Topical Review

Metal ion transporters in mammals: structure, function and pathological implications

Andreas Rolfs and Matthias A. Hediger

Membrane Biology Program and Renal Division, Department of Medicine, Brigham & Women's Hospital and Harvard Medical School, and Department of Biological Chemistry & Molecular Pharmacology, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, MA 02115, USA

(Received 2 March 1999; accepted after revision 29 April 1999)

Despite the importance of metal ions in several catalytic functions, there has been, until recently, little molecular information available on the mechanisms whereby metal ions are actively taken up by mammalian cells. The classical concept for iron uptake into mammalian cells has been the endocytosis of transferrin-bound Fe^{3+} by the transferrin receptor. Studies with hypotransferrinaemic mice revealed that in the intestine mucosal transferrin is derived from the plasma and that its presence is not required in the intestinal lumen for dietary iron absorption. This suggests that, at least in the intestine, other non-receptor-mediated uptake systems exist. The molecular identification of metal ion transporters is of great importance, in particular since an increasing number of human diseases are thought to be related to disturbances in metal ion homeostasis, including metal ion overload and deficiency disorders (i.e. anaemia, haemochromatosis, Menkes disease, Wilson's disease), and neurodegenerative diseases (i.e. Alzheimer's, Friedreich's ataxia and Parkinson's diseases). Furthermore, susceptibilities to mycobacterial infections are caused by metal ion transporter defects. The pathological implications of disturbed metal ion homeostasis confirm the vital roles these metal ions play in the catalytic function of many enzymes, in gene regulation (zinc-finger proteins), and in free radical homeostasis. Recent insights have significantly advanced our knowledge of how metal ions are taken up or released by mammalian cells. The purpose of this review is to summarize these advances and to give an overview on the growing number of mammalian metal ion transporters.

Functional role of iron

Iron is required in all organisms for growth and crucial metabolic pathways. The redox potential of Fe^{2+}/Fe^{3+} favours its use in a number of protein complexes, especially those involved in electron transfer. A number of proteins require iron for activity in the form of haeme or iron–sulfur clusters to transfer electrons. Iron complexes are not only necessary in the electron transport chain to supply cells with energy, but they are also affected by oxygen radicals ($O_2^{-}\cdot$), and free Fe^{2+} is part of the Fenton reaction to generate reactive oxygen species (Henle & Linn, 1997). Therefore, the maintenance of iron homeostasis in the body as well as in the cells must be balanced, to supply enough iron for the metabolism, and to avoid excessive, toxic levels. Regulation of iron uptake also depends on the state of oxygenation. Studies of duodenal brush-border membranes in rat indicate that iron absorption is increased during chronic hypoxia (O'Riordan *et al.* 1997).

In the presence of oxygen, ferric iron (Fe^{3+}) is the favoured species, but in the organism ferrous iron (Fe^{2+}) is required. The uptake, and transport, of iron under physiological conditions requires special mechanisms, because Fe^{3+} has a very low solubility at neutral pH in oxygenated fluids ($< 10^{-17}$ mol l^{-1} at pH 7·4; Harford, 1994). In daily diet two distinct forms of iron are present, namely non-haeme iron (Fe^{3+}) and haeme iron. The rate-limiting step of iron uptake appears to be in the intestine, where high amounts of iron present in the diet have to be absorbed. In mammals, the best-studied uptake mechanism of iron is the process of transferrin receptor-mediated endocytosis (van Eijk & de Jong, 1992; Harford, 1994; Richardson & Ponka, 1997). However, there are two observations that indicate that this is not the pathway by which iron is taken up into the body. First, apo-transferrin is not available in the intestinal lumen, except from biliary excretion (Green *et al.* 1968; Iancu *et al.* 1995), which is insufficient to account for dietary

iron absorption. Second, experiments with brush-border membrane vesicles suggested that other, non-receptor-mediated iron uptake systems exist in the intestine (Eastham *et al.* 1977; Teichmann & Stremmel, 1990). The acidic pH in the proximal intestine and/or the reduced pH of ~6·0 in the unstirred layer close to the external surface of the intestinal brush-border membrane help to solubilize Fe^{2+}, which is rendered in its reduced form by ascorbate, and a ferrireductase (Wien & Van Campen, 1991; Raja *et al.* 1992; Dorey *et al.* 1993; Inman *et al.* 1994; Jordan & Kaplan, 1994; Han *et al.* 1995; Umbreit *et al.* 1996).

Interestingly, the process of transferrin receptor-mediated endocytosis, thought to be the principal way of uptake of Fe^{3+} into non-intestinal cells, did not lead to an explanation of how iron can cross the endosomal membrane. Studies on the process of transferrin receptor-mediated endocytosis led to the observation that these endosomes need to be acidified. The low endosomal pH is necessary for release of iron from transferrin. Furthermore, the transfer from the endosomes into the cytosol requires the activity of an ferrireductase as well as an Fe^{2+} transporter, because iron exists primarily as Fe^{2+} in the cytosol (Dautry-Varsat, 1986; van Eijk & de Jong, 1992).

The recently cloned plant ferrireductase (Robinson *et al.* 1999) may shed light on eukaryotic iron acquisition. However, a mammalian homologue has not yet been identified. In yeast the combination of a ferrireductase/-oxidase reaction probably makes iron accessible for uptake from stable organic complexes (Askwith & Kaplan, 1998; Dancis, 1998).

Evidence for two types of iron transporters

The activity of more than one transporter for iron in the mammalian intestine was already promoted in 1977 by studies of Eastham and colleagues (Eastham *et al.* 1977) who found that vesicles from the brush-border or basolateral membrane show different kinetics of Fe^{2+} uptake. These findings were supported by those of Teichmann & Stremmel (1990) on Fe^{3+} uptake in intestinal vesicles and by studies of mice suffering from microcytic hypochromic anaemia. This anaemia is indistinguishable from iron deficiency anaemia but is unresponsive to increased dietary iron. In microcytic anaemia (*mk*) mice a transporter located in the brush-border membrane of microvillus cells was thought to be mutated, thereby blocking iron uptake into these cells. In sex-linked anaemia (*sla*) mice iron uptake into the microvillus cells is detectable, but the iron is not released into the serum (Anderson *et al.* 1998). These studies indicate two types of transporter proteins, located in the brush-border and basolateral membranes, respectively (Fig. 1).

In recent years, various iron transporters have been identified for lower organisms, including the Fe^{2+} transporters feoB from *E. coli* (Kammler *et al.* 1993), FET4 from yeast (Dix *et al.* 1994), the plant IRT1 (Eide *et al.* 1996), and the Fe^{3+} transporter FTR1 from yeast (Stearman *et al.* 1996). However, until recently the transporters which mediate direct uptake of iron and metal ions into mammalian cells remained elusive.

Functional cloning of rat DCT1

Using expression cloning with *Xenopus laevis* oocytes, our laboratory isolated the divalent cation/metal ion transporter (DCT1) from a duodenal cDNA library, prepared from mRNA from rats fed a low-iron diet (Gunshin *et al.* 1997). DCT1 was isolated by screening this library using a radiotracer assay of $^{55}Fe^{2+}$ uptake in *Xenopus* oocytes. The isolated cDNA clone encodes a 561 amino acid polypeptide, which, when expressed in oocytes, increases the uptake of $^{55}Fe^{2+}$ more than 200-fold compared with control (water injected) oocytes. The amino acid sequence predicts 12 membrane spanning domains, a glycosylated extracellular loop, a consensus transport motif (CTM) in the fourth intracellular loop, and a topology with N- and C-termini in the cytosol (see Fig. 3). The CTM is thought to interact with ATP coupling subunits, distinct from the nucleotide binding fold of ABC transporters. However, ATP depletion in oocytes did not affect DCT1-mediated iron uptake. DCT1 expression was found to be widespread in rat based on *in situ* hybridization and Northern blot analysis. Comparison of DCT1 expression in tissues from rats fed a normal diet or an iron-deficient diet for 3 weeks showed that iron deprivation triggers a strong increase of DCT1 mRNA levels in intestine and to some extent in all tissues examined. The finding of an iron-responsive element (IRE) in the 3′ untranslated region of the cloned DCT1 cDNA indicates regulation of DCT1 at the RNA stability level, analogous to the transferrin receptor mRNA. Recent studies in our laboratory on the function of the DCT1-IRE support this hypothesis and suggest that the iron-response element binding protein 1 (IRP1) binds to this IRE (M. A. Hediger, unpublished data).

Electrophysiological studies of DCT1 expressed in oocytes revealed that the divalent cation transporter is electrogenic, with Fe^{2+} evoking currents of up to 1000 nA and an apparent K_m for Fe^{2+} of ~6 μM. These currents were both voltage- and pH-dependent, indicating that an acid pH is necessary for functional transport of iron. Furthermore, it was shown by these experiments that DCT1 transports not only Fe^{2+}, but also Zn^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Cd^{2+} and Pb^{2+}.

The cloning and characterization of DCT1 provided the first demonstration of an active, cellular uptake mechanism for divalent cations (including Fe^{2+}) in mammalian cells. The functional properties of DCT1 show the need of an acidic environment, as found in the proximal duodenum, but also in the transferrin receptor-mediated endosomes. Localization studies by immunofluorescence and confocal microscopy show the presence of DCT1 primarily in recycling endosomes and also in the plasma membrane (Gruenheid *et al.* 1999). DCT1 turns out to be the rat homologue of the previously identified mouse and human Nramp2 and these proteins are homologous (~70% identical) to Nramp1, the natural resistance-associated macrophage protein (Vidal *et al.* 1993, 1995*b*). The biological functions of the Nramp proteins

remained unknown, until DCT1 was identified. The name Nramp was given to these proteins because a defect in Nramp1 leads to lack of resistance to infections of macrophages (Vidal *et al.* 1995*b*; Gruenheid *et al.* 1997).

Positional cloning of Nramp2 (DCT1) in mouse and rat

Independently of our cloning of DCT1, Andrews and colleagues (Fleming *et al.* 1997) identified mouse Nramp2/DCT1 as an iron transporter, by using a genetic approach. That approach stems from their search for the gene for microcytic anaemia (*mk*) in mice. The severe microcytic hypochromic anaemia is indistinguishable from iron deficiency anaemia but is unresponsive to increased dietary iron. Iron injections, intended to circumvent the intestinal block, did not reverse the anaemia, suggesting a block of iron entry into red blood cell precursors. The locus for *mk* was found to be linked to an area of the mouse genome where the Nramp2/DCT1 gene was found earlier (Gruenheid *et al.* 1995; Vidal *et al.* 1995*a*). By using RT-PCR analysis for comparison of Nramp2/DCT1 cDNA in wild-type and *mk* mice, a single point-mutation, substituting arginine for glycine (G185R) within transmembrane domain 4 of *mk*-Nramp2/DCT1 was found (Fleming *et al.* 1997). Given the capacity of Nramp2/DCT1 to transport several other divalent heavy metal ions besides iron, the phenotype of the *mk*/*mk* mouse might be explained in terms of deficiencies of other metal ions such as Zn^{2+}, Mn^{2+} and Cu^{2+} (Chua & Morgan, 1997).

Similar to *mk* mice, the Belgrade (*b*) rats have a severe hypochromic, microcytic anaemia that is associated with defects in erythroid iron utilization and intestinal iron uptake in the apical membrane of the enterocytes. Andrews and colleagues have reported that the *b* rats also have the G185R mutation in transmembrane domain 4 (Fleming *et al.* 1998). Expression of this mutant protein in eukaryotic cells did not result in stimulation of iron uptake (Su *et al.* 1998). It is likely that a subset of human patients with congenital anaemia also harbour mutations in DCT1. Indeed, isolated families with an apparently autosomal recessive inherited iron-deficiency anaemia, unresponsive to iron therapy, have been described (Hartman & Barker, 1996). The affected individuals have a phenotype reminiscent of that of the *b* rat and *mk* mouse.

Nramp1 cloning and pathophysiology

Nramp1 (natural resistance-associated macrophage protein 1) was found by its impaired function in macrophages in response to infections with various species of mycobacteria and other intracellular parasites (Atkinson & Barton, 1998). The Nramp1 gene was identified by positional cloning, and found to be almost exclusively expressed in macrophages (Vidal *et al.* 1993, 1995*b*). The amino acid sequence is highly homologous to DCT1/Nramp2 (73%), suggesting a similar metal ion transport function for Nramp1 in macrophages. Expression of Nramp1 in oocytes suggested a lower affinity for iron compared with DCT1 (Gunshin *et al.* 1997). However, expression levels were low in these experiments and further studies are required to elucidate the functional characteristics of Nramp1. In experiments in which Nramp1 was expressed in monkey COS-1 cells it was shown that iron uptake was not increased, but there was a reduced cellular iron content (Atkinson & Barton, 1998). Localization studies show that Nramp1 protein is present in the lysosomal compartment of macrophages, and in phagosomal membranes during phagocytosis (Gruenheid *et al.* 1997). Its localization does not overlap with that of DCT1/Nramp2, which is also expressed in these cells (Gruenheid *et al.* 1999). Given this localization, together with the observed metal ion transport, Nramp1 might play a role in resistance to infections by depleting the phagosome of Fe^{2+}, Mn^{2+} or other essential

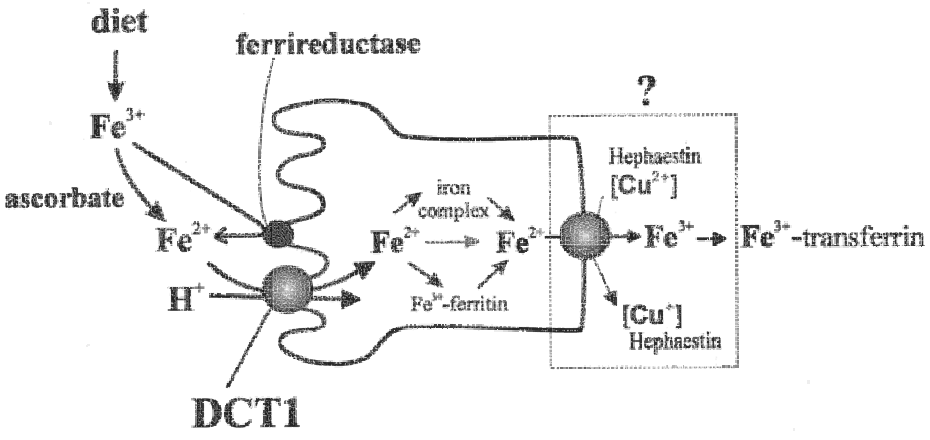

Figure 1. Iron transport in intestinal (villus) cells

Dietary iron (Fe^{3+}) in the lumen is taken up at the apical side by DCT1 after its reduction to Fe^{2+}. In the cytosol, Fe^{2+} is bound to low molecular weight complexes, or proteins, or stored by binding to ferritin. At the basolateral side, Fe^{2+} is released from villus cells into the plasma by a transporter which requires a multicopper oxidase (the *sla* protein). The released Fe^{3+} is instantly transferred to transferrin.

divalent metal ions. Recently, it has been reported that tuberculosis infections in West Africans are linked to polymorphism in the Nramp1 gene (Bellamy *et al.* 1998), indicating that the successful development of treatment strategies for mycobacterial infections requires evaluation of these polymorphisms.

Additional iron transporters

Polarized epithelial cells have two different iron transporters, one in the apical and one in the basolateral membrane (Fig. 1). This is supported by findings in mice with sex-linked anaemia, *sla* mice. These mice suffer from microcytic anaemia similar to *mk* mice. *mk* animals have a disturbed uptake of iron in the intestine, whereas the export out of the intestine is normal. In contrast, the *sla* animals show the opposite disorder. Their uptake of iron into the villus cells appears normal, and is probably mediated by DCT1, but the release into the blood is compromised. Recent attempts to clone the basolateral transporter indicate that it is composed of at least two subunits, one necessary for Fe^{2+} transport, and the other one for oxidation of Fe^{2+} to Fe^{3+} (Anderson *et al.* 1998; McKie *et al.* 1998; Vulpe *et al.* 1999). The recently cloned hephaestin, deficient in *sla* mice, is a membrane-bound homologue of ceruloplasmin, and probably functions as multi-copper ferrioxidase. The protein contains only one transmembrane domain, indicating that hephaestin is not the basolateral iron transporter itself. Most probably it interacts with the transporter and facilitates release of iron into the blood by its ferrioxidase activity (Vulpe *et al.* 1999).

A stimulator of iron transport (SFT) has also been isolated (Gutierrez *et al.* 1997, 1998). This protein was reported to increase the uptake of iron into *Xenopus* oocytes, but it is not clear whether this is due to transport or (as indicated by the name) to stimulation of an endogenous transport protein, and whether Fe^{2+} or Fe^{3+} is the substrate *in vivo* (Yu & Wessling-Resnick, 1998*a*,*b*).

In addition to these iron transporters, frataxin, which is defective in the mitochondria of patients with Friedreich's ataxia has been isolated and shown to be an iron transport exit mechanism for mitochondria (Campuzano *et al.* 1996; Babcock *et al.* 1997; Kispal *et al.* 1997; Koutnikova *et al.* 1997; Wilson & Roof, 1997; Radisky *et al.* 1999). Its defect leads to iron accumulation in the myocardium of patients. Whether the human ABC7, a homologue of the yeast mitochondrial ABC-type iron transporter Atm1p, functions in addition to frataxin as an iron exporter in mitochondria has yet to be proven (Kispal *et al.* 1997; Csere *et al.* 1998).

Sensing body iron, iron transport and hereditary haemochromatosis

One of the most common genetic disorders in man, hereditary haemochromatosis (*HH*), is caused by the mutation of a MHC II protein, HFE. Recently it was reported that the HFE protein interacts with the transferrin receptor (TfR), and in the case of *HH* that this interaction is disrupted, giving rise to the long-lasting iron overload in the body (Parkkila *et al.* 1997; Feder *et al.* 1998; Gross *et al.* 1998; Zhou *et al.* 1998).

As alluded to above, DCT1 mRNA levels in the intestine are tightly controlled by serum iron levels. Intestinal crypt cells express HFE and TfR (Anderson *et al.* 1994; Wood & Han, 1998), whereas mature villus cells express DCT1, but not HFE (Anderson *et al.* 1994; Wood & Han, 1998). These findings led us to speculate that HFE and TfR together sense serum iron in crypt cells. We propose that HFE and TfR in crypt cells regulate the expression of the proteins involved in iron absorption in villus cells, including DCT1, via the IRE/IRP system (Fig. 2) (Waheed *et al.* 1999). In patients suffering from *HH*, this regulation may be disturbed in crypt cells, leading to a higher expression rate of proteins involved in iron absorption in mature villus and in increased intestinal iron absorption. This hypothesis is supported by studies of hypotransferrinaemic mice (Simpson *et al.* 1991). In these animals a hyperabsorption of iron is observed, in conjunction with a reduced level of transferrin expression (1–2% of normal) (Buys *et al.* 1991). A recent report by Feder and coauthors (Roy *et al.* 1999) showed a direct effect of HFE on Tf-mediated, but not on non-Tf-mediated, iron uptake in a non-polarized cell line. In addition, Andrews and colleagues showed that, in heterozygous TfR knock-out mice, reduced levels of TfR expression give rise to microcytic, hypochromic anaemia, due to impaired iron uptake by maturating erythrocytes (Levy *et al.* 1999). The iron content in the liver and spleen of these mice was reduced, probably due to reduced intestinal iron absorption, and possibly as a consequence of disturbed iron sensing by TfR–HFE interaction in crypt cells. This concept is consistent with recent findings by Sly and colleagues addressing regulation of intestinal iron absorption in HFE knock-out mice. These mice were shown to have increased duodenal DCT1/Nramp2 levels, despite high serum iron concentration (Fleming *et al.* 1999).

DCT1 as a metal ion transporter with broad selectivity

The finding that DCT1 mediates active cellular uptake of not only Fe^{2+}, but also Zn^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+} and the toxic metal ions Pb^{2+} and Cd^{2+}, was surprising. The existence of a common intestinal absorptive mechanism for a range of metals has important nutritional implications and emphasizes the potential interplay among these essential trace minerals at the level of their absorption. For example, dietary copper deficiency can cause microcytic anaemia, indistinguishable from iron-deficiency microcytic anaemia, suggesting that a defect in DCT1 may also lead to anaemia due to defective copper uptake. In addition, *mk*/*mk* mice also suffer from skin lesions reminiscent of defective zinc uptake. *b* rats are also reported to have widespread abnormalities in manganese metabolism, including impaired duodenal and reticulocyte uptake (Chua & Morgan, 1997; Savigni & Morgan, 1998). Thus, consistent with the substrate range which we determined for DCT1, the metabolism of a variety of metal

ions including iron, manganese, cobalt (Savigni & Morgan, 1998) and zinc are deranged due to the DCT1 mutation in the *mk* and *b* alleles, underlining the importance of DCT1 in the metabolism of these metals.

The similar affinity of transferrin for other metal ions (Zn^{2+}, Mn^{2+}, Cu^{2+} and Al^{3+}) suggests that this mechanism mediates uptake of a variety of other metal ions. Thus, in non-intestinal tissues, the TfR–DCT1 endocytotic uptake pathway might allow uptake of not only iron but a range of other metal ions.

Uptake of manganese was previously thought to be related to iron uptake (Chua *et al.* 1996). At least three mechanisms were described based on studies of erythroid cells, one using manganese-transferrin, one involving a high-affinity Mn^{2+} transporter, and one involving a low-affinity Mn^{2+} transporter (Chua *et al.* 1996). It is likely that the previously reported high-affinity transporter for Mn^{2+} in erythroid cells involves TfR and DCT1, but this still needs verification (Savigni & Morgan, 1998).

Other metal ion uptake transporters

Thus far, several divalent cation transporters with an affinity for single metal ions have been identified in lower organisms. Most of these are thought to be mono-specific. They include the Fe^{2+} transporters feoB from *E. coli* (Kammler *et al.* 1993), FET4 from yeast (Dix *et al.* 1994), the plant IRT1 (Eide *et al.* 1996), and the Fe^{3+} transporter FTR1 from yeast (Stearman *et al.* 1996). The first Zn^{2+} transporters ZRT1 and 2 were isolated from yeast (Zhao & Eide, 1996*a*,*b*), and a Zn^{2+}-translocating P-type ATPase in *E. coli* has been identified (Rensing *et al.* 1997), which was recently reported to function as a Pb^{2+}-transporting P-type ATPase (Rensing *et al.* 1998). A manganese transporter belonging to the ABC

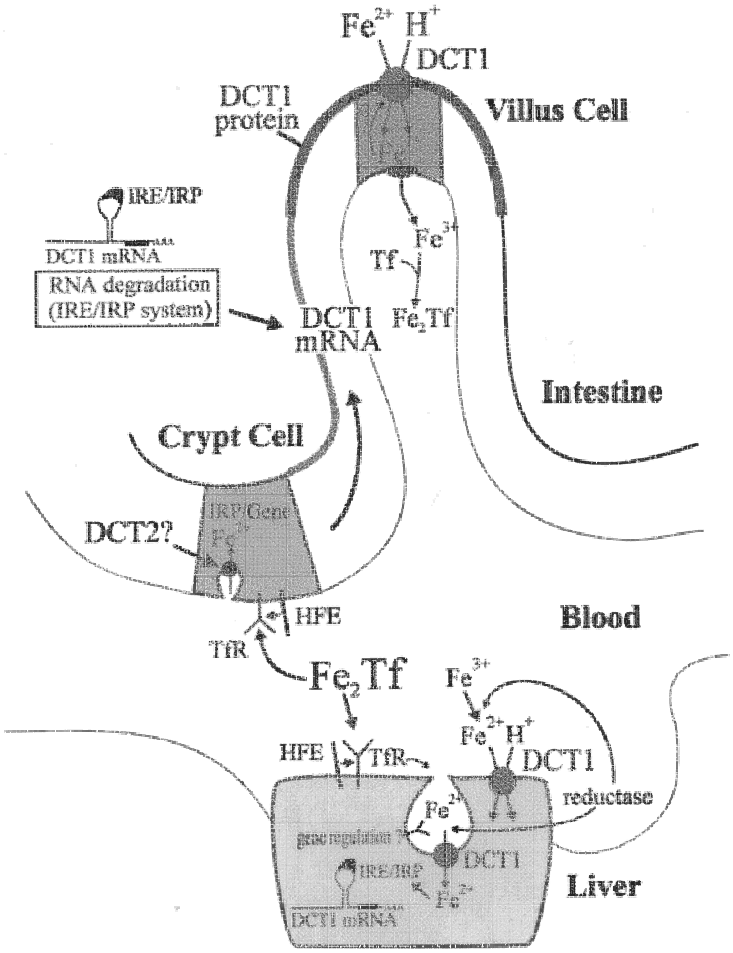

Figure 2. Hypothetical model of iron sensing and uptake in the intestine and liver

Two Fe^{3+} bound to Tf (Fe_2TF) are taken up via the Tf–TfR cycle in the intestinal crypt cells as well as in other cells of the body such as hepatocytes. Intact HFE and TfR are required in crypt cells for iron absorption from the blood, thereby serving as a sensor of body iron. We hypothesize that the amount of iron absorbed in crypt cells determines the stability of DCT1 mRNA along the crypt-to-villus axis, and thereby the amount of DCT1 protein expressed in mature villus cells. A similar regulatory mechanism might exist for other proteins involved in intestinal iron absorption. In patients with atransferrinaemia or haemochromatosis this sensing is predicted to be disturbed, leading to increased iron uptake.

transporter superfamily has been found in a cyanobacterial mutant strain (Sambongi *et al.* 1997). The Zn^{2+} transporters ZIP1 to 4 were identified in *Arabidopsis* (Grotz *et al.* 1998). SMF1 to 3 are yeast homologues of the mammalian Nramp proteins, and recent studies indicated that SMF1 is expressed in the yeast plasma membrane where it mediates Zn^{2+} and Mn^{2+} uptake (Supek *et al.* 1996). Studies in our laboratory indicate that SMF1 isoforms expressed in *Xenopus* oocytes mediate Fe^{2+} transport (X.-Z. Chen & M. A. Hediger, unpublished observations).

Recently, a human gene involved in high-affinity cellular uptake of copper (CTR-1) has been identified by complementation in yeast (Zhou & Gitschier, 1997). In yeast, three copper uptake proteins are described, CTR-1, CTR-2, and CTR-3. Whereas CTR-1 and CTR-3 are high-affinity copper transporters, CTR-2 is thought to be a low-affinity transporter (Askwith & Kaplan, 1998; Eide, 1998). Using CTR-1- and -3-deficient yeast, a mammalian cDNA library was screened for clones that restore copper uptake into these cells. Using this functional complementation approach, human CTR-1, which showed a low level of homology to yeast CTR-1, was isolated. The amino acid sequence of hCTR-1 predicts three transmembrane domains, analogous to the yeast CTR-1. The extracellular N-terminus has several histidine, serine and methionine residues, which are thought to be important for binding of copper ions in bacterial copper ATPases. A functional analysis of hCTR-1 has not yet been reported. The human CTR-1 sequence has been used for homology screening, giving rise to a putative second human copper transporter, hCTR-2. Its cDNA codes for a similar putative transporter, with pronounced differences in the N-terminus (Zhou & Gitschier, 1997). Clearly, more studies are required to establish the function, distribution and physiological relevance of the CTR transporters in human. The structure of these transporters is of particular interest since they only have three transmembrane domains.

There are a number of metal ion transporters identified in yeast, plant and bacteria, for which mammalian homologues have not yet been identified (Paulsen & Saier, 1997; Eng *et al.* 1998; Grotz *et al.* 1998). Thus, much work will be required to fully elucidate the molecular identity and properties of these metal ion transporters.

Metal ion export transporters

Several divalent cation export systems have been reported recently. These include the Zn^{2+} export systems ZnT-1 to ZnT-4 (Palmiter & Findley, 1995; Huang & Gitschier, 1997; Palmiter *et al.* 1996*a*,*b*; review articles: Huang, 1997; McMahon & Cousins, 1998), and two highly homologous P-type copper ATPase exporters, the Menkes (MNK) and Wilson's (WND) disease proteins (Mercer *et al.* 1993; Tanzi *et al.* 1993; Vulpe *et al.* 1993; Petrukhin *et al.* 1994). With respect to the ZnT family members, a naturally occurring gene defect of ZnT-4 in mouse has been reported, leading to the phenotype called 'lethal milk' (Huang & Gitschier, 1997). Targeted gene disruption of ZnT-3 did not lead to a specific phenotype. This was of great interest, since the protein is localized on synaptic vesicles of zinc-containing neurons in the hippocampus of wild-type animals, and is thereby associated with zinc transport into these vesicles (Wenzel *et al.* 1997; Cole *et al.* 1999).

The Menkes disease protein (MNK) is a P-type ATPase Cu^{2+} transporter. The MNK cDNA was the first mammalian heavy metal ion transporter cDNA to be cloned. In patients suffering from Menkes disease, the transport of Cu^{2+} is defective, leading to damage of certain tissues, neurodegeneration, and to death in early childhood. The cloning of the Menkes disease gene (*MNK*) was performed in part by positional cloning (Mercer *et al.* 1993; Chelly *et al.* 1993) and exon trapping (Vulpe *et al.* 1993). The cDNA (8·5 kb) predicts a protein of 1500 amino acids with eight transmembrane domains, six putative copper binding sites within a 600 amino acid N-terminal domain, an ATP-binding motif, a phosphatase domain, and several invariant amino acid residues in the proposed cation transduction channel (Vulpe & Packman, 1995; Lutsenko *et al.* 1997). Mutations in the *MNK* gene which cause Menkes disease give rise to accumulation of copper in cell cultures, and lead to a deficiency of enzymes that need copper for their activity. In patients, Cu^{2+} accumulates predominantly in the intestine and kidney, due to (re)absorption of Cu^{2+} in these organs. MNK is expressed in all tissues except the liver, where its function is probably mediated by the Wilson protein.

The Wilson's disease protein (WND) was thought to be related to a defect in the blood Cu^{2+} transport protein ceruloplasmin, since in this disease the amount of ceruloplasmin and its Cu^{2+} content are reduced. The clinical picture of Wilson's disease is hepatic cirrhosis and neuronal degradation in early childhood, due to the lack of Cu^{2+} release from the bile, and Cu^{2+} overload of hepatic cells. However, cloning of the *WND* gene showed that it encodes a Cu^{2+}-ATPase consisting of 1465 amino acids, found to be expressed in liver and kidney (Tanzi *et al.* 1993). The sequence predicts eight transmembrane domains, six Cu^{2+} binding domains, and the characteristic P-type ATPase features. *WND* was cloned in part by positional cloning, and in part by its homology to *MNK* (54% homology of the proteins). Experiments with hepatic cell lines revealed only low expression of WND on the plasma membrane. Most of its expression was found in the trans-Golgi network (Nagano *et al.* 1998), together with ceruloplasmin. This might indicate that Cu^{2+} is not released as a free ion into the blood, but is bound to ceruloplasmin, a copper transport protein thought to be secreted in the bile only after complete Cu^{2+} loading (Chowrimootoo *et al.* 1996). WND localized in the trans-Golgi network is thought to migrate close to the plasma membrane in a copper-dependent manner. Such a copper-dependent re-localization was also described for the MNK protein (Petris *et al.* 1996, 1998). Cu^{2+}, but not Zn^{2+}, Fe^{2+}, Cd^{2+} or Co^{2+}, was able to stimulate this migration of WND from the Golgi network to the plasma membrane (Hung *et*

al. 1997). This specific effect of copper on the relocalization of WND is interesting, since zinc acetate is very efficient for the treatment of patients suffering from Wilson's disease, with minimal side effects (Brewer *et al.* 1994; Anderson *et al.* 1998). Other therapeutic strategies involve the use of Cu^{2+} chelating agents (*d*-penicillamine and trientine). However, these components show significant side effects. A detailed description of Cu^{2+}-ATPases, their pathophysiological impacts, and their interaction with copper chaperons are presented elsewhere in excellent recent articles (Vulpe & Packman, 1995; DiDonato & Sarkar, 1997; Pufahl *et al.* 1997; Askwith & Kaplan, 1998).

Structure of human metal ion transporters

The diversity of the predicted structures of metal ion transporters from different transporter families is striking. Table 1 summarizes these differences and Fig. 3 shows topology models of selected transporters. Except for the DCT1/NRAMP2 family, metal transporters do not comply with the 12 transmembrane domain (TMs) dogma of transport proteins. The MNK and WND proteins have eight putative TMs consistent with the structure of other ATP-driven transporters. Interestingly, the ZnTs and the SFT have only six TMs and the CTRs only three TMs. The low number of TMs in the CTRs gives rise to the question of

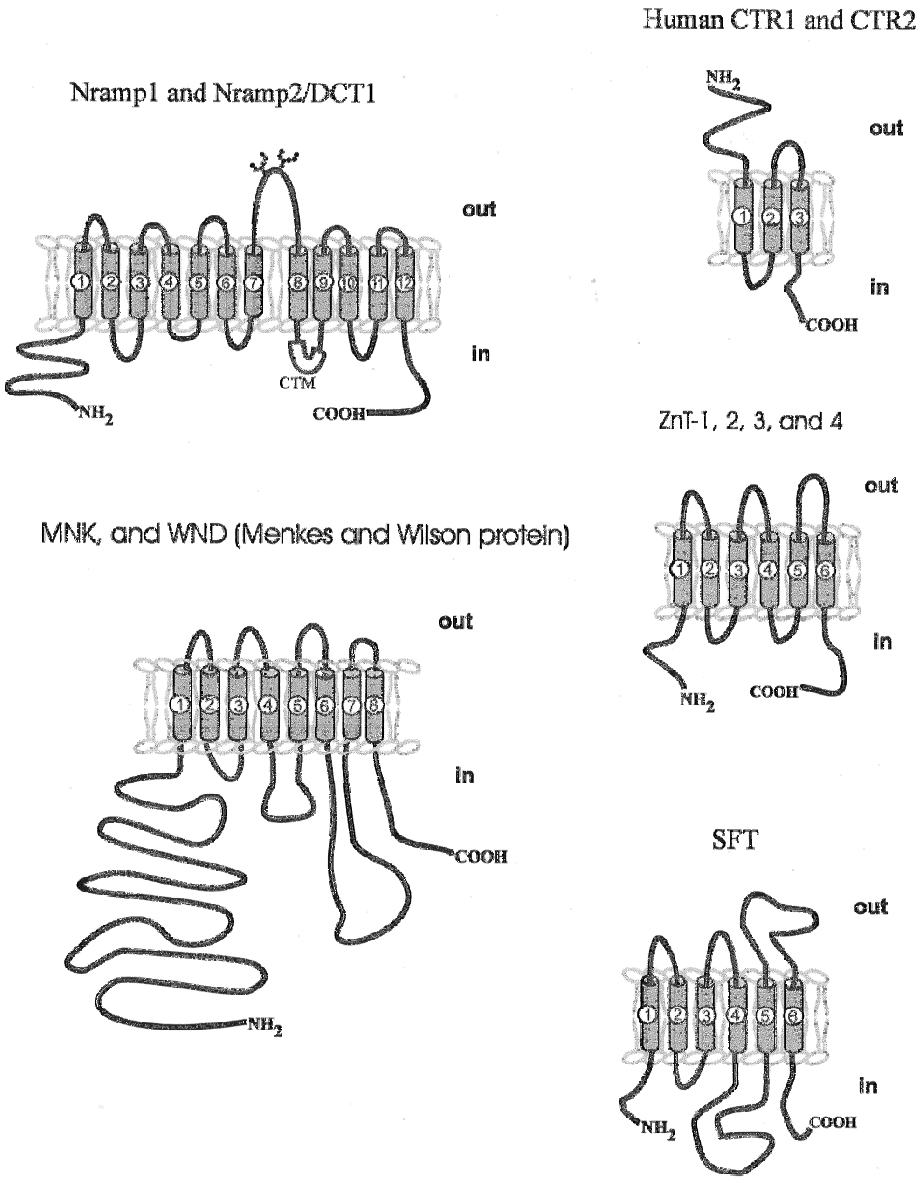

Figure 3. Topology models of selected metal ion transporters

Predicted topology models of transporters mediating metal ion uptake (DCT1/Nramp2: Gunshin *et al.* 1997; CTRs: Zhou & Gitschier, 1997) and export (ZnTs: Palmiter & Findley, 1995; Palmiter *et al.* 1996*a*,*b*; Huang & Gitschier, 1997), WND/MNK (Tanzi *et al.* 1993; Mercer *et al.* 1993; Vulpe *et al.* 1993; Chelly *et al.* 1993; Petrukhin *et al.* 1994) and the iron transporter stimulator SFT (Gutierrez *et al.* 1997).

Table 1. Mammalian metal ion transporters

Name	Metal transported	Function	TM	Tissue distribution	Disease	Reference(s)
DCT1/ Nramp2	Fe^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Cd^{2+}, Pb^{2+}	Uptake/ endosomal exit	12	Widespread (intestine, kidney, liver, neurons, etc.)	Haemochromatosis[A], microcytic anaemia[G]	Gruenheid *et al.* 1995; Vidal *et al.* 1995*a*; Gunshin *et al.* 1997
Nramp1	Fe^{2+}, Mn^{2+}, other?	Phagosomal/ lysosomal exit	12	Macrophages	Infectious susceptibility[G]	Vidal *et al.* 1995*b*; Gunshin *et al.* 1997
SFT	Fe^{2+}/Fe^{3+}	Uptake	6	Ubiquitous	—	Gutierrez *et al.* 1997, 1998
Frataxin/FRDA	Fe^{2+}/Fe^{3+}	Mitochondrial export	n.d.	Neuronal	Friedreich's ataxia[G]	Babcock *et al.* 1997; Koutnikova *et al.* 1997; Wilson & Roof, 1997; Radisky *et al.* 1999
hCTR1	Cu^{2+}	Uptake (yeast)	3	Ubiquitous	—	Zhou & Gitschier, 1997
hCTR2	Cu^{2+}	n.d.	3	Ubiquitous	—	Zhou & Gitschier, 1997
ZnT-1	Zn^{2+}	Basolateral exit	6	Ubiquitous (intestine, kidney)	—	Palmiter & Findley, 1995
ZnT-2	Zn^{2+}	Vacuolar exit	6	n.d.	—	Palmiter *et al.* 1996*a*
ZnT-3	Zn^{2+}	Synaptic vesicles	6	Brain, neurons	—	Palmiter *et al.* 1996*b*
ZnT-4	Zn^{2+}	Export/lactation	6	Mammary gland	Lethal milk[G]	Huang & Gitschier, 1997
MNK	Cu^{2+}	Basolateral exit (intestine)	8	Ubiquitous, except liver	Menkes disease[G]	Mercer *et al.* 1993; Vulpe *et al.* 1993; Chelly *et al.* 1993
WND	Cu^{2+}	Exit, biliary excretion	8	Liver, kidney	Wilson's disease[G]	Tanzi *et al.* 1993; Petrukhin *et al.* 1994

TM, transmembrane domain; A, acquired; G, genetic; FRDA, Friedreich's ataxia; n.d., not determined.

whether these transporters function as homo- or hetero-multimers, since a single protein would probably not be able to form a pore complex sufficient for the translocation of the metal ion.

What are the pharmacological opportunities in the metal ion transporter field?

Treatment of disturbances in metal ion homeostasis often involves supplementation of the missing metal ion, or metal ion chelation. In the case of Cu^{2+} overload in Wilson's disease, treatment with zinc is very effective in preventing symptoms (Anderson *et al.* 1998; Brewer *et al.* 1994). In hereditary haemochromatosis, weekly phlebotomy is the usual treatment in early diagnosis (Barton *et al.* 1998). Blocking of DCT1 in the intestine by suitable pharmaceutical substances might offer an alternative treatment, but the impact on the uptake of other metal ions needs to be considered. An important consideration for dietary metal supplementation is that iron supplementation will down-regulate DCT1. Furthermore, competition between iron and other divalent metal ions for a single absorptive site can be expected when using multiple trace element supplementation. Nramp1 dysfunction leads to failure to protect against pathogen resistance (Bellamy *et al.* 1998; Skamene *et al.* 1998). In addition, an implication of Nramp1 in rheumatoid arthritis has been discussed (Govoni & Gros, 1998). Whether Nramp1 can be used as a therapeutic target in certain diseases needs to be evaluated.

At glutamatergic synapses, glutamate is co-released with zinc which is thought to interact with NMDA receptors, possibly modulating synaptic transmission (Huang, 1997). Although a gene knock-out in mice of ZnT-3, which transports Zn^{2+} into synaptic vesicles, was shown to give no specific phenotype (Cole *et al.* 1999), modulation of Zn^{2+} transport might lead to subtle changes of the transmission process. Thus, the zinc transporter in neurons may be a potential target for modulation of glutamatergic transmission. Whether DCT1 is involved in re-uptake of released Zn^{2+} remains to be determined.

Metal ion transporters might also contribute to inappropriate accumulation of metal ions in affected neurons in the substantia nigra in patients with Parkinson's disease (Jellinger *et al.* 1993; Gerlach *et al.* 1994; Hirsch, 1994; Hirsch & Faucheux, 1998). The dysfunction of metal ion transport may be involved in other neurodegenerative diseases (Gerlach *et al.* 1994; Multhaup *et al.* 1997;

Atwood *et al.* 1998) opening a variety of pharmacological opportunities in the metal ion transporter field.

Anderson, G. J., Murphy, T. L., Cowley, L., Evans, B. A., Halliday, J. W. & McLaren, G. D. (1998). Mapping the gene for sex-linked anemia – An inherited defect of intestinal iron absorption in the mouse. *Genomics* **48**, 34–39.

Anderson, G. J., Powell, L. W. & Halliday, J. W. (1994). The endocytosis of transferrin by rat intestinal epithelial cells. *Gastroenterology* **106**, 414–422.

Anderson, L. A., Hakojarvi, S. L. & Boudreaux, S. K. (1998). Zinc acetate treatment in Wilson's disease. *Annals of Pharmacotherapy* **32**, 78–87.

Askwith, C. C. & Kaplan, J. (1998). Iron and copper transport in yeast and its relevance to human disease. *Trends in Biochemical Sciences* **23**, 135–138.

Atkinson, P. P. & Barton, C. H. (1998). Ectopic expression of NRAMP1 in COS-1 cells modulates iron accumulation. *FEBS Letters* **425**, 239–242.

Atwood, C. S., Moir, R. D., Huang, X., Scarpa, R. C., Bacarra, N. M., Romano, D. M., Hartshorn, M. A., Tanzi, R. E. & Bush, A. I. (1998). Dramatic aggregation of Alzheimer abeta by Cu(II) is induced by conditions representing physiological acidosis. *Journal of Biological Chemistry* **273**, 12817–12826.

Babcock, M., de Silva, D., Oaks, R., Davis-Kaplan, S., Jiralerspong, S., Montermini, L., Pandolfo, M. & Kaplan, J. (1997). Regulation of mitochondrial iron accumulation by Yfh1p, a putative homolog of frataxin. *Science* **276**, 1709–1712.

Barton, J. C., McDonnell, S. M., Adams, P. C., Brissot, P., Powell, L. W., Edwards, C. Q., Cook, J. D. & Kowdley, K. V. (1998). Management of hemochromatosis. Hemochromatosis Management Working Group. *Annals of Internal Medicine* **129**, 932–939.

Bellamy, R., Ruwende, C., Corrah, T., McAdam, K. P., Whittle, H. C. & Hill, A. V. (1998). Variations in the NRAMP1 gene and susceptibility to tuberculosis in West Africans. *New England Journal of Medicine* **338**, 640–644.

Brewer, G. J., Dick, R. D., Yuzbasiyan-Gurkan, V., Johnson, V. & Wang, Y. (1994). Treatment of Wilson's disease with zinc. XIII: Therapy with zinc in presymptomatic patients from the time of diagnosis. *Journal of Laboratory and Clinical Medicine* **123**, 849–858.

Buys, S. S., Martin, C. B., Eldridge, M., Kushner, J. P. & Kaplan, J. (1991). Iron absorption in hypotransferrinemic mice. *Blood* **78**, 3288–3290.

Campuzano, V., Montermini, L., Molto, M. D., Pianese, L., Cossee, M., Cavalcanti, F., Monros, E., Rodius, F., Duclos, F. & Monticelli, A. (1996). Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. *Science* **271**, 1423–1427.

Chelly, J., Tumer, Z., Tonnesen, T., Petterson, A., Ishikawa-Brush, Y., Tommerup, N., Horn, N. & Monaco, A. P. (1993). Isolation of a candidate gene for Menkes disease that encodes a potential heavy metal binding protein. *Nature Genetics* **3**, 14–19.

Chowrimootoo, G. F., Ahmed, H. A. & Seymour, C. A. (1996). New insights into the pathogenesis of copper toxicosis in Wilson's disease: evidence for copper incorporation and defective canalicular transport of caeruloplasmin. *Biochemical Journal* **315**, 851–855.

Chua, A. C. & Morgan, E. H. (1997). Manganese metabolism is impaired in the Belgrade laboratory rat. *Journal of Comparative Physiology* B **167**, 361–369.

Chua, A. C., Stonell, L. M., Savigni, D. L. & Morgan, E. H. (1996). Mechanisms of manganese transport in rabbit erythroid cells. *Journal of Physiology* **493**, 99–112.

Cole, T. B., Wenzel, H. J., Kafer, K. E., Schwartzkroin, P. A. & Palmiter, R. D. (1999). Elimination of zinc from synaptic vesicles in the intact mouse brain by disruption of the ZnT3 gene. *Proceedings of the National Academy of Sciences of the USA* **96**, 1716–1721.

Csere, P., Lill, R. & Kispal, G. (1998). Identification of a human mitochondrial ABC transporter, the functional orthologue of yeast Atm1p. *FEBS Letters* **441**, 266–270.

Dancis, A. (1998). Genetic analysis of iron uptake in the yeast *Saccharomyces cerevisiae*. *Journal of Pediatrics* **132**, S24–S29.

Dautry-Varsat, A. (1986). Receptor-mediated endocytosis: the intracellular journey of transferrin and its receptor. *Biochimie* **68**, 375–381.

DiDonato, M. & Sarkar, B. (1997). Copper transport and its alterations in Menkes and Wilson diseases. *Biochimica et Biophysica Acta* **1360**, 3–16.

Dix, D. R., Bridgham, J. T., Broderius, M. A., Byersdorfer, C. A. & Eide, D. J. (1994). The FET4 gene encodes the low affinity Fe(II) transport protein of *Saccharomyces cerevisiae*. *Journal of Biological Chemistry* **269**, 26092–26099.

Dorey, C., Cooper, C., Dickson, D. P., Gibson, J. F., Simpson, R. J. & Peters, T. J. (1993). Iron speciation at physiological pH in media containing ascorbate and oxygen. *British Journal of Nutrition* **70**, 157–169.

Eastham, E. J., Bell, J. I. & Douglas, A. P. (1977). Iron-transport characteristics of vesicles of brush-border and basolateral plasma membrane from the rat enterocyte. *Biochemical Journal* **164**, 289–294.

Eide, D. J. (1998). The molecular biology of metal ion transport in *Saccharomyces cerevisiae*. *Annual Review of Nutrition* **18**, 441–469.

Eide, D., Broderius, M., Fett, J. & Guerinot, M. L. (1996). A novel iron-regulated metal transporter from plants identified by functional expression in yeast. *Proceedings of the National Academy of Sciences of the USA* **93**, 5624–5628.

Eng, B. H., Guerinot, M. L., Eide, D. & Saier, M. H. J. (1998). Sequence analyses and phylogenetic characterization of the ZIP family of metal ion transport proteins. *Journal of Membrane Biology* **166**, 1–7.

Feder, J. N., Penny, D. M., Irrinki, A., Lee, V. K., Lebron, J. A., Watson, N., Tsuchihashi, Z., Sigal, E., Bjorkman, P. J. & Schatzman, R. C. (1998). The hemochromatosis gene product complexes with the transferrin receptor and lowers its affinity for ligand binding. *Proceedings of the National Academy of Sciences of the USA* **95**, 1472–1477.

Fleming, M. D., Romano, M. A., Su, M. A., Garrick, L. M., Garrick, M. D. & Andrews, N. C. (1998). Nramp2 is mutated in the anemic belgrade (b) rat: evidence of a role for nramp2 in endosomal iron transport. *Proceedings of the National Academy of Sciences of the USA* **95**, 1148–1153.

Fleming, M. D., Trenor, C. C., Su, M. A., Foernzler, D., Beier, D. R., Dietrich, W. F. & Andrews, N. C. (1997). Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. *Nature Genetics* **16**, 383–386.

Fleming, R. E., Migas, M. C., Zhou, X., Jiang, J., Britton, R. S., Brunt, E. M., Tomatsu, S., Waheed, A., Bacon, B. R. & Sly, W. S. (1999). Mechanism of increased iron absorption in murine model of hereditary hemochromatosis: Increased duodenal expression of the iron transporter DMT1. *Proceedings of the National Academy of Sciences of the USA* **96**, 3143–3148.

Gerlach, M., Ben-Shachar, D., Riederer, P. & Youdim, M. B. (1994). Altered brain metabolism of iron as a cause of neurodegenerative diseases? *Journal of Neurochemistry* **63**, 793–807.

Govoni, G. & Gros, P. (1998). Macrophage NRAMP1 and its role in resistance to microbial infections. *Inflammatory Research* **47**, 277–284.

Green, R., Charlton, R., Seftel, H., Bothwell, T., Mayet, F., Adams, B., Finch, C. & Layrisse, M. (1968). Body iron excretion in man: a collaborative study. *American Journal of Medicine* **45**, 336–353.

Gross, C. N., Irrinki, A., Feder, J. N. & Enns, C. A. (1998). Co-trafficking of HFE, a nonclassical major histocompatibility complex class I protein, with the transferrin receptor implies a role in intracellular iron regulation. *Journal of Biological Chemistry* **273**, 22068–22074.

Grotz, N., Fox, T., Connolly, E., Park, W., Guerinot, M. L. & Eide, D. (1998). Identification of a family of zinc transporter genes from *Arabidopsis* that respond to zinc deficiency. *Proceedings of the National Academy of Sciences of the USA* **95**, 7220–7224.

Gruenheid, S., Canonne-Hergaux, F., Gauthier, S., Hackam, D. J., Grinstein, S. & Gros, P. (1999). The iron transport protein NRAMP2 is an integral membrane glycoprotein that colocalizes with transferrin in recycling endosomes. *Journal of Experimental Medicine* **189**, 831–841.

Gruenheid, S., Cellier, M., Vidal, S. & Gros, P. (1995). Identification and characterization of a second mouse Nramp gene. *Genomics* **25**, 514–525.

Gruenheid, S., Pinner, E., Desjardins, M. & Gros, P. (1997). Natural resistance to infection with intracellular pathogens: the Nramp1 protein is recruited to the membrane of the phagosome. *Journal of Experimental Medicine* **185**, 717–730.

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

Gutierrez, J. A., Yu, J. & Wessling-Resnick, M. (1998). Characterization and chromosomal mapping of the human gene for SFT, a stimulator of Fe transport. *Biochemical and Biophysical Research Communications* **253**, 739–742.

Gutierrez, J. A., Yu, J. M., Rivera, S. & Wessling-Resnick, M. (1997). Functional expression cloning and characterization of SFT, a stimulator of Fe transport. *Journal of Cellular Biology* **139**, 895–905.

Han, O., Failla, M. L., Hill, A. D., Morris, E. R. & Smith, J. C. J. (1995). Reduction of Fe(III) is required for uptake of nonheme iron by Caco-2 cells. *Journal of Nutrition* **125**, 1291–1299.

Harford, J. B. (1994). Cellular iron homeostasis: a paradigm for mechanisms of posttranscriptional control of gene expression. *Progress in Liver Disease* **12**, 47–62.

Hartman, K. R. & Barker, J. A. (1996). Microcytic anemia with iron malabsorption: an inherited disorder of iron metabolism. *American Journal of Hematology* **51**, 269–275.

Henle, E. S. & Linn, S. (1997). Formation, prevention, and repair of DNA damage by iron/hydrogen peroxide. *Journal of Biological Chemistry* **272**, 19095–19098.

Hirsch, E. C. (1994). Biochemistry of Parkinson's disease with special reference to the dopaminergic systems. *Molecular Neurobiology* **9**, 135–142.

Hirsch, E. C. & Faucheux, B. A. (1998). Iron metabolism and Parkinson's disease. *Movement Disorders* **13**, 39–45.

Huang, E. P. (1997). Metal ions and synaptic transmission: think zinc. *Proceedings of the National Academy of Sciences of the USA* **94**, 13386–13387.

Huang, L. & Gitschier, J. (1997). A novel gene involved in zinc transport is deficient in the lethal milk mouse. *Nature Genetics* **17**, 292–297.

Hung, I. H., Suzuki, M., Yamaguchi, Y., Yuan, D. S., Klausner, R. D. & Gitlin, J. D. (1997). Biochemical characterization of the Wilson disease protein and functional expression in the yeast *Saccharomyces cerevisiae*. *Journal of Biological Chemistry* **272**, 21461–21466.

Iancu, T. C., Shiloh, H., Raja, K. B., Simpson, R. J., Peters, T. J., Perl, D. P., Hsu, A. & Good, P. F. (1995). The hypotransferrinaemic mouse: ultrastructural and laser microprobe analysis observations. *Journal of Pathology* **177**, 83–94.

Inman, R. S., Coughlan, M. M. & Wessling-Resnick, M. (1994). Extracellular ferrireductase activity of K562 cells is coupled to transferrin-independent iron transport. *Biochemistry* **33**, 11850–11857.

Jellinger, K. A., Kienzl, E., Rumpelmaier, G., Paulus, W., Riederer, P., Stachelberger, H., Youdim, M. B. & Ben-Shachar, D. (1993). Iron and ferritin in substantia nigra in Parkinson's disease. *Advances in Neurology* **60**, 267–272.

Jordan, I. & Kaplan, J. (1994). The mammalian transferrin-independent iron transport system may involve a surface ferrireductase activity. *Biochemical Journal* **302**, 875–879.

Kammler, M., Schon, C. & Hantke, K. (1993). Characterization of the ferrous iron uptake system of *Escherichia coli*. *Journal of Bacteriology* **175**, 6212–6219.

Kispal, G., Csere, P., Guiard, B. & Lill, R. (1997). The ABC transporter Atm1p is required for mitochondrial iron homeostasis. *FEBS Letters* **418**, 346–350.

Koutnikova, H., Campuzano, V., Foury, F., Dolle, P., Cazzalini, O. & Koenig, M. (1997). Studies of human, mouse and yeast homologues indicate a mitochondrial function for frataxin. *Nature Genetics* **16**, 345–351.

Levy, J. E., Jin, O., Fujiwara, Y., Kuo, F. & Andrews, N. C. (1999). Transferrin receptor is necessary for development of erythrocytes and the nervous system. *Nature Genetics* **21**, 396–399.

Lutsenko, S., Petrukhin, K., Cooper, M. J., Gilliam, C. T. & Kaplan, J. H. (1997). N-terminal domains of human copper-transporting adenosine triphosphatases (the Wilson's and Menkes disease proteins) bind copper selectively *in vivo* and *in vitro* with stoichiometry of one copper per metal-binding repeat. *Journal of Biological Chemistry* **272**, 18939–18944.

McKie, A. T., Wehr, K., Simpson, R. J., Peters, T. J., Hentze, M. W. & Farzaneh, F. (1998). Molecular cloning and characterisation of a novel duodenal-specific gene implicated in iron absorption. *Biochemical Society Transactions* **26**, S264.

McMahon, R. J. & Cousins, R. J. (1998). Mammalian zinc transporters. *Journal of Nutrition* **128**, 667–670.

Mercer, J. F., Livingston, J., Hall, B., Paynter, J. A., Begy, C., Chandrasekharappa, S., Lockhart, P., Grimes, A., Bhave, M. & Siemieniak, D. (1993). Isolation of a partial candidate gene for Menkes disease by positional cloning. *Nature Genetics* **3**, 20–25.

Multhaup, G., Ruppert, T., Schlicksupp, A., Hesse, L., Beher, D., Masters, C. L. & Beyreuther, K. (1997). Reactive oxygen species and Alzheimer's disease. *Biochemical Pharmacology* **54**, 533–539.

Nagano, K., Nakamura, K., Urakami, K. I., Umeyama, K., Uchiyama, H., Koiwai, K., Hattori, S., Yamamoto, T., Matsuda, I. & Endo, F. (1998). Intracellular distribution of the Wilson's disease gene product (ATPase7B) after *in vitro* and *in vivo* exogenous expression in hepatocytes from the LEC rat, an animal model of Wilson's disease. *Hepatology* **27**, 799–807.

O'Riordan, D. K., Debnam, E. S., Sharp, P. A., Simpson, R. J., Taylor, E. M. & Srai, S. S. (1997). Mechanisms involved in increased iron uptake across rat duodenal brush-border membrane during hypoxia. *Journal of Physiology* **500**, 379–384.

Palmiter, R. D., Cole, T. B. & Findley, S. D. (1996*a*). ZnT-2, a mammalian protein that confers resistance to zinc by facilitating vesicular sequestration. *EMBO Journal* **15**, 1784–1791.

Palmiter, R. D., Cole, T. B., Quaife, C. J. & Findley, S. D. (1996*b*). ZnT-3, a putative transporter of zinc into synaptic vesicles. *Proceedings of the National Academy of Sciences of the USA* **93**, 14934–14939.

Palmiter, R. D. & Findley, S. D. (1995). Cloning and functional characterization of a mammalian zinc transporter that confers resistance to zinc. *EMBO Journal* **14**, 639–649.

Parkkila, S., Waheed, A., Britton, R. S., Bacon, B. R., Zhou, X. Y., Tomatsu, S., Fleming, R. E. & Sly, W. S. (1997). Association of the transferrin receptor in human placenta with HFE, the protein defective in hereditary hemochromatosis. *Proceedings of the National Academy of Sciences of the USA* **94**, 13198–13202.

Paulsen, I. T. & Saier, M. H. Jr (1997). A novel family of ubiquitous heavy metal ion transport proteins. *Journal of Membrane Biology* **156**, 99–103.

Petris, M. J., Camakaris, J., Greenough, M., Lafontaine, S. & Mercer, J. B. (1998). A C-terminal di-leucine is required for localization of the Menkes protein in the trans-Golgi network. *Human Molecular Genetics* **7**, 2063–2071.

Petris, M. J., Mercer, J. F., Culvenor, J. G., Lockhart, P., Gleeson, P. A. & Camakaris, J. (1996). Ligand-regulated transport of the Menkes copper P-type ATPase efflux pump from the Golgi apparatus to the plasma membrane: a novel mechanism of regulated trafficking. *EMBO Journal* **15**, 6084–6095.

Petrukhin, K., Lutsenko, S., Chernov, I., Ross, B. M., Kaplan, J. H. & Gilliam, T. C. (1994). Characterization of the Wilson disease gene encoding a P-type copper transporting ATPase: genomic organization, alternative splicing, and structure/function predictions. *Human Molecular Genetics* **3**, 1647–1656.

Pufahl, R. A., Singer, C. P., Peariso, K. L., Lin, S. J., Schmidt, P. J., Fahrni, C. J., Culotta, V. C., Penner-Hahn, J. E. & O'Halloran, T. V. (1997). Metal ion chaperone function of the soluble Cu(I) receptor Atx1. *Science* **278**, 853–856.

Radisky, D. C., Babcock, M. C. & Kaplan, J. (1999). The yeast frataxin homologue mediates mitochondrial iron efflux. Evidence for a mitochondrial iron cycle. *Journal of Biological Chemistry* **274**, 4497–4499.

Raja, K. B., Simpson, R. J. & Peters, T. J. (1992). Investigation of a role for reduction in ferric iron uptake by mouse duodenum. *Biochimica et Biophysica Acta* **1135**, 141–146.

Rensing, C., Mitra, B. & Rosen, B. P. (1997). The zntA gene of *Escherichia coli* encodes a Zn(II)-translocating P-type ATPase. *Proceedings of the National Academy of Sciences of the USA* **94**, 14326–14331.

Rensing, C., Sun, Y., Mitra, B. & Rosen, B. P. (1998). Pb(II)-translocating P-type ATPases. *Journal of Biological Chemistry* **273**, 32614–32617.

Richardson, D. R. & Ponka, P. (1997). The molecular mechanisms of the metabolism and transport of iron in normal and neoplastic cells. *Biochimica et Biophysica Acta* **1331**, 1–40.

Robinson, N. J., Procter, C. M., Connolly, E. L. & Guerinot, M. L. (1999). A ferric-chelate reductase for iron uptake from soils. *Nature* **397**, 694–697.

Roy, C. N., Penny, D. M., Feder, J. N. & Enns, C. A. (1999). The hereditary hemochromatosis protein, HFE, specifically regulates transferrin-mediated iron uptake in HeLa cells. *Journal of Biological Chemistry* **274**, 9022–9028.

Sambongi, Y., Wakabayashi, T., Yoshimizu, T., Omote, H., Oka, T. & Futai, M. (1997). *Caenorhabditis elegans* cDNA for a Menkes/Wilson disease gene homologue and its function in a yeast CCC2 gene deletion mutant. *Journal of Biochemistry* **121**, 1169–1175.

Savigni, D. L. & Morgan, E. H. (1998). Transport mechanisms for iron and other transition metals in rat and rabbit erythroid cells. *Journal of Physiology* **508**, 837–850.

Simpson, R. J., Lombard, M., Raja, K. B., Thatcher, R. & Peters, T. J. (1991). Iron absorption by hypotransferrinaemic mice. *British Journal of Haematology* **78**, 565–570.

Skamene, E., Schurr, E. & Gros, P. (1998). Infection genomics: Nramp1 as a major determinant of natural resistance to intracellular infections. *Annual Review of Medicine* **49**, 275–287.

Stearman, R., Yuan, D. S., Yamaguchi-Iwai, Y., Klausner, R. D. & Dancis, A. (1996). A permease-oxidase complex involved in high-affinity iron uptake in yeast. *Science* **271**, 1552–1557.

Su, M. A., Trenor, C. C., Fleming, J. C., Fleming, M. D. & Andrews, N. C. (1998). The G185R mutation disrupts function of the iron transporter Nramp2. *Blood* **92**, 2157–2163.

Supek, F., Supekova, L., Nelson, H. & Nelson, N. (1996). A yeast manganese transporter related to the macrophage protein involved in conferring resistance to mycobacteria. *Proceedings of the National Academy of Sciences of the USA* **93**, 5105–5110.

Tanzi, R. E., Petrukhin, K., Chernov, I., Pellequer, J. L., Wasco, W., Ross, B., Romano, D. M., Parano, E., Pavone, L. & Brzustowicz, L. M. (1993). The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. *Nature Genetics* **5**, 344–350.

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. *Journal of Clinical Investigation* **86**, 2145–2153.

Umbreit, J. N., Conrad, M. E., Moore, E. G., Desai, M. P. & Turrens, J. (1996). Paraferritin: a protein complex with ferrireductase activity is associated with iron absorption in rats. *Biochemistry* **35**, 6460–6469.

van Eijk, H. G. & de Jong, G. (1992). The physiology of iron, transferrin, and ferritin. *Biological Trace Element Research* **35**, 13–24.

Vidal, S., Belouchi, A. M., Cellier, M., Beatty, B. & Gros, P. (1995*a*). Cloning and characterization of a second human NRAMP gene on chromosome 12q13. *Mammalian Genome* **6**, 224–230.

Vidal, S., Gros, P. & Skamene, E. (1995*b*). Natural resistance to infection with intracellular parasites: molecular genetics identifies Nramp1 as the Bcg/Ity/Lsh locus. *Journal of Leukocyte Biology* **58**, 382–390.

Vidal, S. M., Malo, D., Vogan, K., Skamene, E. & Gros, P. (1993). Natural resistance to infection with intracellular parasites: isolation of a candidate for Bcg. *Cell* **73**, 469–485.

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. *Nature Genetics* **3**, 7–13.

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

Vulpe, C. D. & Packman, S. (1995). Cellular copper transport. *Annual Review of Nutrition* **15**, 293–322.

Waheed, A., Parkkila, S., Saarnio, J., Fleming, R. E., Zhou, X. Y., Tomatsu, S., Britton, R. S., Bacon, B. R. & Sly, W. S. (1999). Association of HFE protein with transferrin receptor in crypt enterocytes of human duodenum. *Proceedings of the National Academy of Sciences of the USA* **96**, 1579–1584.

Wenzel, H. J., Cole, T. B., Born, D. E., Schwartzkroin, P. A. & Palmiter, R. (1997). Ultrastructural localization of zinc transporter-3 (ZnT-3) to synaptic vesicle membranes within mossy fiber boutons in the hippocampus of mouse and monkey. *Proceedings of the National Academy of Sciences of the USA* **94**, 12676–12681.

Wien, E. M. & van Campen, D. R. (1991). Ferric iron absorption in rats: relationship to iron status, endogenous sulfhydryl and other redox components in the intestinal lumen. *Journal of Nutrition* **121**, 825–831.

Wilson, R. B. & Roof, D. M. (1997). Respiratory deficiency due to loss of mitochondrial DNA in yeast lacking the frataxin homologue. *Nature Genetics* **16**, 352–357.

Wood, R. J. & Han, O. (1998). Recently identified molecular aspects of intestinal iron absorption. *Journal of Nutrition* **128**, 1841–1844.

Yu, J. M. & Wessling-Resnick, M. (1998*a*). Influence of copper depletion on iron uptake mediated by SFT, a stimulator of Fe transport. *Journal of Biological Chemistry* **273**, 6909–6915.

Yu, J. & Wessling-Resnick, M. (1998*b*). Structural and functional analysis of SFT, a stimulator of Fe transport. *Journal of Biological Chemistry* **273**, 21380–21385.

Zhao, H. & Eide, D. (1996*a*). The yeast ZRT1 gene encodes the zinc transporter protein of a high-affinity uptake system induced by zinc limitation. *Proceedings of the National Academy of Sciences of the USA* **93**, 2454–2458.

Zhao, H. & Eide, D. (1996*b*). The ZRT2 gene encodes the low affinity zinc transporter in *Saccharomyces cerevisiae*. *Journal of Biological Chemistry* **271**, 23203–23210.

Zhou, B. & Gitschier, J. (1997). hCTR1 — A human gene for copper uptake identified by complementation in yeast. *Proceedings of the National Academy of Sciences of the USA* **94**, 7481–7486.

Zhou, X. Y., Tomatsu, S., Fleming, R. E., Parkkila, S., Waheed, A., Jiang, J., Fei, Y., Brunt, E. M., Ruddy, D. A., Prass, C. E., Schatzman, R. C., O'Neill, R., Britton, R. S., Bacon, B. R. & Sly, W. S. (1998). HFE gene knockout produces mouse model of hereditary hemochromatosis. *Proceedings of the National Academy of Sciences of the USA* **95**, 2492–2497.

Corresponding author

M. A. Hediger: Harvard Institutes of Medicine, Rm 570, 77 Avenue Louis Pasteur, Boston, MA 02115, USA.

Email: mhediger@rics.bwh.harvard.edu