Chapter 6
Intestinal Iron Absorption

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1 Introduction

The last 15 years has seen tremendous advances made in our understanding, at the molecular level, of the proteins involved in iron transport and regulation of iron metabolism with the major players involved in intestinal non-heme iron transport now identified. This achievement comes after several decades had seen little progress in this area and it was the application of modern molecular biology techniques starting about 25 years ago that has revolutionized our understanding. Advances have also been made in understanding the regulation of iron absorption with the iron hormone hepcidin emerging as the key systemic regulator. Moreover, the regulation of hepcidin production by several well-known signal transduction pathways has been shown and the stage has now been reached where intervention treatments based on molecules which regulate hepcidin represent viable approaches to the treatment of iron disorders. The molecular regulation of the main intestinal proteins involved in iron absorption is well advanced. The present chapter will bring together recent findings on physiology, molecular biology, and biochemistry of iron absorption and attempt to provide an integrated view of the regulation of this process.

2 Overview of Iron Metabolism in Mammals

In man, the normal diet should contain 13–18 mg of iron per day, of which only 1 mg is absorbed. In iron deficiency, absorptive capacity may be increased to 2–4 mg and in iron overload it is reduced to 0.5 mg. Under normal circumstances, the intestine takes up more iron than is required and, depending on the body's demand for iron, a certain amount is transferred to the circulation, the rest being retained by the enterocyte and lost when the villus cells are exfoliated. The principle site of iron absorption is the duodenum and proximal jejunum. Once iron is absorbed into the bloodstream, it is conserved and there is little excretion via the kidneys. This was established by pioneering work of McCance and Widdowson who were the first to suggest that body iron stores were determined by

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regulation of intestinal iron absorption [1]. They found that when various transition metals were intravenously injected into the body, iron was the only metal not rapidly excreted in the urine.

The formation of red blood cells requires about 30 mg of iron daily and this is balanced by an equal flux of iron from the breakdown of senescent red blood cells by the reticuloendothelial (RE) cells in the spleen, liver (Kupffer cells), and bone marrow. Body iron losses are small in comparison and are associated with the sloughing of epithelial cells (skin, gastrointestinal cells, urinary tract cells) and the loss of fluids (e.g., tears, sweat, and particularly in menstruating women, blood). This accounts for the loss of 1 mg/day of iron. In man, dietary iron intake consists of two components: heme iron (predominantly in red meat) and non-heme or inorganic iron (also abundant in meat, but the main form of iron in vegetables, cereals, etc.). The absorption of both is discussed below.

3 Heme Iron Absorption

Intestinal absorption of heme is not yet well understood and it is not clear how heme iron enters the intestinal mucosa. Early work established that heme is released by digestion of hemoproteins in the stomach and duodenal lumen [2–4]. Heme differs from non-heme iron in its solubility and availability profile. Heme tends to form oligomeric aggregates in acid solution and this is promoted if ligands can bridge between the iron atoms chelated within the heme or if neutral molecules such as water are the iron ligands. If high enough concentrations of charged ligands are present, bridging does not occur and heme becomes soluble. Examples of good ligands for heme iron are amines such as arginate and importantly, hydroxide. Thus, solubility of heme is promoted by high concentrations of hydroxide (i.e., higher pH values) or amino acids or peptides that can act as ligands to the iron within heme. As with non-heme iron absorption (see below), the machinery for heme iron absorption is most active in the proximal intestine [5] and involves breakdown of the heme within the mucosa by heme oxygenase [6] with release of iron that is then transported to the blood, probably by the same mechanism used for non-heme iron (i.e., via ferroportin as discussed in detail below). The mucosal uptake of heme appears to involve a receptor on the brush border membrane [7] and transport of the heme into the enterocytes. HCP1 was identified as a candidate transporter for heme [8]; however, this protein was subsequently shown to be more active as a folate transporter and renamed PCFT [9, 10]. Loss of function mutations in PCFT/HCP1 are associated with hereditary folate deficiency and further work is needed to clarify whether this protein plays any significant role in heme absorption. It is noteworthy that absorption of heme iron is not regulated as tightly by iron stores as non-heme iron absorption [7], presumably relating to a less tightly regulated mucosal uptake step. The oxidation state of heme iron does not seem to affect its solubility and the absorption of heme iron is not affected by food factors that alter non-heme iron absorption. Thus, heme iron has a relatively high bioavailability (15–30%) that is relatively constant. One result of this weaker regulation and high bioavailability is that higher iron stores are associated with high intakes of heme iron [11–13].

4 Non-Heme Iron Absorption

Knowledge of non-heme iron absorption is much more extensive than that of heme absorption as the former is more highly regulated and more associated with iron deficiency. The remainder of this chapter focuses on non-heme iron absorption. Non-heme (or inorganic) iron is present in the diet as either the reduced ferrous (Fe(II), Fe^{2+}) ionic form or the oxidized ferric (Fe(III), Fe^{3+}) form. Under normal physiological conditions (i.e., neutral pH and in the presence of oxygen), ferrous iron is

rapidly oxidized to the ferric form which has a strong tendency to precipitate as iron hydroxide. Several luminal factors, both in the diet and secreted by the gut, can have marked effects on the absorption of dietary iron. In studies of iron absorption in animals and humans, subjects are normally fasted before administering radioactively labeled iron into the intestine. The presence of food drastically reduces the bioavailability of non-heme iron due to components of the diet (such as phytates, polyphenols) binding the iron to form complexes that are not available for intestinal uptake [14]. In contrast, the presence of luminal reducing agents, such as ascorbate, is known to enhance iron absorption. Iron binding or complexing agents, forming weaker, soluble low-molecular-mass complexes (such as citrate, ascorbate, and perhaps some amino acids and peptides and other organic acids) can also enhance iron absorption. Non-heme iron is absorbed early in digestion mainly in the duodenum where the low pH favors solubility and reduction of iron. Further down the intestine, it is likely that the formation of insoluble ferric complexes reduces bioavailability. The transport of non-heme iron across the duodenal mucosa has been studied intensively over the years and is highly adaptive to changes in iron requirements (low iron stores, pregnancy, erythropoiesis, hypoxia). Much progress has been made in the last few years in identifying the proteins involved in this process and these will be described below.

5 Anatomical Location of Iron Absorption

It has been shown that iron in the stomach is relatively soluble and significant amounts are reduced to Fe (II) by dietary and secreted factors [15, 16]. The low pH of the stomach is a major factor in the release of ionized, soluble iron from the food, and loss of gastric acid secretion can compromise iron absorption, leading to anemia [17]. Early studies of the iron absorption mechanism and its regulation established that this process was mainly confined to the duodenum and proximal jejunum where the small intestinal lumenal pH is most suited to maintaining non-heme iron in a soluble form (reviewed in [18]). With the exception of hephaestin, whose mRNA is more uniformly expressed along the length of the gut, the duodenum and proximal jejunum coincides with highest expression of various iron transport molecules – DCYTB, DMT1, and FPN. However, more recent data have shown that the colon also expresses relatively high levels of FPN, DMT1, and hephaestin, but not DCYTB [19, 20]. This would suggest that the colon may also be important for iron metabolism and, given the low expression of FPN in the ileum, it is possible that some iron lost through ileal epithelial cell sloughing is reabsorbed in the colon. The lack of DCYTB in the colon is interesting and may reflect the fact that the environment in the colonic lumen is reducing and therefore a surface reductase is not required, that another reductase is present, or that another form of iron is absorbed. Due to its high iron absorptive capacity with a high degree of regulation, the remainder of this chapter focuses on proximal intestine and especially the duodenum.

6 Iron Absorption: A Two-Step Process

Manis and Schachter showed in 1961 that the absorption process in the proximal intestine can be divided into two steps, namely, *uptake* of lumenal iron into the mucosa and *transfer* of iron from mucosa to the blood [21]. This terminology has been almost universally adopted in subsequent work, as it fits not only with iron absorption kinetic parameters that have been extensively measured in vivo, but also with the major sites of localization of the principle proteins involved in iron absorption (below).

Early work provided evidence for the regulation of iron absorption at both the uptake and transfer steps, with a few experiments suggesting distinct mechanisms operated on the two steps. For a review of the early studies on iron absorption, please see [22]. Simple principles of metabolic regulation would lead one to expect the primary regulated step in a pathway would be the first committed step. In many metabolic pathways, this is indeed observed. In the case of a potentially toxic nutrient like iron, things get more complicated. Older ideas ("mucosal block") focused on iron as a potentially toxic metal and the body had to be protected from excess absorption. Such ideas could fit with the primary regulated step being the basolateral transfer step. However, iron is essential as well as being potentially toxic, not only for the body but also for the enterocytes responsible for absorbing it from the diet. The latter consideration means that mucosal uptake also needs to be regulated and some regulated coordination of the two steps is needed to maintain an efficient flux across the enterocyte and prevent enterocyte iron levels dropping too low or building up too high. Elegant molecular mechanisms have been identified which can bring about this complex regulation and these will be described in more detail below. Current molecular evidence supports earlier kinetic studies and indicates that the basolateral transport of iron is rate-limiting for iron entry into the circulation.

7 Proteins Involved in Uptake: DMT1 and DCYTB

7.1 DMT1

Divalent metal-ion transporter 1 (DMT1; also known as NRAMP2 or DCT1) was the first mammalian iron transporter to be identified by two groups working independently. Gunshin et al. [23] used the *Xenopus* oocyte expression cloning system to identify a cDNA that caused a rapid inward current in the presence of ferrous iron in the external medium in comparison with a water-injected control. The transport of iron into the oocyte was highly dependent on an inward-directed proton gradient. The mechanism of iron transport is therefore likely to be proton coupled and requires a pH gradient [24]. *DMT1* mRNA was shown to be highly expressed in the duodenum and strongly upregulated in iron deficiency. The mRNA was later shown to contain a functional iron responsive element (IRE) in its 3′ untranslated region which can mediate iron-dependent regulation [25]. In the other study, Fleming et al. were working on the genetic basis of the microcytic anemia (mk/mk) mouse [26]. This mouse strain has a hypochromic, microcytic anemia and affected animals have both defective intestinal iron absorption and reduced iron uptake by developing erythroid cells. The causative gene was identified as *Nramp2* [26]. The same group later showed an identical mutation was present in Dmt1 in the Belgrade rat which has a similar microcytic anemia [27]. Thus, the expression pattern along with transport data and powerful genetic evidence are consistent with DMT1 being responsible for the regulated step of duodenal iron uptake. Genetic knockout experiments have since shown that DMT1 is essential for life [28] and human DMT1 mutations that cause anemia have been identified [29–31]. DMT1 plays a role in reticulocyte iron uptake (and indeed in iron uptake by most body cells) as well as intestinal iron uptake, and mice with global Dmt1 knockout die within a few days of birth with severe anemia [28]. Complete loss of Dmt1 is more severe than the mk or Belgrade mutations mentioned above, indicating that these mutants retain some Dmt1 function. In an intestine-specific Dmt1 knockout, survival is improved but mice rapidly become iron deficient after weaning, indicating DMT1 is the major pathway for iron absorption in adults [28]. A detailed study of iron absorption in Dmt1 knockout mice has not been published; however, there are sufficient published data [28] to estimate total body iron levels (and therefore dietary iron absorption) in such mice. The fact that the mice grow normally for at least 3 days after birth strongly suggests normal iron absorption from dam's milk. Thus, neonatal iron absorption from the mother's milk seems to be Dmt1 independent. Mice start to consume adult foods at about 15 days of age and wean at about 21 days and a progressive

decline in body iron seems to occur from about 15 days of age in the Dmt1 intestinal knockout. Intestine-specific Dmt1 knockout mice still grow from 4 to 8 weeks of age and therefore their calculated total body iron increases, suggesting that a non-DMT1 absorption pathway may be present [28]. The assumptions needed for the total body iron calculation, however, make this increase debatable. Suggestions have been made that in mice in which both the *Dmt1* (intestine-specific) and *Hfe* genes have been knocked out, an alternative iron absorption pathway exists [32]. However, the data supporting this possibility have not been published and the conclusion is based on the improved survival of the double knockout mice [28]. This increased survival could be explained by increased prenatal and neonatal iron absorption associated with the *Hfe* deletion, leading to increased iron stores at weaning that allow the Dmt1 intestinal knockout animals to survive a little longer. In addition, the tissue-specific gene knockout may not be 100% efficient in all the mice. The question of alternate (i.e., non-DMT1) iron absorption pathways that may make a minor contribution to adult iron absorption therefore remains to be resolved and more detailed studies of the Dmt1 intestinal knockout mouse need to be carried out.

DMT1 has been shown to transport other divalent metals such as Zn, Mn, Co, and Cd. However, whether this property of DMT1 has any physiological relevance is open to question. In the case of zinc, absorption from the diet is controlled by members of the ZIP and ZNT family. There is also evidence that ZIP4 and ZIP14 can transport iron and may play a role in iron uptake in some tissues [33, 34]. It has been demonstrated that manganese absorption is impaired in Belgrade rats (presumably as a result of the mutation in Dmt1 [35]). Iron deficiency or anemia cause increases in tissue cadmium levels, suggesting that these conditions could be risk factors for cadmium toxicity [36]. Interestingly, the increased Cd uptake may not be mediated by DMT1 [37, 38]. On the other hand, Zn and Cu have been reported to regulate DMT1 expression [39, 40], providing another possible mechanism for metal interactions with iron absorption.

7.2 DCYTB

DMT1 transports only ferrous iron whereas dietary iron is likely to be mostly in the ferric form. Thus, a ferric reductase was predicted to be present in the duodenal mucosa [41]. The presence of such a surface ferric reductase activity in the duodenum was first described some time before the discovery of DMT1 [42]. The reductase activity was strongly stimulated by hypoxia and iron deficiency, both of which stimulate iron absorption, especially of ferric iron [42]. In addition, it was found that the activity was highest in the duodenum and lowest in the ileum, compatible with the profile of iron absorption along the gut. Attempts to purify the protein responsible for this activity provided evidence that it was associated with a b-type heme center that was immunologically distinct from the NADPH oxidase GP91-Phox [43]. The protein was, however, never successfully purified using biochemical methods as the heme was lost early in the purification [44]. The gene responsible for this activity, Dcytb (for duodenal cytochrome b; also called Cybrd1), was eventually identified using a subtractive cloning strategy [45]. The protein sequence of DCYTB was homologous to cytochrome b561, a b-type heme transmembrane dehydroascorbate reductase highly expressed in chromaffin granule membranes in the adrenal medulla [46, 47]. The role of b561 is to reduce granular dehydroascorbate to ascorbate by transporting an electron donated by cytoplasmic ascorbate across the granule membrane [48]. In addition to b561, DCYTB was identical to the N terminus of a protein called P30 [49, 50]. When expressed in either *Xenopus* oocytes or cultured cells, Dcytb induces ferric reductase activity. The protein has recently been found at high concentrations in the membrane of mature red blood cells of scorbutic species such as human and guinea pig, but is absent from those of non-scorbutic species (those able to synthesize ascorbic acid) such as mice and rats [51]. This has led to the hypothesis that DCYTB plays a physiological role in ascorbate regeneration; however, this has yet to be confirmed.

7.3 Dcytb Knockout Mouse

In 2005, Gunshin et al. described the phenotype of a Dcytb knockout mouse in which Dcytb was deleted in all tissues [52]. The Dcytb knockout mice on the 129 background displayed a relatively mild phenotype – relative to loss of either DMT1 or FPN – with a small reduction in liver iron, normal hematological parameters with no overt anemia. The authors therefore concluded that DCYTB was not required for iron absorption in mice. However, the study was limited to measurement of liver iron and no measurements of iron absorption itself or duodenal reductase activity were made to rule out compensatory effects from other ferric reductases [53]. The study did not address possible confounding strain effects, e.g., the fact that the 129 strain are highly resistant to iron deficiency due to their high liver iron stores (relative to other strains such as C57) and do not become anemic even when chronically fed iron-restricted diets. Hence the effect of lack of DCYTB may be more obvious in another background such as C57. We have now examined the same DCYTB knockout mice used by Gunshin et al. and measured duodenal ferric reductase activity. We found that Dcytb is the only iron-regulated duodenal ferric reductase and that in Dcytb knockout mice inclusive there were significant decreases in spleen iron compared to WT mice (Choi et al. 2010, unpublished). We conclude that Dcytb is required for optimal iron metabolism likely by increasing the bioavailability of ferrous iron for the transporter DMT1, a process that is likely to be important under dietary iron-limited conditions.

7.4 Proteins Involved in Iron Transfer: Ferroportin and Hephaestin

The basolateral iron transporter was identified by three groups working independently and therefore surfaces in the literature under three different aliases: Ireg1 [54], ferroportin [55], and MTP1 [56]. The more descriptive appellation of ferroportin (FPN) is now most widely used. FPN was first isolated and reported using the same strategy which identified Dcytb [54]. The predominant mRNA for FPN contains a functional IRE sequence, is highly localized to the duodenum and is regulated by several independent stimulators of iron absorption. The presence of an alternatively spliced, non-IRE FPN mRNA has been recently described and resolves some issues regarding FPN regulation (see below) [57]. The gene encodes a highly hydrophobic membrane protein with 10–12 transmembrane spanning domains, which bears little sequence identity with any other transporter family. Transfecting polarized epithelial cells with a tagged FPN showed the protein was targeted to the basolateral membrane. This finding led to the hypothesis that FPN was an iron-regulated protein involved in the transfer of iron to the circulation across this membrane. The *Xenopus* oocyte expression system demonstrated that FPN did indeed stimulate efflux of iron [54]. Donovan et al. [55] used positional cloning to identify the gene responsible for the *wiessherbst* (weh) mutant phenotype in zebrafish, so called because their lack of hemoglobin gives them a pale appearance. The gene they identified was the zebrafish homologue of FPN. In zebrafish, FPN was found to be expressed in the yolk sac where it is likely to be responsible for transfer of iron from the maternally derived iron stores to the embryonic circulation. In the third report, an approach of enriching for cDNAs containing IREs [56] was used.

In addition to its role in the transfer of iron from the intestine to the circulation, FPN likely also plays an important role in iron transport in other cells, notably the macrophages where iron efflux is required to recycle iron back into the circulation from the breakdown of hemoglobin. These red cell recycling macrophages are found in the liver (Kupffer cells), bone marrow, and splenic red pulp. FPN knockout mice die early in life confirming that FPN is essential in mammals [58]. Selective knockout of the gene in the intestine confirmed that FPN was required for intestinal iron absorption [58].

Mutations in FPN cause type IV hemochromatosis [59–61] also known as Ferroportin disease with an autosomal dominant inheritance distinct from HFE mutations. The clinical data from these patients shows distinct differences from patients with HFE mutations [59]. In some patients with FPN mutations, serum ferritin levels are very high and reticuloendothelial (RE) cells are severely iron loaded. In contrast, in HFE patients macrophages are low in iron [59]. These data suggest a fault in the recycling of macrophage iron in patients with FPN mutations, consistent with the high expression of FPN in these cells. In other patients, transferrin saturation is high and the defect appears to be the inability of FPN to recognize hepcidin, so-called hepcidin resistance [62, 63]. Hereditary defects causing diseases of iron metabolism are discussed extensively elsewhere in this volume.

Mice with sex-linked anemia (sla) develop anemia due to a defect in the intestinal transfer of iron to the circulation. As a consequence, iron builds up in the enterocytes and the animals become anemic [64, 65]. Using a positional cloning approach, the defective gene was identified by Anderson, Vulpe, and coworkers [66]. Interestingly, the protein sequence encoded by this novel gene (hephaestin) was very similar to ceruloplasmin, a copper-containing protein with ferroxidase activity. However, unlike ceruloplasmin, which is a secreted protein, hephaestin contains a single putative transmembrane domain at its C terminus, which may serve to anchor the protein into a membrane. Hephaestin is highly expressed in small intestine, though it does not show the regional predominance in the duodenum that FPN1, Dcytb, or DMT1 display. In fact, hephaestin is expressed along the length of the gut with no obvious gradient. This suggests hephaestin may have additional roles in the intestine unrelated to iron absorption. The subcellular location of hephaestin is also puzzling for a protein implicated in transfer of iron to the circulation. Studies indicate the protein is found not only on the basolateral membrane but also in intracellular perinuclear vesicles [20, 67]. The transport of iron through the enterocyte itself is an important aspect of the absorptive process about which little has been confirmed [68]. It is possible that hephaestin has a role in this process or has a separate function in basolateral cytoplasmic vesicles (see below for further discussion of vesicle trafficking in iron absorption).

8 The Regulation of Iron Absorption

The two key membrane iron transporters, DMT1 and FPN, show remarkably complex regulation, with the critical levels of each in their respective membranes determined by a combination of transcriptional, translational/mRNA stability and protein trafficking and turnover mechanisms (Fig. 6.1). The regulation of FPN in particular shows an apparently inconsistent set of regulatory mechanisms, with transcription being increased by low iron but translation of the resulting major mRNA being blocked by IRPs under the same conditions. A third level of regulation operates through the action of hepcidin to downregulate FPN protein at the basolateral membrane when iron levels are high. The presence of multiple control mechanisms leads to some degree of redundancy and knockout and mutant mice show varying degrees of loss of iron absorption regulation. In order to understand this complexity, one needs to consider the fact that the enterocyte has intrinsic (housekeeping) iron requirements. Although these requirements may be quantitatively small compared to overall body requirements, they are nevertheless important to the enterocytes and may conflict with body iron requirements and the need to regulate flux of iron across the enterocyte. DMT1 regulation seems relatively straightforward in that the enterocyte's intrinsic need to control its housekeeping iron levels operates in the same direction as the body's iron requirements, so both enterocyte and body need to increase iron uptake at times of iron depletion. There are again three levels of regulation – transcriptional, regulation of the stability and therefore the level of translatable mRNA by IRPs, and the trafficking of the protein to/from the BBM by iron levels. DCYTB regulation seems much simpler with primarily a transcriptional regulation. Hephaestin regulation is less well understood. We will describe the various mechanisms by which iron absorption gene expression is controlled, then describe an integrated model that explains the functional significance of these various mechanisms.

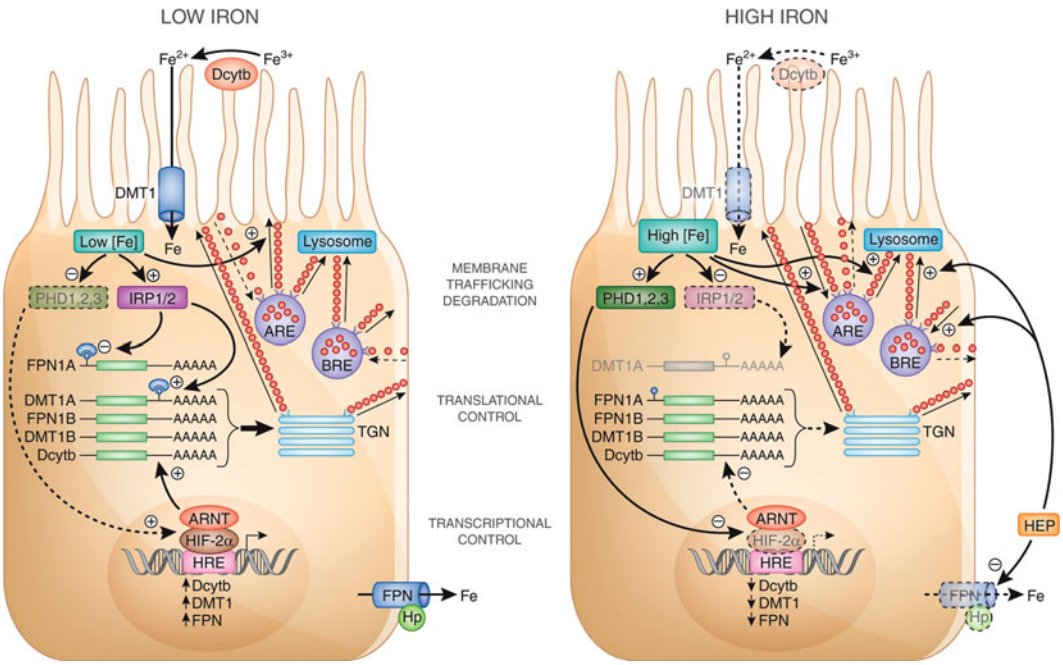

Fig. 6.1 Iron absorption regulation in enterocytes. Regulation operates at three levels (**a**) transcription of iron absorption genes in the nucleus is controlled by HIF2α; (**b**) levels and translation rates of some mRNAs are controlled by the IRP/IRE system in the cytosol and rough endoplasmic reticulum; and (**c**) the localization and degradation of some proteins in their target plasma membranes is also regulated. These differing mechanisms of regulation operate on differing timescales: transcription affects protein levels after some hours, protein turnover in membranes can be directly altered in minutes, and effects on mRNA can lead to changes in protein levels from minutes to hours later. ARE-Apical recyling endosome, BRE-basolateral recyling endosome

8.1 Transcriptional Control of DMT1, DCYTB, and FPN by HIF2α

Until recently, an iron-dependent mechanism for controlling transcription of the key iron absorption genes was lacking and no iron-sensitive transcription factors had been identified in the enterocyte. However, hypoxia-inducible factors (HIFs) have recently been identified as potential iron sensors in the duodenum. HIFs have long been known to be key oxygen-sensitive transcription factors identified as regulating erythropoietin expression [69], but they are now known to regulate a host of genes including many iron metabolism genes [70]. At the heart of the oxygen-sensing mechanism lie the prolyl hydroxylases, (PHDs of which there are three isoforms PHD1-3) which hydroxylate HIF and potentially respond to oxygen, iron, and ascorbate levels. Thus, these enzymes have the potential to act as iron sensors [71] and HIF could operate as an iron-sensitive transcription factor.

HIF2α has been identified as a key transcription factor that regulates genes encoding proteins involved in iron absorption in landmark studies by Shah et al. [70] and Mastrogiannaki et al. [72, 73]. Dcytb does not have a recognizable IRE and hence its strong regulation by iron and hypoxia therefore seemed likely to be transcriptionally controlled. Both studies show that DMT1 and Dcytb contain hypoxia response elements (HREs) within their promoters, which preferentially bind HIF2α, rather than HIF-1α, and activate transcription. Selective deletion of HIF2α in the enterocytes of mice results in low levels of DMT1 and Dcytb (as well as FPN1 and hephaestin, suggesting that these genes are also dependent on HIF2α), leading to low serum and liver iron and anemia. Interestingly, this effect could not be counteracted by reduced hepcidin levels in these mice. These findings help resolve a long standing observation that increased iron absorption (and in particular iron uptake) is an early (6–8 h) response to hypoxia [74, 75], preceding alterations in plasma iron, erythropoiesis, or liver iron levels.

8.2 Posttranscriptional Control by IRPs

Although several of the iron transporters (DMT1 and FPN) contain IREs, the control of iron absorption by the IRP/IRE mechanism has never been adequately explained. The IRE in the 5′ region of FPN has been shown to bind IRPs [54], and this strongly inhibits protein translation as intestinal Irp knockout mice have massive increases in FPN protein levels [76]. DMT1 protein, on the other hand, contains a 3′ IRE which stabilizes the mRNA, resulting in increased protein levels in iron deficiency [25, 76]. Dcytb and hephaestin, however, do not appear to contain IREs despite (in the case of Dcytb) being highly upregulated by iron deficiency.

However, some clarity may now be emerging with recent studies suggesting that non-IRE splice forms of DMT1 and FPN may be of greater relative importance for iron absorption. The IRE forms, on the other hand, may regulate iron for housekeeping purposes and prevent the enterocyte from becoming too iron deficient or iron loaded. Zhang and colleagues showed that two splice variants of FPN exist, one containing a 5′ IRE (FPN1A) and a non-IRE FPN1B [57]. Importantly, the FPN1B transcript appears to make up over 20% of the total duodenal FPN. The FPN1B transcript was highly responsive to iron, implying transcriptional regulation. DMT1 also exists as a non-IRE form which is upregulated by iron deficiency [76]. Hence it appears that transcriptional regulation of the non-IRE forms of FPN and DMT1, which leads to an upregulation of these genes under iron-deficient conditions and enables them to evade regulation by the IRP/IREs is of primary importance in regulating iron absorption. Precisely why FPN mRNA has a splice variant that can be translationally blocked in iron deficiency is unclear. Perhaps this mechanism prevents the enterocyte from becoming iron deficient to such an extent that its normal metabolism is compromised.

8.3 Control of Protein Trafficking by Iron (DMT1) and Hepcidin (FPN)

It has been known for many years that high oral doses of iron downregulate iron absorption. Part of this downregulation is associated with loss of DMT1 from the brush border membrane due to altered protein trafficking [77, 78]. The details of how this occurs have not been worked out. In contrast, the trafficking of ferroportin away from the basolateral membrane is known to be mediated by hepcidin binding [79] and this effect is thought to be central to the action of hepcidin to downregulate iron absorption. Hepcidin action is considered in detail in Chap. 9.

It has been suggested that some fraction of iron absorption proceeds via vesicular transport mechanisms [68]. The possibility that some or all of the intestinal absorption of iron is mediated by transcytosis, or pathways partly involving endocytosis or exocytosis of iron in vesicles was first raised by Johnson et al. [80] who showed that microtubule poisons are effective inhibitors of iron absorption in vivo in rats. We were not able to reproduce these findings in mice ([81]; also Simpson RJ, unpublished data). More recently, Peres et al. [82] showed in rats that absorption of iron complexed to caseinophosphopeptide was partially inhibited by the endocytosis inhibitor phenylarsine oxide. The inhibition was <30% and absorption of iron complexed to gluconate was not inhibited at all. It is possible that the chemical form of iron given in such experiments affects the outcome, with some iron complexes transported by vesicular transport pathways.

Much more evidence that iron transport across epithelial cells depends on vesicular transport has been obtained in studies of cultured cells [68, 83]. The limitations of such studies as predictors of in vivo iron absorption mechanisms have been discussed by Sharp and Srai [84]. It should be noted that early data on in vivo iron absorption has frequently shown low-affinity iron absorption that is regulated by iron [85–88]. It is difficult to explain such low-affinity pathways by a high-affinity membrane transporter such as DMT1. Such a low-affinity pathway might be explained by a gated pinocytotic transcytosis pathway triggered by iron interacting with DMT1, although it is difficult to

envisage a requirement for FPN for such a pathway. Given the requirement that any physiological pathway in adults must involve DMT1 and FPN, one may suggest that low-affinity pathways are not of physiological interest but may be important pharmacologically and may not require DMT1 or FPN. Studies of absorption of high doses of iron have not yet been carried out in intestine-specific Dmt1 or Fpn knockout mice. Others have found that the rapid phase of iron absorption seems to proceed with iron gaining some access to cytosolic ferritin [89, 90]. On the other hand, others have found in a recent study that iron absorption was little affected by increased cytosolic ferritin [91]. In the absence of more detailed studies in vivo, it is not possible to rule out transcytosis; however, the available data remain most consistent with a membrane transport-based mechanism with iron traversing the cytosol. This latter pathway could also be affected by microtubule inhibitors if the localization of any of the key transport components (FPN, Dcytb, Heph, or DMT1) was affected by such inhibitors.

Overall, these protein trafficking regulation mechanisms can be seen as rapidly acting controls that can downregulate iron uptake or iron absorption within minutes. This contrasts with transcription regulation which likely acts on a timescale of hours to alter DMT1 and FPN levels. Translational control via IRPs can act on an intermediary timescale.

9 Systemic Regulation

9.1 Hepcidin

The identification of hepcidin as a key regulator of iron absorption and iron distribution in health and disease has greatly advanced our understanding of iron homeostasis in humans. Hepcidin, synthesized in the liver, circulates in plasma before binding to FPN on enterocytes, macrophages, and other cells, promoting degradation of the transporter and thereby inhibiting iron release from these cells [79]. Hepcidin can be regulated positively (by increased iron stores and inflammatory cytokines such as IL6) [92, 93] or negatively (by anemia and hypoxia) [93], although the mechanism(s) involved have not been fully elucidated. The relative importance and crosstalk between these factors and the signaling pathways involved in determining net levels of plasma hepcidin is not clear. Hepcidin regulation is discussed in more detail in Chap. 9.

9.2 Liver Iron Sensing (TfR2, HFE, TfR1, HJV, BMPs)

It is thought that the level of transferrin saturation or the level of diferric transferrin is an important determinant of hepcidin levels, but how this system operates is not completely understood [94]. Primary mouse hepatocytes increase hepcidin levels in response to increasing transferrin saturation [95]. TfR2 has been implicated in hepcidin regulation via changes in levels of transferrin and/or transferrin saturation. TfR2 shares 45% sequence identity with TfR1, is abundantly expressed on hepatocytes, and binds diferric transferrin with 20–30-fold lower affinity than TfR1. Whereas TfR1 is inversely regulated by iron expression, TfR2 protein is upregulated in cell lines and animal models in response to increased levels of diferric transferrin, but not apotransferrin or NTBI [96], suggesting that this receptor could serve as an iron sensor on the hepatocyte cell membrane. Furthermore, the finding that humans with mutations in TfR2 develop iron overload [97] associated with hepcidin deficiency and mouse models of whole body knockout (or targeted hepatocyte disruption) of Tfr2 [98] are also hepcidin deficient, is consistent with the idea that this receptor is an iron-sensing molecule on the pathway regulating hepcidin synthesis by the liver.

HFE is clearly involved in regulating hepcidin synthesis as hepcidin deficiency characterizes human and mouse models of HFE-related hemochromatosis [99]; however, its role in iron sensing is unclear. HFE has been shown to bind to TFR1 and to the same domain that also binds diferric transferrin; therefore, HFE and transferrin compete for binding to TfR1 [100]. HFE also binds to TfR2 and binding has been shown to stabilize the receptor [101, 102]. It has been proposed that HFE displaced from TfR1 by diferric transferrin could bind to TfR2 and signal an increase in hepcidin expression, but this mechanism has yet to be proven in vivo.

Hemojuvelin (HJV) contains a C-terminal glycosylphosphatidylinositol anchor and has been found as both cell-associated and soluble forms. The cell-associated form positively regulates hepcidin gene transcription through the BMP signal transduction pathway while the soluble form, demonstrated in cell culture medium and in the circulation in vivo, has been found to suppress hepcidin mRNA in primary cultures of mouse hepatocytes [103]. Ganz and colleagues have proposed that through competitive binding at the hepatocyte membrane, the two forms of HJV reciprocally regulate hepcidin expression in response to changes in extracellular iron concentration [103].

Hence, iron sensing in hepatocytes is likely to be extremely complex and involve HJV, BMPs, TfR2, and HFE. Recently, a liver-specific type II transmembrane serine protease (TMPRSS6, or matriptase-2) has been shown to be required for the appropriate response of hepcidin to iron deficiency [104, 105]. The enzyme has been shown to cleave membrane-bound HJV and therefore act as a negative regulator of hepcidin [106]. In addition, BMP6 has recently been shown to be essential for hepcidin production [107, 108] with Bmp6 KO mice becoming highly iron loaded due to lack of hepcidin [107]. Further work in this area is clearly required.

10 Integrated Control of Iron Flux Across the Enterocyte

Balancing iron flux across the mucosa to provide for body iron requirements and at the same time satisfy the enterocyte's own iron needs is achieved by the above control mechanisms. In addition to these, as in all cells, enterocytes possess ferritin to protect themselves from excess iron and this is regulated by IRPs via a 5′ UTR translation repression mechanism. The rapid downregulation of DMT1 from the apical membrane seems also to operate as a defense against sudden excess iron entry into enterocytes.

Making sense of all these various control mechanisms needs consideration of their differing purposes as well as the differing timescales they operate on. Regulation of iron absorption requires coordinated up/downregulation of all the primary iron-sensitive genes responsible for moving iron across the enterocytes and the HIF system plays a key role in this regulation. On the other hand, the IRP/IRE posttranscriptional control mechanisms perform a housekeeping role which serves to protect the enterocyte from becoming too iron deficient or iron loaded. This is illustrated by the findings with Irp knockout mice. Double knockout of both Irp1 and Irp2 in intestinal cells only (global double knockout is embryonic lethal) leads to severe derangement of enterocytes early in life [76], thus emphasizing the importance of the IRP system for fundamental cell metabolism. Furthermore, Galy et al. noticed that Dmt1 non-IRE mRNA was upregulated and Dmt1 IRE was only mildly decreased in these double knockout mice. This, taken with more drastic effects of the Irp knockout on other IRE-regulated proteins, implied some transcriptional upregulation of Dmt1 gene. The enterocytes of the Irp double intestinal knockout mice were likely to be severely functionally iron deficient due to upregulation of FPN and ferritin protein and the Hif2 transcriptional control provides a molecular mechanism for a compensatory increase in Dmt1 gene transcription that can balance the increased degradation of the Dmt1 IRE mRNA. Irp2 is believed to be the main IRP for iron regulation in enterocytes and selective knockout of this gene leads to increased ferritin and FPN production [91] and therefore functional enterocyte iron deficiency. Once again, Dmt1 is relatively unchanged,

presumably because of increased transcription of the Dmt1 gene in response to the relative enterocyte iron deficiency. Overall iron absorption was found to be unchanged [91], suggesting that the IRP system does not affect regulation of iron absorption *per se*.

The hepcidin/FPN system acts as a signaling mechanism to communicate body iron requirements to the duodenal enterocyte. However, hepcidin itself can be seen primarily to function as the regulator of plasma iron levels. Regulating plasma iron is particularly important. This pool of iron turns over rapidly, yet must supply the erythroid marrow (and other cells) with sufficient iron for their metabolic needs while at the same time preventing excess (and potentially toxic) iron accumulating.

In situations where iron accesses the duodenum in bursts (meal feeding, as in humans, or when high doses of iron are given to animals), the varying timescales of regulatory mechanisms become more apparent. It has long been known that transfer of an oral dose of iron to the carcass of rats follows two phases, an early burst of iron transfer to the plasma and a slower phase of transfer over several hours. During the early phase, existing FPN protein acts to efflux iron to the plasma. Excess iron in the cytosol is taken up by ferritin but also stimulates translation of ferritin and FPN mRNAs and shuts down transcription of the FPN, Dcytb, and DMT1 genes. The arrival of iron in the plasma subsequently leads to increased hepcidin production that will tend to block further efflux from the enterocytes; however, the translation of more FPN1A mRNA will leave some capacity for efflux, albeit at a slower rate.

11 Conclusion

The above description highlights the complexity of iron absorption regulation and the difficulties of providing a simple explanation of this complexity. The most likely explanation is that the various regulatory mechanisms have been acquired at different times through the evolutionary history of mammals, the resulting system being, like many biological structures, not designed from scratch but a make-do-and-mend system that has evolved to meet various physiological requirements as best it can.

References

1. McCance RA, Widdowson EM. Absorption and excretion of iron. Lancet. 1937;2:680–4.
2. Conrad ME, Weintraub LR, Sears DA, Crosby WH. Absorption of hemoglobin iron. Am J Physiol. 1966;211:1123–30.
3. Conrad ME, Benjamin BI, Williams HL, Foy AL. Human absorption of hemoglobin–iron. Gastroenterology. 1967;53:5–10.
4. Weintraub LR, Conrad ME, Crosby WH. Absorption of hemoglobin iron by the rat. Proc Soc Exp Biol Med. 1965;120:840–3.
5. Uzel C, Conrad ME. Absorption of heme iron. Semin Hematol. 1998;35:27–34.
6. Raffin SB, Woo CH, Roost KT, Price DC, Schmid R. Intestinal absorption of hemoglobin iron–heme cleavage by mucosal heme oxygenase. J Clin Invest. 1974;54:1344–52.
7. Latunde-Dada GO, Takeuchi K, Simpson RJ, McKie AT. Haem carrier protein 1 (HCP1): expression and functional studies in cultured cells. FEBS Lett. 2006;580:6865–70.
8. Shayeghi M, Latunde-Dada GO, Oakhill JS, Laftah AH, Takeuchi K, Halliday N, et al. Identification of an intestinal heme transporter. Cell. 2005;122:789–801.
9. Qiu A, Jansen M, Sakaris A, Min SH, Chattopadhyay S, Tsai E, et al. Identification of an intestinal folate transporter and the molecular basis for hereditary folate malabsorption. Cell. 2006;127:917–28.
10. Zhao R, Min SH, Qiu A, Sakaris A, Goldberg GL, Sandoval C, et al. The spectrum of mutations in the PCFT gene, coding for an intestinal folate transporter, that are the basis for hereditary folate malabsorption. Blood. 2007;110:1147–52.
11. Rossi E, Bulsara MK, Olynyk JK, Cullen DJ, Summerville L, Powell LW. Effect of hemochromatosis genotype and lifestyle factors on iron and red cell indices in a community population. Clin Chem. 2001;47:202–8.

12. Cade JE, Moreton JA, O'Hara B, Greenwood DC, Moor J, Burley VJ, et al. Diet and genetic factors associated with iron status in middle-aged women. Am J Clin Nutr. 2005;82:813–20.
13. Greenwood DC, Cade JE, Moreton JA, O'Hara B, Burley VJ, Randerson-Moor JA, et al. HFE genotype modifies the influence of heme iron intake on iron status. Epidemiology. 2005;16:802–5.
14. Turnbull A, Cleton F, Finch CA. Iron absorption. IV. The absorption of hemoglobin iron. J Clin Invest. 1962;41:1897–907.
15. Bergheim O, Kirch ER. Reduction of iron in the human stomach. J Biol Chem. 1949;177:591–6.
16. Simpson RJ, Peters TJ. Forms of soluble iron in mouse stomach and duodenal lumen: significance for mucosal uptake. Br J Nutr. 1990;63:79–89.
17. Golubov J, Flanagan P, Adams P. Inhibition of iron absorption by omeprazole in rat model. Dig Dis Sci. 1991;36:405–8.
18. Miret S, Simpson RJ, McKie AT. Physiology and molecular biology of dietary iron absorption. Annu Rev Nutr. 2003;23:283–301.
19. Takeuchi K, Bjarnason I, Laftah AH, Latunde-Dada GO, Simpson RJ, McKie AT. Expression of iron absorption genes in mouse large intestine. Scand J Gastroenterol. 2005;40:169–77.
20. Frazer DM, Vulpe CD, McKie AT, Wilkins SJ, Trinder D, Cleghorn GJ, et al. Cloning and gastrointestinal expression of rat hephaestin: relationship to other iron transport proteins. Am J Physiol Gastrointest Liver Physiol. 2001;281:G931–9.
21. Manis JG, Schachter D. Active transport of iron by intestine: features of the two-step mechanism. Am J Physiol. 1962;203:73–80.
22. McKie AT, Simpson RJ. Basolateral transport of iron in mammalian intestine: from physiology to molecules. In: Templeton DM, editor. Molecular and cellular iron transport. New York: Marcel Dekker; 2002. p. 175–88.
23. Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, et al. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature. 1997;388:482–8.
24. Tandy S, Williams M, Leggett A, Lopez-Jimenez M, Dedes M, Ramesh B, et al. Nramp2 expression is associated with pH-dependent iron uptake across the apical membrane of human intestinal Caco-2 cells. J Biol Chem. 2000;275:1023–9.
25. Gunshin H, Allerson CR, Polycarpou-Schwarz M, Rofts A, Rogers JT, Kishi F, et al. Iron-dependent regulation of the divalent metal ion transporter. FEBS Lett. 2001;509:309–16.
26. Fleming MD, Trenor CC, Su MA, Foernzler D, Beier DR, Dietrich WF, et al. Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. Nat Genet. 1997;16:383–6.
27. Fleming MD, Romano MA, Su MA, Garrick LM, Garrick MD, Andrews NC. Nramp2 is mutated in the anemic Belgrade (b) rat: evidence of a role for Nramp2 in endosomal iron transport. Proc Natl Acad Sci USA. 1998;95:1148–53.
28. Gunshin H, Fujiwara Y, Custodio AO, Direnzo C, Robine S, Andrews NC. Slc11a2 is required for intestinal iron absorption and erythropoiesis but dispensable in placenta and liver. J Clin Invest. 2005;115:1258–66.
29. Mims MP, Guan Y, Pospisilova D, Priwitzerova M, Indrak K, Ponka P, et al. Identification of a human mutation of DMT1 in a patient with microcytic anemia and iron overload. Blood. 2005;105:1337–42.
30. Iolascon A, d'Apolito M, Servedio V, Cimmino F, Piga A, Camaschella C. Microcytic anemia and hepatic iron overload in a child with compound heterozygous mutations in DMT1 (SCL11A2). Blood. 2006;107:349–54.
31. Beaumont C, Delaunay J, Hetet G, Grandchamp B, de Montalembert M, Tchernia G. Two new human DMT1 gene mutations in a patient with microcytic anemia, low ferritinemia, and liver iron overload. Blood. 2006;107:4168–70.
32. Mackenzie B, Garrick MD. Iron imports. II. Iron uptake at the apical membrane in the intestine. Am J Physiol Gastrointest Liver Physiol. 2005;289:G981–6.
33. Lichten LA, Cousins RJ. Mammalian zinc transporters: nutritional and physiologic regulation. Annu Rev Nutr. 2009;29:153–76.
34. Liuzzi JP, Aydemir F, Nam H, Knutson MD, Cousins RJ. Zip14 (Slc39a14) mediates non-transferrin-bound iron uptake into cells. Proc Natl Acad Sci USA. 2006;103:13612–7.
35. Chua AC, Morgan EH. Manganese metabolism is impaired in the Belgrade laboratory rat. J Comp Physiol B. 1997;167:361–9.
36. Min KS, Ueda H, Kihara T, Tanaka K. Increased hepatic accumulation of ingested Cd is associated with upregulation of several intestinal transporters in mice fed diets deficient in essential metals. Toxicol Sci. 2008;106:284–9.
37. Raja K, Jafri S, Peters T, Simpson R. Iron and cadmium uptake by duodenum of hypotransferrinaemic mice. Biometals. 2006;19:547–53.
38. Suzuki T, Momoi K, Hosoyamada M, Kimura M, Shibasaki T. Normal cadmium uptake in microcytic anemia mk/mk mice suggests that DMT1 is not the only cadmium transporter in vivo. Toxicol Appl Pharmacol. 2008;227:462–7.
39. Yamaji S, Tennant J, Tandy S, Williams M, Singh Srai SK, Sharp P. Zinc regulates the function and expression of the iron transporters DMT1 and IREG1 in human intestinal Caco-2 cells. FEBS Lett. 2001;507:137–41.

40. Tennant J, Stansfield M, Yamaji S, Srai S, Sharp P. Effects of copper on the expression of metal transporters in human intestinal Caco-2 cells. FEBS Lett. 2002;527:239.
41. Crane FL, Sun IL, Clark MG, Grebing C, Low H. Transplasma-membrane redox systems in growth and development. Biochim Biophys Acta. 1985;811:233–64.
42. Raja KB, Simpson RJ, Peters TJ. Investigation of a role for reduction in ferric iron uptake by mouse duodenum (published erratum appears in Biochim Biophys Acta 1993; 1176: 197). Biochim Biophys Acta. 1992;1135:141–6.
43. Pountney DJ, Raja KB, Simpson RJ, Wrigglesworth JM. The ferric-reducing activity of duodenal brush–border membrane vesicles is associated with a b-type haem. Biometals. 1999;12:53–62.
44. Pountney DJ, Raja KB, Bottwood MJ, Wrigglesworth JM, Simpson RJ. Mucosal surface ferricyanide reductase activity in mouse duodenum. Biometals. 1996;9:15–20.
45. McKie AT, Barrow D, Latunde-Dada GO, Rolfs A, Sager G, Mudaly E, et al. An iron-regulated ferric reductase associated with the absorption of dietary iron. Science. 2001;291:1755–9.
46. Srivastava M, Duong LT, Fleming PJ. Cytochrome b561 catalyzes transmembrane electron transfer. J Biol Chem. 1984;259:8072–5.
47. Duong LT, Fleming PJ, Russell JT. An identical cytochrome b561 is present in bovine adrenal chromaffin vesicles and posterior pituitary neurosecretory vesicles. J Biol Chem. 1984;259:4885–9.
48. Fleming PJ, Kent UM. Secretory vesicle cytochrome b561: a transmembrane electron transporter. Ann N Y Acad Sci. 1987;493:101–7.
49. Escriou V, Laporte F, Garin J, Brandolin G, Vignais PV. Purification and physical properties of a novel type of cytochrome b from rabbit peritoneal neutrophils. J Biol Chem. 1994;269:14007–14.
50. Escriou V, Laporte F, Vignais PV, Desbois A. Differential characterization of neutrophil cytochrome p30 and cytochrome b-558 by low-temperature absorption and resonance Raman spectroscopies. Eur J Biochem. 1997;245:505–11.
51. Su D, May JM, Koury MJ, Asard H. Human erythrocyte membranes contain a cytochrome b561 that may be involved in extracellular ascorbate recycling. J Biol Chem. 2006;281:39852–9.
52. Gunshin H, Starr CN, Direnzo C, Fleming MD, Jin J, Greer EL, et al. Cybrd1 (duodenal cytochrome b) is not necessary for dietary iron absorption in mice. Blood. 2005;106:2879–83.
53. Frazer DM, Wilkins SJ, Vulpe CD, Anderson GJ. The role of duodenal cytochrome b in intestinal iron absorption remains unclear. Blood. 2005;106:4413.
54. McKie AT, Marciani P, Rolfs A, Brennan K, Wehr K, Barrow D, et al. A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. Mol Cell. 2000;5:299–309.
55. Donovan A, Brownlie A, Zhou Y, Shepard J, Pratt SJ, Moynihan J, et al. Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. Nature. 2000;403:776–81.
56. Abboud S, Haile DJ. A novel mammalian iron-regulated protein involved in intracellular iron metabolism. J Biol Chem. 2000;275:19906–12.
57. Zhang DL, Hughes RM, Ollivierre-Wilson H, Ghosh MC, Rouault TA. A ferroportin transcript that lacks an iron-responsive element enables duodenal and erythroid precursor cells to evade translational repression. Cell Metab. 2009;9:461–73.
58. Donovan A, Lima CA, Pinkus JL, Pinkus GS, Zon LI, Robine S, et al. The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. Cell Metab. 2005;1:191–200.
59. Montosi G, Donovan A, Totaro A, Garuti C, Pignatti E, Cassanelli S, et al. Autosomal-dominant hemochromatosis is associated with a mutation in the ferroportin (SLC11A3) gene. J Clin Invest. 2001;108:619–23.
60. Devalia V, Carter K, Walker AP, Perkins SJ, Worwood M, May A, et al. Autosomal dominant reticuloendothelial iron overload associated with a 3-base pair deletion in the ferroportin 1 gene (SLC11A3). Blood. 2002;100:695–7.
61. Roetto A, Merryweather-Clarke AT, Daraio F, Livesey K, Pointon JJ, Barbabietola G, et al. A valine deletion of ferroportin 1: a common mutation in hemochromastosis type 4. Blood. 2002;100:733–4.
62. Drakesmith H, Schimanski LM, Ormerod E, Merryweather-Clarke AT, Viprakasit V, Edwards JP, et al. Resistance to hepcidin is conferred by hemochromatosis-associated mutations of ferroportin. Blood. 2005;106:1092–7.
63. Schimanski LM, Drakesmith H, Merryweather-Clarke AT, Viprakasit V, Edwards JP, Sweetland E, et al. In vitro functional analysis of human ferroportin (FPN) and hemochromatosis-associated FPN mutations. Blood. 2005;105:4096–102.
64. Bannerman RM, Cooper RG. Sex-linked anemia: a hypochromic anemia of mice. Science. 1966;151:581–2.
65. Peppriell JE, Edwards JA, Bannerman RM. The kinetics of iron uptake by isolated intestinal cells from normal mice and mice with sex-linked anemia. Blood. 1982;60:635–41.
66. Vulpe CD, Kuo YM, Murphy TL, Cowley L, Askwith C, Libina N, et al. Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. Nat Genet. 1999;21:195–9.

67. Kuo YM, Su T, Chen H, Attieh Z, Syed BA, McKie AT, et al. Mislocalisation of hephaestin, a multicopper ferroxidase involved in basolateral intestinal iron transport, in the sex linked anaemia mouse. Gut. 2004;53:201–6.
68. Ma Y, Yeh M, Yeh Ky, Glass J. Iron imports. V. Transport of iron through the intestinal epithelium. Am J Physiol Gastrointest Liver Physiol. 2006;290:G417–22.
69. Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol. 1992;12:5447–54.
70. Peyssonnaux C, Nizet V, Johnson RS. Role of the hypoxia inducible factors HIF in iron metabolism. Cell Cycle. 2008;7:28–32.
71. Berra E, Ginouves A, Pouyssegur J. The hypoxia-inducible-factor hydroxylases bring fresh air into hypoxia signalling. EMBO Rep. 2006;7:41–5.
72. Shah YM, Matsubara T, Ito S, Yim SH, Gonzalez FJ. Intestinal hypoxia-inducible transcription factors are essential for iron absorption following iron deficiency. Cell Metab. 2009;9:152–64.
73. Mastrogiannaki M, Matak P, Keith B, Simon MC, Vaulont S, Peyssonnaux C. HIF-2alpha, but not HIF-1alpha, promotes iron absorption in mice. J Clin Invest. 2009;119:1159–66.
74. Hathorn MK. The influence of hypoxia on iron absorption in the rat. Gastroenterology. 1971;60:76–81.
75. Raja KB, Simpson RJ, Pippard MJ, Peters TJ. In vivo studies on the relationship between intestinal iron (Fe3+) absorption, hypoxia and erythropoiesis in the mouse. Br J Haematol. 1988;68:373–8.
76. Galy B, Ferring-Appel D, Kaden S, Grone HJ, Hentze MW. Iron regulatory proteins are essential for intestinal function and control key iron absorption molecules in the duodenum. Cell Metab. 2008;7:79–85.
77. Ma Y, Specian RD, Yeh Ky, Yeh M, Rodriguez-Paris J, Glass J. The transcytosis of divalent metal transporter 1 and apo-transferrin during iron uptake in intestinal epithelium. Am J Physiol Gastrointest Liver Physiol. 2002;283:G965–74.
78. Frazer DM, Wilkins SJ, Becker EM, Murphy TL, Vulpe CD, McKie AT, et al. A rapid decrease in the expression of DMT1 and Dcytb but not Ireg1 or hephaestin explains the mucosal block phenomenon of iron absorption. Gut. 2003;52:340–6.
79. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science. 2004;306:2090–3.
80. Johnson G, Jacobs P, Purves LR. The effects of cytoskeletal inhibitors on intestinal iron absorption in the rat. Biochim Biophys Acta. 1985;843:83–91.
81. Simpson RJ, Osterloh KRS, Raja KB, Snape SD, Peters TJ. Studies on the role of transferrin and endocytosis in the uptake of Fe3+ from Fe-nitrilotriacetate by mouse duodenum. Biochim Biophys Acta. 1986; 884:166–71.
82. Peres JM, Bouhallab S, Bureau F, Neuville D, Maubois JL, Devroede G, et al. Mechanisms of absorption of caseinophosphopeptide bound iron. J Nutr Biochem. 1999;10:215–22.
83. Moriya M, Linder MC. Vesicular transport and apotransferrin in intestinal iron absorption, as shown in the Caco-2 cell model. Am J Physiol Gastrointest Liver Physiol. 2006;290:G301–9.
84. Sharp P, Srai SK. Molecular mechanisms involved in intestinal iron absorption. World J Gastroenterol. 2007;13:4716–24.
85. Beutler E, Kelly BM, Beutler F. The regulation of iron absorption: II. Relationship between iron dosage and iron absorption. Am J Clin Nutr. 1962;11:559–67.
86. Wheby MS, Jones LG, Crosby WH. Studies on iron absorption. Intestinal regulatory mechanisms. J Clin Invest. 1964;43:1433–42.
87. Gitlin D, Cruchard A. On the kinetics of iron absorption in mice. J Clin Invest. 1962;41:344–50.
88. Hahn PF, Carothers EL, Darby WJ, Martin M, Sheppard CW, Cannon RO, et al. Iron metabolism in human pregnancy as studied with radioactive isotope, Fe59. Am J Obstet Gynecol. 1951;61:477–86.
89. Snape S, Simpson RJ, Peters TJ. Subcellular localization of recently-absorbed iron in mouse duodenal enterocytes: identification of a basolateral membrane iron-binding site. Cell Biochem Funct. 1990;8:107–15.
90. Osterloh K, Snape S, Simpson RJ, Grindley H, Peters TJ. Subcellular distribution of recently absorbed iron and of transferrin in the mouse duodenal mucosa. Biochim Biophys Acta. 1988;969:166–75.
91. Galy B, Ferring D, Minana B, Bell O, Janser HG, Muckenthaler M, et al. Altered body iron distribution and microcytosis in mice deficient in iron regulatory protein 2 (IRP2). Blood. 2005;106:2580–9.
92. Pigeon C, Ilyin G, Courselaud B, Leroyer P, Turlin B, Brissot P, et al. A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. J Biol Chem. 2001;276:7811–9.
93. Nicolas G, Chauvet C, Viatte L, Danan JL, Bigard X, Devaux I, et al. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. J Clin Invest. 2002;110:1037–44.
94. Frazer DM, Anderson GJ. The orchestration of body iron intake: how and where do enterocytes receive their cues? Blood Cells Mol Dis. 2003;30:288–97.

95. Lin L, Valore EV, Nemeth E, Goodnough JB, Gabayan V, Ganz T. Iron transferrin regulates hepcidin synthesis in primary hepatocyte culture through hemojuvelin and BMP2/4. Blood. 2007;110:2182–9.
96. Robb A, Wessling-Resnick M. Regulation of transferrin receptor 2 protein levels by transferrin. Blood. 2004;104:4294–9.
97. Camaschella C, Roetto A, Cali A, De Gobbi M, Garozzo G, Carella M, et al. The gene TFR2 is mutated in a new type of haemochromatosis mapping to 7q22. Nat Genet. 2000;25:14–5.
98. Fleming RE, Ahmann JR, Migas MC, Waheed A, Koeffler HP, Kawabata H, et al. Targeted mutagenesis of the murine transferrin receptor-2 gene produces hemochromatosis. Proc Natl Acad Sci USA. 2002;99:10653–8.
99. Fleming RE, Sly WS. Mechanisms of iron accumulation in hereditary hemochromatosis. Annu Rev Physiol. 2002;64:663–80.
100. Lebron JA, West Jr AP, Bjorkman PJ. The hemochromatosis protein HFE competes with transferrin for binding to the transferrin receptor. J Mol Biol. 1999;294:239–45.
101. Chen J, Chloupkova M, Gao J, Chapman-Arvedson TL, Enns CA. HFE modulates transferrin receptor 2 levels in hepatoma cells via interactions that differ from transferrin receptor 1-HFE interactions. J Biol Chem. 2007;282:36862–70.
102. Gao J, Chen J, Kramer M, Tsukamoto H, Zhang AS, Enns CA. Interaction of the hereditary hemochromatosis protein HFE with transferrin receptor 2 is required for transferrin-induced hepcidin expression. Cell Metab. 2009;9:217–27.
103. Lin L, Goldberg YP, Ganz T. Competitive regulation of hepcidin mRNA by soluble and cell-associated hemojuvelin. Blood. 2005;106:2884–9.
104. Du X, She E, Gelbart T, Truksa J, Lee P, Xia Y, et al. The serine protease TMPRSS6 is required to sense iron deficiency. Science. 2008;320:1088–92.
105. Finberg KE, Heeney MM, Campagna DR, Aydinok Y, Pearson HA, Hartman KR, et al. Mutations in TMPRSS6 cause iron-refractory iron deficiency anemia (IRIDA). Nat Genet. 2008;40:569–71.
106. Silvestri L, Pagani A, Nai A, De Domenico I, Kaplan J, Camaschella C. The serine protease matriptase-2 (TMPRSS6) inhibits hepcidin activation by cleaving membrane hemojuvelin. Cell Metab. 2008;8:502–11.
107. Meynard D, Kautz L, Darnaud V, Canonne-Hergaux F, Coppin H, Roth MP. Lack of the bone morphogenetic protein BMP6 induces massive iron overload. Nat Genet. 2009;41:478–81.
108. Andriopoulos Jr B, Corradini E, Xia Y, Faasse SA, Chen S, Grgurevic L, et al. BMP6 is a key endogenous regulator of hepcidin expression and iron metabolism. Nat Genet. 2009;41:482–7.