1 (DMT1) at the apical membrane of enterocytes takes up iron from the lumen of the duodenum after DCYTB reduces Fe(III) to Fe(II). Ferroportin at the basolateral membrane cooperates with hephaestin that oxidizes Fe(II) to Fe(III). Iron-loaded (diferric) transferrin (Tf-Fe_2), indicated by red dots, supplies iron to all cells by binding to the transferrin receptor 1 (TfR1) and subsequent endocytosis. TfR1 is highly expressed on hemoglobin-synthesizing erythroblasts. Hepatocytes sense transferrin saturation/iron stores and release hepcidin accordingly. Red cell iron is recycled by macrophages via ferroportin and the ferroxidase ceruloplasmin. In iron overload (left), high hepcidin levels inhibit ferroportin-mediated iron export by triggering internalization and degradation of the complex to reduce transferrin saturation. Hepcidin expression is high. In iron deficiency (right), iron is released by ferroportin into the circulation. Hemoglobin-derived heme is catabolized in macrophages by hemoxygenase-1 (HOX1). Hepcidin expression is low.

Because iron cannot be excreted from the organism in a regulated way, iron absorption represents the critically controlled process. Normally, only 1–2 mg of iron per day are absorbed to compensate for iron losses, for example by sloughing of intestinal epithelial cells, desquamation of skin and urinary cells, blood loss, or sweat. Iron absorption can be enhanced when the needs are higher (for example, because of increased erythropoiesis or pregnancy) and suppressed in iron overload. The lack of an active mechanism for iron excretion accounts for the development of iron overload when the regulation of iron absorption is defective or bypassed (as occurs in blood transfusions).

Iron Utilization: Erythropoiesis

The vast majority of recycled iron (~25 mg/day) is dedicated to hemoglobin synthesis. TfR1 mediates erythroid iron acquisition, and its expression parallels the maturation of erythroid progenitors. Mouse embryos lacking TfR1 die because of severe anemia (and with neurologic abnormalities), whereas *Tfr1* haploinsufficiency or dysfunction of other components of the TfR1 endocytotic cycle (such as DMT1, STEAP3, or EXOC6, see below) cause microcytic anemia (characterized by abnormally small red blood cells) as a result of defective iron utilization in mice; mutations of DMT1 in humans elicit a similar phenotype and cause liver iron accumulation (Table S1 available online) (see review by Iolascon et al., 2009). A proposed additional route of erythroblast iron acquisition is ferritin released from macrophages in the so-called "erythroblastic islands" (Leimberg et al., 2008). However, the severe iron deficiency anemia of both mice and patients with transferrin deficiency (Table S1) suggests that this process can at best make a minor contribution to erythroid iron acquisition.

Erythroblasts not only acquire but also handle large amounts of iron. Potentially, iron may be directly transported from endosomes into mitochondria by a "kiss-and-run mechanism" through a direct contact between both organelles, effectively bypassing the cytosol (Sheftel et al., 2007). Iron is imported into mitochondria by the inner membrane protein mitoferrin 1 (Mfrn1/ SLC25A37, solute carrier family 25, member 37) (Shaw et al., 2006). This process is facilitated by the ABCB10 (ATP-binding cassette, subfamily B, member 10) protein, which is thought to stabilize Mfrn1 (Chen et al., 2009). Mfrn2/SLC25A28 may represent the housekeeping Mfrn1 homolog.

To coordinate the synthesis of the heme precursor protoporphyrin IX with iron availability, δ-aminolevulinic acid synthase 2 (ALAS2), the erythroid-specific first enzyme of protoporphyrin IX synthesis, is posttranscriptionally regulated by iron via the iron-responsive element/iron-regulatory protein (IRE/IRP) system (see below). Genetic defects in *ALAS2* cause sideroblastic

anemia (Table S1), whereas haploinsufficiency of other enzymes in the pathway cause porphyrias, due to accumulation of toxic heme precursors (see review by Puy et al., 2010). How heme is exported from mitochondria remains to be defined.

Mitochondrial iron uptake without the ability to use it for heme or Fe/S cluster biogenesis causes iron accumulation, because the excess import is not properly balanced by the export of products. Such mitochondrial iron depositions lead to the appearance of "ringed" sideroblasts (erythroblasts with perinuclear iron accumulations) and occur in conditions such as X-linked sideroblastic anemia due to ALAS2 deficiency or in the autosomal recessive deficiency of SLC25A38 (solute carrier family 25, member 38), a mitochondrial transporter likely involved in the import of ALA substrate glycine (Table S1). Anemia and ringed sideroblasts also appear when proteins involved in Fe/S cluster biogenesis are defective, such as impairment of GLRX5 (glutaredoxin 5) (Ye et al., 2010) or the ATP-binding cassette protein ABCB7, which leads to sideroblastic anemia with ataxia (Table S1).

Although erythroblasts consume large amounts of iron, they have to maintain safety mechanisms to avoid iron and/or heme excess: iron can be stored in ferritin or exported by ferroportin. Erythroblasts express a *ferroportin* messenger RNA (mRNA) isoform (1b) that lacks the 5′ IRE and thus evades potential translational repression by IRPs (see below) (Zhang et al., 2009). This isoform is susceptible to hepcidin degradation and may endow erythroid precursors with a mechanism to respond to systemic iron availability. Additionally, erythroblasts have the capacity to export excess heme (for example, when globin synthesis is limiting). The proposed exporter, FLVCR (feline leukemia virus subgroup C cellular receptor), is a multitransmembrane protein, a member of the major facilitator superfamily, and a receptor for a virus that causes severe aplastic anemia in cats. Accumulation of toxic heme at the proerythroblast stage can cause apoptosis, and mice with neonatal FLVCR deficiency develop severe hyperchromic, macrocytic anemia, reticulocytopenia, and a block in erythroid maturation at the proerythroblast stage (Keel et al., 2008).

Iron Recycling

Macrophages have to shoulder the lion's share of the burden of maintaining adequate levels of plasma iron. Given that less than 10% of the daily iron needs are met by intestinal absorption, the rest is covered by macrophages that recycle iron internally. The amount of plasma iron is just over 10% of the amount used daily, which means that plasma iron is turned over many times each day.

Macrophages phagocytose aged or damaged erythrocytes and catabolize heme using hemoxygenase. NRAMP1 (natural resistance-associated macrophages protein 1), a divalent metal transporter homologous to DMT1, is expressed within phagolysosomal membranes and participates in iron export from phagocytic vesicles (Soe-Lin et al., 2009). Export of ferrous iron from macrophages occurs via ferroportin (Figure 1). Reflecting its central role in systemic iron homeostasis, ferroportin expression in macrophages is regulated at multiple levels: ferroportin transcription is induced by erythrophagocytosis and heme iron, its translation is regulated by the IRE/IRP system, and its protein stability by hepcidin (see below). Ferroportin-mediated iron export is coupled to the function of the multicopper oxidase ceruloplasmin, a protein synthesized and secreted by the liver. Ceruloplasmin-deficient mice and humans show hepatocyte and macrophage iron accumulation. Aceruloplasminemia causes anemia (highlighting the critical role of iron release for erythropoiesis), diabetes, a late-onset disorder of the basal ganglia, and retinal degeneration (Table S1).

Systemic Iron Homeostasis: The Iron Hormone Hepcidin

Hepcidin has emerged as the central regulatory molecule of systemic iron homeostasis. It is a defensin family member with strong links to innate immunity. The bioactive, mature 25 amino acid peptide is generated from an 84 amino acid prepropeptide by furin cleavage. Hepcidin is secreted from hepatocytes and circulates in plasma bound to α2-macroglobulin (Peslova et al., 2009). Hepcidin clearance occurs via the kidney or by codegradation with ferroportin. Hepcidin forms a hairpin structure with four intramolecular disulfide bonds (Jordan et al., 2009) and exhibits modest antimicrobial activity in vitro, which has not yet been demonstrated in vivo.

Hepcidin binds to ferroportin, triggers its internalization, ubiquitination, and subsequent lysosomal degradation (Nemeth et al., 2004). Ferroportin binding is mediated by the N terminus of the peptide; Jak2 (Janus kinase 2) has been reported to bind to the hepcidin-ferroportin complex and to phosphorylate ferroportin before internalization (De Domenico et al., 2009).

Iron Overload

Research into the molecular mechanisms that underlie hereditary hemochromatosis in patients and murine models has been instrumental to decipher hepcidin regulation in mammals. Hereditary hemochromatosis is an autosomal recessive disease that leads to iron overload of the liver and other organs. Complications, which are preventable by iron depletion therapies, can be fatal and include liver cirrhosis, cancer, diabetes, hypogonadism, heart failure, and arthritis. Family studies implicate four genes in the disorder (Table S1): the most common type, which has a carrier frequency of $\sim$1:8 in Caucasian populations, is due to a homozygous missense mutation of the *HFE* gene (C282Y) (Feder et al., 1996). Less common but clinically more severe forms of hereditary hemochromatosis are caused by mutations of the *TfR2*, *hemojuvelin* (*HJV*), or hepcidin (*HAMP*) genes. All recessive forms of the disease represent molecular defects of hepatocytes and are caused by inappropriately low hepcidin expression (Figure 2A): the disease severity and the age of onset roughly correlate with the degree of hepcidin deficiency (see review by Camaschella, 2005).

HFE encodes a ubiquitously expressed major histocompatibility complex class 1-like molecule. The C282Y mutation abrogates β2-microglobulin binding and HFE surface expression; other HFE mutations are relatively rare. Hepcidin levels in patients may be normal, but are inadequately low for the degree of iron loading (Piperno et al., 2007; Ganz et al., 2008) and display a blunted response to acute oral iron challenges (Piperno et al., 2007). The penetrance of HFE mutations is low and clinical manifestations occur most commonly in middle

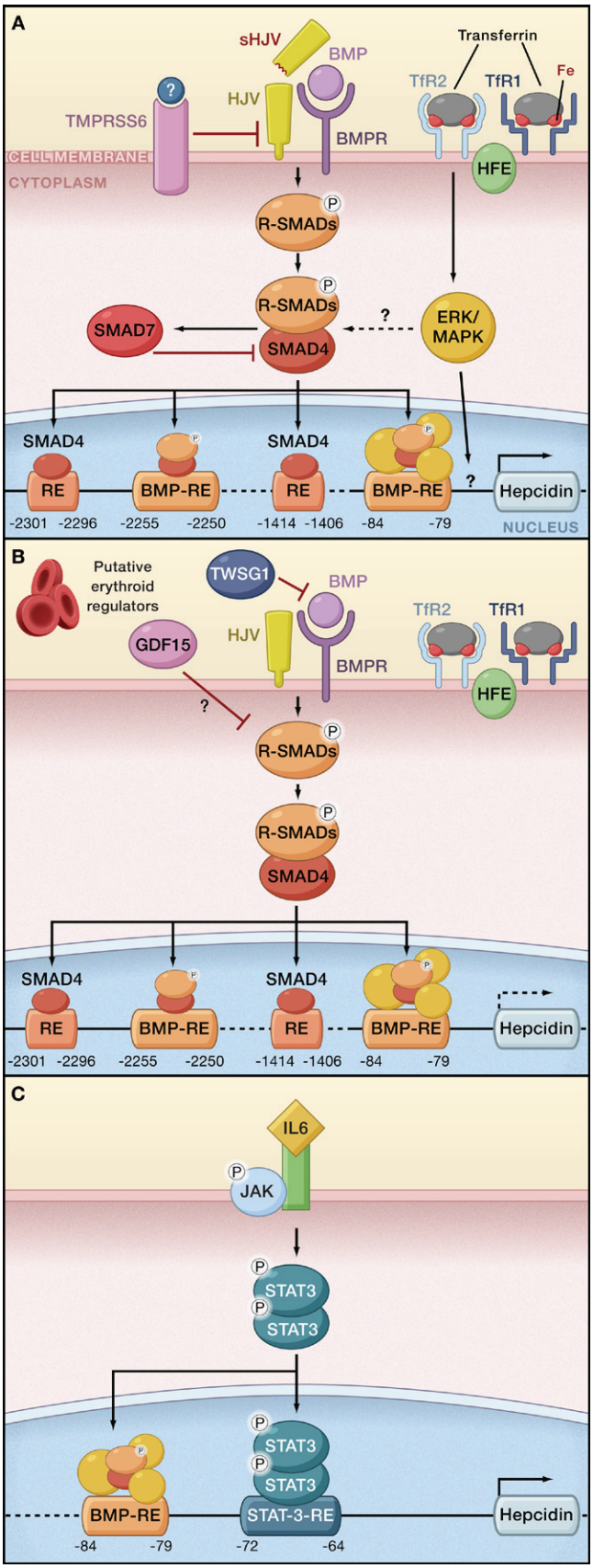

Figure 2. Regulation of Hepcidin Expression

(A) Hepcidin regulation by systemic iron availability. High concentrations of transferrin-Fe_2 (Tf-Fe_2) displace HFE from TfR1 to promote its interaction with transferrin receptor 2 (TfR2). The HFE-TfR2 complex then activates hepcidin transcription via ERK/MAPK and bone morphogenetic protein (BMP)/SMAD signaling. The BMP coreceptor hemojuvelin (HJV) interacts with type I and type II BMP receptors (BMPR) at the plasma membrane to induce phosphorylation of receptor-activated SMAD (R-SMAD) proteins, and subsequent formation of active transcriptional complexes involving the co-SMAD factor SMAD4. This signaling is inhibited by soluble HJV (sHJV). TMPRSS6 physically interacts with HJV and causes HJV fragmentation. SMAD7 interferes with SMAD4-controlled hepcidin activation. Sequence motifs critical for SMAD-mediated control of the hepcidin promoter are shown.

(B) Hepcidin regulation by erythropoietic signals. GDF15 and TWSG 1 are released by erythroid precursors to inhibit BMP/SMAD activation of hepcidin. This situation characterizes iron-loading anemias.

(C) Hepcidin regulation by inflammatory stimuli. Interleukin 6 (IL6) activates the Janus kinase (JAK)/ signal transducer and activator of transcription (STAT) signaling pathway and stimulates the hepcidin promoter via a STAT-binding motif close to the transcription start site. The BMP signaling pathway also contributes to the inflammatory response via SMAD4.

aged males, indicating the importance of environmental and/or additional genetic factors for disease expression (Beutler et al., 2002).

Juvenile hereditary hemochromatosis due to mutations in the *HAMP* or *HJV* genes may lead to irreversible hypogonadism, refractory heart failure, and even death in the second to third decades of life. Patients with *HAMP* or *HJV* mutations are phenotypically similar and have virtually undetectable hepcidin levels. HJV is a glycophosphatidlyinositol-linked protein, homologous to repulsive guidance molecules, and mostly expressed in liver, skeletal muscle, and heart. HJV is a bone morphogenetic protein (BMP) coreceptor (Babitt et al., 2006) that is required to drive hepcidin transcription via SMAD proteins (Figure 2A) (see below). *HAMP* mutations are extremely rare. Mice lacking HAMP and HJV recapitulate the organ iron loading observed in humans (Table S1).

Hereditary hemochromatosis due to *TfR2* mutations may present early, but with a less severe phenotype than the juvenile form (see review by Camaschella, 2005). TfR2 is a type II transmembrane protein that binds transferrin with lower affinity than TfR1 (Figure 2A). Targeted *Tfr2* gene deletion in mice causes iron overload with low basal hepcidin levels (Table S1); similar observations have been reported in humans with *TFR2* mutations.

A dominant form of hereditary hemochromatosis is caused by *missense* mutations in ferroportin (Table S1). Ferroportin is the only known cellular iron exporter and represents the "hepcidin receptor." Mutations that reduce its membrane localization or its ability to export iron cause macrophage iron retention, normal/low plasma iron levels, and in some cases iron-restricted erythropoiesis. A hemochromatosis-like disease with high plasma iron and hepatocyte iron accumulation is caused by hepcidin-resistant ferroportin mutations either because hepcidin fails to bind ferroportin (C326S) or the internalization and degradation of ferroportin following hepcidin binding is impaired (Fernandes et al., 2009).

Hepcidin levels are also inappropriately low in "iron-loading anemias" in which erythropoietic signals suppress hepcidin transcription (Figure 2B) even when systemic iron load is high.

The prototype of these anemias is β-thalassemia intermedia, characterized by transfusion-independent iron overload and low to absent hepcidin levels. Growth differentiation factor 15 (GDF15) and twisted gastrulation 1 (TWSG1) released by erythroblasts have been proposed to be involved in hepcidin suppression (Tanno et al., 2007, Tanno et al., 2009). In patients with homozygous β-thalassemia or other anemias with ineffective erythropoiesis, elevated serum GDF15 correlates with diminished hepcidin levels and increased iron absorption.

Iron Deficiency

Iron (blood) losses and/or insufficient iron intake/absorption from dietary sources can cause iron deficiency that most commonly manifests as microcytic anemia. Likewise, inappropriately high hepcidin expression lowers plasma iron levels (due to diminished iron release by macrophages and lower iron absorption) and causes anemia. In this context, the common acquired anemia of chronic diseases (ACD) and the genetic iron-refractory iron deficiency anemia (IRIDA) are most interesting.

Related to its evolutionary origin, hepcidin transcription is activated by inflammatory cytokines, especially interleukin 6 (Figure 2C). Hypoferremia develops rapidly as a result of decreased macrophage iron release and represents a defense mechanism against (iron-dependent) pathogens. Excessive hepcidin production is also seen in patients with infections, malignancies, chronic kidney diseases, or any type of inflammation. If prolonged, it leads to ACD. In rare cases, hepcidin can be expressed ectopically by hepatic adenomas, which results in microcytic anemia with some features of ACD (Weinstein et al., 2002), but this anemia is fully reversible after removal of the adenoma.

Patients with iron deficiency normally have low or undetectable levels of hepcidin. This is not the case in patients with IRIDA, who suffer from a microcytic anemia that is unresponsive to oral and partially refractory to parenteral iron, because of inappropriately high hepcidin levels. IRIDA is caused by mutations in *TMPRSS6* (matriptase-2), a gene that encodes a protease that negatively regulates hepcidin expression (Du et al., 2008) (see below). Interestingly genetic variants in *TMPRSS6*, frequent in the general population, may modulate the ability to absorb iron and to synthesize hemoglobin for maturing erythroid cells (Andrews, 2009). Whether TMPRSS6 variants may contribute to sporadic iron deficiency by increased hepcidin levels and decreased dietary iron absorption remains to be explored.

Hepcidin Regulation

Hepcidin expression in hepatocytes is regulated by multiple, in part opposing signals, including systemic iron availability (such as diferric transferrin, Tf-Fe_2), hepatic iron stores, erythropoietic activity, hypoxia, and inflammatory/infectious states (Figure 2). These different regulatory inputs are integrated transcriptionally.

Regulation by Systemic Iron Availability

After the discovery of the biological relevance of hepcidin, important progress has been made toward understanding the molecules and pathways that control hepcidin expression in response to iron and the role of the membrane proteins mutated in hereditary hemochromatosis (HFE, HJV, and TfR2) in this process (Figure 2A).

HFE has been suggested to act as a bimodal switch between two sensors of the concentration of Tf-Fe_2, TfR1, and TfR2, on the plasma membrane of hepatocytes (Goswami and Andrews, 2006). This model is supported by the following findings: HFE binds the ubiquitously expressed TfR1 at a site that overlaps the transferrin binding domain, and Tf-Fe_2 thus competes with HFE binding to TfR1. By contrast, TfR2 can bind both HFE and Tf-Fe_2 simultaneously (Gao et al., 2009). Mice bearing an engineered TfR1 mutation with increased HFE binding show low hepcidin expression and systemic iron overload similar to HFE-deficient mice, suggesting that the TfR1 sequesters HFE to prevent its participation in hepcidin activation. Conversely, mutations that abolish the HFE-TfR1 interaction or mice with increased HFE levels display elevated hepcidin expression and succumb to iron deficiency (Schmidt et al., 2008). Hepcidin activation by holotransferrin requires both HFE and TfR2 (Gao et al., 2010). These observations support a model in which high concentrations of Tf-Fe_2 displace HFE from TfR1 to promote its interaction with TfR2, which is further stabilized by increased Tf-Fe_2 binding to the lower-affinity TfR2. The HFE-TfR2 complex then activates hepcidin transcription. Future research is needed to establish the stoichiometry of the proteins involved in this "Tf-Fe_2-sensing complex" and to clarify whether HJV is part of it.

Although HFE and TfR2 clearly contribute to hepcidin activation, the BMP signaling pathway is quantitatively the most critical. By as yet only partially understood mechanisms, it integrates signals from the "Tf-Fe_2-sensing complex" and the hepatocytic iron stores. Central to the latter is BMP6, which is positively regulated by iron. How *BMP6* mRNA expression is activated by increased iron levels and repressed by iron deficiency requires further investigation. Bmp6 knockout mice show hepcidin deficiency and tissue iron overload (Andriopoulos et al., 2009; Meynard et al., 2009), although BMP2 and BMP4 can also bind to HJV. BMP6 is thought to act in an autocrine manner analogous to its role in chondrocyte differentiation (Grimsrud et al., 1999) to induce signaling via HJV, the BMP coreceptor that adapts BMP receptors for iron regulation (Babitt et al., 2006). The BMP/HJV complex joins the type I (Alk2 and Alk3) and the type II (ACTRIIA) BMP receptors to induce phosphorylation of receptor activated SMAD (R-SMAD) proteins and subsequent formation of active transcriptional complexes involving the co-SMAD factor, SMAD4 (Wang et al., 2005) (Figure 2A).

Two sequence motifs (the proximal BMP-RE1 and the distal BMP-RE2) of the hepcidin promoter are critical for transcription via HJV, BMP6, and SMAD4 (Casanovas et al., 2009), and the promoter region that contains BMP-RE2 confers iron responsiveness to the hepcidin promoter (Truksa et al., 2007). Multiple lines of evidence highlight the importance of HJV/BMP/SMAD signaling for hepcidin activation: (1) mice lacking HJV show attenuated R-SMAD phosphorylation in the liver (Babitt et al., 2006), (2) administration of BMP2 and BMP6 to mice induces *hepcidin* mRNA and decreases serum iron levels, (3) BMP antagonists (such as dorsomorphin) inhibit *hepcidin* mRNA expression and increase serum iron levels (Yu et al., 2008), (4) liver-specific disruption of the co-SMAD4

causes severe iron overload with diminished hepcidin transcription (Wang et al., 2005), and (5) the inhibitory iSMAD7 potently suppresses hepcidin transcription in cellular models (Mleczko-Sanecka et al., 2010). Interestingly, R-SMAD phosphorylation is also attenuated in mice lacking HFE, suggesting that HJV and HFE act together to activate hepcidin transcription (Corradini et al., 2009; Kautz et al., 2009). Crosstalk between the BMP/SMAD and p38-MAPK signaling pathways activates *hepcidin* mRNA expression in response to Tf-Fe_2 in primary hepatocytes. Activation of p38-MAPK and Erk1/2 depends on both HFE and TfR2, as this pathway is attenuated in mice lacking HFE or TFR2 and in double-knockout mice (Wallace et al., 2009).

Apart from mutations of the hepcidin gene itself, only HJV mutations lead to a near absence of hepcidin expression and the most severe form of hereditary hemochromatosis. Thus, HJV is central for hepcidin expression, and the point of convergence of multiple regulatory inputs. The membrane-associated protease TMPRSS6 that is mutated in IRIDA (see above) physically interacts with HJV and cleaves HJV when both proteins are expressed on the cell surface, suggesting that HJV is the major TMPRSS6 target for iron regulation (Silvestri et al., 2008a). Genetically, the combined deficiency of HJV and TMPRSS6 causes iron overload, suggesting that TMPRSS6 acts upstream of HJV (Truksa et al., 2009, Finberg et al., 2010). Increased HJV surface expression has however yet to be confirmed in *Tmprss6*-deficient mice or IRIDA patients.

Furin-mediated cleavage releases HJV from cells to generate soluble HJV (sHJV), which antagonizes BMP-dependent hepcidin activation. *Furin* mRNA expression is regulated by iron and hypoxia, conferring another level of control (Silvestri et al., 2008b). Because of the high HJV expression in skeletal muscle, it is tempting to speculate that sHJV is released as a muscle signal in iron deficiency. Importantly, cleavage of HJV by other proteases does not seem to be redundant with that by TMPRSS6, as lack of TMPRSS6 activity causes iron deficiency in humans and mice. Future work needs to address how TMPRSS6 expression and activity are regulated, and the relative contributions of TMPRSS6 and furin to the regulation of HJV and systemic iron homeostasis need to be further defined.

Neogenin, a DCC (deleted in colorectal cancer) family member, appears to stabilize HJV to enhance BMP signaling and hepcidin expression. Consistently, mice lacking neogenin exhibit hepatic iron overload, low hepcidin levels, and reduced BMP signaling (Lee et al., 2010).

Regulation by Erythropoietic Signals

Erythropoiesis requires considerable quantities of iron, and the inhibition of hepcidin expression by erythropoietic signals (Figure 2B) thus is of great physiological importance. Nonetheless, the molecular mechanisms and factors responsible are still poorly understood. Hepcidin suppression in response to phlebotomy or hemolysis depends on intact erythropoietic activity in mouse models: irradiation and cytotoxic inhibition of erythropoiesis prevent hepcidin suppression (Pak et al., 2006). GDF15 and TWSG1 are both released by erythroid precursors. High doses of GDF15 are detectable in the serum of patients with ineffective erythropoiesis such as β-thalassemia (Tanno et al., 2007). Such pathological concentrations of GDF15 can suppress hepcidin transcription in cell models, but the underlying molecular mechanism has not yet been characterized. By contrast, lower GDF15 concentrations fail to suppress hepcidin in cellular models and are apparently ineffective in patients with sickle cell anemia, myelodysplastic syndrome, and ACD. TWSG1 expression is increased in thalassemic mice, where it is produced during early erythroblast maturation. In cellular models, the BMP-binding protein TWSG1 inhibits BMP-dependent activation of Smad-mediated signal transduction that leads to hepcidin activation (Tanno et al., 2009). Correlations between TWSG1 expression, serum iron parameters, and hepcidin levels have not yet been studied in human anemias.

Regulation by Hypoxia

Liver-specific stabilization of the hypoxia-inducible factor 1 (HIF1) and HIF2 decreases hepcidin expression, and chemical HIF stabilizers can suppress *hepcidin* mRNA expression in hepatoma cells (Peyssonnaux et al., 2007). These findings have raised the possibility that iron-dependent prolyl hydroxylases involved in HIF degradation may act as hepatic iron sensors. Whether or not HIFs directly bind to the hepcidin promoter is currently controversial.

In vivo, hypoxia induces erythropoietin (EPO) synthesis, which in turn stimulates erythropoiesis. EPO injection into mice reduces hepcidin levels in a dose-dependent manner and can override signals that activate hepcidin expression. Even low dose EPO injections in human volunteers promptly decrease urinary excretion of hepcidin (Robach et al., 2009). Because experimental blockade of erythropoietic activity prevents its effect, EPO likely suppresses hepcidin by stimulation of erythropoiesis rather than more directly (Pak et al., 2006).

Regulation by Inflammatory and Stress Signals

The inflammatory cytokines IL1 and IL6 are both potent inducers of hepcidin expression, a response whose clinical importance for ACD has been discussed above. IL6 activates the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway, which activates the hepcidin promoter via a STAT-binding motif close to the transcription start site (Figure 2C) (Fleming 2007). The BMP signaling pathway also contributes to the inflammatory response via SMAD4 (Casanovas et al., 2009; Wang et al., 2005). Mice injected with lipopolysaccharide (LPS) augment hepcidin transcription even in the context of iron overload; likewise, LPS counteracts the diminished hepcidin expression in response to iron deficiency, suggesting that the two signals are integrated at the hepcidin promoter and that inflammatory and iron stores regulators operate independently rather than following a strict hierarchy (Huang, et al., 2009a). Hepcidin expression is also increased by endoplasmic reticulum (ER) stress. This stress response can be controlled by the transcription factor cyclic AMP response element-binding protein H (CREBH) (Vecchi et al., 2009) or by the stress-inducible transcription factors CHOP and C/EBPalpha (Oliveira et al., 2009). It has also been suggested that increased hepcidin transcription and iron deprivation may represent defense mechanisms against excessive cell proliferation and cancer, possibly by binding of the p53 tumor suppressor protein to a response element in the hepcidin promoter (Weizer-Stern et al., 2007).

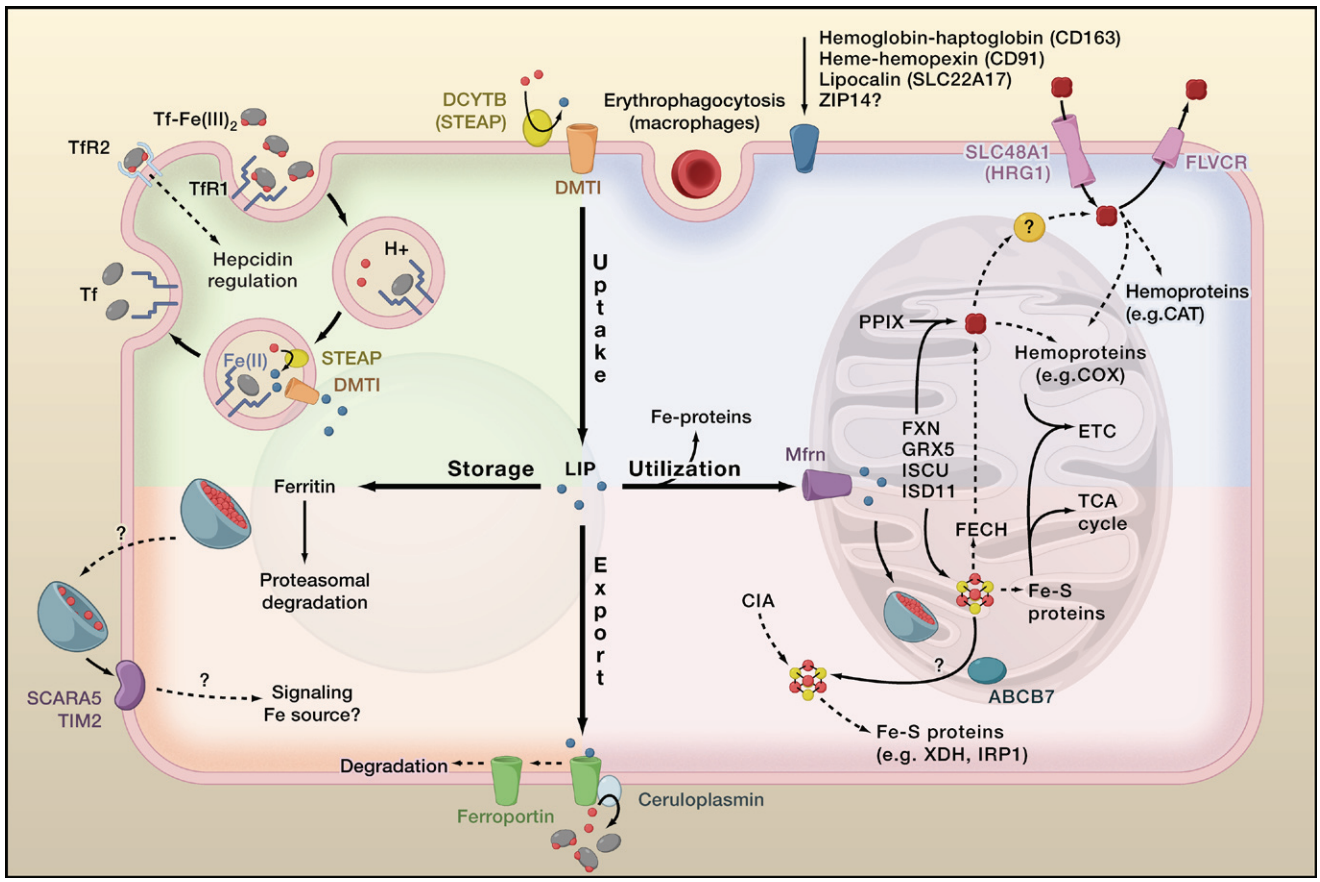

Figure 3. Cell Biology of Iron Metabolism

A generic cell is depicted. Most cells acquire plasma iron via transferrin receptor 1 (TfR1)-mediated endocytosis of transferrin-bound iron. In endosomes, iron is freed from transferrin and reduced to Fe(II) by STEAP metalloreductases prior its release into cytosol via divalent metal transporter 1 (DMT1); transferrin and TfR1 return to the plasma membrane to be used for further cycles. DMT1 also functions in the apical absorption of dietary iron after reduction by DCYTB and possibly other ferrireductases. Other iron acquisition pathways are symbolized (e.g., acquisition of heme iron from red blood cells by macrophages). Iron uptake systems feed the so-called labile iron pool (LIP). The LIP is utilized for direct incorporation into iron proteins or iron transport to mitochondria via mitoferrin (Mfrn), where the metal is inserted into heme and Fe/S cluster prosthetic groups. Proteins promoting heme transport into and out of cells have been identified. The fraction of the LIP that is not utilized for metalation reactions can be exported via ferroportin, which works together with ferroxidases for iron loading onto transferrin, or stored in a nontoxic form in ferritin shells. Ferritin can be released into the extracellular milieu by unknown mechanisms and interact with specific receptors on the cell surface. Some cells also express a mitochondrial form of ferritin to protect the organelle against iron-induced toxicity. The size of the LIP is determined by the rate of iron uptake, utilization, storage, and export; these processes must be coordinately regulated to avoid detrimental iron deficiency and prevent iron excess.

Therapeutic Opportunities

The increasing understanding of the role of hepcidin in iron overload and deficiency (inclucing ACD) opens new therapeutic avenues in a field that at present is essentially limited to iron depletion or substitution therapy. Both hepcidin agonists and antagonists would be useful drug prospects to treat iron-related disorders.

Current treatment options for diseases hallmarked by insufficient hepcidin expression would be complemented by hepcidin agonists. A proof-of-principle study has shown that transgenic hepcidin expression in mice lacking HFE can prevent iron overload (Nicolas et al., 2003). However, it is not clear whether hepcidin substitution (similar to insulin treatment of type 1 diabetes) would complement the current treatment of hereditary hemochromatosis with phlebotomy (or iron chelators). However, small molecules to augment hepcidin synthesis (via transcription) or mimic its effects on ferroportin are attractive candidates for the treatment of thalassemias, other iron-loading anemias, and the iron overload induced by hepatitis C infection.

Hepcidin antagonists would be useful for the treatment of patients with iron-restricted anemias as a consequence of hepcidin excess (e.g., ACD, IRIDA). Hepcidin depletion by neutralizing antibodies or by hepcidin small interfering RNAs (siRNAs) was shown to restore normal hemoglobin levels in a mouse model of anemia of inflammation when applied in combination with EPO (Sasu et al., 2010). Here, inhibitors of the stimulatory pathways for hepcidin transcription could offer one class of candidate compounds. Agents like the BMP signaling inhibitor dorsomorphin (Yu et al., 2008) or sHJV, which decrease baseline expression of hepcidin in mice, might prevent iron-deficiency anemias due to excess hepcidin. Alternatively, one can envisage blocking the effect of hepcidin on its only known target ferroportin, at the level of binding or the downstream effects that it triggers.

Cellular Iron Homeostasis: The IRE/IRP System

The maintenance of iron homeostasis by cells involves tasks that are very similar to those addressed at the systemic level: coordination of iron uptake, utilization, and storage to assure the availability of appropriate supplies and to prevent toxicity. Remarkably, the machinery and the mechanisms are entirely different. In contrast to systemic iron metabolism, cellular iron traffic also involves regulated iron excretion (Figure 3).

Cellular Iron Uptake

Tf-Fe_2 is a major iron source for mammalian cells, which they take up via the high-affinity TfR1. The Tf-Fe_2/TfR1 complex is internalized by clathrin-dependent endocytosis. Subsequent acidification of early endosomes triggers conformational changes in both transferrin and its receptor that promote the release of iron (Dautry-Varsat et al., 1983). The freed iron is then reduced to Fe(II) by members of the STEAP familly of metalloreductases (Ohgami et al., 2005, 2006) for transport into the cytosol via DMT1; thus, DMT1 plays a dual role in iron metabolism as an apical membrane protein of enterocytes to mediate systemic iron absorption as well as a ubiquitous endosomal protein involved in iron transfer from endosomes to the cytosol. Apo-transferrin and the TfR1 are then largely recycled to the cell surface. Optimal kinetics of the transferrin cycle are important for efficient acquisition of transferrin-bound iron and require the EXOC6 member of the exocyst, a protein complex involved in vesicular trafficking (Lim et al., 2005). Although TfR1 is ubiquitously expressed, the transferrin cycle is particularly important for the massive iron delivery to erythroid precursors. Humans and mice lacking transferrin expression accumulate iron in nonhematopoietic tissues such as the liver (Table S1), and targeted disruption of the *Tfr1* locus in the mouse shows that TfR1 is required for the differentiation of erythroid, lymphoid, and neuroepithelial cells, but it is dispensable for the development of other tissues, at least during fetal life. This implies that at least some cells can acquire iron independently of the transferrin cycle.

Biochemical and genetic studies support the existence of transferrin-independent routes of iron uptake. DMT1 was once thought to be responsible for non-transferrin-bound iron uptake by liver cells, but iron loading of DMT1-deficient mouse hepatocytes indicates that at least one alternative transferrin-independent uptake pathway must exist (Gunshin et al., 2005); the metal transporter ZIP14 is a candidate for this (Liuzzi et al., 2006), but awaits functional validation in vivo.

Under conditions of systemic iron overload, the L-type voltage-gated calcium channel mediates transferrin-independent iron entry into cardiomyocytes (Oudit et al., 2003); calcium channels may also play a role in iron delivery to neuronal cells (Gaasch et al., 2007). Interestingly, calcium channel blockers such as nifedipine mobilize liver iron and enhance urinary excretion in iron-loaded mice, probably by increasing DMT1-mediated iron transport (Ludwiczek et al., 2007). These findings suggest new therapeutic opportunities for the treatment of systemic iron overload. Receptor-mediated endocytosis of other forms of protein-bound iron represents an additional means for specific cell types to take up iron: lipocalin 2-dependent endocytosis of an iron-laden siderophore via the SLC22A17 lipocalin receptor has been proposed to modulate the survival of kidney cells in culture (Devireddy et al., 2005), but the physiological relevance of this iron uptake pathway remains uncertain as lipocalin 2 knockout mice develop normally. Serum ferritin can enter cells via the Scara5 (scavenger receptor class A, member 5) and TIM-2 (T cell immunoglobulin and mucin domain containing 2) ferritin receptors (Li et al., 2009, Chen et al., 2005).

Finally, specialized cells are able to acquire iron in the form of heme. The nature of the enterocytic heme importer remains uncertain; SLC48A1 is the only bona fide heme import molecule identified so far (Rajagopal et al., 2008), and gene inactivation in mice should help to evaluate its in vivo functions in mammals.

Cells also acquire heme indirectly. Macrophages obtain heme by phagocytosis and processing of dying red blood cells. In plasma, hemoglobin and free heme arising from intravascular hemolysis are cleared by specific scavenger systems: hemoglobin forms a complex with haptoglobin that is delivered to reticuloendothelial cells via CD163-mediated endocytosis. Among other plasma molecules, free heme binds to hemopexin and the complex is endocytosed via the CD91 receptor present on the surface of macrophages, hepatocytes, and other cell types. Thus, cells meet their iron needs via different uptake systems optimized to serve the specific cellular iron demands (Figure 3).

Cellular Iron Export

Iron export occurs from many cells including neuronal and erythroid cells, but it is particularly important in cells that maintain plasma iron levels. Such cells include macrophages and duodenal enterocytes, and in fetal develoment iron export is mediated by cells of the extraembryonic visceral endoderm (ExVE) and placental syncythiotrophoblasts. These cells express relatively high levels of ferroportin (SLC40A1), and the effects of targeted disruption of the *Slc40a1* locus in the mouse reflects the unique, nonredundant functions of ferroportin in iron release from these cell types (Donovan et al., 2005). As mentioned above, ferroportin transports Fe(II) and acts in concert with either of the ferroxidases hephaestin (enterocytes) or ceruloplasmin (other cell types) that facilitate iron extraction from the ferroportin channel and subsequent loading onto plasma transferrin (De Domenico et al., 2007). The fact that both ceruloplasmin and hephaestin are copper dependent explains the importance of the copper status for iron metabolism.

In addition to ferroportin-mediated release of elemental iron, cells appear to be able to export iron in the form of heme; overexpression and viral interference studies suggest that FLVCR1 could promote heme efflux. The physiological role of heme export remains unclear, but FLVCR1 is essential in the mouse and is required for proerythroblast differentiation and macrophage heme iron recycling (Keel et al., 2008). A small fraction of cellular iron can also exit the cell bound to ferritin, but the mechanisms and physiological role of ferritin release remain to be better defined.

Cellular Iron Storage

Iron from the cytoplasmic "labile iron pool" (LIP) that is not utilized for metalation reactions or exported is stored within the nanocavity of ferritin heteropolymers made of 24 subunits of heavy (FtH1) and light (FtL) chains (see review by Arosio and

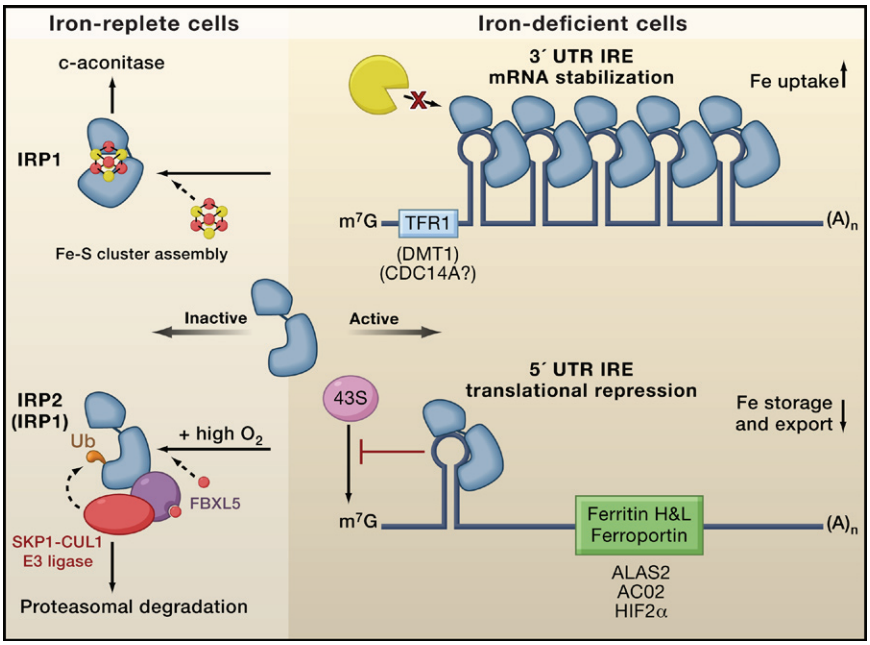

Figure 4. Regulation of Cellular Iron Metabolism

In iron-deficient cells (right), iron regulatory protein 1 (IRP1) or IRP2 bind to *cis*-regulatory hairpin structures called iron-responsive elements (IREs), present in the untranslated regions (UTRs) of mRNAs encoding proteins involved in iron transport and storage (Muckenthaler et al., 2008). The binding of IRPs to single IREs in the 5′ UTRs of target mRNAs inhibits their translation, whereas IRP interaction with multiple 3′ UTR IREs in the *transferrin receptor 1* (*TfR1*) transcript increases its stability. As a consequence, TfR1-mediated iron uptake increases whereas iron storage in ferritin and export via ferroportin decrease, thereby increasing the LIP. In iron-replete cells (left), the FBXL5 iron-sensing F-box protein interacts with IRP1 and IRP2 and recruits the SKP1-CUL1 E3 ligase complex that promotes IRP ubiquitination and degradation by the proteasome; IRP1 is primarily subject to regulation via the assembly of a cubane Fe/S cluster that triggers a conformational switch precluding IRE-binding and conferring aconitase activity to the holoprotein. IRPs also modulate the translation of the mRNAs encoding the erythroid-specific ALAS2 heme synthesis enzyme, the mitochondrial aconitase (ACO2), and the HIF2α hypoxia-inducible transcription factor. Single 3′ UTR IRE motifs are present in the *DMT1* and *CDC14A* mRNAs, but their role and mechanism of function are not yet fully defined.

Levi, 2010). Both ferritin subunits are ubiquitously expressed, but their expression ratios vary depending on the cell type and in response to stimuli such as inflammation or infection. FtH1 carries the ferroxidase activitiy that is necessary for iron deposition into the nanocage, while FtL facilitates iron nucleation and increases the turnover of the ferroxidase site. Little is known about how iron is extracted from the LIP and delivered to ferritin. Poly(rC)-binding protein 1 (PCBP1), an RNA-binding protein mostly known for its role in posttranscriptional regulation, is required for ferritin iron loading in cultured cells and can promote ferritin iron loading in vitro (Shi et al., 2008); future work will address whether PCBP1 is the metallochaperone responsible for ferritin mineralization in vivo.

Ferritin provides cells with a means to lock up excess iron in a redox inactive form to prevent iron-mediated cell and tissue damage; it also constitutes an iron store whose mobilization involves both proteasomal and lysosomal ferritin degradation. Ferritin is essential, as shown by the early embryonic lethality of FtH1 knockout mice (Ferreira et al., 2000). Mutations of the *FtL* 5′ IRE (see below) cause the dominant hyperferritinemia-cataract syndrome. C-terminal mutations of *FtL* cause hereditary ferritinopathy, an adult-onset autosomal dominant neurodegenerative disease characterized by the presence of ferritin inclusion bodies and iron deposition in the brain (Table S1).

Homopolymers of a nuclear gene-encoded H-type ferritin are present in mitochondria (see review by Arosio and Levi, 2010). Akin to its cytosolic counterpart, mitochondrial ferritin (FtMt) is thought to protect the organelle against iron-mediated toxicity. In contrast to FtH1 and FtL, FtMt is not ubiquitously expressed; it has been detected in tissues such as the testis, heart, endocrine pancreas, and kidney, but not in spleen, gut, or liver. Another major difference between cytosolic and mitochondrial ferritins is that FtMt expression is not (directly) controlled by the IRPs (see below).

Regulation of Cellular Iron Metabolism

While key aspects of systemic iron metabolism are regulated transcriptionally (hepcidin expression) and posttranslationally (ferroportin function by hepcidin), cellular iron homeostasis is coordinately regulated posttranscriptionally by iron regulatory protein 1 (IRP1) and IRP2 (also known as ACO1 and IREB2, respectively) (Figure 4). The two orthologous RNA-binding proteins interact with conserved *cis*-regulatory hairpin structures known as IREs, which are present in the 5′ or 3′ untranslated regions (UTRs) of target mRNAs. Either of the two IRPs inhibits translation initiation when bound to the single 5′ UTR IREs of *ferritin H-* or *L-chain* (iron storage), *ferroportin* (export), *ALAS2* (utilization), *mitochondrial aconitase* (*ACO2*), or *hypoxia-inducible factor 2α* (*HIF2α*/*EPAS1*) mRNAs, whereas their binding to the multiple IRE motifs within the 3′ UTR of *TFR1* (uptake) mRNA prevents its endonucleolytic cleavage and subsequent degradation (see reviews by Muckenthaler et al., 2008, and Recalcatti et al., 2010). The IRPs also appear to positively regulate *DMT1* (uptake) mRNA expression via a single 3′ UTR IRE motif, but the molecular mechanism is not known. Single IRE-like structures with restricted phylogenetic conservation have been identified in the 3′ UTR of the *CDC14A* (*cell division cycle 14 homolog A*), *HAO1* (*hydroxyacid oxidase 1*), and *CDC42BPA* (*CDC42 binding protein kinase α*)/*MRCKa* mRNAs, but their functional roles are not yet clear.

A canonical IRE is defined by both RNA sequence and structure and consists of an unpaired cytosine separated from a CAGUGN loop (where N = U, C, or A) by a 5 base pair upper stem plus a lower stem of variable length (Muckenthaler et al., 2008); the IREs of the *HIF2α* and DMT1 mRNAs have a noncanonical additional bulge on the 3′ strand of the upper stem. The high specificity and affinity of the IRP1/IRE interaction results from two spatially distant sites that establish multiple RNA-protein contacts clustered around the terminal loop and the C bulge, respectively, of the IRE (Walden et al., 2006); sequence

variability of the upper and lower stems also influences IRP1 binding affinity, potentially contributing to the graded response of IRE-controlled mRNAs to IRP regulation (Goforth et al., 2010). Approaches based on mutagenesis and systematic evolution of ligands by exponential enrichment (SELEX) have yielded high-affinity RNA binders that are substantially different from the canonical IRE (Henderson et al., 1994, Butt et al., 1996), suggesting that the IRP regulon may extend beyond the current list of IRE-containing mRNAs.

IRP-binding to IREs responds to cellular iron levels (Figure 4). In iron-replete cells, IRP2 (and apo-IRP1, see below) interacts with the FBXL5 (F-box and leucine-rich repeat protein 5) adaptor protein that recruits a SCF (SKP1-CUL1-F-box) E3 ligase complex, promoting IRP ubiquitination and subsequent degradation by the proteasome (Salahudeen et al., 2009, Vashisht et al., 2009); in iron-deficient cells, the FBXL5-dependent degradation of IRPs decreases. Iron regulation of IRP turnover involves a hemerythrin-like domain of FBXL5 that acts as an iron sensor: direct binding of iron to this domain stabilizes FBXL5 (hence IRPs are degraded), whereas FBXL5 is otherwise degraded.

IRP1-binding to IREs is subject to an additional layer of regulation. In iron-replete cells, IRP1 (but not IRP2) ligates a cubane 4Fe-4S cluster that precludes IRE binding (Walden et al., 2006; Muckenthaler et al., 2008); remarkably, 4Fe-4S IRP1 functions as a cytosolic aconitase and the protein is hence bifunctional. In iron-deficient cells, IRP1 loses its Fe/S cluster and aconitase activity and adopts its IRE-binding (apo-IRP1) conformation. The molecular details of this iron-regulated Fe/S cluster assembly/disassembly are not yet known, but disruption of critical components of Fe/S cluster biogenesis stimulate the IRE-binding activity of IRP1 (see review by Sheftel and Lill, 2009). It is thus possible that the ratio of holo- to apo-IRP1 depends primarily on mitochondrial iron availability and Fe/S cluster synthesis, whereas cytosolic iron sensing may primarily involve IRP2.

Genetic ablation of both IRPs in the mouse causes embryonic lethality (Smith et al., 2006, Galy et al., 2008). By contrast, animals lacking either protein are viable and fertile. IRP2 knockout mice show a mild microcytic anemia, a tendency toward increased neurodegeneration, and abnormal body iron distribution (Cooperman et al., 2005, Galy et al., 2005). IRP1 knockout mice are asymptomatic under laboratory conditions, demonstrating that the cytosolic aconitase activity is not essential (Meyron-Holtz et al., 2004a). Taken together, IRP expression is essential, but the two proteins are largely redundant.

Experiments with animals and cultured cells show that the two IRPs respond differentially to non-iron signals. For example, hypoxic conditions inactivate IRP1 by favoring holo-IRP1 formation (Meyron-Holtz et al., 2004b) while stabilizing IRP2 as a result of the oxygen requirement for iron-mediated FBXL5 degradation. Furthermore, reactive oxygen species selectively activate IRP1 by causing disassembly of Fe/S clusters, likely via a membrane-initiated signaling pathway (Pantopoulos and Hentze, 1998). Phosphorylation of IRP1 and IRP2 by specific kinases can also regulate their activity. Although the exact physiological and pathophysiological functions of the differential regulation of IRP1 and IRP2, respectively, remain to be determined, it could in principle allow cells to finely control iron metabolism over a wide range of conditions and to alter the set points of the two regulatory proteins independently from iron control.

Intracellular Iron Trafficking and Utilization

One of the least well understood problems in iron biology is how iron moves within cells. In the cytoplasm, iron is present in di-iron centers directly bound to proteins such as ribonucleotide reductase, but most iron trafficks to mitochondria, where it is incorporated into bioactive heme and Fe/S cluster prosthetic groups. Recent work identifies 2,5 dihydroxybenzoic acid as the iron-binding moiety of a mammalian siderophore related to bacterial enterobactin. Disruption of its biosynthesis causes mitochondrial iron deficiency, implicating its importance for intracellular iron transport to mitochondria (Devireddy et al., 2010). In erythroblasts, the main mitochondrial iron importer for heme and Fe/S cluster biogenesis is Mfrn1. Mfrn1 is required for primitive and definitive erythropoiesis (Shaw et al., 2006).

Iron management within mitochondria also remains poorly understood. The metal is inserted into protoporphyrin IX by ferrochelatase to form heme, or delivered to the Fe/S cluster biosynthetic machinery possibly by the iron chaperone frataxin (FXN), a matrix protein that is defective in patients with Friedreich's ataxia (Table S1) (for details on the biogenesis of Fe/S cluster proteins, see review by Sheftel and Lill, 2009).

Whether a fraction of elemental iron exits mitochondria is not known, but heme is exported from the organelle via a yet undefined mechanism and incorporated into proteins throughout the cell. Likewise, Fe/S clusters are utilized in multiple subcellular compartments; whether they all originate from mitochondria is still debated as components of the Fe/S cluster assembly machinery have been detected in the cytosol and could promote extramitochondrial Fe/S cluster synthesis or repair (Tong and Rouault., 2006; Sheftel and Lill, 2009). The central role of mitochondria in Fe/S cluster metabolism is also underscored by the inner membrane protein ABCB7 that is required for the maturation of cytosolic but not mitochondrial Fe/S cluster proteins; how exactly ABCB7 contributes to the maturation of extramitochondrial Fe/S cluster proteins is not known, as its function has not yet been defined.

By making heme and Fe/S clusters, mitochondria represent the major subcellular site of iron utilization and as such play a central role in the control of cellular iron metabolism. The IRPs are essential to secure mitochondrial iron supplies and function in vivo (Galy et al., 2010). When Fe/S cluster biogenesis is impaired, iron accumulates in mitochondria, potentially harming the organelle. A current model of how mitochondria influence cellular iron metabolism posits that cells sense mitochondrial iron insufficiency via an Fe/S cluster-dependent factor and respond by increasing mitochondrial iron levels; a heme intermediate could also be involved given that mitochondrial iron loading also occurs in erythroid cells with heme deficiency stemming from mutations in ALAS2 or SLC25A38. Diversion of iron to mitochondria would deplete the cytosol, thereby stimulating IRP binding to IREs; in addition, perturbation of Fe/S cluster metabolism would activate IRP1. This in turn increases cellular iron uptake (TfR1, DMT1) and diminishes iron storage (ferritin) and export (ferroportin), so that more iron becomes

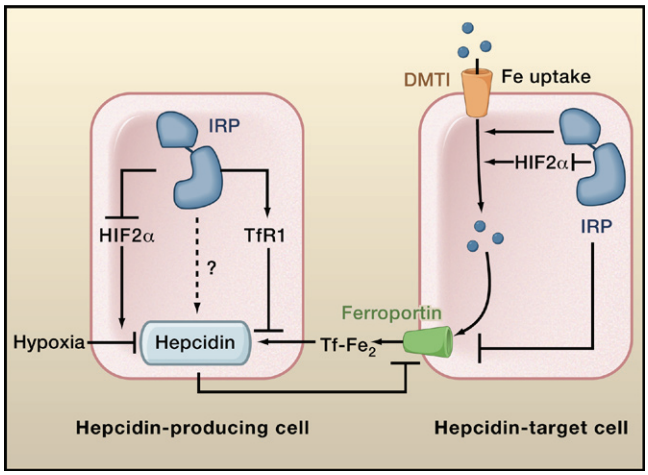

Figure 5. Interplay between Systemic and Cellular Iron-Regulatory Systems

(Left) In hepcidin-producing cells (for example, hepatocytes), iron-regulatory proteins (IRPs) can influence *Hepcidin* gene regulation by modulating levels of transferrin receptor 1 (TfR1) and/or hypoxia-inducible factor 2α (HIF2α); the iron-responsive element (IRE)/IRP system may also impact on *Hepcidin* expression by changing intracellular iron levels (dashed line).

(Right) Ferroportin expression is regulated by both hepcidin and IRPs. Furthermore, IRPs can potentially exert a direct positive effect on iron uptake via divalent metal transporter 1 (DMT1) or TfR1, or an indirect negative effect via HIF2α repression.

available; IRPs protect mitochondria against detrimental iron deficiency (Galy et al., 2010), although additional regulatory pathways could contribute (Huang et al., 2009b).

In erythroid cells, IRP activation furthermore inhibits ALAS2 translation and heme synthesis to avoid the accumulation of toxic metabolic intermediates until mitochondrial iron sufficiency is restored. By contrast, impaired Fe/S cluster biogenesis with abnormally high IRP activity seems to maintain constitutively high levels of iron, triggering mitochondrial iron (over) loading and, as observed in erythroblasts with glutaredoxin 5 deficiency, block ALAS2 expression and heme synthesis (Table S1). Whether and how mitochondria can protect themselves against iron overload remains to be elucidated.

Crosstalk between Cellular and Systemic Regulators

As described above, systemic and cellular iron homeostasis are maintained by distinct control systems: hepcidin/ferroportin and IRE/IRP. It seems very likely that there is higher level coordination between these two systems, and future work will define in more detail how the two systems tango in harmony (Figure 5). Three interconnections have already been identified.

First, the ferroportin connection: The expression of ferroportin 1, which is critical for plasma iron levels, is subject to regulation by both systems: the systemic iron status is communicated posttranslationally via hepcidin, whereas intracellular iron availability regulates ferroportin synthesis via the 5′ UTR IRE of the *ferroportin* mRNA. Mice doubly deficient for IRP1 and IRP2 in enterocytes are unable to limit ferroportin expression in these iron exporting cells, leading to cellular iron deficiency. This shows that both control mechanisms are required to assure regulated iron export (Galy et al., 2008). The IRE/IRP system has to protect the cells exporting iron against detrimental iron losses, whereas hepcidin protects the organism against systemic overload. The creation of ferroportin mouse mutants in which hepcidin and IRP-dependent regulation are dissociated may help to more clearly define the relative contribution of the two regulatory systems to the control of cellular iron export under different physiological and pathological conditions.

Second, the HIF2α connection: *HIF2α* mRNA is an IRP target (Sanchez et al., 2007), and the encoded transcription factor regulates DMT1 expression at the apical surface of duodenal enterocytes (Mastrogiannaki et al., 2009). Mice lacking intestinal HIF2α have decreased expression of DMT1 and ferroportin and thus fail to promote iron absorption even upon lowering of hepcidin expression. How exactly hepcidin, HIF2α, and IRP activity depend on each other to assure adequate systemic iron supplies requires further research. Cross-breeding of mouse lines with tissue-specific ablations of IRPs, HIF2α, and/or hepcidin may prove to be informative. It has also been discussed that hepcidin transcription may be controlled by HIF2α in response to hypoxia or iron deficiency.

Third, the TfR connection: Hepcidin expression is regulated by the signaling receptor TfR2 and the "switch factor" HFE that also binds to TfR1 in competition with plasma Tf-Fe_2 (see above). TfR1 expression is promoted by high IRP activity. The equilibrium between the amount of plasma iron "sensing" TfR1 and "signaling" TfR2 is thought to be important for hepcidin activation, and IRP activity may thus indirectly affect hepcidin expression by regulating TfR1 levels in hepatocytes.

Perspectives

The 1980s and 90s brought us the foundational discoveries in cellular iron metabolism and its regulation, and we are now just leaving a decade of research during which systemic iron metabolism has progressed from enigma to significant understanding. What lies ahead?

As discussed above, an urgent issue is to further unravel how systemic and cellular iron control mechanisms talk to each other. We should not lose sight of the fact that fundamental questions of the cell biology of iron remain unanswered. How does iron traffic inside cells, and what is the importance of the recently identified 2,5 dihydroxybenzoic acid-containing mammalian siderophore? How is iron (and Fe/S clusters and heme) incorporated into iron containing proteins? We also need to focus research efforts to a greater degree on iron metabolism of different organ systems and of cell-cell interactions. For example, little is known about how the kidney handles iron: iron undergoes glomerular filtration and reabsorption, which have important physiological and potentially therapeutic implications. Similarly, the nervous system poses grand challenges: For example, why does iron deficiency affect the central nervous system, although hereditary hemochromatosis patients do not accumulate iron in the brain even in the presence of extremely high plasma and systemic iron levels? How do local alterations of iron metabolism contribute to neurodegeneration, and can we devise strategies to prevent or ameliorate its course? What role do changes in iron metabolism play in

interactions between inflamed tissues and cells of the immune system? Responses to the latter questions will also have implications for common disorders such as atherosclerosis and cancer.

Increasingly, our quest to understand basic cell biological phenomena will be complemented by exploration of the iron biology of disease states. Progress in this area will help to devise new therapeutic concepts. The impressive progress that has been made during the past few years in unraveling the regulation of hepcidin expression and its function may well translate into new drugs to treat iron overload diseases and some forms of anemia.

Supplemental Information

Supplemental Information includes one table and can be found with this article online at doi:10.1016/j.cell.2010.06.028.

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