REVIEW ARTICLE

Iron homeostasis: new players, newer insights

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Abstract

Although iron is a relatively abundant element in the universe, it is estimated that more than 2 billion people worldwide suffer from iron deficiency anemia. Iron deficiency results in impaired production of iron-containing proteins, the most prominent of which is hemoglobin. Cellular iron deficiency inhibits cell growth and subsequently leads to cell death. Hemochromatosis, an inherited disorder results in disproportionate absorption of iron and the extra iron builds up in tissues resulting in organ damage. As both iron deficiency and iron overload have adverse effects, cellular and systemic iron homeostasis is critically important. Recent advances in the field of iron metabolism have led to newer understanding of the pathways involved in iron homeostasis and the diseases which arise from alteration in the regulators. Although insight into this complex regulation of the proteins involved in iron homeostasis has been obtained mainly through animal studies, it is most likely that this knowledge can be directly extrapolated to humans.

Iron is an essential element for virtually all-living organisms. It is required as a cofactor for a multitude of proteins of diverse biological function. Iron plays a central role in the formation of hemoglobin and myoglobin and in many vital biochemical pathways and enzyme systems including energy metabolism, neurotransmitter production, collagen formation and immune system function. As a transition metal, iron also has useful ligand-binding and redox properties. At the same time, iron poses enormous problems because of the easy inter-conversion of Fe(II) and Fe(III), the very property that makes it attractive for biological redox processes (1). The reduced iron, Fe(II) can produce highly reactive hydroxyl and lipid radicals, which can damage lipid membranes, nucleic acids and proteins. In the million years of evolution, nature has not developed a pathway for excretion of iron in humans, so the body concentration of iron can only be regulated by absorption to match losses of iron.

Under normal circumstances 1–2 mg of iron enters and leaves the body each day. In the body, iron is distributed in three pools – functional, storage and transport iron. The absorbed iron circulates in the plasma bound to transferrin (Tf) (transport iron) and the excess iron gets stored in the parenchymal cells of the liver and reticuloendothelial macrophages as ferritin (storage iron). About 0.1% of total body iron is circulating bound to Tf. Nearly 80% of iron is utilized for hemoglobin synthesis in the bone marrow. Approximately 10–15% is present in muscle fibers (myoglobin) and in other tissues as enzymes and cytochromes (functional iron).

Homeostatic mechanisms regulating the absorption, transport, storage and mobilization of cellular iron are therefore of critical importance in iron metabolism, and a rich biology and chemistry underlie all of these mechanisms. The last decade has seen a rapid advancement of knowledge in iron metabolism. Modern techniques in molecular biology and biochemistry are giving new insights through the identification and characterization of proteins involved in iron homeostasis. This review focuses on the current developments in the understanding of iron homeostasis and the delicate mechanisms involved in the regulation of iron in the body.

Iron absorption

Iron absorption occurs predominantly in the apical surface of the duodenum and upper jejunum. The two forms of dietary iron namely heme and non-heme iron are absorbed by the enterocyte non-competitively.

Heme iron is not chelated and not precipitated by numerous constituents of the diet that render non-heme iron difficult to-absorb. Shayegi *et al.* (2) have identified a membrane protein called heme carrier protein 1 which mediates heme uptake by cells. Once heme enters the cell, it is opened up by heme oxygenase to release Fe^{2+} and thereafter the fate of both heme and non-heme iron are the same.

The non-heme iron mainly exists in the Fe^{3+} state. The ferric iron is reduced to ferrous iron before it is transported across the intestinal epithelium. This is accomplished by dietary components and duodenal cytochrome b reductase (Dcytb) which is highly expressed in the brush border of enterocytes (3). Once the insoluble Fe^{3+} is converted to Fe^{2+}, it enters the mucosal phase. Fe^{2+} is transported across the apical membrane into the cell through a divalent metal transporter (DMT1). DMT1 is expressed at the duodenal brush border where it controls uptake of dietary iron. A pathway for Fe^{3+} transport has also been proposed, where it is transported as an integrin–mobilferrin (IM) complex across the enterocyte. This Fe^{3+}–IM complex combines with flavine mono oxygenase and β2-microglobulin in the cytosol to form paraferritin. The Fe^{3+} is then converted to Fe^{2+} by the inherent ferrireductase activity of paraferritin and is transported out as Fe^{2+} by DMT1, which is also present in the paraferritin (4).

The Fe^{2+} inside the cell can undergo two fates – either it can be stored as ferritin or transported across the basolateral surface into the blood stream. The mechanism by which Fe^{2+} reaches the basolateral membrane is poorly understood. The factors that decide whether iron will be stored in the intestinal cell or transported into the plasma are not yet clear but are being slowly understood.

The iron inside the cell is exported out of the cell by a transport protein called ferroportin (FPN). Hephaestin, a multicopper ferroxidase, works in concert with FPN in the export of iron from the enterocytes (5,6). It is a transmembrane-bound ceruloplasmin homolog highly expressed in the intestine (7). It oxidizes Fe^{2+} and enables it to be taken up by Tf. Ceruloplasmin also plays a role in oxidation under conditions of stress (8).

Studies have shown that plant and animal ferritin can also be absorbed in the intestine (9,10). Receptors for lactoferrin, an iron binding protein are found in fetal enterocytes (11). Lactoferrin is viewed as the primary source of iron in infants and it may be a source in adult females also (12).

Regulation of iron absorption is complex and depends on dietary factors, body iron stores and erythropoiesis. The changes in the dietary iron are sensed by the villus enterocytes and there is a rapid regulation of transporters. Cells in the crypt express HFE and Tf receptor (TfR) on the basolateral surface (13), but their exact role in regulation of iron absorption is not clear. In addition, regulation may be exerted by several humoral and tissue derived factors like hepcidin and cytokines. Crosstalk between the epithelial cells and other cells like macrophages, neutrophils and intra-epithelial lymphocytes also play an important role in iron absorption. The way in which the erythropoietic demand controls iron absorption has not been elucidated. Hepcidin levels are decreased by high erythropoietic activity (14) and it has been postulated that erythroid regulation of absorption is controlled through Hepcidin. Growth differentiation factor 15, is a member of the transforming growth factor beta family. Recent evidence suggests that when it is over expressed because of an expanded erythroid compartment, this contributes to iron overload in thalassemia syndromes by inhibiting hepcidin expression (15).

Transport, uptake and storage of iron

Tf receives and binds iron for delivery to receptors at recipient cells. Apotransferrin is bilobular and each of the duplicated lobes binds one atom of Fe(III) and one carbonate anion to become Tf. This Tf then delivers iron to cells by binding to TfRs, after which the apotransferrin is returned to the plasma to again function as an iron transporter (16). The autosomal recessive disorder, classic hemochromatosis (HH), is most often caused by mutation in a gene designated HFE on chromosome 6p21.3. It has been shown that the HFE protein competes with Tf for binding to the receptor, thereby impeding the uptake of iron from Tf. Mutation of the HFE gene abolishes this competition, thereby increasing the access of Tf and its iron to cells (17).

Iron uptake by cells is essential for cellular growth and proliferation and is mainly carried out via TfR. TfR1 has been studied extensively (18) while the role of TfR2 is not clear (19). TfR1 mediates the uptake of Tf-bound iron and remains the major regulatory site for iron homeostasis (20,21). When iron-loaded Tf attaches to TfR1, this interaction triggers clathrin-mediated invagination of the cell membrane and formation of intracellular Tf-TfR1-containing endosomes. The proton pumps present in the endosomal membrane pumps H^+ ions into the endosome from the cytoplasm, which induces a conformational change of Tf and its receptor resulting in the release of iron. The released Fe^{3+} is converted to Fe^{2+} by STEAP3, a metalloreductase present in the endosomes (22,23). The Fe^{2+} is then transported out of the

endosomes by DMT1. Human TfR1 expression is regulated by altering the mRNA stability (24) or by the binding of HFE (25), and also by hypoxia (26). Erythroid cells contain the highest number of TfRs which are released from reticulocytes during their maturation to erythrocytes (27). Human TfR shedding is caused by an integral membrane metalloprotease releasing a soluble form, soluble TfR (sTfR) into the plasma. This process is controlled by Tf (28) and the level of sTfR is an indicator of available functional iron, independent of the iron stores.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been identified on the macrophage cell surface where it acts as a novel TfR. The GAPDH–Tf complex is subsequently internalized into the endosomes. Iron depletion increases the expression of GAPDH on the cell surface (29).

There is compelling evidence that although, under normal circumstances, Tf-bound iron uptake is the predominant form of iron uptake by cells, there are alternative mechanisms of iron transport. In normal individuals, the plasma concentration of non-Tf-bound iron (NTBI) is extremely small (30–32). A candidate iron transport protein has been identified in the brush border cells of the small intestine (33). Recently, TfR2 in CHO cells and Zip14 (Slc39a14) in HEK 293H cells and Sf9 insect cells have been identified as transporters of NTBI. (34,35). DMT1 has also been implicated in transport of NTBI. Penetration of NTBI into mitochondria from cytosol occurs very rapidly and is resistant to high-affinity iron-binding chelators (36).

Other iron uptake mechanisms also exist. Calcium channels also act as transporters of iron into cardiomyocytes under iron-overloaded conditions (37). A member of the lipocalin super family (24p3/Ngal) delivers iron to the cytoplasm where it activates or represses iron-responsive genes during organogenesis (38). Protein bound forms of iron such as isoferritin (39) and hemoglobin can be taken up by the cells. Hemoglobin released by intravascular hemolysis is bound by haptoglobin and then taken up by the scavenger receptor (CD163) present on monocytes and macrophages (40). Studies have identified H-ferritin as an iron transport protein and suggest the presence of an H-ferritin receptor for mediating iron delivery in brain and multiple organs (41). The presence of different iron uptake mechanisms helps in accommodating different forms of iron and also aids in differential regulation. Macrophages acquire iron from old senescent RBCs which at the end of their functional lifetime are phagocytosed and catabolized at extra vascular sites by Kupffer cells and splenic macrophages.

Ferritin is an ubiquitous, water soluble protein for iron storage. The level of serum ferritin parallels the concentration of storage iron within the body, regardless of the cell type in which it is stored. Normally, 95% of the stored iron in liver tissue is found in hepatocytes as ferritin. Hemosiderin constitutes the remaining 5% and is found predominately in Kupffer cell lysosomal remnants. Ferritin expression is regulated mainly at the post-transcriptional level by iron regulatory proteins (IRP1 and IRP2) (42). Pro-oxidants induce the expression of ferritin H subunit through an antioxidant/electrophile response element (43).

Iron and mitochondria

Although excess iron is stored in the cytoplasm, most of the metabolically active iron is processed in the mitochondria of the cell for the synthesis of iron-sulfur clusters (Fe-S) and heme. Little is known about how these organelles regulate iron homeostasis and toxicity. Data obtained from studies using yeast strains show that iron delivery is mediated through Mrs3/4p found in the inner membrane of mitochondria in yeast (44). Knock out models of Mrs3/4p revealed that there may be additional routes. A human homolog of Mrs3/4p has been identified (45). Studies from a zebra fish mutant, frascati helped to identify mitoferrin, which functions as a principal iron importer in vertebrate erythroblasts (46). Recently it has been shown that the endosomes come in contact with mitochondria and facilitate direct transfer of the metal from cytosol to mitochondria (47). Once inside the mitochondria, iron can be used for a variety of metabolic pathways. Frataxin, a mitochondrial matrix protein enhances the bioavailability of iron for Fe-S cluster biosynthesis and heme synthesis (48,49). It is also postulated to play a role in iron export and storage (50). The mitochondrial ferritin (MtF) acts as the storage site. MtF has a high degree of sequence homology with human H-chain ferritin. MtF and cellular ferritin reveal striking differences in their iron oxidation and hydrolysis chemistry despite their similar ferroxidase centers (51). Experimental over expression of MtF results in mitochondrial iron accumulation, decreased cellular ferritin, and increased TfR1 expression. MtF plays a protective role against iron mediated toxicity in the mitochondria. Export of heme from mitochondria is through an unknown transporter, whereas the [Fe-S] clusters may be exported to the cytosol via the mitochondrial inner membrane transporter, ABCB7 (52). The ATP-binding cassette transporter (ABCB6) present in the outer mitochondrial membrane has also been associated with iron homeostasis and porphyrin transport (53,54). It is proposed that an Fe-S protein may act as a sensor of mitochondrial iron status. Iron transport into mitochondria is directly coupled to its uptake at the cell membrane

and iron transport out of mitochondria depends on adequate iron–sulfur cluster synthesis. Regulatory mechanisms in the cytosol would then sense a postmitochondrial iron pool (55). Defects in mitochondrial Fe-S cluster assembly and export can also affect iron uptake in mitochondria (56). GTP in the mitochondrial matrix is also involved in the mitochondrial iron homeostasis. Mutations in the GTP/GDP carrier protein Ggc1p, results in accumulation of iron within mitochondria (57).

Regulation of iron homeostasis

The major pathways and the key players involved in iron absorption, transport and storage have been illustrated (Fig. 1). Cellular and systemic iron imbalance is detrimental and so these processes require a tight regulation. As iron cannot be effectively excreted, regulation is mainly at the absorption level. A tight control of iron homeostasis is also imperative as resistance to infection is in part dependant on the outcome of a tug-of-war over
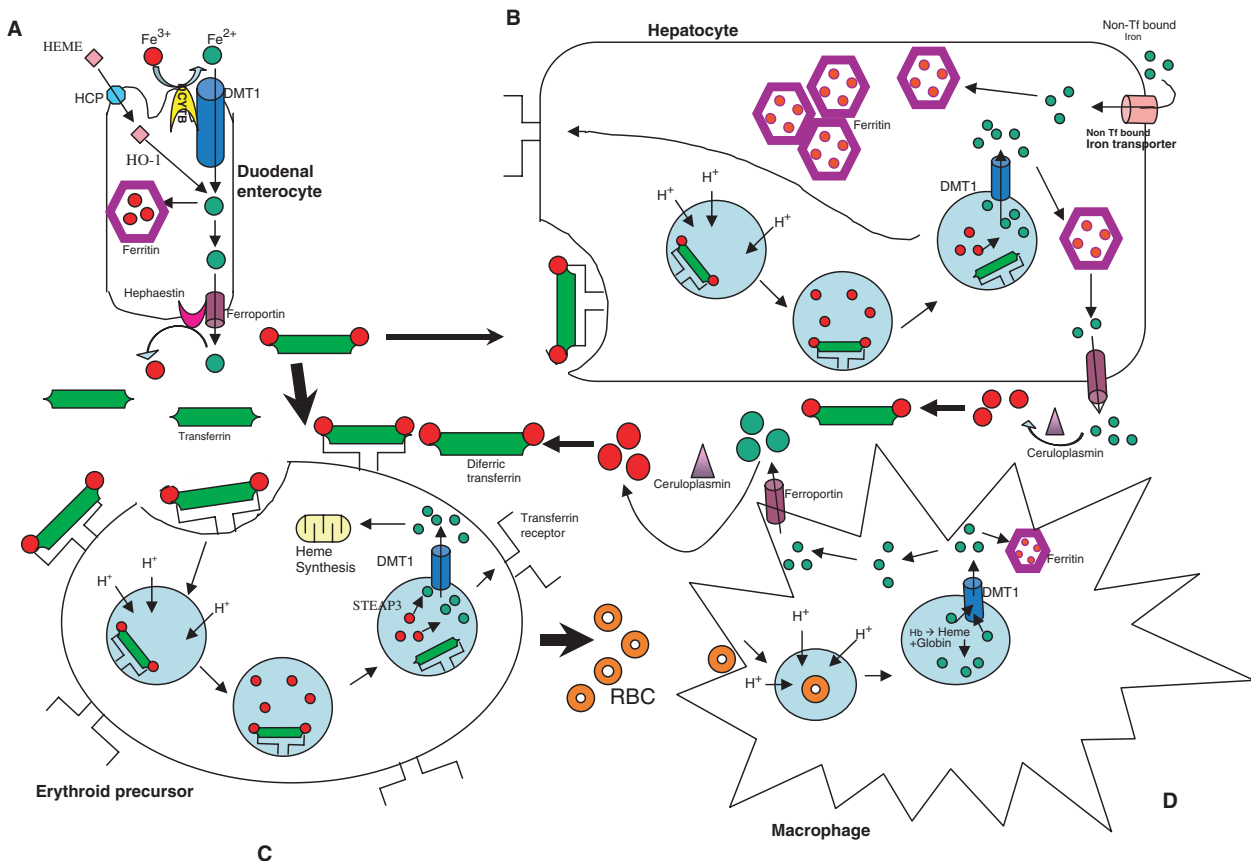

Figure 1 Proteins and pathways involved in non-heme iron absorption, transport and storage, (A) Non-heme iron in the food exists mainly in the Fe^{3+} state. It is first reduced to the bio-available Fe^{2+} state by duodenal cytochrome b reductase and dietary components before it is transported inside the enterocyte by divalent metal transporter (DMT1). Inside the cell it can be stored as ferritin or transported out with the help of ferroportin (FPN). Heme iron enters the cell through a heme carrier protein. Inside the cell it is opened up by heme oxygenase-1 and the released Fe^{2+} takes a similar path as of non-heme iron. The Fe^{2+} leaves the enterocyte through FPN and it is again oxidized to Fe^{3+} by Hephaestin before being bound by transferrin (Tf) and transported to different tissues. (B) The storage site of iron is the liver. The Tf-bound iron is taken up by transferrin receptors (TfR) present on the cell surface. Excess iron is stored as ferritin in the hepatocytes. The mechanism of transport of non-Tf-bound iron into the hepatocytes remains obscure although several pathways have been hypothesized. The storage iron is released in times of iron requirement by hydrolysis of ferritin to yield Fe^{2+} ions. The Fe^{2+} is converted to Fe^{3+} by ceruloplasmin before it is transported by Tf. (C) The majority of iron is utilized by erythroid cells which take up Tf-bound iron via TfR 1. The TfR are endocytosed and Tf-bound iron is released due to a change in pH inside the endosomes. The released Fe^{3+} is converted to Fe^{2+} by STEAP3. The Fe^{2+} is transported out of the endosomes by DMT1. Fe^{2+} is transported to mitochondria for heme synthesis, a crucial step for Hb formation. (D) Macrophages engulf senescent RBCs and recirculate the iron. The hemoglobin released is cleaved into heme and globin. The heme molecule is degraded and Fe^{2+} is transported out by DMT1. The Fe^{2+} released can be stored either as ferritin or transported out through FPN depending on the iron stores. In circulation, iron (Fe^{3+}) is bound to Tf. Each Tf molecule can bind two molecules of Fe^{3+}. Ceruloplasmin helps in the oxidation of Fe^{2+} to Fe^{3+} and facilitates the transport of iron released from macrophages and hepatocytes.

iron between the host and the invading bacteria. This dual challenge of avoiding iron deficiency and iron overload involves distinct regulatory mechanisms to achieve iron homeostasis both at the cellular and systemic levels.

Regulation of cellular iron homeostasis

Cellular iron homeostasis depends on co-ordinated regulation of proteins involved in iron uptake, storage, utilization and export. Cells react to iron deficiency by up regulating TfRs to maximize internalization of Tf-bound iron. Conversely, synthesis of iron storage protein is halted to enhance metal availability. Intracellular iron metabolism is mainly controlled at the post-transcriptional level by the iron-responsive elements (IRE)/IRP system and this remains the best characterized system of post-transcription gene expression (58).

Post-transcriptional regulation of intracellular iron levels involves interaction between transacting IRP and structural motifs known as IRE. These are found in the mRNAs encoding proteins which are involved in storage, erythroid heme synthesis, the TCA cycle, iron export and iron uptake. IREs were first identified in the 5′-UTR of ferritin H- and L-chain mRNAs and were noted to mediate inhibition of ferritin mRNA in iron deprived cells (59). The formation of IRE–IRP complexes in the 5′-UTR results in inhibition of the translation process because of proximity of the IRE to the transcription start site (60). IRE/IRP complexes in such cases act as steric inhibitors of 43 S preinitiation complex binding. Alternatively the interaction between IRP and IRE in the 3′-UTR results in stabilization of the mRNA of the TfR1. In iron deficient cells the IREs become targets for the IRPs which bind to them with high affinity resulting in stabilization of the unstable TfR1 mRNA and translational inhibition of mRNAs coding ferritin chains. This enables the cells to increase the uptake of Tf-bound iron by TfR and minimize sequestration into ferritin. Conversely, in cells with enough iron, the IRE/IRP interaction fails resulting in decreased iron uptake and increased storage.

IRPs exist in two isoforms – IRP1 and IRP2 that share extensive sequence homology and belong to the family of iron–sulfur cluster isomerases (61). IRP-1 and IRP-2 exhibit similar high affinities for wild type IREs and are strongly induced in iron deficient cells with rapid loss of IRE binding after iron administration (62). IRP1 is ubiquitously expressed and this may also be the case with IRP2. IRPs may be regulated by effectors other than the cytoplasmic labile iron pool resulting in modulation of cellular iron metabolism. Reactive oxygen species and serine phosphorylation (of IRP1) can lead to disassembly of Fe-S clusters leading to IRP1 regulation (63,64). Nitric oxide and hypoxia can affect both IRPs

(65). The complex regulation of IRPs by environmental stimuli at the post-translational level in turn results in fine tuning of important metabolic pathways in the human body. IRPs have evolved in such a way that they sense and control cellular iron levels so as to maintain the critical balance between ensuring adequate iron supply and avoiding toxic iron excess (66).

In addition to post-transcriptional modifications in iron-related genes, cytokines also play a major role in cellular iron homeostasis. Cytokines like tumor necrosis factor-α, interleukein-1, interleukin-6 and interferon-γ stimulate H-ferritin and TfR1 expression (67,68). The finding of IREs in two duodenal transport proteins, FPN and DMT1 strongly suggests that the IRE/IRP regulatory system may have a role in systemic iron regulation (69). IRP1$^{-/-}$ mice have a mild phenotype suggesting that IRP2 can fully compensate for the loss of IRP1 function. IRP2$^{-/-}$ mice however develop a neurodegenerative phenotype secondary to iron overload (70).

Regulation of systemic iron homeostasis

Systemic regulators control iron entry and mobilization from the stores to fulfill the erythropoietic needs and to scavenge the previously used iron. Iron balance is maintained by meticulous regulation of iron absorption because of the paucity of well developed regulated pathways for iron excretion. Based on segregation of iron requirements within an organism, several 'regulators' for iron homeostasis have been hypothesized. Iron absorption is modulated by the amount of iron consumed in the diet, a mechanism referred to as the 'dietary' regulator (71). This phenomenon was previously called the 'mucosal block'. A second regulatory mechanism termed the 'stores' regulator, senses iron level in the circulation and responds to the total amount of body iron (72). The 'stores' regulator may involve regulation at the level of crypt programming and facilitates a slow accumulation of non-heme iron rather than heme-iron. As this regulator must signal between the liver, muscle and intestine a soluble component is hypothesized. A third regulatory mechanism, that modulates iron absorption in response to the erythropoietic demands, is known as the 'erythropoietic' regulator. The 'erythropoietic' regulator is a stronger inducer of iron absorption in comparison to the 'stores' regulator (72). Iron absorption is increased in response to acute hypoxia through a humoral 'hypoxia' regulator. Whether this regulatory pathway is truly distinct from the one induced by the erythropoietic regulator is uncertain. Cellular iron retention in a setting of infection and/or inflammation may be a mechanism to withhold a nutrient from an invading organism. Such iron accumulation in macrophages may be considered as an 'inflammatory' regulator under normal circumstances.

The different regulators need not be different and may represent differential responses mediated by the same molecules (73). The search for regulatory effectors that modulate iron absorption and release from tissue stores has made considerable progress with the identification of genes involved in hemochromatosis (HFE, TfR2, and HJV) and the discovery of hepcidin. Hepcidin (HAMP, LEAP) is thought to be the central regulator of iron homeostasis and acts as a final point for all other regulators.

Hepcidin which is secreted by the liver and excreted by the kidneys appears to be the central regulator of iron metabolism (74,75). It is also produced in small amounts by inflammatory monocytes and macrophages (76). Synthesis of hepcidin is greatly stimulated by inflammation and iron overload (dietary and parenteral) as shown in murine models (77,78). Based on USF-2 gene knockout models hepcidin is believed to be the predominant negative regulator of iron absorption in the small intestine and iron release from the macrophage (79). Transgenic mice constructed to over-express hepcidin died shortly after birth with iron deficiency, indicating that hepcidin was a negative regulator of placental transport of iron to the fetus (80). Hepcidin acts on the iron exporter of the enterocytes and macrophages and suppresses iron uptake and iron release (81). It also inhibits DMT1 expression (82). Hepcidin expression is influenced by a variety of factors and molecules – hypoxia, erythroid demand, iron, inflammation, copper deficiency, bone morphogenic proteins (BMP), and p53. Erythropoietic demand suppresses hepcidin expression and GDF 15 is thought to play a key role in this (15).

HFE, HJV and TfR2 are upstream modulators of hepcidin. The discovery that HFE was associated with the TfR was the first indication that HFE may regulate iron metabolism (83). HFE-associated hereditary HH has been described as a disorder of enterocyte iron regulation wherein there is an increase in the iron regulatory set point resulting in chronic increase in the rate of iron transfer from enterocyte to blood. It is postulated that HFE interacts with a protein in a signal transduction pathway that stimulates hepcidin (84,85). HFE's role in regulating iron balance remains enigmatic and more investigations are required to elucidate the exact mechanism.

Hemojuvelin (HJV) is an important upstream regulator of hepcidin which was discovered recently. The role of HJV in iron homeostasis has been confirmed by knockout models, which manifest increased iron deposition in liver, pancreas and heart (86). Mutated HJV suppresses hepcidin expression and this has been observed in both juvenile HH patients and in HJV$^{-/-}$ mice (87,88). Recent studies indicate that HJV alters hepcidin expression through a BMP-mediated signaling pathway (89).

BMPs bind to HJV as co-receptors and regulate the transcription of hepcidin through SMAD/SMAD4 complexes (90).

Unlike TfR1, the role of TfR2 is not clearly elucidated. It also binds ferric-Tf but plays a major role in systemic iron homeostasis. It is highly expressed in liver and mutations in this gene result in HH (91). As disruption of TFR2 causes decreased hepcidin production, it appears to be involved in the upstream regulation of hepcidin. Increased serum Tf saturation results in the release of HFE from HFE–TfR1 complex and binding to TFR2. This initiates a cascade signaling which results in the production of hepcidin. Mutation or absence of HFE or TFR2 affects this signal cascade leading to dysregulation of systemic iron homeostasis (92).

Applied aspects – understanding human disease

The advances in iron metabolism and regulation have revolutionized the understanding of the molecular basis of iron related diseases and may lead to the identification of potential therapeutic targets and novel treatment strategies. Animal models have contributed greatly in understanding human diseases of iron metabolism. (Table 1).

Anemia of chronic disease

Anemia of inflammation or anemia of chronic disease (ACD), is commonly observed in patients with chronic infections, malignancy, trauma, and inflammatory disorders, and is a well-known clinical entity. It is characterized by low serum iron levels (hypoferremia), low serum iron-binding capacity and normal to elevated ferritin concentrations. ACD is immune driven and iron homeostasis is altered by cytokines and cells of the reticuloendothelial system. Myeloid cells, when challenged by bacterial pathogens, produce hepcidin in a TLR4 (toll-like receptor 4)-dependent manner. Hepcidin expression is induced by lipopolysaccharide and interleukin-6 and is inhibited by TNF-α (93). Hepcidin is involved in the diversion of iron and acts as a link between iron homeostasis and inflammation. It reduces iron absorption and release from macrophages, thereby reducing iron supply for erythropoiesis. It decreases iron absorption and iron release from the macrophages. Bacterial stimulation also triggers a TLR4-dependent reduction in the expression of FPN (76). IL-6, a cytokine released during inflammation mediates the release of hepcidin through STAT3 (94,95). TNF-α, another cytokine, causes iron sequestration in the spleen, reduces duodenal iron transfer by increasing ferritin levels in the enterocyte and leads to loss of function of FPN in mice (96). HJV is also thought to act in concert with hepcidin in the pathophysiology of ACD (97).

Table 1 Animal models associated with iron metabolism and their corresponding human phenotypes

Animal model	Human disease	Gene	Phenotype	Reference
HFE$^{-/-}$, HFE$^{C282Y/C282Y}$	Hemochromatosis (type 1)	HFE	Hepatocellular iron deposition decreased macrophage iron, elevated Tf saturation and ferritin	(126)
Hepc 1$^{-/-}$	Juvenile hemochromatosis	HAMP	Hepatocellular iron deposition Decreased macrophage iron	(127)
Hjv$^{-/-}$	Juvenile hemochromatosis	HJV	Hepatocellular iron deposition Decreased macrophage iron Elevated Tf saturation	(87)
TfR2$^{-/-}$	hemochromatosis (type 3)	TFR2	Hepatocellular iron deposition Decreased macrophage iron Elevated Tf saturation	(128)
Weh (zebra fish)	ND	Ferroportin	Hypochromic anemia, impaired iron transfer from yolk sac to embryo	(5)
Nm 1054	ND	Not known	Hypochromic microcytic anemia Failure to thrive, infertility Hydrocephaly	(121)
hpx	Atransferrinemia	TFR	Microcytic hypochromic anemia Tissue iron deposition	(129, 130)
TfR$^{+/-}$ Chianti (zebra fish)	ND	TFR1	Microcytic hypochromic RBCs Decreased iron stores	(114, 131)
Belgrade rat	Iron deficiency anemia with tissue iron overload	DMT1	Systemic iron deficiency, impaired iron uptake in duodenum and erythroid precursors Hepatic iron overload	(132)
Shiraz (zebra fish)	ND	Grx5	Anemia, loss of iron cluster assembly	(123)
Cp$^{-/-}$	Aceruloplasminemia	Cp	Iron accumulation in hepatocyte and macrophages	(133)
sla mouse	ND	HEPH	Microcytic hypochromic anemia	(122)
Frda– tissue specific k.o neuron/heart	Friedreich ataxia	Frataxin	Mitochondrial iron deposits, neurodegeneration, cardiomyopathy	(134)
Hemoglobin deficit mouse	ND	Sec15l1	Microcytic hypochromic anemia	(135)

ND, not described.

Hemochromatosis

Hemochromatosis refers to a clinical disorder of iron excess resulting in end organ damage. The disease manifestations include cirrhosis, diabetes mellitus, hypogonadism, cardiomyopathy, and arthropathy and skin pigmentation. Identification of genes which are mutated has resulted in the introduction of molecular tests that allow early and presymptomatic diagnosis. HH is a genetically heterogeneous disorder and may result from mutations in at least four genes. In the first three, systemic hepcidin deficiency is the major pathogenetic mechanism.

Mutations in the HFE gene are responsible for type 1 HH and this accounts for more than 90% of patients with HH. C282Y and H63D are the two most common mutations observed in population screening (98). The C282Y mutation replaces a cysteine, required for a disulfide bridge, which is necessary for the binding of HFE to β2-microglobulin (99). The HFE$^{-/-}$ mice exhibit profound abnormalities in iron homeostasis. The abnormally high Tf saturations and excessive accumulation of iron in

liver, seen even on a standard diet, demonstrate that the HFE gene is a key regulator of iron homeostasis. Emerging data relate HFE to hepcidin activation in liver. Hepcidin levels are inappropriately low for the level of iron overload in HFE HH (100). In one study, variable levels of hepcidin ranging to almost normal values in serum and urine were found in HFE-HH patients. The reason remains unknown (101).

Juvenile HH (type 2) is a recessive iron overload disorder where hypogonadism and cardiac failure are more frequent presenting symptoms. It results from mutations in the HJV (type 2A) gene or the gene coding for hepcidin, HAMP (type 2B) (102,103). However, the commonest gene involved, however is the HJV gene on the long arm of chromosome 1. The clinical picture of TfR2 HH (type 3) is more severe than HFE related HH and has an earlier age of presentation. TfR2 senses the body iron status by sensing saturation of Tf (104). TfR2 protein may also be involved in the erythroid regulator pathway.

FPN (SCL40A1) – FPN Disease (type 4) is a dominant form of iron overload characterized by iron

accumulation in the macrophages but low/normal Tf saturation. Heterozygous mutations of the SCL40A1, the gene encoding FPN, have been documented (105,106). FPN mutants may reach the membrane but are resistant to the action of hepcidin, resulting in hepcidin resistance, and a HH like phenotype (107).

Rare genetic defects of iron loading and iron deficiency

There are rare genetic conditions leading to iron deficiency and iron overload in the tissues that pose diagnostic and therapeutic difficulties (Table 2). Divalent metal transporter is responsible for iron transport at the brush border of the enterocyte and from endosomes to cytosol. Mutations in DMT1 are a rare cause for iron deficiency and overload. Iron deficiency is caused by defective absorption while iron overload may be related to the upregulation of the heme iron absorption pathway that bypasses the DMT1 defect. Both mk mice and Belgrade rats, which carry an identical DMT1 mutation (G185R), exhibit severe microcytic anemia at birth, defective intestinal iron absorption and erythroid iron utilization. Mutations in DMT1 in humans are characterized by hypochromic microcytic anemia and hepatic iron overload. The first case of homozygous DMT1 mutation (1285G > C exon 12 skipping) in humans has been reported recently. The patient had hypochromic microcytic anemia with significant hepatic iron overload (108). Another novel mutation, described subsequently, is a 3bp deletion in intron 4 (c.310–315delCTT) resulting in a splicing abnormality and a C → T transition at nucleotide 1246(p. R416C) (109). Two more reports of DMT1 mutations have also been published (110,111).

Another gene identified in iron refractory iron deficiency anemia is TMPRSS6. It encodes a type II trans-membrane serine protease which is produced by the liver and regulates the expression of the systemic iron regulatory hormone, hepcidin. It is essential for normal systemic iron homeostasis in humans. Mutations in this gene have been associated with iron deficiency anemia refractory to iron. The key features are: a congenital hypochromic, microcytic anemia; a low Tf saturation; abnormal iron absorption, characterized by no hematological improvement following treatment with oral iron; abnormal iron utilization characterized by a sluggish, incomplete response to parenteral iron (112) .

Hypotransferrinemia is a rare recessive disease characterized by an extremely low Tf level (< 10 mg/L), severe iron deficiency anemia and iron loading of the liver and other non-erythroid organs (113). This condition emphasizes the importance of the Tf-TfR1 pathway for erythroid iron uptake. Alternative pathways for internalization of non-Tf-bound iron result in the iron overload. Extremely low hepcidin levels in the hypotransferrinemic mouse hpx explain the increased intestinal iron absorption. Low hepcidin may be secondary to anemia or lack of TfR2 signaling (104) . Tf replacement by plasma transfusion is the treatment of choice in these patients.

The Tf cycle is essential for iron uptake. Homozygous TfR mutant mice die between 9.5 and 11.5 d postconception. Heterozygote mutants present with microcytosis though they are not anemic (114). This interesting phenotype suggests that some humans with unexplained microcytosis may be heterozygous for TfR1 gene mutations.

Aceruloplasminemia is a rare autosomal recessive disorder resulting from deficiency of the ferroxidase activity of ceruloplasmin that is responsible along with FPN for iron export out of the macrophage and hepatocytes. The

Table 2 Rare causes of iron deficiency and iron overload in humans

Gene	Inheritance	Defect	Findings	Reference
DMT1	Autosomal recessive	Iron deficiency anemia	Hypochromic microcytic anemia Hepatic iron overload	(108–111)
Tf	Autosomal recessive	Hypotransferrinemia	Severe iron deficiency anemia Iron loading of the liver Low hepcidin levels	(113)
TfR	Autosomal recessive	Heterozygous for TfR1 mutation	Microcytosis	(136)
Cp	Autosomal recessive	Aceruloplasminemia	Anemia Diabetes mellitus Iron loading of liver, pancreas Neurological and retinal degeneration	(115)
ABCB7	X-linked	X-linked sideroblastic anemia with ataxia	Anemia Iron accumulation in mitochondria	(116, 117)
TMPRSS6		Iron refractory iron deficiency anemia		(112)

DMT1, divalent metal transporter; TfR, transferrin receptor.

disease is characterized by diabetes mellitus, iron loading of the liver and pancreas and neurological and retinal degeneration (115). Mild anemia is present in early life. Serum iron and Tf saturation are low but elevated ferritin should raise the suspicion. Murine models indicate that the iron excess may be related to defective cellular iron flux.

Defects in ABCB7, a transporter which is responsible for transport of iron and Fe-S clusters from mitochondria, causes X-linked sideroblastic anemia with ataxia (116,117). This is characterized by iron accumulation in the mitochondria, iron which is therefore not available for heme synthesis. Mutations in the Frataxin gene are responsible for mitochondrial iron deposits, neurodegeneration and cardiomyopathy in Friedreich ataxia (118). A functional deficiency of frataxin affects intracellular iron distribution and Fe-S cluster biogenesis.

The hemoglobin deficit mouse, suffers from a microcytic, hypochromic anemia and exhibits a defective iron transport in the endocytosis cycle. An exon deletion in the candidate gene Sec15l1, a key protein in vesicle docking has been described as the cause of defective endocytosis (119,120). A recessive, hypochromic, microcytic anemia mouse mutant, nm1054 has been reported. It is caused by an intrinsic hematopoietic defect, resulting in inefficient Tf-dependent iron uptake by erythroid precursors (121).

Sex-linked sla mice, carry mutations in the hephaestin gene-ferrous oxidase, which along with FPN is responsible for basolateral iron transport (122). These mice are anemic at birth because of defective placental iron transfer. The anemia disappears with age though low iron stores persist. These two mutations have not been described in humans.

The hypochromic anemia in shiraz (sir) zebrafish mutants is caused by deficiency of glutaredoxin 5 (grx5), a gene required in yeast for Fe-S cluster assembly (123). Loss of Fe-S cluster assembly activates IRP1 and blocks heme biosynthesis leading to anemia.

The constellation of microcytic anemia, increased tissue iron deposition, and neurodegeneration has been documented in IRP2-deficient mice. Here, the iron limited erythropoiesis is because of reduced TfR expression in erythroid precursors (124). Hyperferritinemia-cataract syndrome is caused by a heterozygous mutation of the IRE of L-ferritin resulting in deregulated synthesis of ferritin. The only pathological consequence appears to be early cataract (125). The pathogenesis of neonatal iron overload and reticuloendothelial iron overload in African-American individuals (described initially in Bantu population) is as yet unknown.

Recent advances in identifying specific proteins involved in iron metabolism and their regulation have provided glimpses of the elaborate inter-connected circuitry operational in the human body. These new insights have had a major impact in understanding the pathophysiology of iron-related genetic disorders. However, the urgent need is to further our understanding of intestinal iron absorption at the molecular level and how it is linked to cellular iron balance. Polymorphisms in many of these genes may underlie individual susceptibility to both iron deficiency and iron overload in the presence of environmental excess or decreased availability of iron. New molecules to treat iron overload are urgently required, both for genetic and acquired disorders, where iron overload has devastating consequences and they may be developed earlier because of this new understanding of the players which regulate iron homeostasis in the body.

Acknowledgements

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