Annu. Rev. Nutr. 2000. 20:129–51

IRON TRANSPORT

Marianne Wessling-Resnick
Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts 02115; e-mail: wessling@hsph.harvard.edu

Key Words ferrireductases, ferroxidases, transferrin, transferrin receptors

■ **Abstract** Iron homeostasis is maintained by regulating its absorption: Under conditions of deficiency, assimilation is enhanced but iron uptake is otherwise limited to prevent toxicity due to overload. Iron deficiency remains the most important micronutrient deficiency worldwide, but increasing awareness of the genetic basis for iron-loading diseases points to iron overload as a major public health issue as well. Recent identification of mutant alleles causing iron uptake disorders in mice and humans provides new insights into the mechanisms involved in iron transport and its regulation. This article summarizes these discoveries and discusses their impact on our current understanding of iron transport and its regulation.

CONTENTS

OVERVIEW

Iron homeostasis is maintained by regulating its absorption. Unlike other essential minerals, our bodies do not have a regulated mechanism to excrete excess iron. Therefore, although iron uptake can be stimulated when necessary, its absorption must be otherwise limited to prevent toxicity due to iron overload. As a consequence of its regulation, iron absorption varies inversely with an individual's iron status. Only about 1 mg of dietary iron is taken up per day because of its relatively low absorption, but this is sufficient to replace the amount estimated to be lost through nonspecific mechanisms (e.g. exfoliation of gut cells, urinary and biliary secretions, menstruation, etc). Thus, the maintenance of iron homeostasis reflects not only the regulation of dietary absorption to limit uptake, but also the economic reutilization of existing stores by the body.

The major depot for body iron is the circulating hemoglobin content of red cells, where it fulfills its primary function in oxygen delivery. Combined with iron present in myoglobin, the oxygen reservoir of muscle, this pool represents some 85% of the total body iron content (50 mg/kg in men and 40 mg/kg in women). Although only a relatively modest amount (∼0.1–0.2%) is bound to circulating transferrin, the turnover of this transported pool is dynamic (∼30–35 mg/day) (179). About 80% of transferrin iron is delivered to bone marrow for heme synthesis within newly developing erythrocytes; these cells replace senescent erythrocytes phagocytosed by the reticuloendothelial system. Conversely, heme iron is retrieved from the latter cell population to be released to transferrin and, hence, reutilized (148).

Iron is also required for a number of important biological functions in nonerythroid cells. This transition metal undergoes a reversible one-electron oxidation/reduction cycle, a reaction that contributes to ATP production involving a number of heme-containing cytochromes of the electron transport chain. Other enzymes that require iron as a cofactor include cytochrome P450, xanthine oxidase, catalase, NADH dehydrogenase, ribonucleotide reductase, and aconitase. Finally, excess iron is stored within ferritin. Synthesis of this storage protein is therefore tightly regulated in a manner that reflects cellular iron levels, as reviewed by Eisenstein (53a).

Iron deficiency remains a primary nutritional deficiency worldwide despite the fortification of food supplies, increased use of supplements, and improvements in diet. It has been estimated that 15% of the world's population has anemia resulting from iron deficiency (19). Iron deficiency also contributes to impaired immune function, poor cognitive development, and other pathologies (20, 43, 147). Paradoxically, too much iron produces oxidative injury, resulting in lipid peroxidation and DNA damage (112). The relatively recent discovery that a mutation in the *HFE* gene is responsible for hereditary hemochromatosis has led to the confirmation that ∼1 in 400 Caucasians is homozygous for this iron overload disorder, which can cause cirrhosis, hepatomas, congestive heart failure, endocrinopathies, and premature death (57). Recognition of the prevalence of this particular mutation has triggered an increased awareness of the genetic basis for other iron-loading disorders. For example, it is now clear that iron overload in sub-Saharan Africa, which has long been associated with the consumption of large amounts of iron in home-brewed beer, involves a genetic component that is unrelated to *HFE* (115, 120, 121). A non-*HFE*–associated form of iron loading appears to be common among African-Americans as well (69, 117), and it is postulated that a Mediterranean form of hereditary hemochromatosis exists that is independent of mutations known for the *HFE* allele (145). Thus, the fact that both iron deficiency and iron overload are major public health problems underscores the critical importance of understanding how iron is transported and how this process is regulated. The purpose of this review is to summarize important new information about iron transport and its regulation that has recently emerged from studies of iron disorders in mice and man.

DIETARY IRON ABSORPTION AND ITS REGULATION

The intestine is the primary site of regulation for iron homeostasis. Dietary uptake occurs in the proximal small intestine, which adapts to promote iron absorption during iron deficiency and in response to both hypoxia and enhanced erythropoiesis (25, 37, 88, 116, 134). Both heme and non-heme iron uptake are regulated, but in general, non-heme iron absorption is more predominantly influenced by iron status (182). On entering the enterocyte, heme iron is released by the action of heme oxygenase (166), entering a common pool with inorganic iron such that their efflux shares a common pathway. Thus, assimilation of dietary iron occurs in three sequential stages: uptake across the apical mucosa, intracellular processing and translocation, and efflux across the basolateral or serosal membrane.

Crypt enterocytes acquire iron from circulating transferrin, but as they differentiate and migrate to the villus tip, they initiate their function to absorb dietary iron across the apical mucosa (5, 6, 37, 129, 134, 135). Ferritin mRNA is found in crypt cells; however, protein appears to be produced in the absorptive villus cells and in a manner that reflects dietary iron loading (130). This observation is consistent with the "mucosal block" theory, which has been advanced to explain the limited absorption of iron (71, 81). In this model, excess enteral iron is deposited in the intestinal epithelia within this iron storage protein (or some other acceptor) to prevent its transfer to across the serosa. The trapped iron is then lost upon exfoliation or apoptosis of gut cells (82).

Despite the close relationship between ferritin levels and intestinal iron absorption (198), it is not clear whether this protein plays a passive or active role in transport regulation (28, 29). From their early studies, Conrad & Crosby (37) postulated that enterocytes are programmed in the crypt to subsequently modulate iron absorption at the villus. Ferritin synthesis is regulated by the activity of iron regulatory proteins (IRPs), which in turn are regulated by enterocyte iron levels, and in Caco-2 intestinal cells, iron absorption and IRP activity are known to be regulated over the same cellular iron concentration range (20–200 μM) (7). In vitro studies of rat and mouse intestinal mucosa confirm that IRP activity along the entire crypt-villus axis is enhanced by iron depletion (152, 176). When iron is administered intravenously, IRP activity is reduced in crypt but not villus enterocytes, consistent with the idea that crypt enterocytes "sense" plasma levels of iron to modulate gene expression as they differentiate (176). However, the villus IRP pool can be activated in response to a challenge of luminal iron. Concomitantly, iron absorption is lowered and reduced uptake is observed within 2 h of iron administration (81, 176). Schumann et al (176) argue that within this short time period, it is unlikely that the amount of ferritin synthesized would be sufficient to produce the putative "mucosal block." These investigators theorize that modulation of the transporter's activity in direct response to dietary iron load would be necessary to account for the rapid down-regulation of absorption (176). Thus, although it is likely that the number of transporters and the amount of ferritin present in cells at the villus tip reflect an iron-responsive pattern of gene expression established early

in enterocyte differentiation and during dietary iron loading, additional regulatory elements must provide functional control of the absorptive process.

McKie et al (113) have also studied the regulation of gene expression in response to intestinal iron levels. Unlike translational control of ferritin synthesis, transferrin receptor synthesis is regulated by IRP stabilization of its message (see 53a). As predicted, receptor transcript levels change in response to iron deficiency and overload in a manner that would correspond to iron-responsive IRP function and up- or down-regulation of iron absorption. However, although hypoxia promotes iron absorption, it fails to induce any change in transferrin receptor transcript levels, leading McKie et al (113) to conclude that regulation of iron absorption and IRP activity do not necessarily need to be coordinated. This view is in general agreement with the model that multiple pathways exist to control iron transport. Although recent kinetic studies indicate that enhanced mucosal iron uptake is responsible for increased dietary iron absorption during iron deficiency (175), other studies have suggested that hypoxia induces the up-regulation of both apical absorption and transfer of iron across the intestine (144). Thus, the entry of iron into the body can be governed by (*a*) the expression level of transport factors, (*b*) regulation of their functional activity, (*c*) control of intracellular processing steps, and (*d*) modulation of iron release from intestinal enterocytes.

TRANSFERRIN-MEDIATED IRON DELIVERY AND ITS REGULATION

The serum iron-binding protein transferrin is a ~80-kDa glycoprotein that binds two atoms of Fe(III) with high affinity ($K_d \sim 10^{-23}$ M) and in a reversible manner. The liver is the predominant site of transferrin synthesis, and circulating levels reflect the body's iron status, i.e. serum levels increase with iron deficiency. Transferrin not only obtains iron from dietary absorption, it also can acquire iron from heme that has been degraded because of the phagocytosis of sensecent red cells. How the latter pool is rapidly turned over to circulating transferrin is not precisely clear. It is known that copper-deficient animals have an iron deficiency anemia despite adequate levels of iron. Instead, iron accumulates in duodenal enterocytes and reticuloendothelial cells of these animals, observations that led to the idea that copper is required for the release of iron to the circulation (105, 136). It is proposed that loss of the serum ferroxidase ceruloplasmin activity due to copper-deficiency results in failure to mobilize iron because the oxidation of Fe(II) to Fe(III) is required. Indeed, when ceruloplasmin activity is restored, the release of iron to the circulation is promoted in copper-deficient animals (105). Perfusion studies by Osaki et al (136) further supported a direct role for the serum ferroxidase in iron release. More recent genetic evidence has strengthened this model: For certain patients, aceruloplasminemia has been linked to a mutation in the ceruloplasmin gene, and these individuals display iron loading in a number of different tissues (86, 203). Finally, studies of $Cp^{-/-}$ knockout mice confirm the essential role of

ceruloplasmin because they have significant impairments in the mobilization of iron from reticuloendothelial cells and hepatocytes (85).

Circulating transferrin associates with specific cell surface receptors that are constitutively endocytosed. Although its iron-binding capacity is high at neutral pH, transferrin releases its cargo upon entering the acidic milieu of endosomal compartments (47, 102). The pH-dependent intracellular release of iron appears to be facilitated through interactions with the transferrin receptor (12–14, 181). The transferrin receptor is an integral membrane glycoprotein that functions as a homodimer with each ~90-kDa subunit capable of binding one molecule of transferrin. Its synthesis is posttranscriptionally controlled in response to cellular iron levels such that when the demand for iron is high, the pool of receptors available to bind transferrin is increased. This effect is mediated through iron-responsive elements (IREs) in the 3′ untranslated region of the receptor message to which IRPs can bind and stabilize the mRNA against degradation (see 53a). Recently, a second isoform has been identified with structural and functional features related to the well-characterized transferrin receptor; however, its transcript appears to lack the necessary IREs to promote iron-dependent regulation by this mechanism (100). Full characterization of transferrin receptor-2 has yet to be accomplished, but the relative abundance of transcripts found in liver suggests it may confer tissue-specific function to transferrin-mediated iron uptake.

Recent studies of transferrin receptor knockout mice ($Trfr^{-/-}$) provide new insights regarding the physiologic role of the transferrin/transferrin receptor cycle (107). Of particular issue is the relative necessity for transferrin-mediated iron uptake because mice that have hypotransferrinemia (*hpx*) survive with little or no circulating transferrin. The observation that *hpx* mice take up excessive amounts of dietary iron to become iron loaded despite suffering from severe anemia suggests that transferrin is not required for iron delivery to tissues other than erythroid cells (23, 31, 41). In contrast, homozygous $Trfr^{-/-}$ animals die in utero with impaired erythropoiesis and defective neurological development. These results differ from those reported for homozygous *hpx* mice because the latter have an increased pool of nontransferrin-bound iron that is available for iron assimilation by alternative mechanisms (see below). In the homozygous $Trfr^{-/-}$ animals, transferrin is present to chelate free iron, but its ligand can not be delivered to cells in the absence of the transferrin receptor (107). Thus, the $Trfr^{-/-}$ mice provide a convincing demonstration that the transferrin/transferrin receptor cycle plays a central role in the maintenance of normal iron metabolism.

A second advance in our understanding of the transferrin/transferrin receptor cycle has been obtained by the molecular identification of the mutation responsible for hereditary hemochromatosis (57). More than 80% of patients with this disorder carry a single point mutation (C282Y) that disrupts the function of *HFE*, a major histocompatibility complex class I–like molecule (24, 57, 94, 97). A second mutation, H63D, is observed in many individuals, but its role in the disease remains unclear (59, 97). *HFE* knockout mice recapitulate the human iron-overload disorder, confirming the relationship of this gene and the disease (108, 206).

Like other major histocompatibility complex class I molecules, HFE associates with β2-microglobulin, and this interaction is disrupted by the C282Y mutation such that the protein fails to be expressed at the cell surface (59, 196). Thus, β2-microglobulin knockout animals also suffer from iron overload because they fail to produce functional *HFE* (48, 168). Immunoprecipitation (58) and in vitro binding experiments (104, 142) further demonstrate that not only does HFE associate with β2-microglobulin, it also binds to the transferrin receptor. Thus, current models of transferrin/transferrin receptor trafficking must be revised to include these interactions with the HFE–β2-microglobulin complex.

How HFE may regulate transferrin receptor activity is still unknown. The binding of HFE to the receptor reduces the affinity for transferrin ~10-fold (58, 104). The physiological consequences of this effect are uncertain, however, because micromolar levels of transferrin are present in serum, i.e. receptor binding sites would remain saturated despite the increased K_d for transferrin. It has been shown that HFE rapidly associates with transferrin receptors in the biosynthetic pathway (72, 171), but the subsequent association with receptors during membrane trafficking steps is still unclear, and contradictory results have been reported. Enns and colleagues (72, 169) have demonstrated that when HFE is overexpressed, it is cointernalized with transferrin receptors without any effect on endocytosis or recycling. Salter-Cid et al (171) have provided contradictory data that suggest that overexpression of HFE reduces the number of functional cell surface transferrin binding sites and impairs endocytosis. Although the true mechanistic details of receptor interactions must still be ascertained, it is clear that overexpression of HFE lowers transferrin-mediated iron uptake (169). It is interesting to note that HFE has an intracellular localization overlapping with transferrin receptors in intestinal enterocytes (143, 195). Because patients with hereditary hemochromatosis suffer from iron overload due to excessive intestinal uptake (114), it is possible that HFE-transferrin receptor interactions within the endosome of the enterocyte provide a regulatory cue to maintain iron homeostasis at the level of dietary iron absorption (100).

NONTRANSFERRIN-BOUND IRON UPTAKE

Micromolar levels of non–transferrin-bound iron (NTBI) occur in hereditary hemochromatosis and other iron-overload disorders (18, 80). Additionally, NTBI is found in cord blood and plasma of premature and full-term infants (22, 54), in serum from patients with fulminant hepatic failure (55) or undergoing chemotherapy (33), in synovial fluid from arthritic patients (78), and in cerebral spinal fluid (79). Specific mechanisms for the uptake of NTBI have been described for many different cell types (8, 9, 16, 27, 35, 39, 52, 89, 91, 92, 98, 118, 122, 132, 137, 140, 141, 150, 151, 156, 184, 199, 200, 201, 205). Both high- and low-affinity uptake mechanisms for NTBI uptake have been characterized (0.5 to 20 μM K_m) with

differential sensitivity to various other transition metals (8, 92, 98, 173, 184). For example, low-affinity iron uptake can be mediated by the Na^+/Mg^{2+} antiport system in erythroid cells (183). In general, NTBI uptake appears to require energy (76, 150, 151), but exactly how this is coupled to transbilayer movement of iron is unclear. There is limited evidence to suggest that NTBI transport is regulated in response to iron loading. Ferric ammonium citrate (FAC) appears to enhance the V_{max} of transport and thereby induce uptake of iron (98, 139, 156). However, it has also been shown that free radical scavengers can suppress FAC-induced uptake, leading to the proposal that NTBI uptake may be mediated through the FAC-mediated production of hydroxyl radicals rather than through increased transporter synthesis or recruitment of the cell surface (162). NTBI uptake also appears to be influenced by other trivalent metals, but how these effects are exerted is unknown (36, 133).

In contrast to the import of NTBI iron, relatively little is known about its cellular export. As noted above, the copper-binding ferroxidase ceruloplasmin appears to stimulate this process, but several investigations evaluating this hypothesis have led to contradictory results (85, 122, 204). Clinically, iron chelators have long been utilized to reduce iron overload, and in vitro studies have revealed that this process is time, temperature, and energy dependent (10, 159). After erythrophagocytosis by mononuclear phagocytes, iron may be released as ferritin, hemoglobin, and a low-molecular-weight species (42, 103, 170); it is interesting to note that the efflux of the latter form is two times greater from monocytes collected from patients with hereditary hemochromatosis (119). Unfortunately, a comprehensive understanding of iron homeostasis will be lacking until the mechanism(s) of iron efflux are better defined: The mobilization or release of iron is not only critical for the reutilization of iron scavenged by the reticuloendothelial system, it is also essential for the assimilation of dietary iron across the intestinal epithelia (see below).

Additional transferrin-independent mechanisms exist to transport iron. Found to be associated with human melanomas, melanotransferrin (p97) has one intact transferrin-like Fe(III)-binding site (11, 30) and is linked to the surface of cells via a glycosylphosphatidylinositol anchor (65). When transfected to express melanotransferrin, CHO cells internalize the protein and acquire iron (95, 101). A similar activity endogenous to the SK-MEL-28 melanoma cell line has been studied, and it has been concluded that iron uptake can be mediated from this pathway, albeit to a limited extent (157, 160, 161). It is of interest to note that melanotransferrin is found on the apical brush border of fetal intestine (2, 46), which suggests a possible function in gut iron delivery. A second transferrin homolog implicated in alternative pathways for iron uptake is lactoferrin. Like transferrin, lactoferrin can deliver iron to hepatocytes via an endocytic process (111). Lactoferrin also appears to bind to the intestinal brush border membrane and may contribute to dietary iron assimilation (110).

Finally, transferrin-dependent iron transport can occur independently of the transferrin receptor (35, 138, 158, 188–190). This absorptive process does not

appear to involve the transferrin receptor because (*a*) transferrin-mediated iron delivery continues in the presence of blocking antibodies against the receptor (189), (*b*) an N-terminal "half-transferrin," which binds poorly to the transferrin receptor, still delivers iron to hepatocytes (187), and (*c*) antisense suppression of transferrin receptor synthesis does not abolish transferrin-mediated iron uptake (172, 188, 190). The mechanism by which cells acquire transferrin-bound iron in the absence of transferrin receptors may be related to other NTBI pathway(s) (70, 188); however, the recent discovery of the second transferrin receptor isoform indicates that it will be important for future experiments to evaluate the possible contributions of this factor in transferrin-dependent uptake by the liver.

MEMBRANE IRON TRANSPORT SYSTEMS

The handling and transport of iron by the duodenum reflects the ultimate complexity in membrane iron transport, as elements must be coordinated to import the cation across the brush border, to target its transcellular passage, and to mediate its exsorption across the serosal surface for release to circulation. Recent biochemical and genetic studies have greatly enhanced our understanding of all three elements of intestinal iron absorption. Brush border uptake most likely involves iron-binding moieties and a ferrireductase activity, as well as specific transport molecules. A number of brush border iron-binding proteins have been described, (38, 40, 46, 125, 131, 186), including a 520-kDa membrane complex called paraferritin. This complex contains integrin, mobilferrin (a calreticulin homolog), and flavin monooxygenase and is theorized to participate in uptake of dietary iron bound in the gut lumen by mucin (191). How these various iron-binding moieties mediate uptake and what their relative contributions are to the dietary absorptive process remain unclear. However, the idea that iron binding is the first step in intestinal uptake is supported by properties of iron-binding and NTBI uptake observed in other cells (1, 17, 70, 123, 174).

Dietary iron is predominantly found in the ferric form, but Fe(III) is highly insoluble under physiologic conditions. As a result, the fact that Fe(II) is more effectively absorbed may be a consequence of its greater solubility in the gut lumen rather than a reflection of the properties of a specific transport mechanism. Nonetheless, the ability of intestinal mucosa to reduce Fe(III) to Fe(II) has been documented (155, 163), and a ferrireductase activity has been characterized for the intestinal Caco-2 and HuTu-80 cell lines (53, 163). A functional role for Fe(III) reduction in iron transport across the brush border is implicated by the fact that inhibition of ferrireductase activity reduces Caco-2 cell apical iron uptake (83, 126). Furthermore, increased ferrireductase activity correlates with enhanced iron uptake induced by iron deficiency and hypoxia (155). Duodenal fragments from patients with hemochromatosis also display higher reductase activity as well as transport capacity, which suggests a relationship between these two functions in

the disease state (154). Finally, the fact that ferrireduction appears prerequisite for NTBI uptake by other mammalian cells further supports this model for intestinal iron absorption (21, 68, 91, 96, 156, 177).

Although our knowledge of the mammalian enzymes responsible for ferric iron reduction necessary for iron uptake is limited, recent studies of iron transport in yeast and plants have defined molecular factors contributing to this process. Genetic analysis of iron acquisition by *Saccharomyces cerevisiae* led to the identification of the *FRE1* (44, 45) and *FRE2* (67) ferrireductase genes, both of which are under control of the iron-inducible transcriptional regulator Aft1p (202). Thus, together with other factors involved in iron transport, expression of the Fre1p and Fre2p proteins is up-regulated under iron deficiency conditions. Fre1p, Fre2p, and their *Saccharomyces pombe* homolog Frp1p all display sequence similarity to the large subunit $gp91^{phox}$ of the mammalian cytochrome b_{558} (45, 67, 165, 178). When phagocytes are stimulated, $gp91^{phox}$ associates with $gp22^{phox}$ to form the b_{558} flavocytochrome of the NADPH oxidoreductase complex. This membrane complex then transfers electrons from NADPH across the membrane to reduce molecular oxygen, thus producing superoxide anions necessary for host defense. Based on their homologies with the FAD and NADPH binding domains of the NADPH oxidoreductase, Shatwell and colleagues (61, 178) theorize that Fre1p, Fre2p, and Frp1 are also membrane-associated flavocytochromes that move electrons across the bilayer, an idea that is supported by spectroscopic studies. Like yeast, plants also appear to utilize a ferrireductase that becomes induced under iron-poor conditions to help make Fe(III) more bioavailable. Based on the functional data in yeast, Robinson et al (164) used degenerate primers common to the FAD-binding site of $gp91^{phox}$ and Fre1p/Fre2p to successfully identify the *FRO2* gene, which is predicted to encode an *Arabidopsis* ferrireductase.

Although whether or not the brush border ferrireductase is a flavocytochrome like these other eukaryotic factors must still be determined, a candidate Fe(II) transporter has been identified. By functional expression cloning using *Xenopus* oocytes to screen for Fe(II) uptake activity, Gunshin et al (75) identified that iron uptake could be mediated by a homolog of Nramp1 (74, 192). Using a genetic approach, Fleming et al (63) defined that *mk* mice harbor a mutation in this same factor. These animals suffer from a microcytic anemia because of well-characterized defects in dietary iron assimilation (15, 49, 87), implicating a functional role for this transporter in apical iron absorption.

Also known as Nramp2 or DCT1 (divalent cation transporter 1), DMT1 (divalent metal transporter 1) is a member of the Nramp family, with 12 predicted transmembrane spanning domains (34). Other Nramp family members include the yeast transporters Smf1 and Smf2, and expression of DMT1 can functionally complement *smf1/smf2* mutants (146). The symport activity of DMT1 is proton-coupled with a stoichiometry of 1 Fe^{2+}:1 H^+ and apparent affinities of 6 μM and 1–2 μM, respectively, have been defined (75). In order of substrate preference, DMT1 can mediate import of Fe^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Cd^{2+}, Cu^{2+},

Ni^{2+}, and Pb^{2+} (75). The idea that the transporter responsible for dietary iron absorption recognizes other divalent cations agrees well with the observations that Mn^{2+}, Zn^{2+}, Cu^{2+}, and Cd^{2+} can all inhibit this process (93, 167). This may also help to explain why hemochromatosis patients accumulate other metals in addition to iron (124). DMT1 mRNA is found in many different tissues (74, 75), but the protein and its mRNA are most abundant in the proximal duodenum, with decreasing expression along the distal axis, consistent with a function in intestinal iron absorption (32, 75).

A remarkable link between apical iron import by enterocytes and transferrin iron assimilation by erythroid cells was made by the discovery that Belgrade (*b*) rats have the exact same DMT1 mutation as is found in *mk* mice (62). Although impaired erythroid iron assimilation by *mk* animals has been reported (15, 87), defects in transferrin-mediated iron transport are better characterized for Belgrade rats (26, 50, 51). Transferrin is internalized by Belgrade reticulocytes, but its cargo iron fails to be captured because of a loss of transmembrane transport activity in the endosome (56, 66, 173). Cytolocalization studies support the role of DMT1 in this endocytic process because the protein has been localized to transferrin-positive compartments in HEK293T cells, CHO cells, and RAW macrophages (73, 185). Thus, DMT1 apparently functions in both the import of dietary iron and the acquisition of transferrin-bound iron by erythroid cells.

Because *mk* mice and *b* rats survive despite their iron deficiency anemias, additional pathways for membrane iron transport are predicted. One factor called SFT (stimulator of Fe transport) has been identified to stimulate uptake of both NTBI and transferrin-bound iron (77). In contrast to DMT1, which selectively imports Fe(II) in a pH-dependent manner (75), SFT-stimulated uptake of either ferric or ferrous iron occurs at pH 7.4 (205). Furthermore, SFT function has been linked to a cell surface ferrireductase activity (205), although reduction of Fe(III) by exogenously added ascorbate is necessary to enable DMT1 function (75, 185). In addition to the SFT pathway, a low-affinity Fe(II) uptake system is found in erythroid cells that is unimpaired in Belgrade reticulocytes (173). Finally, Attieh et al (8) have characterized a ceruloplasmin-stimulated iron uptake mechanism that appears to be specific for trivalent metals, although the physiological relevance of this process remains to be ascertained (85).

Although the major route for dietary iron absorption is likely to be mediated by DMT1, this transporter is found only at the apical surface of enterocytes (32). Thus, other factors must be involved in the transfer across the intestinal epithelium. It is possible that either holo- or apo-transferrin internalized by enterocytes from the basolateral surface acquires iron imported from the apical brush border (3, 4, 90, 127, 128). This model is consistent with the observation that transferrin localizes to apical endosomes within enterocytes where association of the receptor with HFE could potentially modulate dietary iron assimilation. However, transferrin does not appear to regulate iron uptake across the duodenum (180), and the *hpx* mouse actually accumulates iron in many tissues despite the loss of transferrin

(31). Thus, the transcellular passage of iron through the enterocyte still remains poorly defined.

How iron is released from the intestinal enterocyte also remains ambiguous, but the recent discovery of the gene responsible for sex-linked anemia (*sla*) in mice may provide new clues about this process. These animals accumulate iron within enterocytes but fail to release this pool to the circulation. The *sla* defect has been found to reside in a ceruloplasmin homolog called hephaestin (194). Hephaestin mRNA is abundant in the small intestine and colon, with lower levels in other tissues. Like ceruloplasmin, this protein is predicted to have ferroxidase activity, but unlike the serum protein, hephaestin is anchored to the cell surface via a single transmembrane-spanning domain. Nonetheless, the idea that hephaestin is involved in efflux of iron from the enterocyte is compatible with the model that ceruloplasmin provides ferroxidase activity necessary to release iron to circulating transferrin. The discovery of hephaestin may also explain why anemia is not typically found in Menkes disease, an inherited disorder of copper metabolism in man affecting a copper-transporting ATPase (193). The latter problem results in failure to export copper from the intestine; however, dietary copper would still be available to maintain enterocyte hephaestin function. Thus, these patients would still be able to mobilize iron absorbed from the diet, thereby preventing anemia (194).

MOLECULAR MECHANISMS OF IRON TRANSPORT REGULATION

As discussed above, how dietary iron absorption is regulated by the intestine in response to the body's iron demands remains ambiguous. Based on the new information that has emerged from molecular studies of iron disorders in mice and humans, some limited insights have been gained. Two DMT1 transcripts have been found, one of which harbors an iron-responsive element (IRE) in its 3′ untranslated region (75, 106). Iron depletion results in increased DMT1 message levels in the intestine (32, 75), which suggests that IRPs bind and stabilize the DMT1 mRNA. This model is supported by studies by Han et al (84), which reveal that the DMT1 message is reciprocally regulated in response to the iron status in the intestinal Caco-2 cell line. However, unlike the transferrin receptor, which possesses five IREs, DMT1 transcripts contain only one, and an instability determinant has not yet been identified (see 53a). Furthermore, although the loop of its IRE contains the consensus 5′-CAGUGN-3′, the bulge in the putative DMT1 stem differs from other IRE structures. DMT1 mRNA does appear to bind IRPs present in LMTK-cell lysates, but these cells fail to display iron-dependent regulation of DMT1 transcript levels or iron transport (197). Thus, although IRPs are predicted to sense iron status in the intestine (7, 130, 176), whether or not DMT1 expression is posttranscriptionally regulated through the IRE/IRP system is equivocal. Nevertheless, because DMT1 transcript levels are higher in *HFE*

knockout animals (64) and in the intestine of hemochromatosis patients (207), it is likely that regulation of its gene expression is necessary to prevent iron loading. What also remains to be rectified is how intestinal transport function can be rapidly down-modulated in response to luminal iron load because it is arguable whether IRP function is involved in this rather immediate regulatory effect (176).

Studies of iron metabolism in mice have also contributed to our understanding of the regulation of heme iron absorption and metabolism. The activity of intestinal heme oxygenase, which releases iron from heme absorbed from the diet, is known to be regulated by iron status, but the molecular basis for this effect is not clear (153, 166). The generation of animals deficient in heme oxygenase 1 (Hmox1) has led to the recent discovery that it is required for iron efflux from certain cells (149). *Hmox1* mice develop serum iron deficiency but display iron loading in liver and kidney, which suggests that this enzyme contributes to the release of iron from these tissues. The idea that heme oxygenase plays a critical role in iron metabolism is consistent with the observation that patients treated with inhibitors of this enzyme have a reversible anemia (99). Recently, Ferris et al (60) demonstrated that cells lacking heme oxygenase 1 accumulate iron and that when expression of the enzyme is restored, iron efflux is enhanced. Whether or not the intestinal heme oxygenase contributes to the control of intestinal iron assimilation in a similar fashion must still be explored, but the fact that its activity is up-regulated by iron deficiency fits with this model. Once heme is degraded, the inorganic iron released to the enterocyte joins a common pool for transfer across the basolateral surface. This point of entry also appears to be affected in hemochromatosis, such that excessive amounts of iron become assimilated (114); indeed, patients with this disease have been found to absorb increased amounts of heme iron as well as inorganic iron, which suggests that defects in regulation lie on this common pathway (198).

As discussed earlier, multiple and hierarchical regulatory mechanisms must exist to control dietary absorption of iron. The enterocyte is unique in that it must not only respond to iron loading and deficiency through the regulatory function of IRPs, it must also serve as a gated channel to permit sufficient entry of iron into the body. In unicellular organisms like yeast, iron-responsive transcription factors are found to coordinate control of the genes involved in maintaining iron homeostasis (202). Although not yet identified, a similar regulatory mechanism can readily be envisioned for mammalian cells. It is also conceivable that posttranslational control mechanisms participate in the regulation of mammalian iron transport. It is intriguing that the Nramp homolog Smf1 can be regulated by protein degradation in yeast (109), which suggests the potential for this sort of regulation for mammalian iron transport systems. It is clear that future investigations must focus not only on the identification of molecules involved in mammalian cell iron transport (e.g. ferrireductases and ferroxidases) but also on how the regulation of iron homeostasis is maintained via transcriptional, posttranscriptional, and/or posttranslational control mechanisms.

LITERATURE CITED

1. Akompong T, Inman RS, Wessling-Resnick M. 1995. Phorbol esters stimulate non-transferrin iron uptake by K562 cells. *J. Biol. Chem.* 270:20937–41
2. Alemany R, Vila MR, Franci C, Egea G, Real FX, Thomson TM. 1993. Glycosyl phosphatidylinositol membrane anchoring of melanotransferrin (p97): apical compartmentalization in intestinal epithelial cells. *J. Cell Sci.* 104:1155–62
3. Alvarez-Hernandez X, Smith M, Glass J. 1994. Regulation of iron uptake and transport by transferrin in Caco-2 cells, an intestinal cell line. *Biochim. Biophys. Acta* 1192:215–22
4. Alvarez-Hernandez X, Smith M, Glass J. 1998. The effect of apotransferrin on iron release from Caco-2 cells, an intestinal epithelial cell line. *Blood* 91:3974–79
5. Anderson GJ, Powell LW, Halliday JW. 1994. The endocytosis of transferrin by rat intestinal epithelial cells. *Gastroenterology* 106:414–22
6. Anderson GJ, Walsh MD, Powell LW, Halliday JW. 1991. Intestinal transferrin receptors and iron absorption in the neonatal rat. *Br. J. Haematol.* 77:229–36
7. Arredondo M, Orellana A, Garate MA, Nunez MT. 1997. Intracellular iron regulates iron absorption and IRP activity in intestinal epithelial (Caco-2) cells. *Am. J. Physiol.* 273:G275–80
8. Attieh ZK, Mukhopadhyay CK, Seshadri V, Tripoulas NA, Fox PL. 1999. Ceruloplasmin ferroxidase activity stimulates cellular iron uptake by a trivalent cation-specific transport mechanism. *J. Biol. Chem.* 274:1116–23
9. Baker E, Baker SM, Morgan EH. 1998. Characterisation of non-transferrin-bound iron (ferric citrate) uptake by rat hepatocytes in culture. *Biochim. Biophys. Acta* 1380:21–30
10. Baker E, Vicary FR, Huehns ER. 1981. Iron release from isolated hepatocytes. *Br. J. Haematol.* 47:493–504
11. Baker EN, Baker HM, Smith CA, Stebbins MR, Kahn M, et al. 1992. Human melanotransferrin (p97) has only one functional iron-binding site. *FEBS Lett.* 298:215–18
12. Bali PK, Aisen P. 1991. Receptor-modulated iron release from transferrin: differential effects on N- and C-terminal sites. *Biochemistry* 30:9947–52
13. Bali PK, Aisen P. 1992. Receptor-induced switch in site-site cooperativity during iron release by transferrin. *Biochemistry* 31: 3963–67
14. Bali PK, Zak O, Aisen P. 1991. A new role for the transferrin receptor in the release of iron from transferrin. *Biochemistry* 30:324–28
15. Bannerman RM, Edwards JA, Kreimer-Birnbaum M, McFarland E, Russell ES. 1972. Hereditary microcytic anaemia in the mouse; studies in iron distribution and metabolism. *Br. J. Haematol.* 23:235–45
16. Barisani D, Berg CL, Wessling-Resnick M, Gollan JL. 1995. Evidence for a low Km transporter for non-transferrin-bound iron in isolated rat hepatocytes. *Am. J. Physiol.* 269:G570–76
17. Barisani D, Wessling-Resnick M. 1996. Fe-nitrilotriacetic acid–binding proteins associated with rat liver plasma membranes. *Hepatology* 24:934–38
18. Batey RG, Fong PLC, Shamir S, Sherlock S. 1980. A non-transferrin-bound serum iron in idiopathic hemochromatosis. *Dig. Dis. Sci.* 25:340–46
19. Baynes RD, Bothwell TH. 1990. Iron deficiency. *Annu. Rev. Nutr.* 10:133–48
20. Beard JL, Dawson H, Pinero DJ. 1996. Iron metabolism: a comprehensive review. *Nutr. Rev.* 54:295–317

21. Berczi A, Sizensky JA, Crane FL, Faulk WP. 1991. Diferric transferrin reduction by K562 cells: a critical study. *Biochim. Biophys. Acta* 1073:562–70
22. Berger HM, Mumby S, Gutteridge JM. 1995. Ferrous ions detected in iron-overloaded cord blood plasma from preterm and term babies: implications for oxidative stress. *Free Radic. Res.* 22:555–59
23. Bernstein SE. 1987. Hereditary hypotransferrinemia with hemosiderosis, a murine disorder resembling human atransferrinemia. *J. Lab. Clin. Med.* 110:690–705
24. Beutler E, Gelbart T, West C, Lee P, Adams M, et al. 1996. Mutation analysis in hereditary hemochromatosis. *Blood Cells Mol. Dis.* 22:187–94
25. Bothwell TH, Pirzio-Biroli G, Finch CA. 1958. Iron absorption I. Factors influencing absorption. *J. Lab. Clin. Med.* 51:24–36
26. Bowen BJ, Morgan EH. 1987. Anemia of the Belgrade rat: evidence for defective membrane transport of iron. *Blood* 70:38–44
27. Brissot P, Wright TL, Ma WL, Weisiger RA. 1985. Efficient clearance of non-transferrin-bound iron by rat liver. *J. Clin. Invest.* 76:1463–70
28. Brittin GM, Raval D. 1971. Duodenal ferritin synthesis in iron-replete and iron-deficient rats: response to small doses of iron. *J. Lab. Clin. Med.* 77:54–58
29. Brown EB, Dubach R, Moore CV. 1958. Studies on iron transportation and metabolism. XI. Critical analysis of mucosal block by large doses of inorganic iron in human subjects. *J. Lab. Clin. Med.* 52:335–55
30. Brown JP, Hewick RM, Hellstrom I, Hellstrom KE, Doolittle RF, Dreyer WJ. 1982. Human melanoma-associated antigen p97 is structurally and functionally related to transferrin. *Nature* 296:171–73
31. Buys SS, Martin CB, Eldridge M, Kushner JP, Kaplan J. 1992. Iron absorption in hypotransferrinemic mice. *Blood* 80:2432–33
32. Canonne-Hergaux F, Gruenheid S, Ponka P, Gros P. 1999. Cellular and subcellular localization of the Nramp2 iron transporter in the intestinal brush border and regulation by dietary iron. *Blood* 93:4406–17
33. Carmine TC, Evans P, Bruchelt G, Evans R, Handgretinger R, et al. 1995. Presence of iron catalytic for free radical reactions in patients undergoing chemotherapy: implications for therapeutic management. *Cancer Lett.* 94:219–26
34. Cellier M, Prive G, Belouchi A, Kwan T, Rodrigues V, et al. 1995. Nramp defines a family of membrane proteins. *Proc. Natl. Acad. Sci. USA* 92:10089–93
35. Chan RYY, Ponka P, Schulman HM. 1992. Transferrin-receptor-independent but iron-dependent proliferation of variant Chinese hamster ovary cells. *Exp. Cell Res.* 202:326–36
36. Chitambar CR, Wereley JP. 1997. Resistance to the antitumor agent gallium nitrate in human leukemic cells is associated with decreased gallium/iron uptake, increased activity of iron regulatory protein-1, and decreased ferritin production. *J. Biol. Chem.* 272:12151–57
37. Conrad ME, Crosby WH. 1963. Intestinal mucosal mechanisms controlling iron absorption. *Blood* 22:406–15
38. Conrad ME, Umbreit JN, Moore EG, Peterson RD, Jones MB. 1990. A newly identified iron binding protein in duodenal mucosa of rats. Purification and characterization of mobilferrin. *J. Biol. Chem.* 265:5273–79
39. Conrad ME, Umbreit JN, Moore EG, Uzel C, Berry MR. 1994. Alternate iron transport pathway. Mobilferrin and integrin in K562 cells. *J. Biol. Chem.* 269:7169–73
40. Conrad ME, Umbreit JN, Peterson RD, Moore EG, Harper KP. 1993. Function of integrin in duodenal mucosal uptake of iron. *Blood* 81:517–21
41. Craven CM, Alexander J, Eldridge M, Kushner JP, Bernstein S, Kaplan J. 1987. Tissue distribution and clearance kinetics

of non-transferrin-bound iron in the hypotransferrinemic mouse: a rodent model for hemochromatosis. *Proc. Natl. Acad. Sci. USA* 84:3457–61
42. Custer G, Balcerzak S, Rinehart J. 1982. Human macrophage hemoglobin-iron metabolism in vitro. *Am. J. Hematol.* 13:23–36
43. Dallman PR. 1986. Biochemical basis for the manifestations of iron deficiency. *Annu. Rev. Nutr.* 6:13–40
44. Dancis A, Klausner RD, Hinnebusch AG, Barriocanal JG. 1990. Genetic evidence that ferric reductase is required for iron uptake in *Saccharomyces cerevisiae. Mol. Cell. Biol.* 10:2294–301
45. Dancis A, Roman DG, Anderson GJ, Hinnebusch AG, Klausner RD. 1992. Ferric reductase of *Saccharomyces cerevisiae*: molecular characterization, role in iron uptake and transcriptional control by iron. *Proc. Natl. Acad. Sci. USA* 89:3869–73
46. Danielsen EM, van Deurs B. 1995. A transferrin-like GPI-linked iron-binding protein in detergent-isolated noncaveolar microdomains at the apical surface of fetal intestinal epithelial cells. *J. Cell Biol.* 131:939–50
47. Dautry-Varsat A, Ciechanover A, Lodish HF. 1983. pH and the recycling of transferrin during receptor-mediated endocytosis. *Proc. Natl. Acad. Sci. USA* 80:2258–62
48. De Sousa M, Reimao R, Lacerdo R, Hugo P, Kaufmann SHE, Porto G. 1994. Iron overload in β2-microglobulin-deficient mice. *Immunol. Lett.* 39:105–11
49. Edwards JA, Hoke JE. 1972. Defect of intestinal mucosal iron uptake in mice with hereditary microcytic anemia. *Proc. Soc. Exp. Biol. Med.* 141:81–84
50. Edwards JA, Huebers H, Kunzler C, Finch C. 1986. Iron metabolism in the Belgrade rat. *Blood* 67:623–28
51. Edwards JA, Sullivan AL, Hoke JE. 1980. Defective delivery of iron to the developing red cell of the Belgrade laboratory rat. *Blood* 51:645–48
52. Egyed A. 1988. Carrier-mediated iron transport through erythroid cell membrane. *Br. J. Haematol.* 68:483–86
53. Ekmekcioglu C, Feyertag J, Marktl W. 1996. A ferric reductase activity is found in brush border membrane vesicles isolated from Caco-2 cells. *J. Nutr.* 126: 2209–17
53a. Eisenstein RS. 2000. Iron: molecular regulation and viability. *Annu. Rev. Nutr.* 20:627–62
54. Evans PJ, Evans R, Kovar IZ, Holton AF, Halliwell B. 1992. Bleomycin-detectable iron in the plasma of premature and full-term neonates. *FEBS Lett.* 303:210–12
55. Evans PJ, Evans RW, Bomford A, Williams R, Halliwell B. 1994. Metal ions catalytic for free radical reactions in the plasma of patients with fulminant hepatic failure. *Free Radic. Res.* 20:139–44
56. Farcich EA, Morgan EH. 1992. Uptake of transferrin-bound and nontransferrin-bound iron by reticulocytes from the Belgrade laboratory rat: comparison with Wistar rat transferrin and reticulocytes. *Am. J. Hematol.* 39:9–14
57. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, et al. 1996. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat. Genet.* 13:399–408
58. Feder JN, Penny DM, Irrinki A, Lee VK, Lebron JA, et al. 1998. The hemochromatosis gene product complexes with the transferrin receptor and lowers its affinity for ligand binding. *Proc. Natl. Acad. Sci. USA* 95:1472–77
59. Feder JN, Tsuchihashi Z, Irrinki A, Lee VK, Mapa FA, et al. 1997. The hemochromatosis founder mutation in HLA-H disrupts β2-microglobulin interaction and cell surface expression. *J. Biol. Chem.* 272:14025–28
60. Ferris CD, Jaffrey SR, Sawa A, Takahashi M, Brady SD, et al. 1999. Haem oxygenase-1 prevents cell death by regulating cellular iron. *Nat. Cell Biol.* 1:152–57

61. Finegold AA, Shatwell KP, Segal AW, Klausner RD, Dancis A. 1996. Intramembrane bis-heme motif for transmembrane electron transport conserved in a yeast iron reductase and the human NADPH oxidase. *J. Biol. Chem.* 271:31021–24
62. Fleming MD, Romano MA, Su MA, Garrick LM, Garrick MD, Andrews NC. 1998. Nramp2 is mutated in the anemic Belgrade (b) rat: evidence of a role for Nramp2 in endosomal iron transport. *Proc. Natl. Acad. Sci. USA* 95:1148–53
63. Fleming MD, Trenor CC III, Su MA, Foernzler D, Beier DR, et al. 1997. Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. *Nat. Genet.* 16:383–86
64. Fleming RE, Migas MC, Zhou XY, Jiang J, Britton RS, et al. 1999. Mechanism of increased iron absorption in murine model of hereditary hemochromatosis: increased duodenal expression of the iron transporter DMT1. *Proc. Natl. Acad. Sci. USA* 96:3143–48
65. Food MR, Rothenberger S, Gabathuler R, Haidl ID, Reid G, Jefferies WA. 1994. Transport and expression in human melanomas of a transferrin-like glycosylphosphatidylinositol-anchored protein. *J. Biol. Chem.* 269:3034–40
66. Garrick LM, Dolan KG, Romano MA, Garrick MD. 1999. Non-transferrin-bound iron uptake in Belgrade and normal rat erythroid cells. *J. Cell Physiol.* 178:349–58
67. Georgatsou E, Alexandraki D. 1994. Two distinctly regulated genes are required for ferric reduction, the first step of iron uptake in *Saccharomyces cerevisiae. Mol. Cell. Biol.* 14:3065–73
68. Goldenberg H, Dodel B, Seidl D. 1990. Plasma membrane Fe2-transferrin reductase and iron uptake in K562 cells are not directly related. *Eur. J. Biochem.* 192:475–80
69. Gordeuk VR, McLaren CE, Looker AC, Hasselblad V, Brittenham GM. 1998. Distribution of transferrin saturations in the African-American population. *Blood* 91: 2175–79
70. Graham RM, Morgan EH, Baker E. 1998. Ferric citrate uptake by cultured rat hepatocytes is inhibited in the presence of transferrin. *Eur. J. Biochem.* 253:139–45
71. Granick S. 1946. Ferritin. IX. Increase of the protein apoferritin in the gastrointestinal mucosa as a direct response to iron feeding. The function of ferritin in the regulation of iron absorption. *J. Biol. Chem.* 164:737–46
72. Gross CN, Irrinki A, Feder JN, Enns CA. 1998. Co-trafficking of *HFE*, a nonclassical major histocompatibility complex class I protein, with the transferrin receptor implies a role in intracellular iron regulation. *J. Biol. Chem.* 273:22068–74
73. Gruenheid S, Canonne-Hergaux F, Gauthier S, Hackam DJ, Grinstein S, Gros P. 1999. The iron transport protein NRAMP2 is an integral membrane glycoprotein that colocalizes with transferrin in recycling endosomes. *J. Exp. Med.* 189:831–41
74. Gruenheid S, Cellier M, Vidal S, Gros P. 1995. Identification and characterization of a second mouse Nramp gene. *Genomics* 25:514–25
75. Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, et al. 1997. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* 388:482–88
76. Gutierrez JA, Inman RS, Akompong T, Yu J, Wessling-Resnick M. 1998. Metabolic depletion inhibits the uptake of non-transferrin-bound iron by K562 cells. *J. Cell Physiol.* 177:585–92
77. Gutierrez JA, Yu J, Rivera S, Wessling-Resnick M. 1997. Functional expression cloning and characterization of SFT, a stimulator of Fe transport. *J. Cell Biol.* 139:895–905
78. Gutteridge JM. 1987. Bleomycin-detectable iron in knee-joint synovial fluid from arthritic patients and its relationship to the extracellular antioxidant activities of

caeruloplasmin, transferrin and lactoferrin. *Biochem. J.* 245:415–21
79. Gutteridge JM. 1992. Ferrous ions detected in cerebrospinal fluid by using bleomycin and DNA damage. *Clin. Sci.* 82:315–20
80. Gutteridge JMC, Rowley DA, Griffiths E, Halliwell B. 1985. Low-molecular-weight iron complexes and oxygen radical reactions in idiopathic haemochromatosis. *Clin. Sci.* 68:463–67
81. Hahn PF, Bale WF, Ross JF, Balfour WF, Whipple GF. 1943. Radioactive iron absorption by the gastro-intestinal tract. Influence of anemia, anoxia, and antecedent feeding distribution in growing dogs. *J. Exp. Med.* 78:169–88
82. Hall PA, Coates PJ, Ansari B, Hopwood D. 1994. Regulation of cell number in the mammalian gastrointestinal tract: the importance of apoptosis. *J. Cell Sci.* 107: 3569–77
83. Han O, Failla ML, Hill AD, Morris ER, Smith JCJ. 1995. Reduction of Fe(III) is required for uptake of nonheme iron by Caco-2 cells. *J. Nutr.* 125:1291–99
84. Han O, Fleet JC, Wood RJ. 1999. Reciprocal regulation of *HFE* and Namp2 gene expression by iron in human intestinal cells. *J. Nutr.* 129:98–104
85. Harris ZL, Durley AP, Man TK, Gitlin JD. 1999. Targeted gene disruption reveals an essential role for ceruloplasmin in cellular iron efflux. *Proc. Natl. Acad. Sci. USA* 96:10812–17
86. Harris ZL, Takahashi Y, Miyajima H, Serizawa M, MacGillivray RT, Gitlin JD. 1995. Aceruloplasminemia: molecular characterization of this disorder of iron metabolism. *Proc. Natl. Acad. Sci. USA* 92:2539–43
87. Harrison DE. 1972. Marrow transplantation and iron therapy in mouse hereditary microcytic anemia. *Blood* 40:893–901
88. Hathorn MK. 1971. The influence of hypoxia on iron absorption in the rat. *Gastroenterology* 60:76–81
89. Hodgson LL, Quail EA, Morgan EH. 1995. Iron transport mechanisms in reticulocytes and mature erythrocytes. *J. Cell Physiol.* 162:181–90
90. Huebers HA, Huebers E, Csiba E, Rummel W, Finch CA. 1983. The significance of transferrin for intestinal iron absorption. *Blood* 61:283–90
91. Inman RS, Coughlan MM, Wessling-Resnick M. 1994. Extracellular ferrireductase activity of K562 cells is coupled to transferrin-independent iron transport. *Biochemistry* 33:11850–57
92. Inman RS, Wessling-Resnick M. 1993. Characterization of transferrin-independent iron transport in K562 cells. Unique properties provide evidence for multiple pathways of iron uptake. *J. Biol. Chem.* 268:8521–28
93. Iturri S, Nunez MT. 1998. Effect of copper, cadmium, mercury, manganese and lead on Fe2+ and Fe3+ absorption in perfused mouse intestine. *Digestion* 59:671–75
94. Jazwinska EC, Cullen LM, Busfield F, Pyper WR, Webb SI, et al. 1996. Haemochromatosis and HLA-H. *Nat. Genet.* 14: 249–51
95. Jefferies WA, Gabathuler R, Rothenberger S, Food M, Kennard ML. 1996. Pumping iron in the '90s. *Trends Cell. Biol.* 6:223–28
96. Jordan I, Kaplan J. 1994. The mammalian transferrin-independent iron transport system may involve a surface ferrireductase activity. *Biochem. J.* 302:875–79
97. Jouanolle AM, Gandon G, Jezequel P, Blayau M, Campion ML, et al. 1996. Haemochromatosis and HLA-H. *Nat. Genet.* 14:251–52
98. Kaplan J, Jordan I, Sturrock, A. 1991. Regulation of the transferrin-independent iron transport system in cultured cells. *J. Biol. Chem.* 266:2997–3004
99. Kappas A, Drummond GS, Galbraith RA. 1993. Prolonged clinical use of a heme oxygenase inhibitor: hematological evidence for an inducible but reversible irondeficiency state. *Pediatrics* 91:537–39

100. Kawabata H, Yang R, Hirama T, Vuong PT, Kawano S, et al. 1999. Molecular cloning of transferrin receptor 2. A new member of the transferrin receptor-like family. *J. Biol. Chem.* 274:20826–32
101. Kennard ML, Richardson DR, Gabathuler R, Ponka P, Jefferies WA. 1995. A novel iron uptake mechanism mediated by GPI-anchored human p97. *EMBO J.* 14:4178–86
102. Klausner RD, Ashwell G, Van Renswoude J, Harford JB, Bridges KR. 1983. Binding of apotransferrin to K562 cells: explanation of the transferrin cycle. *Proc. Natl. Acad. Sci. USA* 80:2263–66
103. Kondo H, Saito K, Grasso JP, Aisen P. 1988. Iron metabolism in the erythrophagocytosing Kupffer cell. *Hepatology* 8:32–38
104. Lebron JA, Bennett MJ, Vaughn DE, Chirino AJ, Snow PM, et al. 1998. Crystal structure of the hemochromatosis protein *HFE* and characterization of its interaction with transferrin receptor. *Cell* 93:111–23
105. Lee GR, Nacht S, Lukens JN, Cartwright GE. 1968. Iron metabolism in copper-deficient swine. *J. Clin. Invest.* 47:2058–69
106. Lee PL, Gelbart T, West C, Halloran C, Beutler E. 1998. The human Nramp2 gene: characterization of the gene structure, alternative splicing, promoter region and polymorphisms. *Blood Cells Mol. Dis.* 24:199–215
107. Levy JE, Jin O, Fujiwara Y, Kuo F, Andrews NC. 1999. Transferrin receptor is necessary for development of erythrocytes and the nervous system. *Nat. Genet.* 21:396–99
108. Levy JE, Montross LK, Cohen D, Fleming MD, Andrews NC. 1999. The C282Y mutation causing hereditary hemochromatosis does not produce a null allele. *Blood* 94:9–11
109. Liu XF, Culotta VC. 1999. Post-translation control of Nramp metal transport in yeast. Role of metal ions and the BSD2 gene. *J. Biol. Chem.* 274:4863–68
110. Lonnerdal B, Iyer S. 1995. Lactoferrin: molecular structure and biological function. *Annu. Rev. Nutr.* 15:93–110
111. McAbee DD. 1995. Isolated rat hepatocytes acquire iron from lactoferrin by endocytosis. *Biochem. J.* 311:603–9
112. McCord JM. 1998. Iron, free radicals, and oxidative injury. *Semin. Hematol.* 35:5–12
113. McKie AT, Raja KB, Peters TJ, Farzaneh F, Simpson RJ. 1996. Expression of genes involved in iron metabolism in mouse intestine. *Am. J. Physiol.* 271:G772–79
114. McLaren GD, Nathanson MH, Jacobs A, Trevett D, Thomson W. 1991. Regulation of intestinal iron absorption and mucosal iron kinetics in hereditary hemochromatosis. *J. Lab. Clin. Med.* 117:390–401
115. McNamara L, MacPhail AP, Gordeuk VR, Hasstedt SJ, Rouault T. 1998. Is there a link between African iron overload and the described mutations of the hereditary haemochromatosis gene? *Br. J. Haematol.* 102:1176–78
116. Mendel GA. 1961. Studies on iron absorption. I. The relationships between the rate of erythropoiesis, hypoxia, and iron absorption. *Blood* 18:727–36
117. Monaghan KG, Rybicki BA, Shurafa M, Feldman GL. 1998. Mutation analysis of the *HFE* gene associated with hereditary hemochromatosis in African Americans. *Am. J. Hematol.* 58:213–17
118. Morgan EH. 1988. Membrane transport of non-transferrin-bound iron by reticulocytes. *Biochim. Biophys. Acta* 943:428–39
119. Moura E, Noordermeer MA, Verhoeven N, Verheul AF, Marx JJ. 1998. Iron release from human monocytes after erythrophagocytosis in vitro: an investigation in normal subjects and hereditary hemochromatosis patients. *Blood* 92: 2511–19
120. Moyo VM, Gangaidzo IT, Gomo ZA,

Khumalo H, Saungweme T, et al. 1997. Traditional beer consumption and the iron status of spouse pairs from a rural community in Zimbabwe. *Blood* 89:2159–66
121. Moyo VM, Mandishona E, Hasstedt SJ, Gangaidzo IT, Gomo ZA, et al. 1998. Evidence of genetic transmission in African iron overload. *Blood* 91:1076–82
122. Mukhopadhyay CK, Attieh ZK, Fox PL. 1998. Role of ceruloplasmin in cellular iron uptake. *Science* 279:714–17
123. Musilkova J, Kriegerbeckova K, Krusek J, Kovar J. 1998. Specific binding to plasma membrane is the first step in the uptake of non-transferrin iron by cultured cells. *Biochim. Biophys. Acta* 1369:103–8
124. Nichols GM, Bacon BR. 1989. Hereditary hemochromatosis: pathogenesis and clinical features of a common disease. *Am. J. Gastroenterol.* 84:851–62
125. Nichols GM, Pearce AR, Alverez X, Bibb NK, Nichols KY, et al. 1992. The mechanisms of nonheme iron uptake determined in IEC-6 rat intestinal cells. *J. Nutr.* 122:945–52
126. Nunez MT, Alvarez X, Smith M, Tapia V, Glass J. 1994. Role of redox systems on Fe3+ uptake by transformed human intestinal epithelial (Caco-2) cells. *Am. J. Physiol.* 267:C1582–88
127. Nunez MT, Nunez-Millacura C, Beltran M, Tapia V, Alvarez-Hernandez X. 1997. Apotransferrin and holotransferrin undergo different endocytic cycles in intestinal epithelia (Caco-2) cells. *J. Biol. Chem.* 272:19425–28
128. Nunez MT, Tapia V. 1999. Transferrin stimulates iron absorption, exocytosis, and secretion in cultured intestinal cells. *Am. J. Physiol.* 276:C1085–90
129. Oates PS, Morgan EH. 1996. Effects of dietary iron loading with carbonyl iron and of iron depletion on intestinal growth, morphology, and expression of transferrin receptor in the rat. *Anat. Rec.* 246:364–71
130. Oates PS, Morgan EH. 1997. Ferritin gene expression and transferrin receptor activity in intestine of rats with varying iron stores. *Am. J. Physiol.* 273:G636–46
131. O'Donnell M, Cox TM. 1982. Microvillar iron-binding glycoproteins isolated from the rabbit small intestine. *Biochem. J.* 202:107–15
132. Olakanmi O, Stokes JB, Britigan BE. 1994. Acquisition of iron bound to low molecular weight chelates by human monocyte-derived macrophages. *J. Immunol.* 153:2691–703
133. Olakanmi O, Stokes JB, Pathan S, Britigan BE. 1997. Polyvalent cationic metals induce the rate of transferrin-independent iron acquisition by HL-60 cells. *J. Biol. Chem.* 272:2599–606
134. O'Riordan DK, Debnam ES, Sharp PA, Simpson RJ, Taylor EM, Srai SK. 1997. Mechanisms involved in increased iron uptake across rat duodenal brush-border membrane during hypoxia. *J. Physiol.* 500:379–84
135. O'Riordan DK, Sharp P, Sykes RM, Srai SK, Epstein O, Debnam ES. 1995. Cellular mechanisms underlying the increased duodenal iron absorption in rats in response to phenylhydrazine-induced haemolytic anaemia. *Eur. J. Clin. Invest.* 25:722–27
136. Osaki S, Johnson DA, Frieden E. 1971. The mobilization of iron from the perfused mammalian liver by a serum copper enzyme, ferroxidase I. *J. Biol. Chem.* 246:3018–23
137. Oshiro S, Nakajima H, Markello T, Krasnewick D, Bernardini I, Gahl WA. 1993. Redox, transferrin-independent, and receptor-mediated endocytosis iron uptake systems in cultured human fibroblasts. *J. Biol. Chem.* 268:21586–91
138. Page MA, Morgan EH, Baker E. 1984. Transferrin binding and iron uptake by rat hepatocytes in culture. *Am. J. Physiol.* 246:G26–G33
139. Parkes JG, Hussain RA, Olivieri NF, Templeton DM. 1993. Effects of iron loading

on uptake, speciation, and chelation of iron in cultured mycardial cells. *J. Lab. Clin. Med.* 122:36–47

140. Parkes JG, Olivieri NF, Templeton DM. 1997. Characterization of Fe2+ and Fe3+ transport by iron-loaded cardiac myocytes. *Toxicology* 117:141–51
141. Parkes JG, Templeton DM. 1994. Iron transport and subcellular distribution in HepG2 hepatocarcinoma cells. *Ann. Clin. Lab. Sci.* 24:509–20
142. Parkkila S, Waheed A, Britton RS, Bacon BR, Zhou XY, et al. 1997. Association of the transferrin receptor in human placenta with *HFE*, the protein defective in hereditary hemochromatosis. *Proc. Natl. Acad. Sci. USA* 94:13198–202
143. Parkkila S, Waheed A, Britton RS, Feder JN, Tsuchihashi Z, et al. 1997. Immunohistochemistry of HLA-H, the protein defective in patients with hereditary hemochromatosis, reveals unique pattern of expression in gastrointestinal tract. *Proc. Natl. Acad. Sci. USA* 94:2534–39
144. Peters TJ, Raja KB, Simpson RJ, Snape S. 1988. Mechanisms and regulation of intestinal iron absorption. *Ann. NY Acad. Sci.* 526:141–47
145. Pietrangelo A. 1998. Hemochromatosis 1998: Is one gene enough? *J. Hepatol.* 29:502–9
146. Pinner E, Gruenheid S, Raymond M, Gros P. 1997. Functional complementation of the yeast divalent cation transporter family SMF by NRAMP2, a member of the mammalian natural resistance-associated macrophage protein family. *J. Biol. Chem.* 272:28933–38
147. Pollitt E. 1993. Iron deficiency and cognitive function. *Annu. Rev. Nutr.* 13:521–37
148. Ponka P, Beaumont C, Richardson DR. 1998. Function and regulation of transferrin and ferritin. *Semin. Hematol.* 35:35–54
149. Poss KD, Tonegawa S. 1997. Heme oxygenase 1 is required for mammalian iron reutilization. *Proc. Natl. Acad. Sci. USA* 94:10919–24
150. Qian ZM, Morgan EH. 1991. Effect of metabolic inhibitors on uptake of non-transferrin-bound iron by reticulocytes. *Biochim. Biophys. Acta* 1073:456–62
151. Quail EA, Morgan EH. 1994. Role of membrane surface potential and other factors in the uptake of non-transferrin-bound iron by reticulocytes. *J. Cell Physiol.* 159:238–44
152. Rabie A, Simpson RJ, Bomford A, Cunninghame-Graham D, Peters TJ. 1995. Relationship between duodenal cytosolic aconitase activity and iron status in the mouse. *Biochim. Biophys. Acta* 1245:414–20
153. Raffin SB, Woo CH, Roost KT, Price DC, Schmid R. 1974. Intestinal absorption of hemoglobin iron-heme cleavage by mucosal heme oxygenase. *J. Clin. Invest.* 54:1344–52
154. Raja KB, Pountney D, Bomford A, Przemioslo R, Sherman D, et al. 1996. A duodenal mucosal abnormality in the reduction of Fe(III) in patients with genetic haemochromatosis. *Gut* 38:765–69
155. Raja KB, Simpson RJ, Peters TJ. 1992. Investigation of a role for reduction in ferric iron uptake by mouse duodenum. *Biochim. Biophys. Acta* 1135:141–46
156. Randell EW, Parkes JG, Olivieri NF, Templeton DM. 1994. Uptake of non-transferrin-bound iron by both reductive and nonreductive processes is modulated by intracellular iron. *J. Biol. Chem.* 269:16046–53
157. Richardson D, Baker E. 1991. The uptake of inorganic iron complexes by human melanoma cells. *Biochim. Biophys. Acta* 1093:20–28
158. Richardson D, Baker E. 1992. Two mechanisms of iron uptake from transferrin by melanoma cells. The effects of desferrioxamine and ferric ammonium citrate. *J. Biol. Chem.* 267:13972–79
159. Richardson DR. 1997. Mobilization of

iron from neoplastic cells by some iron chelators is an energy-dependent process. *Biochim. Biophys. Acta* 1320:45–57
160. Richardson DR, Baker E. 1990. The uptake of iron and transferrin by the human malignant melanoma cell. *Biochim. Biophys. Acta* 1053:1–12
161. Richardson DR, Baker E. 1991. The release of iron and transferrin from the human melanoma cell. *Biochim. Biophys. Acta* 1091:294–302
162. Richardson DR, Ponka P. 1995. Identification of a mechanism of iron uptake by cells which is stimulated by hydroxyl radicals generated via the iron-catalysed Haber-Weiss reaction. *Biochim. Biophys. Acta* 1269:105–14
163. Riedel HD, Remus AJ, Fitscher BA, Stremmel W. 1995. Characterization and partial purification of a ferrireductase from human duodenal microvillous membrane. *Biochem. J.* 309:745–48
164. Robinson NJ, Procter CM, Connolly EL, Guerinot ML. 1999. A ferric-chelate reductase for iron uptake from soils. *Nature* 397:694–97
165. Roman DG, Dancis A, Anderson GJ, Klausner RD. 1993. The fission yeast ferric reductase gene frp1+ is required for ferric iron uptake and encodes a protein that is homologous to the gp91-phox subunit of the human NADPH phagocyte oxidoreductase. *Mol. Cell. Biol.* 13:4342–50
166. Rosenberg DW, Kappas A. 1989. Characterization of heme oxygenase in the small intestinal epithelium. *Arch. Biochem. Biophys.* 274:471–80
167. Rossander-Hulten L, Brune M, Sandstrom B, Lonnerdal B, Hallberg L. 1991. Competitive inhibition of iron absorption by manganese and zinc in humans. *Am. J. Clin. Nutr.* 54:152–56
168. Rothenberg BE, Voland JR. 1996. Beta 2 knockout mice develop parenchymal iron overload: a putative role for class I genes of the major histocompatibility complex in iron metabolism. *Proc. Natl. Acad. Sci. USA* 93:1529–34
169. Roy CN, Penny DM, Feder JN, Enns CA. 1999. The hereditary hemochromatosis protein, HFE, specifically regulates transferrin-mediated iron uptake in HeLa cells. *J. Biol. Chem.* 274:9022–28
170. Saito K, Nishisato T, Grasso JA, Aisen P. 1986. Interaction of transferrin with iron-loaded rat peritoneal macrophages. *Br. J. Haematol.* 62:275–86
171. Salter-Cid L, Brunmark A, Li Y, Leturcq D, Peterson PA, et al. 1999. Transferrin receptor is negatively modulated by the hemochromatosis protein *HFE*: implications for cellular iron homeostasis. *Proc. Natl. Acad. Sci. USA* 96:5434–39
172. Sasaki K, Zak O, Aisen P. 1993. Antisense suppression of transferrin receptor gene expression in a human hepatoma cell (HuH-7) line. *Am. J. Hematol.* 42:74–80
173. Savigni DL, Morgan EH. 1998. Transport mechanisms for iron and other transition metals in rat and rabbit erythroid cells. *J. Physiol.* 508:837–50
174. Schieber B, Goldenberg H. 1998. The surface of rat hepatocytes can transfer iron from stable chelates to external acceptors. *Hepatology* 27:1075–80
175. Schumann K, Elsenhans B, Forth W. 1999. Kinetic analysis of 59Fe movement across the intestinal wall in duodenal rat segments ex vivo. *Am. J. Physiol.* 276:G431–40
176. Schumann K, Moret R, Kunzle H, Kuhn LC. 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.* 260:362–72
177. Schweinzer E, Goldenberg H. 1993. Monodehydroascorbate reductase activity in the surface membrane of leukemic cells, characterization by a ferricyanide-driven redox cycle. *Eur. J. Biochem.* 218:1057–62

178. Shatwell KP, Dancis A, Cross AR, Klausner RD, Segal AW. 1996. The FRE1 ferric reductase of *Saccharomyces cerevisiae* is a cytochrome b similar to that of NADPH oxidase. *J. Biol. Chem.* 271: 14240–44
179. Sherwood RA, Pippard MJ, Peters TJ. 1998. Iron homeostasis and the assessment of iron status. *Ann. Clin. Biochem.* 35:693–708
180. Simpson RJ, Lombard M, Raja KB. Snape SD, Peters TJ. 1986. Studies on the role of transferrin and endocytosis in the uptake of Fe3+ from Fe-nitriloacetate by mouse duodenum. *Biochim. Biophys. Acta* 884:166–71
181. Sipe DM, Murphy RF. 1991. Binding to cellular receptors results in increased iron release from transferrin at mildly acidic pH. *J. Biol. Chem.* 266:8002–7
182. Skikne B, Baynes R. 1994. Iron absorption. In *Iron Metabolism in Health and Disease*, ed. J Bock, J Halliday, M Pippard, L Powell, pp. 151–87. London: Saunders
183. Stonell LM, Savigni DL, Morgan EH. 1996. Iron transport into erythroid cells by the Na+/Mg2+ antiport. *Biochim. Biophys. Acta* 1282:163–70
184. Sturrock A, Alexander J, Lamb J, Craven CM, Kaplan J. 1990. Characterization of a transferrin-independent uptake system for iron in HeLa cells. *J. Biol. Chem.* 265:3139–45
185. Su MA, Trenor CC, Fleming JC, Fleming MD, Andrews NC. 1998. The G185R mutation disrupts function of the iron transporter Nramp2. *Blood* 92:2157–63
186. Teichmann R, Stremmel W. 1990. Iron uptake by human upper small intestine microvillous membrane vesicles: indication for a facilitated transport mechanism mediated by a membrane iron-binding protein. *J. Clin. Invest.* 86:2145–53
187. Thorstensen K, Trinder D, Zak O, Aisen P. 1995. Uptake of iron from N-terminal half-transferrin by isolated rat hepatocytes: evidence of transferrin receptor-independent iron uptake. *Eur. J. Biochem.* 232:129–33
188. Trinder D, Morgan E. 1997. Inhibition of uptake of transferrin-bound iron by human hepatoma cells by nontransferrin-bound iron. *Hepatology* 26:691–98
189. Trinder D, Morgan EH, Baker E. 1988. The effects of an antibody to the rat transferrin receptor and of rat serum albumin on the uptake of diferric transferrin by rat hepatocytes. *Biochim. Biophys. Acta* 943:440–46
190. Trinder D, Zak O, Aisen P. 1996. Transferrin receptor independent uptake of diferric transferrin by human hepatoma cells with antisense inhibition. *Hepatology* 23:1512–20
191. Umbreit JN, Conrad ME, Moore EG, Latour LF. 1998. Iron absorption and cellular transport: the mobilferrin/paraferritin paradigm. *Semin. Hematol.* 35:13–26
192. Vidal SM, Malo D, Vogan K, Skamene E, Gros P. 1993. Natural resistance to infection with intracellular parasites: isolation of a candidate for Bcg. *Cell* 73:469–85
193. Vulpe C, Levinson B, Whitney S, Packman S, Gitschier J. 1993. Isolation of a candidate gene for Menkes disease and evidence that it encodes a copper-transporting ATPase. *Nat. Genet.* 3:7–13
194. Vulpe CD, Kuo YM, Murphy TL, Cowley L, Askwith C, et al. 1999. Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. *Nat. Genet.* 21:195–99
195. Waheed A, Parkkila S, Saarnio J, Fleming RE, Zhou XY, et al. 1999. Association of *HFE* protein with transferrin receptor in crypt enterocytes of human duodenum. *Proc. Natl. Acad. Sci. USA* 96:1579–84
196. Waheed A, Parkkila S, Zhou XY, Tomatsu S, Tsuchihashi Z, et al. 1997. Hereditary hemochromatosis: effects of C282Y and H63D mutations on association with

β2-microglobulin, intracellular processing, and cell surface expression of the *HFE* protein in COS-7 cells. *Proc. Natl. Acad. Sci. USA* 94:12384–89

197. Wardrop SL, Richardson DR. 1999. The effect of intracellular iron concentration and nitrogen monoxide on Nramp2 expression and non-transferrin-bound iron uptake. *Eur. J. Biochem.* 263:41–50
198. Whittaker P, Skikne BS, Covell AM, Flowers C, Cooke A, et al. 1989. Duodenal iron proteins in idiopathic hemochromatosis. *J. Clin. Invest.* 83:261–67
199. Wright TL, Brissot P, Ma ML, Weisiger RA. 1986. Characterization of non-transferrin-bound iron clearance by rat liver. *J. Biol. Chem.* 261:10909–14
200. Wright TL, Fitz JG, Weisiger RA. 1988. Non-transferrin-bound iron uptake by rat liver: role of membrane potential difference. *J. Biol. Chem.* 263:1842–47
201. Wright TL, Lake JR. 1990. Mechanisms of transport of nontransferrin-bound iron in basolateral and canalicular rat liver plasma membrane vesicles. *Hepatology* 12:498–504
202. Yamaguchi-Iwai Y, Dancis A, Klausner RD. 1995. AFT1: a mediator of iron regulated transcriptional control in *Saccharomyces cerevisiae. EMBO J.* 14:1231–39
203. Yoshida K, Furihata K, Takeda S, Nakamura A, Yamamoto K, et al. 1995. A mutation in the ceruloplasmin gene is associated with systemic hemosiderosis in humans. *Nat. Genet.* 9:267–72
204. Young SP, Fahmy M, Golding S. 1997. Ceruloplasmin, transferrin and apotransferrin facilitate iron release from human liver cells. *FEBS Lett.* 411:93–96
205. Yu J, Wessling-Resnick M. 1998. Influence of copper depletion on iron uptake mediated by SFT, a stimulator of Fe transport. *J. Biol. Chem.* 273:6909–15
206. Zhou XY, Tomatsu S, Fleming RE, Parkkila S, Waheed A, et al. 1998. *HFE* gene knockout produces mouse model of hereditary hemochromatosis. *Proc. Natl. Acad. Sci. USA* 95:2492–97
207. Zoller H, Pietrangelo A, Vogel W, Weiss G. 1999. Duodenal metal-transporter (DMT1, NRAMP2) expression in patients with hereditary haemochromatosis. *Lancet* 353:2120–23