Review

Iron from the gut: the role of divalent metal transporter 1

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Mammalian cells contain thousands of metalloproteins and evolved systems to correctly incorporate metal cofactors into their designated sites. Among the transient metals in living cells, iron is the most abundant element that present as an iron sulfur cluster, mono- and dinuclear iron centers or heme for catalytic reactions. Iron homeostasis is tightly regulated by intestinal iron absorption in mammals owing to the lack of an iron excretive transport system, apart from superficial epithelial cell detachment and urinary outflow reabsorptive impairment. In mammals, the central site for iron absorption is in the duodenum, where the divalent metal transporter 1 is essential for iron uptake. The most notable manifestation of mutated *divalent metal transporter 1* presents as iron deficiency anemia in humans. In contrast, the mutation of *ferroportin*, which exports iron, causes iron overload by either gain or loss of function. Furthermore, hepcidin secretion from the liver suppresses iron efflux by internalizing and degrading ferroportin; thus, the hepcidin/ferroportin axis is extensively investigated for its potential as a therapeutic target to treat iron overload. This review focuses on the divalent metal transporter 1-mediated intestinal iron uptake and hepcidin/ferroportin axis that regulate systemic iron homeostasis.

***Key Words*:** **iron, divalent metal transporter 1 (DMT1), ferroportin, hepcidin, oxidative stress**

In mammalian cells, >30% of proteins require a transition metal cofactor that binds to the protein at the active site; thus, a lack of micronutrients (including transition metals) causes clinical symptoms.[1] Among the micronutrients, iron, zinc, and vitamin A are essential in preschool-aged children, while iron, zinc, and folate are required by women of reproductive age due to a high prevalence of deficiency.[2] A worldwide survey revealed the wide spectrum of the micronutrient deficiency or overload, especially iron across different countries, warranting periodical reassessments. In Japan, the recommended iron daily intake is 10 mg for men aged >20 years and menopausal women and 15 mg for menstruating women.[3] On an average, 3–5 g of iron is stored in the adult body, while approximately 25 mg iron/day is released from the reticuloendothelial system. Subsequently, iron is incorporated into the erythron and 1–2 mg iron/day is absorbed to equilibrate the lost iron by detached epithelial cells in the digestive tract and skin, minor blood loss, sweat, and urine.[4] However, the excretory mechanism of active iron transport is lacked; thus, iron balance is tightly regulated by intestinal absorption. These results indicate iron recycling is considered a "semi-closed system".

Iron catalyzes DNA synthesis, metabolic energy production, organic compound biosynthesis, oxygen transport, and reactive oxygen species (ROS) generation, making it an essential transition metal in nearly all organisms.[5] In cells, >95% of iron is protein-bound, either directly by protein residues or iron-containing groups, such as heme or iron sulfur clusters. In cytosolic and organellar components, various states and forms of iron are present with millimolar concentrations of organophosphates, carboxylates, amides, thiolates, and hydroxylates.[6] Iron has two common oxidation states: ferrous (Fe^{2+}) and ferric (Fe^{3+}). Furthermore, more high-valent iron-oxo species, such as ferryl (Fe^{4+}; $Fe^{4+}O^{2+}$) and ferrates (Fe^{5+}; $Fe^{5+}O_4^{3-}$, Fe^{6+}; $Fe^{6+}O_4^{2-}$), are also present, and these oxidize compounds rapidly, becoming the most stable ferric ions.[7–9] Ferryl intermediates are transient versatile complexes involved in substrate catalysis via peroxidases, catalases, and the cytochrome P450 family[7] or molecule deterioration via heme ferryl species or the Fenton reaction.[10,11] Furthermore, iron overload triggers cellular injury, exacerbates atherosclerosis, and dysregulates organ function, ultimately causing fetal impairment or carcinogenesis in rodents and humans. Therefore, appropriate iron management is essential to avoid oxidative damage and iron-mediated lipid oxidation-dependent cell death, namely, ferroptosis.[7,12–16]

In mammals, the intestine was identified as an entry site for iron uptake in the 1930s.[17] Enterocytes have a relatively short lifespan of <4 days in humans,[5] and once detached (spontaneously or cytotoxically), all intracellular contents are lost into the gut lumen; thus, orchestrated transcytosis from the apical side to the bloodstream via enterocytes is critical for iron uptake. Dietary iron is mostly present in the ferric state, which is reduced to ferrous ions by duodenal cytochrome *b* reductase (Dcytb)[18] or dietary reductants, such as ascorbate or microbe-produced short-chain fatty acids,[5] and subsequently absorbed from the duodenum by divalent metal transporter 1 (DMT1). Herein, the mechanisms of intestinal iron absorption, which tightly regulates iron homeostasis, are discussed to clarify the role of iron in the body.

DMT1

Hypochromic microcytic anemia is a common symptom of iron deficiency in the body. Iron deficiency anemia (IDA), caused by the loss of iron due to gastrointestinal or genital bleeding or malnutrition, is treated with oral or intravenous iron replenishment. In contrast, iron refractory iron deficiency anemia (IRIDA) is rarely observed. Mammalian iron influx transporter has been identified using two methods. In 1997, a divalent cation transporter (DCT1) was identified by forced expression of intestinal cDNA in *Xenopus laevis*.[19] In the same year, a positional cloning approach identified the natural resistance-associated macrophage protein 2 (*Nramp2*), which was originally cloned by cross-hybridization to the *Nramp1* gene,[20–22] as a causative gene to develop autosomal recessive hypochromic

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microcytic anemia in *mk* mouse.[23] The following year, a point mutation (G185R) in *Nramp2* was identified as the causative mutation for impaired duodenal iron uptake in Belgrade (*b*) rats, which also developed inherited autosomal recessive hypochromic microcytic anemia.[24] Surprisingly, G185R mutation in *Nramp2* was commonly detected in *mk* mice.

In the 3' untranslated regions (UTR) of *Nramp2* mRNA, two isoforms, that contain an iron responsive element (IRE) or do not contain the IRE (nonIRE), were identified.[19,20] The Dmt1-IRE isoform is predominantly expressed in the duodenal mucosa,[25] and the expression of duodenal iron regulatory protein 1 (Irp1), Irp2, and Dmt1-IRE mRNA and protein axis was more activated in anemic homozygous (*b*/*b*) rats than in phenotypically normal heterozygous (+/*b*) rats, suggesting G185R mutated Dmt1-IRE was induced to compensate iron uptake.[26] Furthermore, two variants in 5' DMT1 mRNA were identified, which revealed four DMT1 mRNA and protein isoforms to date (Fig. 1A).[27]

The mammalian DMT1 structure has 12 transmembrane (TM) domains with two *N*-glycosylation sites in the extracellular loop between TM 7 and 8 and intracytoplasmic N- and C-termini (Fig. 1B).[19] Among the TM domains, TMs 1–5 and 6–10 comprise two pseudosymmetric inverted repeats that intertwine in the tertiary structure, whereas TM12 is lacking in most prokaryotes.[28] An iron-binding site is located at two hinges between TM 1a and 1b, as well as 6a and 6b, which are also occupied by Mn^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, and Pb^{2+}, whereas the hinge between 6a and 6b contains a proton (H^+)-binding site. Among the amino acid methionine substitution with alanine at the iron-binding site in TM6 suppresses Mn^{2+}, Co^{2+}, and Fe^{2+} transport, while Mg^{2+} and Ca^{2+} transport is accelerated, indicative of metal discrimination by the binding site.[29] Furthermore, *DMT1*, which harbors the G185R mutation in TM4, induces selective Ca^{2+} permeability, suggesting a gain of function phenotype in *mk* and *b* rodents.[30] As conformational changes are not detected in the presence or absence of divalent metals, two mechanisms for DMT1-mediated iron transport have been proposed. One model describes iron transport via the movement of the inward-outward-facing conformation.[28] Another model indicates a bulk conformational change to an inwardly open state (Fig. 1C).[31] However, the active transport mechanism of proton and metal symporters, including whether or how the substrates are thermodynamically coupled and released, remains unclear.[32] Nramp homologs are found throughout the tree of life, and the Nramp family is subdivided into several distinct evolutionary cascades. Two Nramp homologs have been identified in mice, rats, and humans. Nramp1 and Nramp2 share 78% sequence similarity with highly conserved primary sequence motifs and secondary structures.[21,25] Polymorphisms in *NRAMP1*, also known as SLC11A1 (solute carrier family 11 member 1), which is expressed in phagocytic cells to aid damage to engulfed microbes, are associated with different rates of bacterial infection, indicating the difference between NRAMP1 and NRAMP2 that specializes in innate immunity and metal acquisition.[32]

DMT1-Mediated Iron Absorption Molecular Mechanisms

Dietary iron starvation induces Dmt1-IRE at the brush border membrane (BBM) of the duodenal villi.[25] After bolus iron feeding, Dmt1-IRE rapidly migrates from the BBM to cytoplasmic vesicles in the duodenum in heterozygous (+/*b*) and homozygous (*b*/*b*) rats, indicating that the G185R mutation does not alter endocytosis signaling.[26] However, *Dcytb*-deficient mice exhibited no change in body iron stores and hematological parameters, suggesting that non-enzymatic reactions are functional in reducing dietary ferric iron.[33] Furthermore, systemic *Dmt1* deletion caused IDA and ultimately death within 7 days, the lifespan of which could be extended by a red blood cell (RBC) transfusion.[34] The intestine-specific *Dmt1* ablation induced IDA and decreased iron storage. However, these iron deficiencies were restored by an intraperitoneal iron injection.[35] In contrast, Dmt1-IRE systemic overexpression elevated duodenal iron absorption.[36]

In the intestine, apical BBM protein mislocation, which is partially maintained by intracellular sorting and trafficking of vesicular endocytosis, is associated with malnutrition, diarrheal disorders, inflammatory bowel diseases, and cancer development,[37] indicating that the orchestrated endocytosis machinery is indispensable for iron uptake. Polarized Caco-2 cells form a tight monolayer and enterocyte-like morphology with brush border enzymes or markers in a Transwell chamber. In these cells, BBM- or basolateral membrane (BLM)- originated endocytic vesicles are transported into specific subcellular compartments.[38,39] In this model, apo-transferin (TF) and ferri-TF undergo different subcellular processes, suggesting different endocytic cycles between apo- and ferri-TF.[40] Indeed, DMT1-IRE containing BBM-derived vesicles significantly co-localized with BLM-derived apo-TF following iron-ascorbate feeding into the apical chamber for 20 min, whereas no co-localization of BLM-derived ferri-TF was detected at 20 min, suggesting that the recycling endosome signal separates iron-containing vesicles.[41] Furthermore, iron supplementation into the apical chamber decreased DMT1 in the apical membrane fraction and, in turn, elevated DMT1 in the basolateral membrane fraction in polarized Caco-2 cells, indicating BBM- and BLM-derived vesicle fusion.[42] The interaction of the DMT1 N-terminal with poly r(C) binding protein 2 (PCBP2) (which also binds to iron)[43] and the C-terminal of DMT1-IRE with peripheral-type benzodiazepine receptor-associated protein 7 (PAP7) (which regulates cellular proliferation)[44] suggests that the DMT1-containing traveling vesicle supplies iron to the intracytoplasmic labile iron pool (LIP) using guiding proteins. In the LIP, the iron concentration was estimated to range between 1–7 μM, with the majority being Fe^{2+} rather than Fe^{3+}, and forming a complex (1:1) with the cysteinyl residue of GSH. From this pool, a designated amount of iron is delivered to ferritin for storage by PCBP1 or 2.[1,45] When enterocytes sense iron requirements from the body, ferrous ions are exported by ferroportin (FPN, also called SLC40A1)[46] and are rapidly oxidized to ferric ions by hephastin (HEPH).[47] The spontaneous oxidation of ferrous to ferric ions in BLM may not be efficient as Heph was isolated from sex-linked anemia (*sla*) mice, which developed intestinal iron malabsorption and IDA at 6–7 weeks of age.[47] Indeed, systemic or intestine-specific knockout (KO) of *Heph* mice suppressed intestinal iron absorption, while *Heph* KO mice survived for up to 76–79 weeks and IDA was resolved at 10–12 weeks, similar to in *sla* mice, suggesting that Heph is important, but not essential, for optimal iron absorption.[48] Interactions between Fpn and Heph were transiently assembled by iron feeding, suggesting that a protein complex is required to enable iron export in rat enterocytes.[49,50] After export, ferric ions were loaded onto apo-TF and transported to iron-deficient locations (Fig. 1D). When *Tf* was ablated in mice, elevated systemic iron deposition with hypochromic microcytic anemia was observed,[51] which recapitulates congenital atransferrinemia.[52]

In addition to nonheme iron, which is usually associated with plants, heme, which is associated with animals, is also available as a dietary iron source. Heme is transported by heme-responsive gene 1 (HRG1, also known as SLC48A1) at the plasma membrane and phagolysosomes to maintain heme homeostasis.[53,54] However, the confirmation of heme importer remains debatable argument.[4,55] Hemin also elicited DMT1-IRE internalization in polarized Caco-2 cells after 2 h, suggesting absorbed hemin-initiated signal processing for DMT1 migration (Fig. 1D).[56] Notably, the extent to which heme is converted to nonheme iron within enterocytes or transferred in intact form to the plasma remains unknown.[57]

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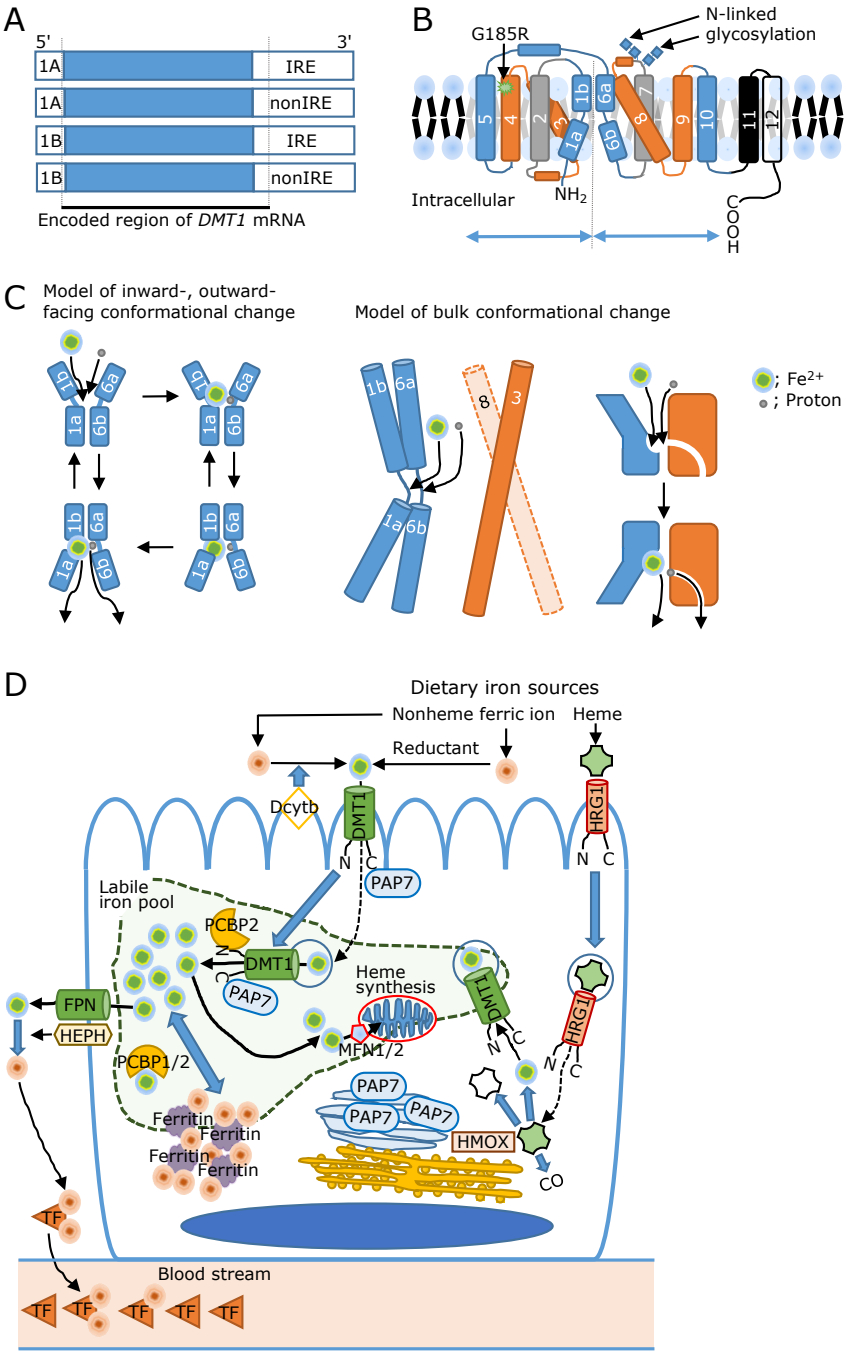

Fig. 1. Divalent metal transporter 1 (DMT1) structure and DMT1-mediated iron transport mechanisms. (A) Four transcriptional variants, which have unique amino acid in each N- or C-terminal, are spliced. (B) The mammalian DMT1 has 12 transmembrane (TM) domains, indicated by number, and both of N- and C-termini are located in intracellular side. Two *N*-glycosylation sites are located in the extracellular loop between TM 7 and 8. The secondary structure of DMT1 comprises two pseudosymmetric inverted repeats, which are separated by intertwining domains to TMs 1–5 and 6–10 in the tertiary structure. (C) The proposed mechanism of DMT1-mediated iron transport via conformational change. The two hinges between TM 1a and 1b, as well as 6a and 6b, contains an iron-binding site, while the hinge between 6a and 6b contains a proton (H^+)-binding site. The left side model indicates iron transport by the movement of the inward-outward-facing conformation.[28] In contrast, the right side model indicates bulk conformational change to the inward-open state.[31] (D) Intestinal iron absorption. Dietary nonheme ferric ion is reduced by Dcytb to a divalent state. The ferrous ion is then uptaken by endocytosis with a DMT1-containing vesicle or transported by membrane-bound DMT1 at the brush border membrane. DMT1 travels with guiding proteins, such as N-terminus interacting PCBP2 or C-terminus interacting PAP7, which is usually located at the Golgi apparatus. The ferrous iron is designated to supply cytoplasmic iron storage, which is termed the labile iron pool (LIP). At the LIP, iron is shuttled into mitochondria for heme synthesis by MFN1/2 or to ferritin for intracytoplasmic iron homeostasis by PCBP1/2. From basolateral membrane, ferrous ion is exported by FPN and subsequently oxidized to ferric ion by HEPH. The ferric ion is bound onto TF and delivered to iron-deficient organs via the blood stream. In contrast, heme is absorbed and transported by HRG1. Heme is degraded by heme oxygenase on the endoplasmic reticulum (ER) and released as ferrous ion. The internalization of DMT1 after heme feeding for 2 h suggests that DMT1 mediates orchestrated iron transport. Dcytb, duodenal cytochrome b reductase; DMT1, divalent metal transporter 1; FPN, ferroportin; HEPH hephastin, HMOX; heme oxygenase; HRG1, heme responsive gene 1; MFN1/2, mitoferrin 1/2; PAP7, peripheral-type benzodiazepine receptor-associated protein 7; PCBP2; poly r(C)-binding protein 2; TF, transferrin.

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The "mucosal block" hypothesis, which opposes transcytosis, is established by diminished intestinal iron absorption from orally administered iron to the body, likely due to exfoliative cell death in enterocytes.[42,58,59] Dietary iron overload significantly induced fetal gastrointestinal erosive hemorrhage in *Dmt1-IRE* transgenic (Tg) mice.[36] In contrast, in intestine-specific *Irp* KO mice, the mucosal block was withheld by a large excess of ferritin, but not by epithelial detachment.[60] Whole body- and intestine-specific *Heph* KO mice developed iron deposition in enterocytes contrastingly, decreased iron storage in hepatocytes was indicative of mucosal block.[48] Furthermore, double KO of *Heph* and *Ceruloplasmin*, which oxidize ferrous ions to ferric ions, developed hypochromic microcytic anemia with iron deposition in the enterocytes, liver, heart, and pancreas and died within 20–30 weeks of age.[61] When *Fpn*, the sole nonheme iron exporter identified in mammals to date, was deleted, embryonal lethality was induced in mice. Conditional *Fpn* deletion leads to iron deposition in enterocytes, which is indicative of the mucosal block phenotype.[62] Notably, not all DMT1 in BBM undergoes endocytosis with a bolus feeding of iron, suggesting a DMT1-mediated non-endocytic iron influx.[58]

Mutated *DMT1* Causes Hypochromic Microcytic Anemia

In humans, 10 published cases of *SLC11A2* mutation have been reported, presenting onset hypochromic microcytic anemia at fetal stage (1 case), birth (6 cases), infancy (1 case), 3 months (1 case), and 13 years of age (1 case), indicating that congenital *SLC11A2* mutation is extremely rare and has an early onset.[63] A single patient, diagnosed with hypochromic microcytic anemia during metrorrhagia treatment, carried compound heterozygosity of G212V and N491S in *SLC11A2* and homozygosity of H63D in *HFE*, while no mutation was detected in *SLC40A1* (ferroportin), *HJV* (homojuvelin), *HAMP* (hepatic antimicrobial peptide; also known as hepcidin), or *TfR2* (transferrin receptor 2).[64] The hyperferritinemia detection in this patient suggested an overlap of hereditary hemochromatosis (HH), which is a disorder of the iron store regulators and is caused by *HFE*, *SLC40A1*, *HAMP*, *HJV*, or *TfR2* mutations.[52,65] Notably, hepatic iron overload is not necessarily observed in *SLC11A2*-mutated patients.[66] The phenotype of the homozygous C282Y *HFE* mutation, which is responsible for >90% of HH cases in people of North European descent, varies markedly from liver cirrhosis to subclinical HH. Previously, three *SLC11A2* polymorphisms, 1245T>C, IVS4+44C>A, and IVS15Ex16-16C>G, were found not to be associated with clinical symptoms in patients carrying the homozygous C282Y *HFE*.[67] An intronic *SLC11A2* polymorphism IVS4+44C>A genotype, which has been associated with an increased risk of type 2 diabetes mellitus,[68] Wilson's disease, age-related macular degeneration, and Parkinson's disease[69] elevated the four-fold risk of IDA, despite the degree of atrophy in patients with celiac disease. These results suggest that patients with celiac disease, who have impaired duodenal mucosal uptake due to reduced absorptive surface as a result of chronic inflammation-induced villous atrophy, may unmask the *SLC11A2* IVS4+44C>A polymorphism-caused IDA.[70] Conclusively, the residual function of mutated SLC11A2 as an iron transporter and presence or absence of other mutated iron-related genes may modulate hematological parameters and present clinical symptoms.

Furthermore, a genome-wide meta-analysis yielded novel variants of *DUOX2* (*dual oxidase 2*), *F5* (*factor V*), and *TMPRSS6* (*transmembrane serine protease 6*), which are associated with IDA onset, in addition to *SLC11A2*.[71] *DUOX2* variants increase infection susceptibility by dysregulating innate immunity, whereas *F5* variants may cause blood loss by hypercoagulable state-induced thrombosis. *TMPRSS6* variants, primarily expressed in the liver and identified as a causative gene for IRIDA due to elevated hepcidin levels,[72] were also associated with IDA.

Hepcidin-Ferroportin Axis

Ferroportin is evolutionarily conserved and is found in plants and humans, whereas hepcidin was first discovered in fish when the hepcidin-binding site in ferroportin was simultaneously detected, indicating that ferroportin and hepcidin co-evolved.[73] During the unhygienic conditions of our evolutionary history, humans were under constant threat from a range of potentially fatal microbes. A host-defense mechanism, which confers extracellular pathogens by depriving iron, was recognized as "hypoferremia of infection" in the 1940s, because nearly all microorganisms are dependent on iron.[17] Hypoferremia of infection is largely mediated by hepcidin, which was invented based on the fact that hepcidin is highly expressed in the liver (hep-) and possesses microbicidal activity (-cidin).[17] Because, hepcidin was initially shown to be a member of the defensin antimicrobial peptide family.[5] In *Hamp*-deficient mice, elevated iron was deposited in liver, pancreas, and heart with high transferrin saturation, and contrastingly, splenic iron deposition was decreased.[74] Despite the common phenotype of iron overload, the mRNA levels of *Dmt1-IRE* in the duodenum were elevated in *Hfe*-null mice,[75] but not in *Hamp*-deficient mice.[74] In *Hamp* Tg mice, severe IDA and neonatal death were observed, which are indicative of negative regulators of intestinal iron absorption.[76] Indeed, among the causative molecules of HH, TfR2 interacts with HFE, which, in turn, interacts with bone morphogenetic protein receptors and the HJV complex in the plasma membrane of hepatocytes to regulate hepcidin secretion, indicating the critical role for the hepcidin-ferroportin axis in intestinal hyperabsorption of dietary iron and iron overload with consequent tissue injuries.[52,77]

Iron efflux suppression by hepcidin is triggered by binding to ferroportin, whose topology firmly establishes that ferroportin is composed of 12 TM domains and intracellular locations of both the N- and C-termini (Fig. 2A), despite the initial controversial results.[78] After binding to ferroportin, hepcidin induces ferroportin occlusion, internalization, and degradation in many cell types (Fig. 2B),[79] including enterocytes, macrophages, erythrons, and hepatocytes, which are central regulators of iron homeostasis. Furthermore, K8R-mutant ferroportin, which inhibits N-terminal ubiquitination, and C326S-mutant ferroportin, which inhibits efflux occlusion, suppress hepcidin-induced internalization and degradation.[80] Furthermore, the binding affinity of hepcidin to ferroportin was elevated to a near 80-fold change in the presence of iron, indicating that hepcidin selectively confers iron-loaded ferroportin (Fig. 2B).[81]

Clinically detectable *ferroportin* (*SLC40A1*) mutations, which are inherited in an autosomal dominant manner, are heterogeneous and classified into two broad phenotypic categories with some overlap. A group with a gain of function ferroportin mutant is caused by partial or complete resistance to hepcidin-induced occlusion or internalization. Here, hyperferritinemia with high transferrin saturation and iron induced toxic damage to the liver, heart, and endocrine organs are often observed at young ages (Fig. 2C). Another group of loss of function ferroportin mutants is characterized by hyperferritinemia without high transferrin saturation or iron-induced tissue damage. Hyperferritinemia is caused by macrophages or histiocytes that phagocytose senescent RBCs to recycle heme and non-heme iron from heme-containing proteins. These cells regulate ferroportin synthesis transcriptionally and translationally, rapidly requiring iron export after erythropagocytosis and in response to infection.[57] They also develop a defense system against iron-induced ROS; thus, symptoms of organ damage are subclinical. Furthermore, systemic hetero *Fpn* KO ($Fpn^{+/-}$), which recapitulates the inherited form in humans, demonstrated age-dependent erythropoiesis disruption and splenic iron overload. At 3 months of age, $Fpn^{+/-}$ mice were indistinguishable from their wild-type littermates, while at 6

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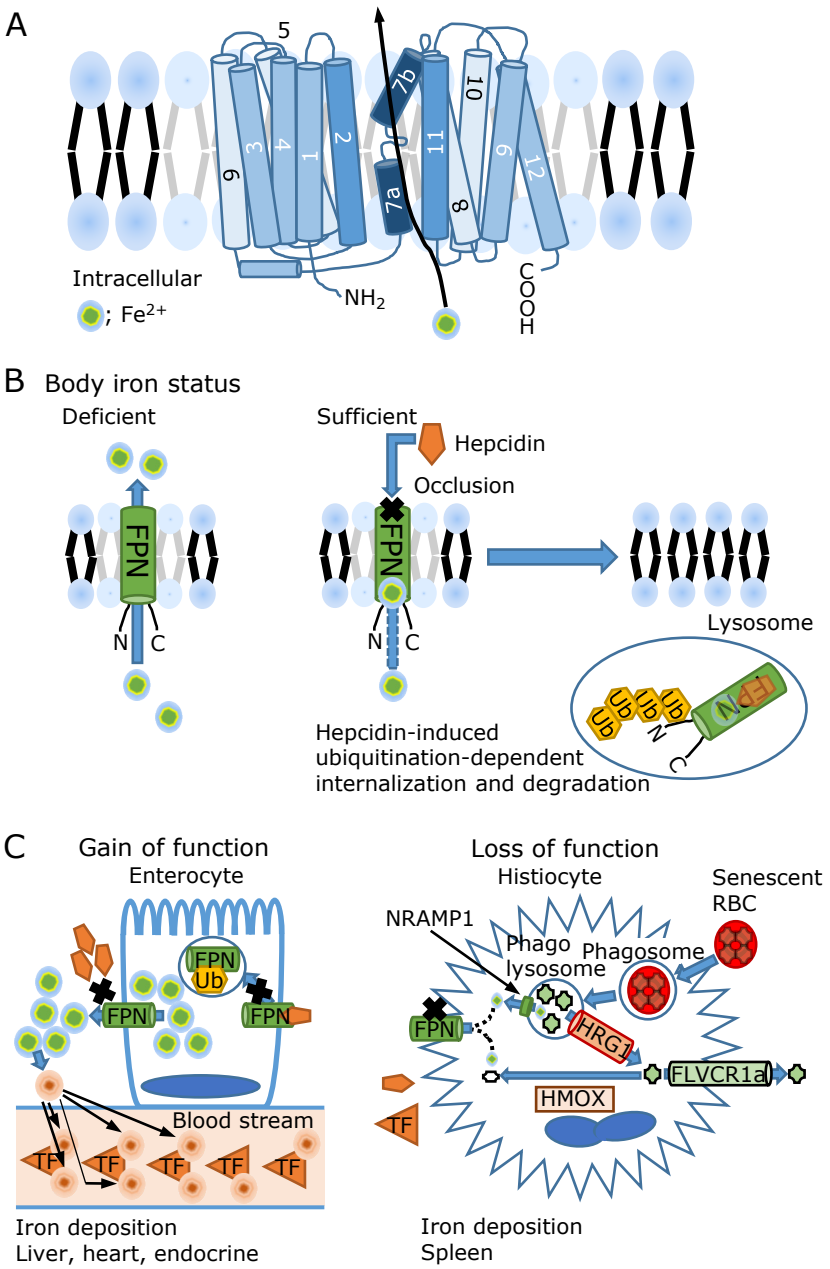

Fig. 2. Ferroportin structure, regulation, and mutation. (A) The mammalian ferroportin (FPN) has 12 transmembrane (TM) domains, indicated by number, and both of N- and C-termini are located on the intracellular side. Iron is exported from TM7a and 7b. (B) With sufficient iron, a hepcidin occludes FPN efflux and initiates N-terminus ubiquitination, subsequently, FPN is internalized and degraded. (C) The gain of function and loss of function in *FPN* causes hereditary hemochromatosis. The gain of function type is characterized by hyperferritinemia with high transferrin (TF) saturation and iron overload in the liver, heart, and endocrine organs. All clinical FPN mutants are functionally resistant to hepcidin, caused by impaired binding or ubiquitination that induces hepcidin overexpression. While the loss of function type is not clinically severe, with hyperferritinemia and iron overload in splenic histiocyte, which phagocytoses senescent red blood cells (RBCs) to recycle iron. The degraded RBC yields heme, which is then transported by the heme responsive gene 1 (HRG1). Some intracytoplasmic heme is degraded by heme oxygenase (HMOX) that yields nonheme iron, while heme is exported by feline leukemia virus subgroup C receptor 1a (FLVCR1a). In this type, the level of hepcidin ranges between low or normal.

months, *Fpn*$^{+/-}$ mice developed low hemoglobin levels and decreased erythrocyte volume without anemia or significant splenic iron deposition. *Fpn*$^{+/-}$ mice exhibited significantly decreased hemoglobin and elevated splenic iron deposition at 1 year of age.[62] In *Dmt1-IRE* Tg mice, elevated iron absorption from the duodenum without significant hepatosplenic iron deposition was observed at 3 months of age,[36] while *Dmt1-IRE* Tg mice >71 weeks of age exhibited increased hepatosplenic iron deposition.[82] Furthermore, nonheme iron and transferrin saturation in sera increased with aging in wild-type rats.[83] Taken together, aged rodents may have changed iron sensors to increase body iron storage, as also observed in humans.[16]

Inflammatory stimuli, such as lipopolysaccharides (LPSs), elevate DMT1 and hepcidin expressions, whereas systemic iron requirement by IDA, phlebotomy, and hypoxia suppresses hepcidin expression.[84] Furthermore, conditional KO of murine

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Hamp elevated mRNA and protein levels of Fpn, Dmt1, and Tfr1 via hypoxia inducible factor 2α (Hif2α) activation in duodenal mucosa, indicating that the liver controls intestinal iron uptake via the hepcidin/ferroportin/Hif2α axis.[85] In contrast, dextran sulfate sodium exposure to induce colitis did not elevate duodenal iron uptake, indicating that deletion of hepcidin and/or tissue iron deposition attenuated the physiological response to intestinal inflammation in *Hamp*-deleted rats.[83] However, microbiota and intestinal inflammation have been shown to regulate hepcidin expression and systemic iron metabolism.[5] Furthermore, another *FPN* mRNA transcript that lacks 5' IRE may modulate the ferroportin protein levels to regulate iron efflux in enterocytes and erythrons.[73] These results indicate that the degree of inflammation and its response may modulate cytokine-induced hepcidin secretion.

Hepcidin is suppressed by ineffective erythropoiesis, resulting in hepatic iron overload due to increased intestinal iron absorption. Urinary hepcidin levels in patients with *DMT1* mutations are normal or moderately low, although hepatic iron is not deficient.[66] Recently, a hepcidin suppressor, erythroferrone, was identified which is induced by phlebotomy and/or erythropoietin treatment of erythroblasts. Indeed, erythroferrone antagonists are therapeutic targets that suppress intestinal iron absorption by increasing hepcidin in patients with hemoglobinopathies without regular transfusion and massive hepatosplenic iron overload.[77] Taken together, dyserythropoiesis-induced erythroferrone may contribute hepcidin suppression due to increased hepatic iron storage in patients with *DMT1* mutation.

DMT1 is indispensable for intestinal iron absorption and recycling for tight iron homeostasis regulation. Recently, four amino acids, Asp, Gln, Glu, and Gly, were found to facilitate iron uptake in iron-deficient mice, indicating an improved oral iron supplementation formula to treat IDA.[86] Furthermore, the $FePO_4$ nanoparticle bioavailability is not toxic to iron deficient women with anemia.[87] Indeed, oral intake of water-soluble iron alters the gut microbiota in patients with inflammatory backgrounds, such as obesity, inflammatory bowel disease, or colorectal cancer that may cause declined absorption.[12] To improve dietary iron supplementation, probiotics and prebiotics are explored to alter iron bioavailability, as impaired gut health-induced dysbiosis results in poor therapeutic effect.[5] Furthermore, microbiotal metabolites, such as 1,3-diaminopropane and reuterin, suppressed intestinal iron absorption via Hif2α suppression, but not Hif1α, indicating metabolic crosstalk between microbiota and the host enterocytes.[88]

Patients with β-thalassemia or sickle cell disease, who experience hemolysis and transfusion-dependent anemia, experience vasculotoxicity and atherosclerosis that may exacerbate ischemic change and cardiovascular disease, ultimately causing fatal heart failure.[13] Recently, a clinical trial on hepcidin-like peptide (LJPC-401) revealed that LJPC-401 significantly reduced the need for phlebotomy in patients with HH. Furthermore, another clinical trial on hepcidin agonists (PTG-300) for polycythemia vera effectively replaced phlebotomy for the hematocrit control.[89] *N*-acetylgalactosamine-modified antisense oligonucleotide, which targets *TMPRSS6* mRNA (Sapablursen), has been analyzed in clinical trials in patients with non-transfusion-dependent β-thalassemia (NCT04059406) and is currently being assessed in an ongoing clinical study in patients with polycythemia vera (NCT05143957). Further studies on iron metabolism are required to develop novel therapeutic approaches.

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Abbreviations

b	Belgrade
BBM	brush border membrane
BLM	basolateral membrane
Dcytb	duodenal cytochrome *b* reductase
DMT1	divalent metal transporter 1
FPN	ferroportin
HEPH	hephastin
HIF2α	hypoxia inducible factor 2α
HJV	hemojuvelin
HRG1	heme responsive gene 1
IDA	iron deficiency anemia
IRE	iron responsive element
IRIDA	iron refractory iron deficiency anemia
IRP	iron responsive protein
KO	knock out
LIP	labile iron pool
NRAMP2	natural resistance-associated macrophage protein 2
PAP7	peripheral-type benzodiazepine receptor-associated protein 7
PCBP2	poly r(C) binding protein 2
RBC	red blood cell
ROS	reactive oxygen species
SLC11A2	solute carrier family 11 member 2
TF	transferrin
TfR2	Tranaferrin receptor 2
Tg	transgenic
TM	transmembrane
TMPRSS6	transmembrane serine protease 6

Conflict of Interest

No potential conflicts of interest were disclosed.

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