BioFactors

Regulatory mechanisms of intestinal iron absorption—Uncovering of a fast-response mechanism based on DMT1 and ferroportin endocytosis

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Abstract.

Knowledge on the intestinal iron transport process and the regulation of body iron stores has greatly increased during the last decade. The liver, through the sensing of circulating iron, is now recognized as the central organ in this regulation. High iron levels induce the synthesis of hepcidin, which in turn decreases circulating iron by inhibiting its recycling from macrophages and its absorption at the intestine. Another mechanism for the control of iron absorption by the enterocyte is an active Iron Responsive Element (IRE)/Iron Regulatory Protein (IRP) system. The IRE/IRP system regulates the expression of iron uptake and storage proteins thus regulating iron absorption. Similarly, increasing evidence points to the transcriptional regulation of both divalent metal transporter 1 (DMT1) and ferroportin expression. A new mechanism of regulation related to a phenomenon called *the mucosal block* is starting to be unveiled. The mucosal block describes the ability of an initial dose of ingested iron to block absorption of a second dose given 2–4 h later. Here, we review the mechanisms involved in the expression of DMT1 and ferroportin, and present recent evidence on the molecular components and cellular processes involved in the mucosal block response. Our studies indicate that mucosal block is a fast-response endocytic mechanism destined to decrease intestinal iron absorption during a high ingest of iron.

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1. Intestinal iron absorption

In the absence of a controlled excretion mechanism, regulation of body iron levels mainly occurs during its absorption by the duodenal epithelium. The process of intestinal iron absorption comprises three sequential steps: the uptake of iron from the intestinal lumen; an intracellular phase, in which iron binds to cytosolic components; and a transfer step, in which iron exits the cells into the blood plasma.

The main components involved in intestinal iron absorption are depicted in Fig. 1. Before transport, Fe^{3+} from foodstuff is reduced to Fe^{2+}, possibly by cytochrome b-like ferrireductase (Dcytb), a hemoprotein present in the apical membrane of the enterocyte [1]. The passage of Fe^{2+} from the lumen of the intestine into the enterocyte is mediated by the product of the *SLC11A2* gene, commonly known as divalent metal transporter 1 (DMT1) [2,3]. DMT1 transports iron into cells by an electrogenic mechanism that involves the cotransport of Fe^{2+} and protons [2,4].

Once inside the enterocyte, iron becomes part of a cytosolic pool of weakly bound iron called the cytosolic labile iron pool (cLIP) [5,6]. The nature of the cLIP-binding counterpart is unknown, but it has been ascribed to diverse low molecular weight compounds such as phosphate, nucleotides, hydroxyl, amino, and sulphydryl groups [7,8]. From the cLIP, iron distributes into ferritin and intra- and extramitochondrial iron-requiring proteins [9,10].

Iron exits the enterocyte through the efflux transporter ferroportin 1 (FPN), the only member of the SLC40 family of transporters and the first reported protein that mediates the exit of iron from cells [11]. The transport mechanism of FPN is unknown, but it is thought that it transports Fe^{2+}, which is reduced at the external face of the basal membrane by the copper-containing ferroxidase hephaestin before binding to circulating transferrin [12,13]. The relevance of FPN to iron absorption was demonstrated in mice carrying a targeted deletion of FPN1, which presented embryonic lethality due to the inability to transfer iron from the mother to FPN1-

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deficient embryos. Similarly, a conditional knockout of FPN1 in the duodenum causes significant anemia in mice [14].

As noted, before binding to transferrin, effluxed iron is oxidized by the ferroxidase hephaestin. Hephaestin cDNA shares 50% sequence identity with ceruloplasmin, including residues involved in copper coordination [12]. The importance of haphaestin in the process of intestinal iron absorption is underlined by the observation that mutations in the murine hephaestin gene produce microcytic, hypochromic anemia that is refractory to oral iron therapy [12,15].

2. Translational regulation of intestinal iron absorption

The most characterized mechanism for the regulation of intestinal iron absorption is carried out by the Iron Responsive Element/Iron Regulatory Protein (IRE/IRP) system (reviewed in [16]). A number of studies during the last decade have evidenced a central role of the RNA-binding proteins IRP1 and IRP2 in controlling, via cis-regulatory mRNA motifs called IREs, the expression of iron metabolism proteins involved in intestinal iron absorption [17–22]. The activities of both IRP1 and IRP2 respond to changes in cellular Fe through different mechanisms. Under iron-replete conditions, IRP1 has a 4S-4Fe cubane structure that renders the protein active as a cytosolic aconitase but inactive for IRE-binding; whereas IRP2 activity is downregulated through iron-induced oxidative damage followed by ubiquitination and proteasome degradation [23].

A low-iron condition either activates IRP1 or stabilizes IRP2, which, by binding to the IRE located within the 5′ untranslated region of the H and L ferritin mRNA leads to the inhibition of their translation. Similarly, the binding of IRP1 or IRP2 to the multiple IRE motifs within the 3′ untranslated region of the transferrin receptor 1 mRNA stabilizes this region, preventing its degradation [24]. The net result of these actions is a decreased intracellular iron level.

Expression of the iron import transporter DMT1 is most probably regulated by the IRE/IRP system. Four isoforms of DMT1 are generated by alternative splicing of the 5′-end exons (exons 1A or 1B) and of the 3′-end exons (exons 16 (or +IRE) or 17 (or −IRE)) [25]. Concurring with the presence of an IRE element in the 3′ flanking region, DMT1 expression is downregulated by high cell iron. Nevertheless, there is no agreement about the specific mechanisms causing this regulation. Expression of the +1A/+IRE isoform makes cells particularly sensitive to cell iron levels, whereas expression of the 1A/−IRE isoform yield cells that respond with less

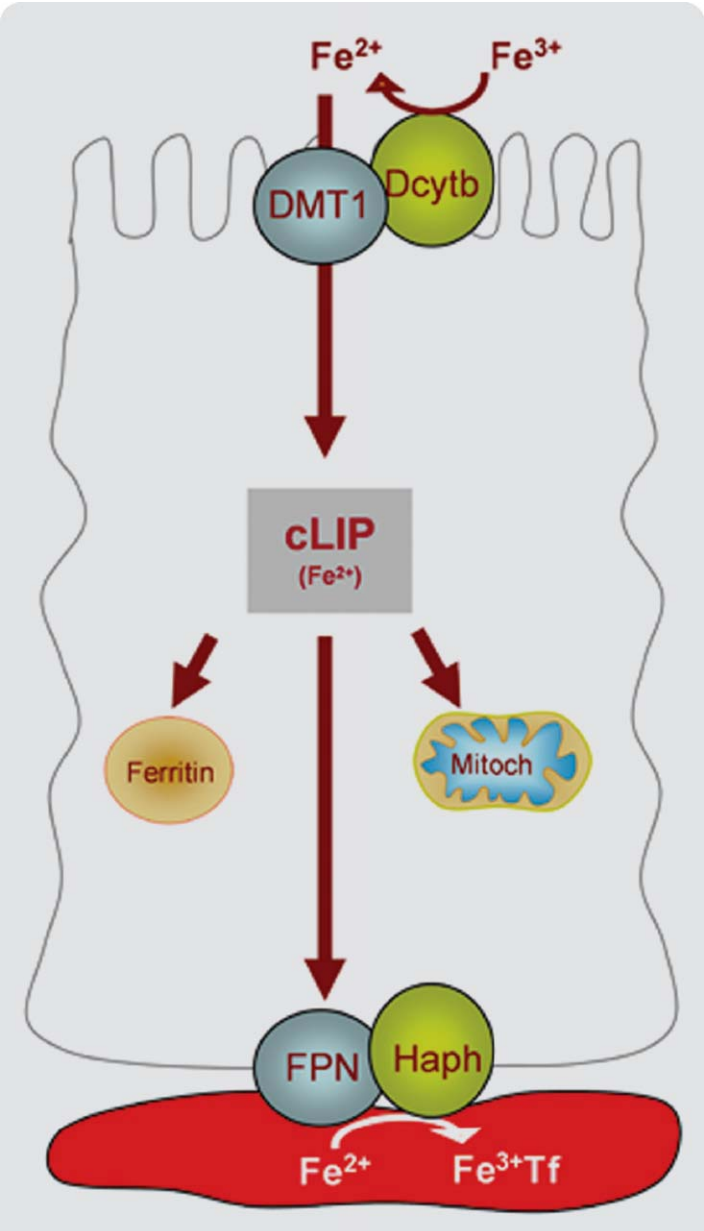

Fig. 1. Main components of intestinal nonheme iron absorption. Inorganic iron from foodstuff is mostly in the oxidized +3 redox state. Before transport, Fe^{3+} is reduced to Fe^{2+}, possibly by duodenal cytochrome b-like ferrireductase (Dcytb), a hemoprotein associated with the apical enterocyte membrane. The transfer of inorganic Fe^{2+} is mediated by DMT1, which transports iron into cells by an electrogenic mechanism that involves the cotransport of Fe^{2+} and protons. Newly entering iron integrates into a pool of weakly bound iron called the cytosolic labile iron pool (cLIP), where it is chelated to substances such as phosphate, nucleotides and amino groups. From the cLIP, iron is either transported into the blood or distributed to ferritin or mitochondria for storage or the synthesis of heme and Fe-S clusters, respectively. The transfer phase involves the transport of intracellular iron, probably as Fe^{2+}, from the cLIP into the blood circulation, a process mediated by the iron-export transporter FPN and the ferrioxidase hephaestin (Haph). Haph re-oxidases iron to the +3 state prior to binding to circulating transferrin (Tf).

intensity to iron changes [25]. In contrast, cells expressing the 1B/+IRE or the 1B/−IRE isoforms do not respond to iron changes. Thus, it is possible that the regulation of DMT1 expression involves two regulatory regions, one contained in exon 1A and another in the IRE-containing 3′ exon.

The relevance of the IRE/IRP system for the regulation of DMT1 expression was further explored using Cre/Lox technology to generate mice lacking IRP1 and IRP2 expression in the intestine. It was shown that IRPs-deficient mice displayed decreased iron uptake capacity, due to TFR1 and DMT1 down regulation, combined with augmented iron sequestration and export capacities [26]. In summary, the evidence accumulated up to date strongly indicates that the IRE/IRP system is an intrinsic part of the regulatory mechanisms involved in intestinal iron absorption.

In the intestine, the expression of the iron exporter ferroportin is upregulated by cell iron levels [27–29] but the mechanism involved in this regulation is poorly understood. Ferroportin (FPN) mRNA has a transferrin-like IRE motif in its 5′ untranslated region, so it could be potentially regulated by the IRE/IRP system [11,30]. A recent study reported that duodenal and erythroid precursor cells utilize an alternative upstream promoter to express a new isoform of FPN named FPN1B, which does not contain an IRE element [31]. The presence in the duodenum of a FPN transcript lacking the IRE element gives an explanation to the observation that FPN mRNA levels are significantly increased in patients with iron deficiency, whereas they are unchanged in patients with secondary iron overload [27]. In summary, the reported evidence shows that the expression of intestinal ferroportin is strongly induced by iron deficiency conditions, but the participation of the IRE/IRP system in this process is obscure.

3. Transcriptional regulation of intestinal iron absorption

DMT1 expression is regulated at the transcriptional level by iron and proinflammatory agents. Treatment of Caco-2 cells with the iron chelator desferioxamine for 36 h resulted in moderate increase in *DMT1* and *FPN1* gene transcription, whereas incubation with $FeCl_3$ led to decreased *DMT1* and *FPN* transcription [28]. The proinflammatory agent LPS and the cytokines TNFα, IFN-γ, and transcription factor NF-κB induce DMT1 expression, indicating a link between inflammation and DMT1 expression [32,33]. The 1B isoform of *DMT1* has a NFκB-responsive element in its 5′ untranslated region, whereas the 1A isoform contains Hif-2α response elements that regulate intestinal DMT1 in response to hypoxia [34,35]. Hif-2α seems to regulate positively duodenal DMT1 expression since a conditional knockout for intestinal Hif-2α showed a strong decrease in duodenal DMT1 transcription coupled to decreased serum and liver iron [34]. These evidences support a mechanism by which hypoxic conditions induce the synthesis of DMT1 through the activation of Hif-2α.

Only a few studies have documented increased ferroportin mRNA expression in response to iron [36–40]. In macrophage J774 cells ferroportin mRNA levels increased by iron treatment reaching six times the control levels after 24 h [37]. The changes in ferroportin mRNA levels were similar to changes in heterogeneous nuclear RNA, the immediate product of ferroportin gene transcription. Moreover, actinomycin D did not stabilize ferroportin mRNA or hnRNA. The authors concluded that iron induces the transcription of the ferroportin gene in these cells [37]. Similarly, in primary cultures of mouse bone marrow-derived macrophages, iron released from the catabolism of artificially aged red blood cells stimulated FPN mRNA and protein expression. This suggests that iron derived from heme catabolism induces gene transcription of *FPN* [36]. Thus, the evidence gathered to date indicates that iron induces *FPN* transcription. Further studies on the mechanism of induction are needed. Similarly, this effect of iron needs to be assessed in cell types other than macrophages since, as discussed above, iron strongly promotes decreased ferroportin expression in intestinal cells.

4. Systemic regulation of intestinal iron absorption: The roles of hepcidin and erythropoietin

At the systemic level, intestinal iron absorption is regulated by two factors: the store regulator, which responds to body iron levels; and the erythron regulator, a putative factor that communicates to the intestine the iron needs for erythropoiesis [41–43].

The stores regulator was identified as hepcidin, a small peptide secreted by the liver in response to elevated plasma iron [44,45]. Consistent with its role as a central regulator of body iron metabolism, hepcidin is regulated by anemia, hypoxia, inflammation, and the level of circulating iron [46]. In intestinal cell lines, hepcidin treatment induces a decrease in mRNA and protein levels of DMT1 and FPN [47–50], whereas in macrophages hepcidin treatment induces internalization and lysosomal degradation of the FPN protein [51]. The adaptive response to an iron challenge mediated by hepcidin is slow. Following a hemolytic stimulus, a 3-day delay was noted before a significant decrease in circulating hepcidin was observed, and a further 24 h period was necessary to observe increased duodenal expression of DMT1, cytochrome b ferrireductase, and FPN [52].

The mechanism by which hepcidin regulates intestinal iron absorption is not fully understood. A model for hepcidin action developed for macrophages, in which Hepc decreased ferroportin protein levels and cell surface localization [51,53], has been extrapolated to explain the effects of Hepc on the absorptive enterocyte [45]. Nevertheless, hepcidin seems to have a cell-specific effect on absorptive enterocytes. In rats, injection of hepcidin significantly reduced mucosal iron uptake and transfer to the body [54], and incubation of Caco-2 cells or intestinal duodenum with hepcidin inhibits DMT1 expression and apical iron uptake with no effect on FPN levels [47,48]. The mechanism by which hepcidin

induces the decrease of DMT1 expression is unknown [47]. Most probably, hepcidin regulates intestinal iron absorption both by inducing FPN degradation and by inhibiting DMT1 expression.

The concept of the erythron regulator was established by Bothwell et al. in 1958 [41] and was later developed by Finch and collaborators [42]. Losses of iron requiring the absorption of 3 or 4 mg, as illustrated by frequent blood donors, deplete stores and activate the erythron regulator. Similarly, subjects with iron deficiency anemia absorb sufficient supplemented iron to increase red blood cell production up to fourfold basal levels. Stimulation of the erythroid marrow starts when anemia or hypoxia increases the output of erythropoietin (EPO) by the kidney. EPO, in turn, stimulates the synthesis of erythroid precursors in the erythroid marrow by inhibiting apoptosis of erythroid progenitors [55,56]. As hepcidin is an effective inhibitor of intestinal iron absorption, it is reasonable to hypothesize that the erythroid regulator inhibits hepcidin production [43]. The observation that patients with thalassemia or ineffective erythropoiesis disorders present abnormally low levels of hepcidin is in tune with this hypothesis [57–61].

The expression of DMT1 responds to erythropoietic stimuli through the inhibition of hepcidin synthesis. In rat intestine, EPO treatment significantly stimulated erythropoiesis, increased iron uptake and decreased liver hepcidin mRNA [50]. In the duodenum, EPO increased the expression of DMT1(+IRE), but not of DMT1(–IRE). However, FPN1 expression presented no detectable changes. The summation of the above evidence indicates that EPO or other factor(s) resulting from stimulated erythropoiesis negatively regulates hepcidin synthesis, leading to increased intestinal iron absorption and increased iron release from recycling macrophages.

5. The vesicular movement of intestinal iron transporters

5.1. The mucosal block

A putative process designed to avoid excess iron absorption is known as the *mucosal block*, which describes the ability of an initial dose of ingested iron to block the absorption of a second dose. When rats are pretreated with an intragastric dose of iron and then with a similar dose containing radioactive iron, the uptake of the second dose of iron decreases in about 33% compared to nontreated animals. The decrease remained constant for 3–9 h after delivery of the blocking dose [62]. It is of interest to note that by 3 h, the shorter time determined, the blocking effect is already maximal. The basolateral transfer of radioactive iron followed a similar pattern, decreasing to about 55% of control after 12 h. Both processes, uptake and transfer, returned to near control levels by 72 h [62]. Variations of this experiment, showing that an initial dose of iron reduces the absorption of a second dose, have been repeatedly reported [62–67]. In another report, it was observed that iron given to fasting rats induced internalization of DMT1 from the apical membrane to intracellular compartment(s), followed by a decrease in DMT1 protein 6 h after dietary iron supplementation [67]. This redistribution was interpreted as evidence of a process whereby vesicles containing DMT1 with bound iron fuse with, and deliver their iron to vesicles containing apo-transferrin [68,69].

The above experiments provide evidence of a cellular regulatory mechanism for the absorption of iron, but do not reveal the mechanisms involved. In particular, given the reported lapses of time, the observed down-regulation could be the result of the IRE/IRP system activity. Decreased DMT1 could explain the mucosal block. In fact, decreased mRNA expression of DMT1(+IRE), but not DMT1(−IRE), was observed 3 h after iron feeding [62]. On the contrary, other investigators found a marked decrease in mRNA for both the +IRE and −IRE isoforms of DMT1 3 h after iron feeding, although in this later case a paradoxical increase in DMT1(+IRE) protein was also reported [67].

5.2. The endocytic movement of membrane transporters

Movement of transporters between the plasma membrane and intracellular domains is an important mechanism to regulate influx of ions and metabolites. Examples of this process are found in the Glut4 transporter [70], aquaporin channels [71], the transient receptor potential family of ion channels [72], and neurotransmitter receptors [73]. Perhaps, Glut4 is the most paradigmatic example of positional regulation. In the basal state, Glut4 undergoes a slow endocytic–exocytic cycling between intracellular compartments and the plasma membrane, with only 5% of the total Glut4 protein localized to the plasma membrane. However, in response to insulin stimulation, the rate of Glut4 exocytosis markedly increases so that approximately 50% of the Glut4 protein is rapidly relocated to the cell surface [74]. Therefore, the vesicular movement of a protein between the plasma membrane and intracellular compartments provides an effective mechanism to regulate its function in relatively short periods of time.

Utilizing a kidney epithelial cell line, Tabuchi et al. showed that the −IRE DMT1 isoform has a recycling endocytic traffic associated to the delivery of iron through transferrin endocytosis, while endocytosis of the +IRE isoform takes the degradation route to lysosomes [75–77]. They concluded that the discrete predominant expression of DMT1 isoforms in a particular cell type may regulate its iron uptake characteristics. The +IRE/−IRE dichotomy has not been studied in enterocytes, but, if it leads to degradation, the endocytosis of DMT1 could have profound repercussions on intestinal iron absorption.

5.3. DMT1 and FPN internalization

Internalization of DMT1 located in the apical membrane could be an effective mechanism operating earlier than the transcriptional or translational regulations of DMT1 expression are in function. Experiments using confocal microscopy

reveal that when polarized Caco-2 cells grown in bicameral inserts are exposed to iron from the apical chamber, about 30% of the DMT1(+IRE) present in the brush-border membrane is internalized within 10 min of exposure [68]. Similarly, in rat duodenum considerable DMT1 internalization was found 20 min after iron feeding. The continued presence of iron in the apical chamber allows for internalization to continue for 40 min, after which an equilibrium appears to be reached [68,69]. Using confocal microscopy, we studied in rat duodenum the effect of iron feeding on the apical/basolateral location of DMT1 and FPN (Fig. 2). We found that under starving conditions DMT1 presented a marked apical distribution, whereas iron feeding induced a large relocalization into basal cytoplasm domains (Fig. 2A). As expected, FPN localized to the basolateral domain, but considerable FPN was also observed in the apical domain (Fig. 2B). Iron gavage induced a reduction of basolateral FPN without apparent effect on apical FPN (Fig. 2B). Determination of DMT1 and FPN abundance in microvilli membrane obtained from fasting and iron-fed rats indicated that iron feeding induced a drastic decrease of the DMT1+IRE isoform present in microvilli membranes (Fig. 2C).

The presence of FPN in the microvilli of the intestinal epithelium has been reported although not functionally assessed [68,79]. Similarly, DMT1 has been noted in the basolateral membrane of rat duodenum [67] and in Caco-2 cells overexpression of the hereditary hemochromatosis protein HFE induces redistribution of DMT1 to the basolateral membrane, possibly a strategy to down-regulate iron absorption [80]. The finding of DMT1 in the basolateral membrane of Caco-2 cells and in the basal domains of enterocytes comes to no surprise, since DMT1 is needed for iron transport out of the endocytic vesicle during basolateral transferrin endocytosis [75].

We characterized the kinetics of DMT1 relocalization by time-lapse confocal microscopy using polarized Caco-2 cells [78]. Cells were transfected with a construct coding for DMT1-CFP, and its cellular localization after the addition of iron was determined from time-lapse apical-to-basal galleries (Fig. 3). Iron induced a time-dependent movement of DMT1 from apical to basal domains (Fig. 3A). The relocalization of DMT1 was relatively fast, being completed within 60–90 min (Fig. 3B). Taken together, the above experiments indicate that iron feeding results in the sequestration of DMT1 from the apical membrane and of FPN from the basolateral membrane. These movements should substantially decrease intestinal iron absorption, as described after gavage of iron-deficient rats.

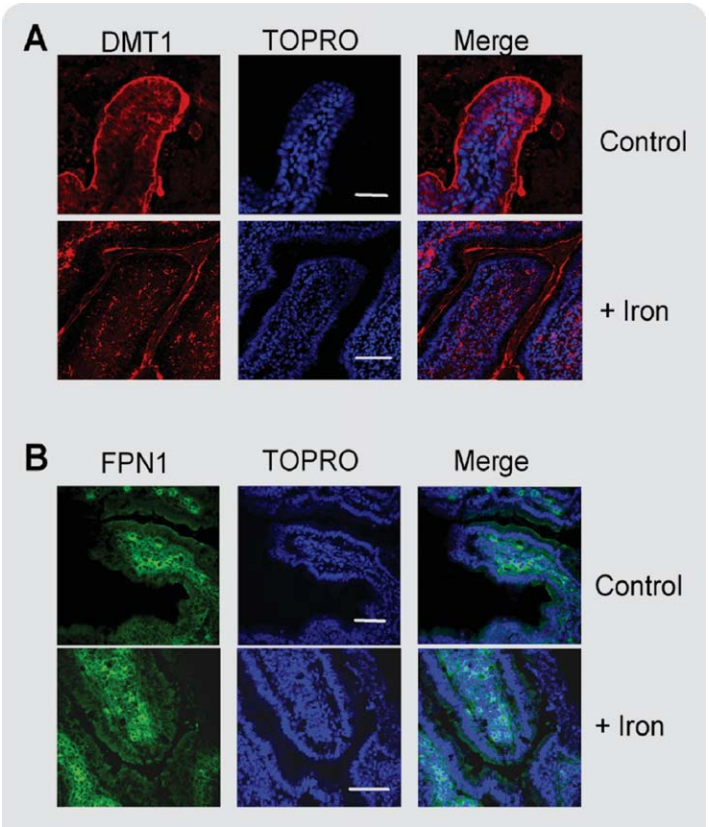

Fig. 2. Effect of iron gavage on the positioning of DMT1 and FNP in rat intestine. A: Fasting Sprague-Dawley rats were given an intragastric dose of iron (20 mg $FeSO_4$ in 250 μL of 0.01 N HCl) and cellular localization of DMT1 and FPN was determined 3.5 h after feeding. Shown is cell localization of DMT1 (red) in the duodenum of fasting (control) and iron-stimulated (+iron) rats 3.5 h after Fe gavage. Nuclear staining (blue, TOPRO) was included as a landmark. B: Localization of FPN (green) under fasting (control) or under iron stimulation (+iron) conditions. Scale bars = 60 μm. Similar results were obtained 2.5 h after gavage. For more experimental details see a similar experiment reported in ref. [78].

5.4. Apical and basolateral iron fluxes

It is widely accepted that DMT1 transports iron from the intestinal lumen into the enterocyte, whereas FPN transports iron from the enterocyte into blood circulation. Thus, the presence of FPN in the apical membrane and the putative presence of DMT1 in the basolateral membrane does not make sense straightaway.

Recently, we demonstrated in Caco-2 cells basolateral iron uptake and apical iron efflux, besides the canonical apical uptake and basolateral efflux [78]. The presence of these fluxes gives support to functionally active apical FPN and basolateral DMT1. These results are compatible with a mechanism in which rapid repositioning of DMT1 and FPN in the apical and basolateral membranes regulate intestinal iron absorption. Figure 4 shows a model in which iron supplementation leads to a fast repositioning of DMT1 and FPN, resulting in decreased intestinal iron absorption. In this model, the direction of the iron movement is determined by the relative activities of DMT1 and FPN at the apical and basolateral membranes. A big question that this model puts

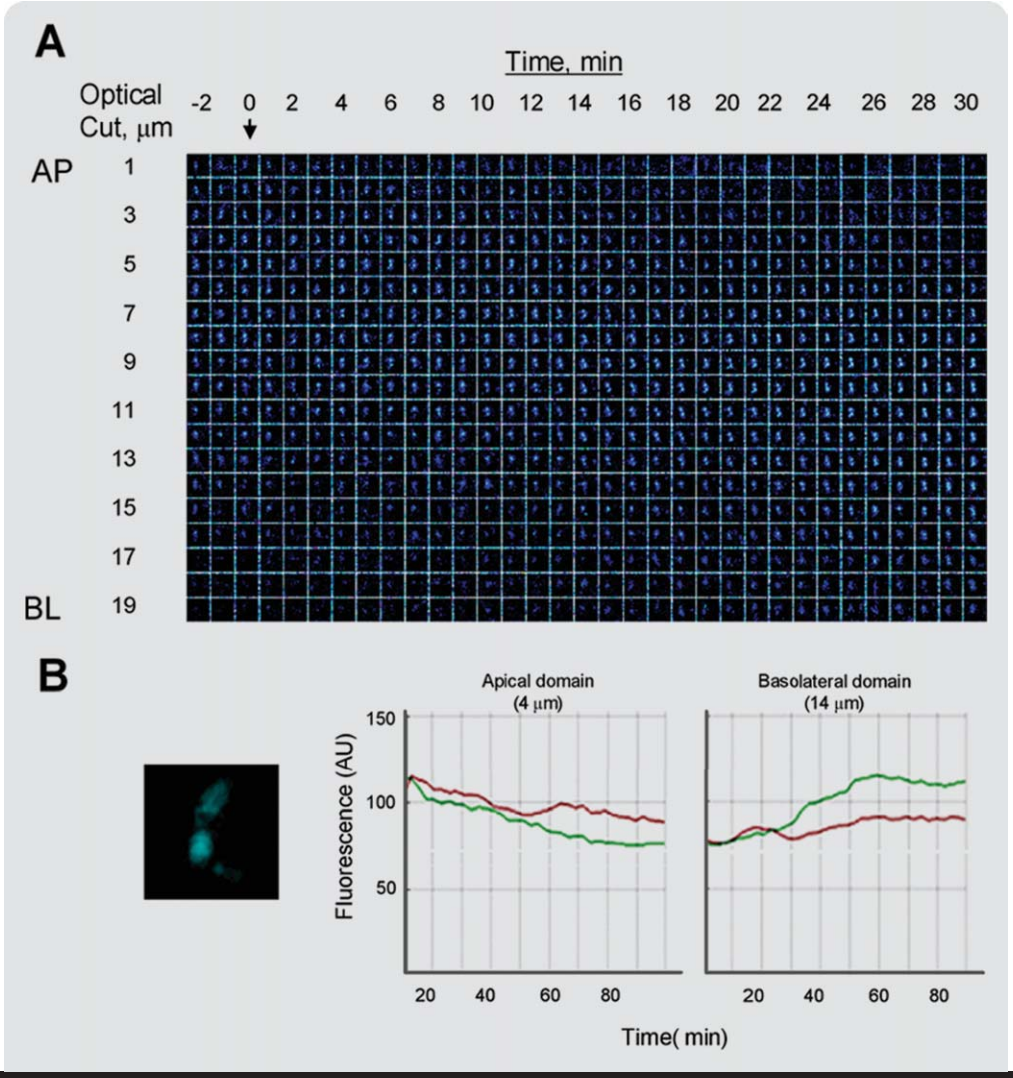

Fig. 3. Kinetics of apical-to basal movement of DMT1 after iron supplementation in Caco-2 cells. A: Caco-2 cells, grown in bicameral inserts for 14 days were transfected with the construct pcDNA3-DMT1(+IRE)-cyano fluorescent protein (CFP) as described [81]. Two days after transfection cells were challenged with iron and 20 apical-to-basal optical cuts of 1 μm were taken every 1 min before (−2 to 0 min) or after (0–30 min) the addition of 20 μM ferrous ammonium sulfate to the apical chamber (arrow). The columns represent galleries of 1 μm confocal cuts in the apical-to-basal axis. A shift in DMT1-CFP fluorescence from apical to basal domains becomes evident with time. B: Quantification of the changes in fluorescence (represented by the green and red lines, right panel) of two DMT1-CFP-expressing cells (shown in the left panel) as a function of time (0–90 min). Total fluorescence was obtained from optical cuts taken every 1 min at fixed distances of 4 μm (apical domain) and 14 μm (basal domain) from the apical border. Modified from ref. [78]. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

forward is about the nature of the molecular signals that direct the vesicular movement of the transporters.

6. Concluding remarks

Transepithelial iron transport by the absorbing enterocyte finely balances the necessity to maximize iron import under iron-deficient conditions with the need to minimize potential toxicity from dietary iron overload. Different mechanisms are in place to regulate intestinal iron absorption. These mechanisms present response times that expand from minutes to days after an iron challenge, as indicated in Table 1. The fastest mechanism are the movements of DMT1 from its apical location and of FPN from its basolateral membrane domain, a response that takes 15–60 min in setting and can last more than 3.5 h. A second response is the translational regulation of DMT1 expression by the IRE/IRP system, with a response time of 0.5–2 h. Further regulation of intestinal iron absorption is achieved by humoral factors such as

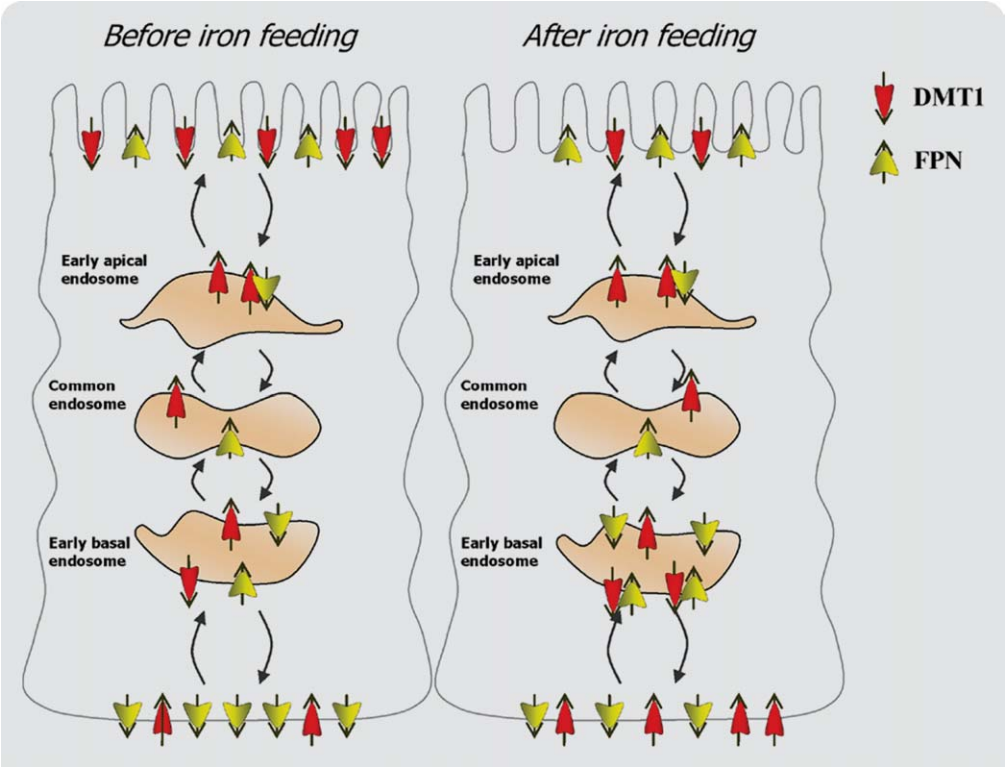

Fig. 4. A model for the fast regulation of intestinal iron absorption by iron-induced DMT1 and FPN movements. The model considers that DMT1 and FPN are located at both the apical (AP) and basolateral (BL) membranes where they are active in iron transport. Through vesicular fluxes, the transporters in each membrane communicate with apical and basolateral early endosomal compartments. Both early compartments connect through the common AP-BL endosome [82–84]. The net flux of iron at each membrane is a function of the relative abundance of the transporters. At low intracellular iron concentrations DMT1 distributes preferentially to the apical membrane while ferroportin has a more even distribution. When the intracellular iron concentration rises, DMT1 undergoes an apical-to-basal flux and FPN leaves the basolateral membrane. The net result is a marked decrease in apical iron uptake coupled with decreased transference of iron to the blood circulation. The set-up time for the repositioning of the transporters is 30–60 min, remaining stable for at least 3.5 h. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Table 1
Response Times

	Transporter	
Process	DMT1	FPN
Endocytosis	30–60 min [68,78]	30–60 min [85][a]
Translational regulation	1.5–3 h [62,67]	–
Transcriptional regulation	12–36 h [28,86]	24–36 h [28,85,86]
Systemic (EPO, Hepc) regulation	3–4 days [49,50,52]	3–4 days [49,52,87]

Response time for the varied processes involved in the regulation of DMT1 and FPN in the intestine. Abbreviations: EPO, erythropoietin; Hepc, hepcidin.
[a] The time for the movement out of an apical compartment.

hepcidin, which induces FPN internalization and degradation and DMT1 inactivation. The response time for the hepcidin-mediated effect, 3–4 days, involves a continuous iron challenge that results in an elevation of serum iron, and the consequent induction of hepcidin synthesis and release to the circulating blood.

The presence of three levels of regulation allows for the modulated response to dietary iron excess or deficiency, destined to ensure adequate iron supply and minimize potential toxicity. Future investigations need to address the integration of these three systems, so new knowledge can contribute to formulate a complete picture of intestinal iron absorption and body iron homeostasis.

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References

[1] McKie, A. T., Barrow, D., Latunde-Dada, G. O., Rolfs, A., Sager, G., Mudaly, E., Mudaly, M., Richardson, C., Barlow, D., Bomford, A., Peters, T. J., Raja, K. B., Shirali, S., Hediger, M. A., Farzaneh, F., and Simpson, R. J. (2001) An iron-regulated ferric reductase associated with the absorption of dietary iron. *Science* **291**, 1755–1759.

[2] Gunshin, H., Mackenzie, B., Berger, U. V., Gunshin, Y., Romero, M. F., Boron, W. F., Nussberger, S., Gollan, J. L., and Hediger, M. A. (1997) Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* **388**, 482–488.

[3] Garrick, M. D., Dolan, K. G., Horbinski, C., Ghio, A. J., Higgins, D., Porubcin, M., Moore, E. G., Hainsworth, L. N., Umbreit, J. N., Conrad, M. E., Feng, L., Lis, A., Roth, J. A., Singleton, S., and Garrick, L. M. (2003) DMT1: a mammalian transporter for multiple metals. *Biometals* **16**, 41–54.

[4] Mackenzie, B., Takanaga, H., Hubert, N., Rolfs, A., and Hediger, M. A. (2007) Functional properties of multiple isoforms of human divalent metal-ion transporter 1 (DMT1). *Biochem. J.* **403**, 59–69.

[5] Epsztejn, S., Kakhlon, O., Glickstein, H., Breuer, W., and Cabantchik, I. (1997) Fluorescence analysis of the labile iron pool of mammalian cells. *Anal. Biochem.* **248**, 31–40.

[6] Kruszewski, M. (2004) The role of labile iron pool in cardiovascular diseases. *Acta. Biochim. Pol.* **51**, 471–480.

[7] Kakhlon, O. and Cabantchik, Z. I. (2002) The labile iron pool: characterization, measurement, and participation in cellular processes(1). *Free Radic. Biol. Med.* **33**, 1037–1046.

[8] Petrat, F., de Groot, H., Sustmann, R., and Rauen, U. (2002) The chelatable iron pool in living cells: a methodically defined quantity. *Biol. Chem.* **383**, 489–502.

[9] El-Shobaki, F. A. and Rummel, W. (1977) Mucosal transferrin and ferritin factors in the regulation of iron absorption. *Res. Exp. Med. (Berl)* **171**, 243–253.

[10] Kolachala, V. L., Sesikeran, B., and Nair, K. M. (2007) Evidence for a sequential transfer of iron amongst ferritin, transferrin and transferrin receptor during duodenal absorption of iron in rat and human. *World J. Gastroenterol.* **13**, 1042–1052.

[11] McKie, A. T., Marciani, P., Rolfs, A., Brennan, K., Wehr, K., Barrow, D., Miret, S., Bomford, A., Peters, T. J., Farzaneh, F., Hediger, M. A., Hentze, M. W., and Simpson, R. J. (2000) A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. *Mol. Cell.* **5**, 299–309.

[12] Vulpe, C. D., Kuo, Y. M., Murphy, T. L., Cowley, L., Askwith, C., Libina, N., Gitschier, J., and Anderson, G. J. (1999) Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. *Nat. Genet.* **21**, 195–199.

[13] Griffiths, T. A., Mauk, A. G., and MacGillivray, R. T. (2005) Recombinant expression and functional characterization of human hephaestin: a multicopper oxidase with ferroxidase activity. *Biochemistry* **44**, 14725–14731.

[14] Donovan, A., Lima, C. A., Pinkus, J. L., Pinkus, G. S., Zon, L. I., Robine, S., and Andrews, N. C. (2005) The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. *Cell Metab.* **1**, 191–200.

[15] Syed, B. A., Beaumont, N. J., Patel, A., Naylor, C. E., Bayele, H. K., Joannou, C. L., Rowe, P. S., Evans, R. W., and Srai, S. K. (2002) Analysis of the human hephaestin gene and protein: comparative modelling of the N-terminus ecto-domain based upon ceruloplasmin. *Protein Eng.* **15**, 205–214.

[16] Muckenthaler, M. U., Galy, B., and Hentze, M. W. (2008) Systemic iron homeostasis and the iron-responsive element/iron-regulatory protein (IRE/IRP) regulatory network. *Annu. Rev. Nutr.* **28**, 197–213.

[17] Arredondo, M., Orellana, A., Garate, M. A., and Núñez, M. T. (1997) Intracellular iron regulates iron absorption and IRP activity in intestinal epithelial (Caco-2) cells. *Am. J. Physiol.* **273**, G275–G280.

[18] Schumann, K., Moret, R., Kunzle, H., and Kuhn, L. C. (1999) Iron regulatory protein as an endogenous sensor of iron in rat intestinal mucosa. Possible implications for the regulation of iron absorption. *Eur. J. Biochem.* **260**, 362–372.

[19] Garate, M. A. and Núñez, M. T. (2000) Overexpression of the ferritin iron-responsive element decreases the labile iron pool and abolishes the regulation of iron absorption by intestinal epithelial (Caco-2) cells. *J. Biol. Chem.* **275**, 1651–1655.

[20] Barisani, D., Parafioriti, A., Bardella, M. T., Zoller, H., Conte, D., Armiraglio, E., Trovato, C., Koch, R. O., and Weiss, G. (2004) Adaptive changes of duodenal iron transport proteins in celiac disease. *Physiol. Genomics* **17**, 316–325.

[21] Schumann, K., Brennan, K., Weiss, M., Pantopoulos, K., and Hentze, M. W. (2004) Rat duodenal IRP1 activity and iron absorption in iron deficiency and after HO perfusion. *Eur. J. Clin. Invest.* **34**, 275–282.

[22] Oates, P. S. (2007) The relevance of the intestinal crypt and enterocyte in regulating iron absorption. *Pflugers Arch.* **455**, 201–213.

[23] Guo, B., Phillips, J. D., Yu, Y., and Leibold, E. A. (1995) Iron regulates the intracellular degradation of iron regulatory protein 2 by the proteasome. *J. Biol. Chem.* **270**, 21645–21651.

[24] Wallander, M. L., Leibold, E. A., and Eisenstein, R. S. (2006) Molecular control of vertebrate iron homeostasis by iron regulatory proteins. *Biochim. Biophys. Acta.* **1763**, 668–689.

[25] Hubert, N. and Hentze, M. W. (2002) Previously uncharacterized isoforms of divalent metal transporter (DMT)-1: implications for regulation and cellular function. *Proc. Natl. Acad. Sci. USA* **99**, 12345–12350.

[26] Galy, B., Ferring-Appel, D., Kaden, S., Grone, H. J., and Hentze, M. W. (2008) Iron regulatory proteins are essential for intestinal function and control key iron absorption molecules in the duodenum. *Cell. Metab.* **7**, 79–85.

[27] Zoller, H., Koch, R. O., Theurl, I., Obrist, P., Pietrangelo, A., Montosi, G., Haile, D. J., Vogel, W., and Weiss, G. (2001) Expression of the duodenal iron transporters divalent-metal transporter 1 and ferroportin 1 in iron deficiency and iron overload. *Gastroenterology* **120**, 1412–1419.

[28] Zoller, H., Theurl, I., Koch, R., Kaser, A., and Weiss, G. (2002) Mechanisms of iron mediated regulation of the duodenal iron transporters divalent metal transporter 1 and ferroportin 1. *Blood Cells Mol. Dis.* **29**, 488–497.

[29] Rolfs, A., Bonkovsky, H. L., Kohlroser, J. G., McNeal, K., Sharma, A., Berger, U. V., and Hediger, M. A. (2002) Intestinal expression of genes involved in iron absorption in humans. *Am. J. Physiol. Gastrointest. Liver Physiol.* **282**, G598–G607.

[30] Abboud, S. and Haile, D. J. (2000) A novel mammalian iron-regulated protein involved in intracellular iron metabolism. *J. Biol. Chem.* **275**, 19906–19912.

[31] Zhang, D. L., Hughes, R. M., Ollivierre-Wilson, H., Ghosh, M. C., and Rouault, T. A. (2009) A ferroportin transcript that lacks an iron-responsive element enables duodenal and erythroid precursor cells to evade translational repression. *Cell Metab.* **9**, 461–473.

[32] Wang, X., Garrick, M. D., Yang, F., Dailey, L. A., Piantadosi, C. A., and Ghio, A. J. (2005) TNF, IFN-gamma, and endotoxin increase expression of DMT1 in bronchial epithelial cells. *Am. J. Physiol. Lung Cell Mol. Physiol.* **289**, L24–L33.

[33] Paradkar, P. N. and Roth, J. A. (2007) Expression of the 1B isoforms of divalent metal transporter (DMT1) is regulated by interaction of NF-Y with a CCAAT-box element near the transcription start site. *J. Cell Physiol.* **211**, 183–188.

[34] Mastrogiannaki, M., Matak, P., Keith, B., Simon, M. C., Vaulont, S., and Peyssonnaux, C. (2009) HIF-2alpha, but not HIF-1alpha, promotes iron absorption in mice. *J. Clin. Invest.* **119**, 1159–1166.

[35] Shah, Y. M., Matsubara, T., Ito, S., Yim, S. H., and Gonzalez, F. J. (2009) Intestinal hypoxia-inducible transcription factors are essential for iron absorption following iron deficiency. *Cell Metab.* **9**, 152–164.

[36] Delaby, C., Pilard, N., Puy, H., and Canonne-Hergaux, F. (2008) Sequential regulation of ferroportin expression after erythrophagocytosis in murine macrophages: early mRNA induction by haem, followed by iron-dependent protein expression. *Biochem. J.* **411**, 123–131.

[37] Aydemir, F., Jenkitkasemwong, S., Gulec, S., and Knutson, M. D. (2009) Iron loading increases ferroportin heterogeneous nuclear RNA and mRNA levels in murine J774 macrophages. *J. Nutr.* **139**, 434–438.

[38] Knutson, M. D., Vafa, M. R., Haile, D. J., and Wessling-Resnick, M. (2003) Iron loading and erythrophagocytosis increase ferroportin 1 (FPN1) expression in J774 macrophages. *Blood* **102**, 4191–4197.
[39] Dupic, F., Fruchon, S., Bensaid, M., Loreal, O., Brissot, P., Borot, N., Roth, M. P., and Coppin, H. (2002) Duodenal mRNA expression of iron related genes in response to iron loading and iron deficiency in four strains of mice. *Gut* **51**, 648–653.
[40] Yang, F., Wang, X., Haile, D. J., Piantadosi, C. A., and Ghio, A. J. (2002) Iron increases expression of iron-export protein MTP1 in lung cells. *Am. J. Physiol. Lung Cell Mol. Physiol.* **283**, L932–L939.
[41] Bothwell, T. H., Pirzio-Biroli, G., and Finch, C. A. (1958) Iron absorption. I. Factors influencing absorption. *J. Lab. Clin. Med.* **51**, 24–36.
[42] Finch, C. (1994) Regulators of iron balance in humans. *Blood* **84**, 1697–1702.
[43] Wrighting, D. M. and Andrews, N. C. (2008) Iron homeostasis and erythropoiesis. *Curr. Top. Dev. Biol.* **82**, 141–167.
[44] Nemeth, E. and Ganz, T. (2006) Regulation of iron metabolism by hepcidin. *Annu. Rev. Nutr.* **26**, 323–342.
[45] Ganz, T. and Nemeth, E. (2006) Iron imports. IV. Hepcidin and regulation of body iron metabolism. *Am. J. Physiol. Gastrointest. Liver Physiol.* **290**, G199–G203.
[46] Nicolas, G., Chauvet, C., Viatte, L., Danan, J. L., Bigard, X., Devaux, I., Beaumont, C., Kahn, A., and Vaulont, S. (2002) The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. *J. Clin. Invest.* **110**, 1037–1044.
[47] Mena, N. P., Esparza, A., Tapia, V., Valdes, P., and Nunez, M. T. (2008) Hepcidin inhibits apical iron uptake in intestinal cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* **294**, G192–G198.
[48] Chaston, T., Chung, B., Mascarenhas, M., Marks, J., Patel, B., Srai, S. K., and Sharp, P. (2008) Evidence for differential effects of hepcidin in macrophages and intestinal epithelial cells. *Gut* **57**, 374–382.
[49] Chung, B., Chaston, T., Marks, J., Srai, S. K., and Sharp, P. A. (2009) Hepcidin decreases iron transporter expression in vivo in mouse duodenum and spleen and in vitro in THP-1 macrophages and intestinal Caco-2 cells. *J. Nutr.* **139**, 1457–1462.
[50] Kong, W. N., Chang, Y. Z., Wang, S. M., Zhai, X. L., Shang, J. X., Li, L. X., and Duan, X. L. (2008) Effect of erythropoietin on hepcidin, DMT1 with IRE, and hephaestin gene expression in duodenum of rats. *J. Gastroenterol.* **43**, 136–143.
[51] Delaby, C., Pilard, N., Goncalves, A. S., Beaumont, C., and Canonne-Hergaux, F. (2005) Presence of the iron exporter ferroportin at the plasma membrane of macrophages is enhanced by iron loading and down-regulated by hepcidin. *Blood* **106**, 3979–3984.
[52] Frazer, D. M., Inglis, H. R., Wilkins, S. J., Millard, K. N., Steele, T. M., McLaren, G. D., McKie, A. T., Vulpe, C. D., and Anderson, G. J. (2004) Delayed hepcidin response explains the lag period in iron absorption following a stimulus to increase erythropoiesis. *Gut* **53**, 1509–1515.
[53] Nemeth, E., Tuttle, M. S., Powelson, J., Vaughn, M. B., Donovan, A., Ward, D. M., Ganz, T., and Kaplan, J. (2004) Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* **306**, 2090–2093.
[54] Laftah, A. H., Ramesh, B., Simpson, R. J., Solanky, N., Bahram, S., Schumann, K., Debnam, E. S., and Srai, S. K. (2004) Effect of hepcidin on intestinal iron absorption in mice. *Blood* **103**, 3940–3944.
[55] Labardini, J., Papayannopoulou, T., Cook, J. D., Adamson, J. W., Woodson, R. D., Eschbach, J. W., Hillman, R. S., and Finch, C. A. (1973) Marrow radioiron kinetics. *Haematologia (Budap)* **7**, 301–312.
[56] Koury, M. J. and Bondurant, M. C. (1990) Erythropoietin retards DNA breakdown and prevents programmed death in erythroid progenitor cells. *Science* **248**, 378–381.
[57] Kattamis, A., Papassotiriou, I., Palaiologou, D., Apostolakou, F., Galani, A., Ladis, V., Sakellaropoulos, N., and Papanikolaou, G. (2006) The effects of erythropoetic activity and iron burden on hepcidin expression in patients with thalassemia major. *Haematologica* **91**, 809–812.
[58] Gardenghi, S., Marongiu, M. F., Ramos, P., Guy, E., Breda, L., Chadburn, A., Liu, Y., Amariglio, N., Rechavi, G., Rachmilewitz, E. A., Breuer, W., Cabantchik, Z. I., Wrighting, D. M., Andrews, N. C., de Sousa, M., Giardina, P. J., Grady, R. W., and Rivella, S. (2007) Ineffective erythropoiesis in beta-thalassemia is characterized by increased iron absorption mediated by down-regulation of hepcidin and up-regulation of ferroportin. *Blood* **109**, 5027–5035.
[59] Jenkins, Z. A., Hagar, W., Bowlus, C. L., Johansson, H. E., Harmatz, P., Vichinsky, E. P., and Theil, E. C. (2007) Iron homeostasis during transfusional iron overload in beta-thalassemia and sickle cell disease: changes in iron regulatory protein, hepcidin, and ferritin expression. *Pediatr. Hematol. Oncol.* **24**, 237–243.
[60] Kearney, S. L., Nemeth, E., Neufeld, E. J., Thapa, D., Ganz, T., Weinstein, D. A., and Cunningham, M. J. (2007) Urinary hepcidin in congenital chronic anemias. *Pediatr. Blood Cancer* **48**, 57–63.
[61] Agarwal, N. and Prchal, J. T. (2009) Anemia of chronic disease (anemia of inflammation). *Acta. Haematol.* **122**, 103–108.
[62] Frazer, D. M., Wilkins, S. J., Becker, E. M., Murphy, T. L., Vulpe, C. D., McKie, A. T., and Anderson, G. J. (2003) A rapid decrease in the expression of DMT1 and Dcytb but not Ireg1 or hephaestin explains the mucosal block phenomenon of iron absorption. *Gut* **52**, 340–346.
[63] O'Neil-Cutting, M. A. and Crosby, W. H. (1987) Blocking of iron absorption by a preliminary oral dose of iron. *Arch. Intern. Med.* **147**, 489–491.
[64] Smith, M. D. and Pannacciulli, I. M. (1958) Absorption of inorganic iron from graded doses: its significance in relation to iron absorption tests and mucosal block theory. *Br. J. Haematol.* **4**, 428–434.
[65] Stewart, W. B., Yuile, C. L., Claiborne, H. A., Snowman, R. T., and Whipple, G. H. (1950) Radioiron absorption in anemic dogs; fluctuations in the mucosal block and evidence for a gradient of absorption in the gastrointestinal tract. *J. Exp. Med.* **92**, 375–382.
[66] Crosby, W. H. (1966) Mucosal block. An evaluation of concepts relating to control of iron absorption. *Semin. Hematol.* **3**, 299–313.
[67] Yeh, K. Y., Yeh, M., Watkins, J. A., Rodriguez-Paris, J., and Glass, J. (2000) Dietary iron induces rapid changes in rat intestinal divalent metal transporter expression. *Am. J. Physiol. Gastrointest. Liver Physiol.* **279**, G1070–G1079.
[68] Ma, Y., Yeh, M., Yeh, K. Y., and Glass, J. (2006) Iron Imports. V. Transport of iron through the intestinal epithelium. *Am. J. Physiol. Gastrointest. Liver Physiol.* **290**, G417–G422.
[69] Ma, Y., Specian, R. D., Yeh, K. Y., Yeh, M., Rodriguez-Paris, J., and Glass, J. (2002) The transcytosis of divalent metal transporter 1 and apo-transferrin during iron uptake in intestinal epithelium. *Am. J. Physiol. Gastrointest. Liver Physiol.* **283**, G965–G974.
[70] Dugani, C. B. and Klip, A. (2005) Glucose transporter 4: cycling, compartments and controversies. *EMBO Rep.* **6**, 1137–1142.
[71] Noda, Y. and Sasaki, S. (2006) Regulation of aquaporin-2 trafficking and its binding protein complex. *Biochim. Biophys. Acta.* **1758**, 1117–1125.
[72] Cayouette, S. and Boulay, G. (2007) Intracellular trafficking of TRP channels. *Cell Calcium* **42**, 225–232.
[73] Shepherd, J. D. and Huganir, R. L. (2007) The cell biology of synaptic plasticity: AMPA receptor trafficking. *Annu. Rev. Cell Dev. Biol.* **23**, 613–643.
[74] Hou, J. C. and Pessin, J. E. (2007) Ins (endocytosis) and outs (exocytosis) of GLUT4 trafficking. *Curr. Opin. Cell Biol.* **19**, 466–473.
[75] Tabuchi, M., Tanaka, N., Nishida-Kitayama, J., Ohno, H. and Kishi, F. (2002) Alternative splicing regulates the subcellular localization of divalent metal transporter 1 isoforms. *Mol. Biol. Cell.* **13**, 4371–4387.
[76] Lam-Yuk-Tseung, S. and Gros, P. (2006) Distinct targeting and recycling properties of two isoforms of the iron transporter DMT1 (NRAMP2, Slc11A2). *Biochemistry* **45**, 2294–2301.
[77] Lam-Yuk-Tseung, S., Touret, N., Grinstein, S., and Gros, P. (2005) Carboxyl-terminus determinants of the iron transporter DMT1/SLC11A2 isoform II (–IRE/1B) mediate internalization from the plasma membrane into recycling endosomes. *Biochemistry* **44**, 12149–12159.
[78] Núñez, M. T., Tapia, V., Rojas, A., Aguirre, P., Gómez, F., and Nualart, F. (2010) Iron supply determines apical/basolateral membrane distribution of intestinal iron transporters DMT1 and ferroportin 1. *Am. J. Physiol. Cell Physiol.*, **298**, C477–485.
[79] Thomas, C. and Oates, P. S. (2004) Ferroportin/IREG-1/MTP-1/SLC40A1 modulates the uptake of iron at the apical membrane of enterocytes. *Gut* **53**, 44–49.
[80] Arredondo, M., Tapia, V., Rojas, A., Aguirre, P., Reyes, F., Marzolo, M. P., and Núñez, M. T. (2006) Apical distribution of HFE-beta2-

microglobulin is associated with inhibition of apical iron uptake in intestinal epithelia cells. *Biometals* **19**, 379–388.

[81] Arredondo, M., Muñoz, P., Mura, C. V., and Núñez, M. T. (2001) HFE inhibits apical iron uptake by intestinal epithelial (Caco-2) cells. *Faseb J.* **15**, 1276–1278.

[82] Knight, A., Hughson, E., Hopkins, C. R., and Cutler, D. F. (1995) Membrane protein trafficking through the common apical endosome compartment of polarized Caco-2 cells. *Mol. Biol. Cell* **6**, 597–610.

[83] Rodriguez-Boulan, E., Kreitzer, G., and Musch, A. (2005) Organization of vesicular trafficking in epithelia. *Nat. Rev. Mol. Cell Biol.* **6**, 233–247.

[84] Hughson, E. J. and Hopkins, C. R. (1990) Endocytic pathways in polarized Caco-2 cells: identification of an endosomal compartment accessible from both apical and basolateral surfaces. *J. Cell. Biol.* **110**, 337–348.

[85] Yeh, K. Y., Yeh, M., Mims, L., and Glass, J. (2009) Iron feeding induces ferroportin 1 and hephaestin migration and interaction in rat duodenal epithelium. *Am. J. Physiol. Gastrointest. Liver Physiol.* **296**, G55–G65.

[86] Frazer, D. M., Wilkins, S. J., Becker, E. M., Vulpe, C. D., McKie, A. T., Trinder, D., and Anderson, G. J. (2002) Hepcidin expression inversely correlates with the expression of duodenal iron transporters and iron absorption in rats. *Gastroenterology* **123**, 835–844.

[87] Yeh, K. Y., Yeh, M., and Glass, J. (2004) Hepcidin regulation of ferroportin 1 expression in the liver and intestine of the rat. Am. J. Physiol. *Gastrointest. Liver Physiol.* **286**, G385–G394.