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REVIEW

Mammalian iron transport

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Abstract Iron is essential for basic cellular processes but is toxic when present in excess. Consequently, iron transport into and out of cells is tightly regulated. Most iron is delivered to cells bound to plasma transferrin via a process that involves transferrin receptor 1, divalent metal-ion transporter 1 and several other proteins. Non-transferrin-bound iron can also be taken up efficiently by cells, although the mechanism is poorly understood. Cells can divest themselves of iron via the iron export protein ferroportin in conjunction with an iron oxidase. The linking of an oxidoreductase to a membrane permease is a common theme in membrane iron transport. At the systemic level, iron transport is regulated by the liver-derived peptide hepcidin which acts on ferroportin to control iron release to the plasma.

Keywords Iron transport · Transferrin · DMT1 · Ferroportin · Hepcidin · Heme transport · Iron oxidoreductase

Introduction

A large number of cellular enzymes depend on iron for their biological function and, consequently, this metal is essential for basic physiological processes. Iron can shuttle between two thermodynamically stable oxidation states, Fe^{3+} or ferric iron and Fe^{2+} or ferrous iron. This makes it ideally suited to the catalysis of biochemical reactions, but it also means that it is able to catalyze reactions leading to the production of toxic oxygen radicals, particularly when it is present in excess. Thus, individual cells and whole organisms must tightly regulate their iron influx and efflux to keep iron levels within the physiologically optimal range, providing sufficient for metabolic functions, while preventing the accumulation of potentially toxic excess iron. This review will focus on mechanisms of iron transport and its regulation in mammalian cells; there have been enormous advances in this area in recent years. There have been equally impressive advances in our knowledge of iron transport in lower eukaryotes and prokaryotes, but it is not possible to comprehensively cover these organisms in a limited space. The reader is referred to several excellent recent reviews for a coverage of these areas [1–3].

There are hundreds of proteins that require iron in the cell. Many of these are involved in enzyme catalysis and electron transport, whereas others are needed for oxygen transport and delivery. Major classes of such proteins include those with heme centers (hemoproteins) and those with iron–sulfur (Fe–S) clusters, but many proteins utilize the co-ordination chemistry of iron in quite different ways, and the list of non-heme, non-iron–sulfur proteins is growing steadily. Quantitatively, the most abundant iron-containing proteins in the body are the hemoproteins hemoglobin and myoglobin, which are produced by immature erythroid cells and myocytes, respectively [4]. These proteins carry the oxygen required for cellular respiration. Other hemoproteins, such as the cytochromes, catalases, and cytochrome oxidase, play central roles in basic cellular metabolism. Proteins containing Fe–S

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clusters play particularly important roles in electron transport and catalysis [5, 6], while proteins with non-heme, non-Fe–S centres include such important enzymes as the lipoxygenases, the amino acid hydroxylases, and the rate limiting enzyme in DNA synthesis, ribonucleotide reductase. Insights into the biology of iron containing proteins can be found in many places (e.g., [7, 8]).

Iron that is taken up by cells and not required immediately for metabolic functions is stored within ferritin [9, 10]. Ferritin consists of a roughly spherical protein shell made up of two types of subunits (H and L) surrounding a central cavity. Iron enters the cavity through pores in the shell and is deposited as ferrihydrite internally. Up to 4,500 atoms of iron can be stored in this way. If cells become heavily iron loaded, another form of storage iron known as hemosiderin may appear [10, 11]. Haemosiderin is derived from the degradation of ferritin aggregates and consists of iron oxide clusters and degraded protein. Both ferritin and hemosiderin iron can be mobilized from cells if the body demand for iron increases.

This review will concentrate on the transport proteins themselves and how they are regulated. A summary of some of the main proteins involved in iron transport is provided in Table 1, and each of these will be considered in more detail below.

Cellular iron uptake

Under normal physiological conditions, mammalian cells acquire most of their iron from the plasma protein transferrin (Tf). Each Tf molecule is able to bind two atoms of ferric iron, and it is this diferric Tf that is most efficiently utilized by cells [12, 13]. However, in certain pathological states, notably iron loading disorders, when the capacity of plasma Tf to bind iron is exceeded, non-transferrin-bound iron (NTBI) can make a major contribution to cellular iron uptake [14, 15]. Iron from circulating heme, hemoglobin and ferritin can also be utilized by some cells, but, like NTBI, these pathways only become significant under pathological conditions.

Transferrin-bound iron uptake

Transferrin is able to deliver its iron to cells via a number of mechanisms, but the high affinity binding of the protein to the plasma membrane protein transferrin receptor 1 (TfR1) is the best characterized [16, 17] (Fig. 1). TfR1 is a disulfide bonded, homodimeric receptor that is found on the surface of most body cells. TfR1 deficiency leads to embryonic lethality in mice, an indication of its physiological essentiality [18]. Cells that are rapidly dividing (e.g., the epithelial cells of the intestinal crypts) or have specialized iron requirements (e.g., immature erythroid cells which require large amounts of iron for hemoglobin synthesis) express particularly high levels of TfR1. The structure of TfR1 ectodomain has been solved to 3.2 Å resolution by X-ray crystallography [19], and the structure of the Tf-TfR1 complex has subsequently been investigated via cryo-electron microscopy [20]. Each TfR1 monomer is a Type II membrane protein that consists of a short, N-terminal cytoplasmic tail, a single transmembrane domain, and a large C-terminal extracellular domain. The cytoplasmic domain contains a consensus internalization motif, while the extracellular domain is able to bind a single molecule of Tf. TfR1 is also heavily modified post-translationally through O- and N-glycosylation, palmitylation and phosphorylation [17].

TfR1 carries Tf and consequently Tf-bound iron into the interior of the cell via receptor-mediated endocytosis [16, 17] (Fig. 1). The receptor binds diferric Tf with 10-fold higher affinity than monoferric Tf at physiological pH, and 2,000-fold higher affinity than apotransferrin [21]. The TfR1-diferric Tf complex is endocytosed through clathrin-coated pits, and the resulting vesicles are then uncoated to become endosomes. The endosomes are acidified by a proton pumping ATPase and, at a pH of around 5.5, iron is released from Tf [22]. Since Tf binds iron with such a high affinity, it is likely that iron release also requires reduction [23], and takes advantage of a conformational change in Tf that accompanies its binding to TfR1 [20, 24]. Proteins of the STEAP (six-transmembrane epithelial antigen of the prostate 3) family are candidate endosomal ferric reductases, and STEAP3 has been shown to perform this function in immature erythroid cells [25]. When the gene encoding this protein is mutated, as in the *nm1054* mouse strain, a hypochromic, microcytic iron deficiency anemia results [26]. STEAP3 is expressed in a range of other tissues where it might be expected to play a similar role, but there are a number of other members of the STEAP family and they may also act as endosomal reductases, particularly in non-erythroid tissues [27, 28]. Members of the STEAP family are membrane proteins with six predicted transmembrane domains and NAD(P)H- and flavin-dependent ferric reductase activity. They also contain a membrane-associated heme group that facilitates electron transfer. The structure of STEAP3 has recently been determined and it is predicted to function as a dimer [29].

Iron released from internalized diferric Tf (now in its reduced or ferrous form) makes its way across the endosomal membrane and into the cytoplasm through the ferrous iron transporter divalent metal-ion transporter 1 (DMT1) [30], a member of the NRAMP (natural resistance-associated macrophage protein) family of metal ion transporters. These proteins show proton dependent transport of metal ions, so DMT1 is well suited to removing iron

Table 1 Some mammalian proteins of iron transport and its regulation

Protein	Protein abbreviation	Gene symbol[a]	Function/role in iron metabolism	Consequence of mutation or deletion
Iron uptake				
Transferrin	Tf	*TF*	Plasma iron transport	Iron deficiency anemia with tissue iron overload
				Human disease: atransferrinemia
Transferrin receptor 1	TfR1	*TFRC*	Internalization of diferric transferrin	Embryonic lethality in homozygote. Mild anemia in heterozygotes.
Divalent metal-ion transporter 1	DMT1	*SLC11A2*	Ferrous iron importer	Iron deficiency anemia
				Human disease: refractory hypochromic, microcytic anemia
Six transmembrane epithelial antigen of prostate protein 3	STEAP3	*STEAP3*	Iron reductase of erythroid cells	Iron deficiency anemia
Exocyst complex component 6	SEC15L1	*EXOC6*	Vesicle trafficking	Iron deficiency anemia
Duodenal cytochrome B	DCYTB	*CYBRD1*	Enterocyte brush border reductase	No overt phenotype
Iron recovery				
Hemopexin	HPX	*HPX*	Heme binding	No phenotype unless stressed by hemolysis; then extensive renal damage
Haptoglobin	HP	*HP*	Hemoglobin binding	No phenotype unless stressed by hemolysis; then extensive renal damage
Iron export				
Ferroportin	FPN	*SLC40A1*	Ferrous iron exporter	Iron overload in heterozygous state; embryonic lethality in homozygotes
				Human disease: hemochromatosis type 4
Ceruloplasmin	Cp	*CP*	Iron oxidase	Iron overload; CNS dysfunction
				Human disease: aceruloplasminemia
Hephaestin	Hp	*HEPH*	Iron oxidase (gut and CNS)	Iron deficiency anemia
Feline leukemia virus, type C, receptor	FLVCR	*FLVCR1*	Heme export protein	Embryonic lethality. Erythropoietic and developmental abnormalities
Mitochondrial iron transport				
Mitoferrin	MFRN	*SLC25A37*	Mitochondrial iron importer	Iron deficiency anemia; Erythroid maturation arrest
ABC transporter type B7	ABCB7	*ABCB7*	Mitochondrial Fe–S export	Mitochondrial iron loading
				Human disease: X-linked sideroblastic anemia
Cellular regulation				
Iron regulatory protein 1	IRP1	*ACO1*	Iron-dependent RNA binding protein	Negligible. Some abnormalities in brown fat and kidney.
Iron regulatory protein 2	IRP2	*IREB2*	Iron-dependent RNA binding protein	Anemia; CNS abnormalities of varying severity
Systemic regulation				
Hepcidin	HEPC	*HAMP*	Regulator of iron release into plasma	Severe iron overload
				Human disease: hemochromatosis type 2B
Hemochromatosis protein	HFE	*HFE*	Regulator of hepcidin	Iron overload
				Human disease: hemochromatosis type 1
Transferrin receptor 2	TfR2	*TFR2*	Regulator of hepcidin	Iron overload
				Human disease: hemochromatosis type 3
Hemojuvelin	HJV	*HFE2*	Regulator of hepcidin	Severe iron overload
				Human disease: hemochromatosis type 2A

[a] Human Genome Organization approved symbol

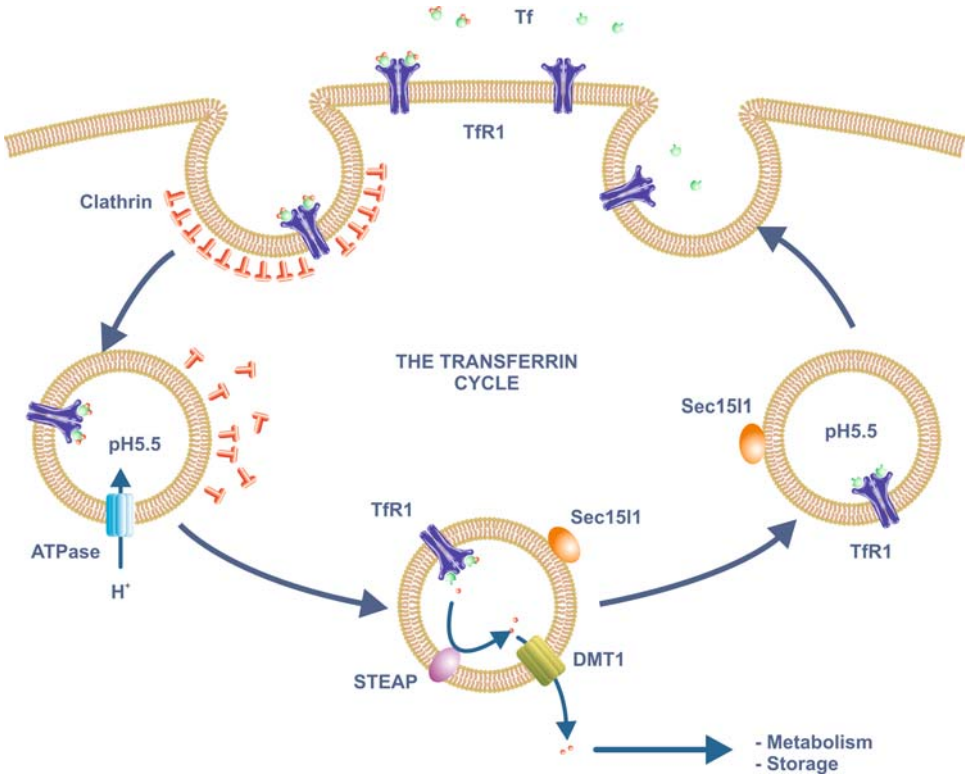

Fig. 1 The transferrin cycle. Plasma ferric iron binds with high affinity to *Tf* which in turn binds to *TfR1* on the plasma membrane. The *Tf/TfR1* complex is internalized through *clathrin*-coated pits by receptor-mediated endocytosis. A proton-pumping *ATPase* lowers the *pH* of the endosome to around 5.5. Iron is released at this low *pH*, a process aided by reduction of the ferric iron by enzymes of the *STEAP* family. The resulting ferrous iron moves across the endosomal membrane via *DMT1* and enters the cytoplasm where it can be utilized for various metabolic functions or stored within ferritin. *ApoTf* remains bound to *TfR1* at the low *pH* of the endosome and is returned to the extracellular medium after the endosome recycles to the plasma membrane. The trafficking protein *Sec15l1* is involved in *Tf* recycling. Abbreviations: see text

from the low pH environment of the endosomes. The NRAMP family will be discussed in more detail in the following section.

After Tf has delivered its iron, it is recycled to the extracellular medium. At the low pH of the endosome, the affinity of TfR1 for apoTf is much higher than at physiological pH, and this explains why the two proteins remain bound as the endosome is recycled to the plasma membrane. However, once the complex reaches the cell surface, the apoTf dissociates [22]. A single round of Tf-mediated iron delivery (the so-called transferrin cycle) is completed in 5–20 min (depending on the cell type), but the half-life of plasma Tf is approximately 8 days in humans [31]. This means that each Tf molecule can potentially undergo hundreds of rounds of iron binding and uptake during its life. Although the basic pathway of Tf recycling has been known for many years, new details continue to be revealed. An example has come from the analysis of the hemoglobin deficit (*hbd*) mouse [32, 33]. These animals carry a microcytic, hypochromic anemia and it has been known for some time that the delivery of Tf-bound iron to immature erythroid cells is defective in this strain [34, 35]. The gene that is mutated in these animals encodes the exocyst protein Sec15l1. Although the precise role of Sec15l1 in the Tf cycle is not known, it has been co-localized with TfR1 in recycling endosomes [36] and is likely involved in regulating the efficiency of endosome recycling. Whether Sec15l1 homologs play similar roles in non-erythroid tissues is unknown.

While the high affinity TfR1-mediated uptake of diferric-Tf is a very efficient means of delivery iron to cells, it is not the only mechanism by which cells can utilize Tf-bound iron, and low affinity uptake has also been described. In fact, the concentrations of Tf and diferric Tf in the extracellular fluid are so high (25–50 and 15–30 μM, respectively), that under normal circumstances the low affinity process is likely to predominate. The relative contributions of receptor-mediated and non-receptor-mediated uptake of diferric-Tf vary from cell type to cell type. In immature erythroid cells, diferric Tf is taken up essentially entirely through the TfR1 route, whereas for many cells, such as hepatocytes and intestinal enterocytes, both the high and low affinity processes are utilized [13, 37].

The low affinity uptake of diferric Tf has been best studied in liver-derived cells and a number of investigations have clearly shown that the process is not mediated by TfR1 [38, 39]. However, a candidate binding protein is the TfR1 homolog, TfR2, at least in the liver where it is most strongly expressed [40–42]. TfR2, like TfR1, is a homodimeric plasma membrane Tf binding protein, but its affinity for diferric Tf is 25-fold lower than that of TfR1, consistent with a possible role in the low affinity uptake of Tf. Also, like TfR1, Tf taken up by the low affinity process appears to enter an endocytic compartment, but ultimately

is recycled to the extracellular medium [38, 43, 44]. Despite this, there may be some differences in the cellular biology of the two uptake processes and much of the Tf taken up by TfR2 appears to remain intracellularly in multivesicular bodies [42]. The demonstration that TfR2 can be detected on both the cell surface and at intracellular sites is consistent with this [45, 46].

While TfR2 appears to be a strong candidate for the low affinity uptake of diferric Tf, other evidence suggests that this may not be the case. Many tissues show low affinity Tf uptake, yet TfR2 has only a limited tissue distribution with particularly high levels being found only in the liver [46]. Some cell types, such as small intestinal crypt enterocytes, do not express TfR2, yet take up much of their Tf-bound iron by the low affinity process [37]. Furthermore, human patients with mutations in *TFR2*, or mice in which the *Tfr2* gene has been deleted, can very effectively deliver iron to their tissues and indeed develop a form of iron loading disease (or hemochromatosis) [47, 48]. In such cases, however, much of the iron taken up may be as NTBI, and not as diferric Tf. Rather than contributing significantly to iron uptake, TfR2 appears to play a more important role in the regulation of body iron homeostasis through its regulation of hepcidin (see below). Other studies have suggested that iron loaded Tf can be taken up by hepatocytes via proteoglycans or fluid phase endocytosis, and by macrophages through surface-bound glyceraldehyde-3-phosphate dehydrogenase [12, 49]. These could also be relevant in other cell types, but whether they represent quantitatively significant pathways of Tf uptake is unclear.

Most work on Tf-bound iron uptake by cells suggests that endocytotic processes are involved, but there is solid evidence that Tf-associated iron can be delivered to cells without endocytosis. This process has been described for several cell types but is most prominent in hepatocytes [50]. Evidence in favor of cell surface uptake includes the demonstration that diferric Tf immobilized on beads too large to be endocytosed is able to deliver its iron to fibroblasts [51], the finding that membrane impermeant iron chelators are able to reduce the uptake of iron from Tf [52, 53] and experiments showing that NTBI can reduce the uptake of Tf-bound iron [54, 55]. This uptake process could also involve a cell surface ferric iron reductase [53], but the mechanism remains poorly characterized.

The NRAMP family of metal-ion transporters

TfR1 will deliver Tf-bound iron to the interior of the cell, but it still remains in the recycling endosomes and is isolated from the cytoplasm and cellular organelles where it is required for metabolic functions. As noted above, the transport of ferrous iron from the endosomes to the cytoplasm is mediated by DMT1, a member of the natural resistance-associated macrophage protein (NRAMP) family [56, 57]. DMT1 is a multispanning membrane protein with 12 predicted transmembrane domains. It efficiently transports ferrous iron but not ferric iron, and this highlights the requirement of a ferric reductase before the ubiquitous environmental ferric iron can be utilized. DMT1 transports not only ferrous iron but a range of other divalent metal irons, including Cu^{2+}, Zn^{2+}, Co^{2+} and Cd^{2+} [56]. Despite this relative lack of specificity, the most obvious phenotype resulting from deletion or mutation in the *SLC11A2* gene (which encodes DMT1) is iron deficiency anemia [30, 58].

Our understanding of how DMT1 functions as an iron transporter remains incomplete, but a large number of mutagenesis studies have been carried out on both this protein and its homologs, and the effects of these mutations on metal transport have been investigated [57, 59]. Transmembrane (TM) domains 1 and 6 appear to be critical for metal binding and uptake as well as proton coupling. Residues in TM segments 3, 4 and 9 are also required for efficient metal transport, and amino acid residues in several other regions have been shown to play some role in iron transport. No crystal structure of DMT1 is available as yet, but recently an NMR structure of a synthetic peptide corresponding to loop 3 and TM domain 4 has been solved [60]. TM4 is the site of several naturally occurring mutations in both DMT1 and its homolog NRAMP1, so this region clearly plays an important functional role.

Since most cells can take up iron via the Tf cycle, it is not surprising that DMT1 is expressed by most body cells [30, 61]. Tissues with high iron requirements, such as immature erythroid cells and cells of the central nervous system (CNS), have particularly high levels of DMT1 [57]. However, in addition to its widespread 'housekeeping' role, DMT1 also plays a specialized role in the uptake of dietary iron across the brush border membrane of intestinal epithelial cells [62] (Fig. 2a). Indeed, it was the search for the protein that mediated the uptake of iron by the intestinal epithelium that initially led to the identification of DMT1 [56, 58]. The placenta must also transport large amounts of iron and it is a prominent site of DMT1 expression, although interestingly it does not appear to be essential for placental iron transport [63]. Iron uptake following TfR2-mediated endocytosis, the surface delivery of Tf-derived iron, and the uptake of NTBI are also likely to utilize DMT1 [12, 54, 64].

The NRAMP family of metal-ion transporters is found in a wide range of organisms from bacteria to mammals, and its members show a high degree of sequence conservation [65]. The first member of the family to be identified in mammals was not DMT1, but the closely related NRAMP1. The gene that encodes NRAMP1 (*SLC11A1*) was identified as being mutated in a strain of mice with

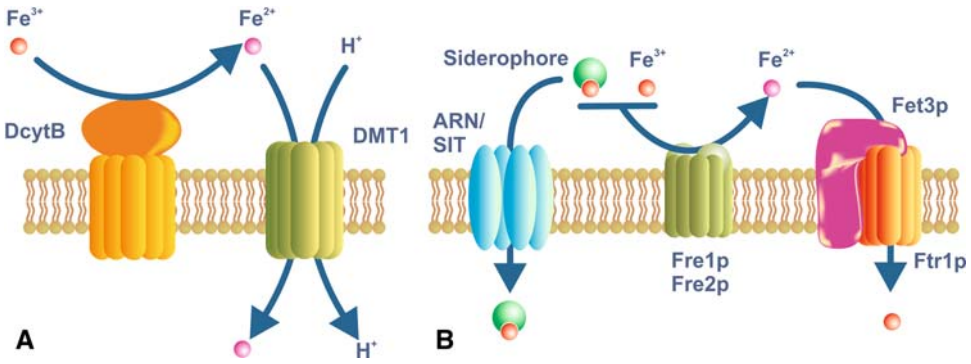

Fig. 2 Iron uptake in enterocytes and yeast. **a** Dietary iron is taken up across the brush border membrane of duodenal enterocytes through the iron/proton symporter *DMT1*. *DMT1* is expressed on most body cells, but it plays a specialized role in iron acquisition from the diet. Most dietary iron is in the Fe^{3+} or ferric form, but it needs to be reduced to the Fe^{2+} or ferrous form before it can be utilized as a substrate by *DMT1*. *DcytB* is an iron-regulated reductase of the brush border membrane that is a candidate for this role. However, whether *DcytB* is the only reductase involved remains to be determined. **b** The yeast *Saccharomyces cerevisiae* is a simple eukaryote that is able to utilize iron in a variety of forms. It also must reduce iron before it can be utilized. The reductases *Fre1p* and *Fre2p* are the principal plasma membrane enzymes involved in this process, but several others have been described. They can utilize both ionic iron or siderophore-bound iron as substrates. The reduced iron is transported into the cell through a complex of the iron permease *Ftr1p* and the iron oxidase *Fet3p*. A variety of siderophores also can be utilized by *S. cerevisiae,* and these are taken up via members of the *ARN* and *SIT* families

increased susceptibility to infections [66]. It was subsequently shown that NRAMP1 is particularly highly expressed in macrophages and resides in the membrane of the phagosome which is involved in the killing of phagocytosed pathogens [67]. The precise function of the protein is unclear, but, like DMT1, it seems to be involved in the transmembrane transport of iron and other divalent metals [68]. Whether it transports iron into or out of the phagosome is unresolved [69]. An increased concentration of redox active iron inside the phagosome could contribute to the enhanced production of toxic oxygen radicals, whereas a reduction in phagosome iron content could contribute to the killing of pathogens by starving them of essential iron. Based on the knowledge that NRAMP1, like DMT1, transports metals down a proton gradient, the export of iron and/other metals from the phagosome seems more likely. Interestingly, it has recently been demonstrated that NRAMP1 may play a role in iron recycling from senescent erythrocytes [70], further evidence that it is involved in metal export from the phagosome.

The uptake of non-transferrin-bound iron

Transferrin is the major source of iron for most body cells under normal physiological conditions. However, in various pathologic states, other forms of iron can be taken up. One of the most important of these is non-transferrin-bound iron (NTBI). While we normally think of Tf as being required to deliver iron to cells, this is not the case. In fact, in the very rare cases of congenital Tf deficiency in humans, or in mice in which the Tf gene has been deleted, iron delivery to the tissues is extremely rapid and heavy iron loading of the organs develops [71, 72]. It is more appropriate to think of Tf as providing a vehicle for regulating iron delivery to cells.

Since Tf has an exceptionally high affinity for iron (K_d $10^{-23}M^{-1}$) [73], the amount of NTBI in the plasma under normal circumstances is vanishingly small. Tf is an abundant plasma protein and only approximately 30% of the total iron binding sites in the plasma Tf pool are occupied at any one time under normal conditions. This provides a large excess of Tf iron binding capacity that helps keep potentially toxic NTBI levels very low. However, when the amount of iron entering the plasma increases, a situation most frequently found in a range of iron loading disorders where dietary iron absorption is inappropriately elevated, the capacity of plasma Tf to bind this iron can be exceeded. It can then be measured readily in the circulation and levels up to 10–15 μM, or even higher is some cases, can be detected [14, 74]. Despite much investigation, the precise chemical form of NTBI has proved difficult to define. Much is thought to be chelated by small organic acids such as citrate, but smaller amounts could be bound loosely to proteins such as albumin [15]. Presumably NTBI is present largely in the oxidized or ferric form.

As the studies of atransferrinemia mentioned above clearly demonstrate, NTBI can enter the tissues very rapidly. This has also been clearly demonstrated in animals where Tf was saturated experimentally [75]. Such studies have shown that the liver has a particularly high avidity for NTBI [75, 76], but other tissues such as the pancreas and heart can also take up considerable amounts. Precisely how NTBI makes its way across the plasma membrane and into cells is unclear, but several candidates have been proposed. DMT1 is perhaps the most feasible of these as its role as a major membrane iron transporter has

been well characterized. However, mice lacking DMT1 can still accumulate hepatic iron [63], suggesting that DMT1 is not the sole route of NTBI entry. Another candidate is SFT, or stimulator of Fe transport. This protein was isolated as an iron transporter from erythroid leukemia cells and has been found to play a role in both Tf-dependent and independent iron uptake, but its role remains poorly characterized [77–79]. Indeed, the original published sequence was incorrect, and the sequence responsible for conferring iron transport activity consists of several smaller open reading frames [77]. Which of these is responsible for the activity is not known. More recently, the zinc transporter ZIP14 (Zrt-Irt-like protein 14) (SLC39A14) has been shown to facilitate NTBI uptake in hepatocytes [80], so it also potentially plays an important role. Finally, L-type voltage-dependent calcium channels may play a role in iron uptake by cardiac myocytes as blockers of these channels will inhibit iron transport [81]. In reality, NTBI may enter cells via multiple routes with the proportion entering through any one pathway varying from tissue to tissue. Since under most circumstances iron crosses cellular membranes in the reduced or ferrous form, it is likely that ferric iron reduction plays a role in NTBI uptake, and early studies indicated that this may indeed be the case [82].

The utilization of iron from proteins other than transferrin

Most of the iron found in the circulation is not bound to Tf but is found within the hemoglobin of circulating red cells. Under normal physiological conditions, this iron can only be utilized when senescent erythrocytes are phagocytosed by macrophages and broken down. Heme is released from the protein and in turn iron is released from heme by heme oxygenases and exported to the plasma where it can be bound by Tf. Quantitatively, this is by far the most important route of iron entry into the plasma. Under a range of pathological conditions, however, when intravascular hemolysis occurs, free hemoglobin and heme can be found in the circulation. These can be cleared very rapidly by several important scavenging pathways and the iron returned to plasma Tf for reutilization. Mice in which these pathways have been disrupted display a normal phenotype, showing that their scavenging function is not required under normal conditions, but when the animals are placed under hemolytic stress, significant pathology results [83, 84].

Hemoglobin released into the circulation is bound by the liver-derived plasma protein haptoglobin [85], and the resulting complex binds to CD163 on the surface of macrophages [86]. CD163 is expressed on the surface of macrophages throughout the body and delivers its cargo by receptor-mediated endocytosis [87, 88]. Subsequently, the complex is degraded and iron is released. Following hemolysis, free heme can also be found in the plasma and, like hemoglobin, it also rapidly binds to a plasma protein, in this case hemopexin [89]. The heme/hemopexin complex can be taken up by cells through both high affinity and low affinity pathways. In the high affinity pathway, the complex binds to low-density lipoprotein receptor-related protein (LRP)/CD91, a heterodimeric complex found on the surface of hepatocytes, macrophages and some other cell types [90, 91]. Recent evidence suggests that the complex is internalized and subsequently degraded in lysosomes [91], but the recycling of hemopexin to the extracellular medium has also been reported [92]. The identity of the low affinity transporter is unknown. Iron from internalized heme is likely released from the porphyrin ring via heme oxygenase [93].

There are several normal physiological situations where heme and its associated iron must cross cellular membranes. The mitochondrial membranes are one prominent location where this occurs, and this will be considered in more detail below. However, another site where heme transport in prominent is across the brush border membrane of small intestinal epithelial cells. Much of the iron in the diet is in the form of heme (principally derived from myoglobin) and this can be very efficiently absorbed by the intestine [94]. The nature of the heme transport pathway has yet to be fully defined. A candidate heme transporter, HCP1 (heme carrier protein 1), was described recently [95], but subsequent investigations have shown that this protein transports folate more efficiently than heme [96], so whether it is the major enterocyte heme transporter remains unclear. Irrespective of how heme is transported into enterocytes, it is likely that iron is released from the porphyrin ring by heme oxygenase within the cells before being exported to the circulation [97]. However, a recently identified heme export protein, FLVCR (feline leukemia virus subgroup c receptor) [98], is expressed in the duodenum, raising the possibility that some intact heme may be exported from the gut.

Iron in excess of cellular requirements is bound within the iron storage protein ferritin, and the intracellular concentration of the protein can reach quite high levels [9]. Small, yet readily detectable, amounts of ferritin are also secreted into the extracellular medium, and measurements of plasma ferritin represent a reliable indicator of body iron levels in non-inflammatory conditions. This form of the protein has a very low iron content and is unlikely to play a significant role in iron transport, although a role for ferritin in the intrahepatic transfer of iron from Kupffer cells (the resident macrophages) to hepatocytes has been proposed [99]. However, in pathological states, particularly when there is damage to the liver, the major iron storage organ,

considerable amounts of iron-rich tissue ferritin can be released. This ferritin can be rapidly cleared from the circulation by the liver [100], but the clearance mechanism is not well defined. A possible ferritin receptor is TIM-2 (T cell immunoglobulin domain and mucin-domain protein 2). This plasma membrane protein is able to bind ferritin and mediate its internalization [101], but its tissue distribution (the bile canaliculi of hepatocytes, bile duct epithelial cells and B cells) seems inconsistent with a role as a ferritin scavenger. More recently, Scara5 (scavenger receptor, member 5) has been demonstrated to bind and internalize ferritin and to deliver ferritin-associated iron to the developing kidney [102]. Scara5 is also expressed in the adult kidney, gonadal epithelia and several other embryonic tissues, and has been shown to preferentially bind L-ferritin, so it likely plays an important role in development and perhaps in adult tissues. Further studies on TIM2, Scara5 and other putative ferritin binding proteins are required. How iron from internalized ferritin is recovered for reutilization is not well characterized, but presumably the protein shell is degraded in lysosomes and the iron released.

Ferric reductases and cellular iron uptake

Most iron in the environment is in the oxidized or ferric form, yet it is the reduced form, ferrous iron, that is more soluble and is more readily transported across membranes. It thus comes as no surprise that ferric iron reductases play a prominent role in cellular iron uptake. Indeed, long before any mammalian cellular iron uptake proteins were identified, cell surface ferric reductase activity had been described [103, 104]. The first eukaryotic ferric reductases were identified in the yeast *Saccharomyces cerevisiae* and now four such proteins are known to be involved in yeast iron acquisition [2, 105] (Fig. 2b).

The role of the STEAP proteins in reducing iron and facilitating its uptake by DMT1 in mammalian cells was noted above [27], but there are other examples. Ferric reductase activity has been described on the brush border membrane of duodenal enterocytes [106], but no STEAP proteins are present. A ferric reductase that may feed ferrous iron to DMT1 in the gut is DCYTB (duodenal cytochrome B) [107]. This membrane-bound protein has iron reductase activity and its expression is enhanced by stimuli that enhance iron absorption. However, when the gene encoding Dcytb was deleted in mice, the animals have no overt phenotype, showing that Dcytb is not essential [108]. Whether one or more other ferric reductases exist remains to be determined. A mammalian iron reductase is also found in the placenta [109]. Thus, the functional linking of an oxidoreductase to an iron transporter is a common theme in iron metabolism.

Iron efflux from cells

The iron exporter ferroportin 1

Cellular iron transport is by no means a unidirectional process and is not restricted to iron uptake. Most cells have the capacity to release iron into the extracellular medium and some cell types are particularly well adapted for doing this. The best examples are macrophages and duodenal enterocytes. Macrophages process senescent erythrocytes and the iron derived from hemoglobin must be released from heme and returned to the circulation where it can be re-utilized, particularly by the erythroid marrow [110]. This recycling of iron is particularly critical as the amount of iron entering the body through dietary absorption is usually insufficient to meet erythroid requirements. Indeed, the erythroid/macrophage recycling system is responsible for approximately 80% of daily iron traffic in the body. Enterocytes are another excellent example of a cell type that must vectorially transport iron. Dietary iron enters the cells across the brush border membrane via DMT1, but is delivered to the circulation by export across the basolateral membrane of the cells [111]. Small amounts or iron are absorbed under normal circumstances, sufficient to replace obligatory passive body losses, but intestinal absorption assumes a particularly important role when iron recycling through the macrophages is insufficient to cater for erythroid demand. Under these same conditions, stored iron can also be drawn from most body cells, so the capacity to export iron is widespread.

The only plasma membrane iron export protein to be described, and by far the most valuable one, is ferroportin 1 (FPN) (also known as IREG1 or MTP1) [112–114]. FPN is expressed at particularly high levels on the surface of cells with a high capacity to export iron, such as macrophages and enterocytes, but it appears to be present on almost all cells. This is consistent with the known capacity of most body cells to release stored iron to the circulation. FPN is also expressed at high concentrations in the placenta, another organ which demonstrates the directional movement of large amounts of iron [115], and appears to be essential for iron transfer to the fetus [116]. Mutations in the FPN gene in humans or deletion of the gene in mice or zebrafish demonstrate the importance of FPN in iron homeostasis [116, 117]. In humans with reduced FPN function, iron rapidly accumulates in macrophages, such as the Kupffer cells of the liver, and, in time, iron concentrations also increase in parenchymal cells. Intestinal iron absorption is decreased and plasma iron levels fall. This leads to a situation where there is iron loading in the tissues but, paradoxically, there is a systemic anemia as reduced iron export into the plasma means reduced iron supply to erythroid progenitors [117]. In most human subjects with

FPN mutations, there remains sufficient FPN activity to allow iron absorption to continue. However, deletion of the Fpn gene solely in the intestinal epithelium in mice is incompatible with postnatal development, confirming the essentiality of the protein in intestinal iron absorption [116].

FPN is a multispanning membrane protein with 12 predicted transmembrane domains and both N and C termini on the cytoplasmic side of the membrane [118]. Like DMT1, it uses ferrous iron as a substrate, but there is very limited information on the mechanism by which the protein transports iron. There remains some debate as to whether FPN functions as a monomer or a dimer. Genetic evidence suggests that a dimer is more likely [119], although recent evidence has shown that purified detergent-solubilized FPN behaves as a monomer [118]. Many mutations in the gene encoding FPN have been identified and these have been well summarized elsewhere [118, 120].

Although FPN is the only known exporter for low molecular weight forms of iron, some iron can also be exported as either heme or ferritin. Feline leukemia virus, subgroup C, receptor (FLVCR) has been shown to act as a heme exporter in mammals, and it is predicted to play a role in reducing excess heme levels in erythroid cell precursors and in heme release from macrophages [98, 121]. As noted above, it could also play a role in other tissues such as the small intestine. Abcg2 (Bcrp) is another heme transport protein in the plasma membrane that appears to facilitate heme efflux from immature erythroid cells [122]. Iron export in the form of ferritin has not been well characterized, but it has been demonstrated that much of the iron released from erythrophagocytosing Kupffer cells (macrophages) is in this form [99].

The requirement of an oxidase for export

Just as efficient cellular iron uptake often requires the concerted actions of a ferrireductase and a ferrous iron transporter, iron efflux also appears to require both a transporter and an enzyme. In this case, the enzyme facilitates iron oxidation rather than reduction. FPN transports iron across the membrane in the ferrous form, but circulating Tf binds ferric iron so oxidation is required. The major plasma ferroxidase has long been known to be the copper-containing protein ceruloplasmin (Cp) [123]. Many years ago, it was shown that animals placed on a copper-deficient diet showed reduced plasma oxidase activity and accumulated iron in their tissues, providing strong evidence that Cp was required for cellular iron efflux. This has since been confirmed with the analysis of human subjects carrying mutations in the Cp gene, or mice in which the Cp gene has been deleted [124, 125]. These subjects have reduced plasma iron levels but increased storage iron in the tissues. While Cp seems to be required for iron release from most tissues, in the small intestine, CNS and placenta, membrane-bound Cp homologs appear to play a similar role. In the small intestine, the Cp homolog hephaestin (HEPH) is required for efficient iron absorption, and animals with defective HEPH function become quite anemic [126, 127]. HEPH consists of an extracellular oxidase domain (which is 50% identical to the Cp amino acid sequence) linked to a single transmembrane domain and a short C terminal tail. Hephaestin is also found in the CNS, and disruption of both Cp and HEPH leads to greater iron accumulation in the brain than deletion of Cp alone [128]. The CNS also expresses a GPI-linked form of Cp [129]. Placenta also contains a Cp homolog which is thought to facilitate iron transfer to the fetus, and it too is presumed to be membrane bound [115]. However, little is known about this protein. It is certainly likely that multiple iron oxidases act on the same tissue to facilitate iron release. The presence of both HEPH and GPI-linked Cp in the CNS supports this concept, as does the demonstration that Cp can contribute to intestinal iron absorption when the stimulus for absorption is particularly high [130]. In the yeast *S. cerevisiae*, an iron oxidase (Fet3p) also acts in conjunction with an iron transporter (the permease Ftrp1) to move iron across the plasma membrane, but in this case it is required for iron uptake rather than iron export [2] (Fig. 2b).

Precisely how Cp or its homologs facilitate iron release from cells is poorly understood, but a recent study has shed some light on the mechanism. De Domenico and colleagues [131] found that Cp-mediated iron oxidation was required to release the metal from FPN. In the absence of oxidation, ferrous iron remains bound to FPN which is then ubiquitinated, internalised and degraded (Fig. 3). Thus, iron oxidation stabilizes FPN on the plasma membrane. Under normal circumstances, the amount of Cp in the circulation is not rate limiting for iron release. Patients with defects in the copper transporter ATP7B (Wilson disease) have low plasma ferroxidase levels as ATP7B is required to load Cp with essential copper. Only when plasma Cp oxidase levels drop to below approximately 5% [132] of the normal level does iron accumulate in the tissues [133]. Whether HEPH functions in the gut in a similar way to the CNS Cp is unknown.

Intracellular iron transport and iron utilization by mitochondria

Cytoplasmic iron trafficking

Iron that is delivered to the cytoplasm, either via endosomes or across the plasma membrane, must be trafficked to intracellular sites where it can be incorporated into the proteins required for various cellular functions. Iron in

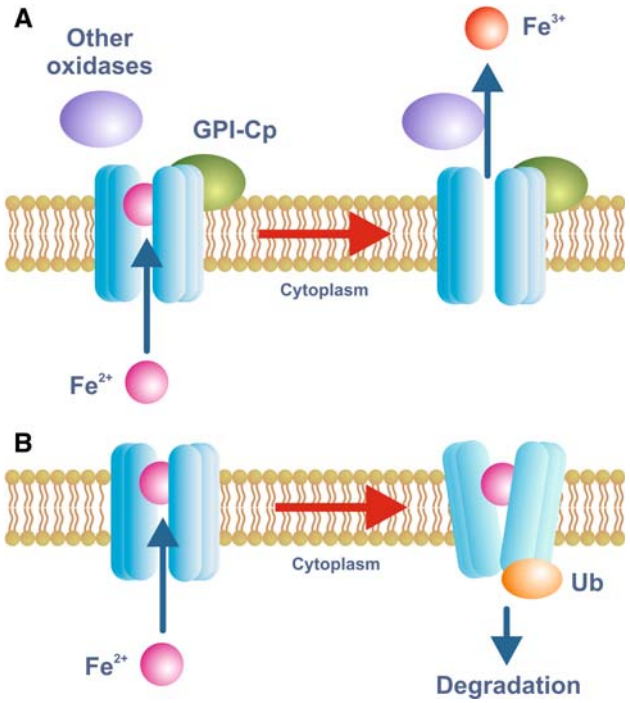

Fig. 3 Model for iron export through FPN. Iron export through FPN requires the action of an iron oxidase and this function is usually fulfilled by either circulating or *GPI*-linked forms of *Cp*. De Domenico et al. [130] have proposed a model for the function of *Cp* whereby the oxidation of FPN-bound ferrous iron by *Cp* is required for its release into the extracellular medium (**a**). In the absence of appropriate oxidase activity, ferrous iron remains bound to FPN and the protein is ubiquitinated and targeted for degradation (**b**). This is only one mechanism by which FPN expression on the plasma membrane can be modulated. The binding of hepcidin to FPN can also facilitate its internalization and degradation

excess of metabolic needs is incorporated into the iron storage protein ferritin, but can subsequently be accessed if metabolic demand increases [9]. How iron is trafficked from sites of uptake to sites of utilization and storage is very poorly understood. As a redox active metal, it makes sense for iron to be sequestered in some way so that it is not available to catalyze reactions leading to oxygen radical production, but its precise intracellular form is unknown. Iron may be bound to low molecular weight organic acids such as citrate or could be bound loosely to intracellular proteins [134]. A third possibility is that iron is bound to specific chaperone proteins. Chaperones have been well described for copper [135], another redox active metal, but until recently no iron chaperones had been identified. However, it was recently demonstrated that poly (rC) binding protein 1 (PCBP1) was able to facilitate iron loading onto human ferritin expressed in the yeast *S. cerevisiae*, and it was subsequently shown to perform a similar function in mammalian cell lines [136]. How PCBP1 receives and subsequently delivers iron is not known. PCBP1 appears to be ubiquitously expressed in mammalian cells, so it could be a universal iron chaperone, but further studies are required to investigate its physiological relevance in mammals.

Mitochondrial iron utilization

A large number of cellular proteins require iron as a cofactor and there is considerable diversity in the types of iron centers found within these proteins. Two of the most widespread iron centers are those involving heme and iron–sulfur (Fe–S) clusters, both of which are predominantly synthesized in mitochondria [5, 137]. In view of this, the mitochondrion plays a critical role in cellular iron homeostasis and is a major sink for iron [138]. How iron traffics from the endosome to the mitochondrion and other intracellular sites is not well understood. As noted above, it may be sequestered by low molecular weight ligands or bound either specifically or non-specifically to cytoplasmic protein, and these could deliver iron to the mitochondria iron import proteins. Another possibility has been suggested for immature erythroid cells which take up large amounts of Tf-bound iron and must deliver it to mitochondria for heme synthesis. It has been proposed that the endosomes may make direct contact with mitochondria and thereby transfer their iron [139]. There is some experimental support for this, but how widespread such a process might be is unclear.

However, iron makes its way to the mitochondrion, it must pass through the mitochondrial membranes to the matrix where it can be utilized. The best candidate for mitochondrial iron import is mitoferrin (Mfrn) [140]. Mfrn is a member of the mitochondrial solute carrier family (SLC25) of transport proteins and is located on the inner mitochondrial membrane. When the Mfrn gene is disrupted in mice, the animals show reduced mitochondrial iron uptake and consequently heme synthesis is reduced. However, mice lacking Mfrn remain viable, suggesting some functional redundancy in iron delivery to this organelle. This is not surprising given its critical role in cellular function. Mfrn is predominantly expressed in erythroid cells, but its homolog mitoferrin 2 (Mfrn2)(65% amino acid identity) is found in many non-erythoid tissues and could play a similar role here [141].

Whether low molecular weight forms of iron can exit the mitochondrion is unclear, but it is well established that both heme and Fe–S clusters can leave the organelle. Heme must be able to leave mitochondria as it is incorporated into various proteins in the cytoplasm. A candidate mitochondrial heme exporter is the ATP-binding cassette (ABC) transporter ABCB10, although this process remains quite poorly understood [142]. The outer mitochondrial membrane protein ABCB6 is also able to transport heme, but in this case it likely acts as a heme importer rather than

an exporter [142]. The inner mitochondrial membrane protein ABCB7 has emerged as a possible Fe–S cluster exporter [143], although this has yet to be confirmed. When the gene encoding ABCB7 is disrupted in humans, X-linked sideroblastic anemia results and iron accumulates in the mitochondrial matrix [144]. This iron accumulation suggests that the mitochondrion may not be able to divest itself of iron unless it is first incorporated in to Fe–S clusters or heme. Indeed, iron accumulation is also seen in mitochondria when Fe–S cluster synthesis is disrupted, as in the progressive neurodegenerative disorder Friedrich ataxia [145–147]. Friedrich ataxia results from disrupted expression of the protein frataxin which is associated with the inner mitochondrial membrane. Although it has been shown to be linked to the synthesis of Fe–S clusters, the precise function of frataxin remains unknown [145, 146].

Regulation of cellular iron transport

Cellular iron levels must be kept within defined limits. Sufficient iron is required to meet metabolic needs, but excess iron can be toxic. Thus, both iron uptake and efflux are tightly regulated processes. When cellular iron requirements are high, the iron import machinery is expressed at higher levels, while iron export is restricted. Thus, concentrations of TfR1 and DMT1 are increased on the cell surface under iron-deficient conditions, and FPN expression is reduced. Also, the expression of the iron storage protein ferritin is decreased under these conditions as iron is preferentially directed for metabolic functions. When cells are iron replete, the opposite pattern is seen, with reduced TfR1 and DMT1 expression and increased ferritin production. However, this is only what might be described as the "classical" pattern of iron transport regulation. In reality, things are not quite so simple. For example, depending on the tissue and the circumstances, the expression of both DMT1 and FPN can be either increased or decreased. Some of the mechanisms underlying this regulation are considered below.

The IRE/IRP system

The best described system for the regulation of proteins of iron metabolism is the post-transcriptional regulation conferred by the RNA-binding iron regulatory proteins (IRPs) [148–150]. When cellular iron levels are low, iron regulatory protein 1 (IRP1) and iron regulatory protein 2 (IRP2) bind to stem loop structures, known as iron responsive elements (IREs), in the mRNAs encoding a range of proteins of iron metabolism. When the IRE is in the 5′ untranslated region (UTR) of the mRNA, the binding of the IRPs blocks translation, but when the IRE is in the 3′ UTR, IRP binding leads to stabilization of the message and thus allows translation to proceed. The regulation of TfR1 and ferritin provides the classic example of the operation of the IRP/IRE system. The TfR1 mRNA has several IREs in its 3′ UTR, whereas the ferritin mRNA contains a single IRE in the 5′ UTR [10, 17, 151]. When iron requirements are high, the binding of the IRPs stabilizes the TfR1 mRNA but blocks translation of the ferritin message. Thus iron uptake is enhanced and iron storage is reduced. When the cell is iron replete, the opposite response is seen.

TfR1 and ferritin are not the only proteins that possess IREs in their mRNAs. The DMT1 message has two 3′ splice variants, and one of these, like TfR1, has an IRE [56, 152]. Thus, under iron-deficient conditions, DMT1 mRNA is stabilized. The IRE-containing splice variant is particularly abundant in the duodenum where its expression may need to increase rapidly to facilitate enhanced iron absorption, but in other tissues, such as the liver, the two splice forms are expressed at comparable levels [153]. Ferroportin has an IRE in its 5' UTR and its translation is blocked when cellular iron levels are low [112]. This pattern of regulation is shown in tissues such as the liver where FPN protein levels correlate directly with iron levels. However, in the intestine, FPN levels vary inversely with iron status, suggesting that its regulation is independent of the IRE/IRP system in this tissue. Despite this, the combined deletion of Irp1 and Irp2 in mice leads to increased FPN expression [154]. Thus, it seems likely that both IRP-dependent and independent (presumably transcriptional) regulation of FPN are occurring in the gut, with the non-IRP mechanism dominating under low iron conditions when FPN levels are particularly high. A murine strain in which the FPN IRE is deleted show severely disturbed iron homeostasis highlighting the importance of this regulatory element [155]. The erythroid form of aminolevulinic acid synthase (eALAS), the rate limiting enzyme in heme synthesis, also has a 5′ IRE in its transcript, so when adequate iron is available its expression is increased [156]. This makes physiological sense and ensures that the protoporphyrin biosynthetic pathway is not stimulated when there is insufficient iron to complete the process and produce heme.

IRP1 and IRP2 respond to iron through different mechanisms [149]. Under iron-replete conditions, IRP1 contains an Fe–S cluster and this gives the protein aconitase activity (in this conformation the protein is known as cytosolic aconitase). However, when the supply of iron is insufficient, the Fe–S cluster disassembles and IRP1 is then able to bind IREs. IRP2 on the other hand does not contain a Fe–S cluster and is regulated by synthesis and degradation. When iron is plentiful, the protein is ubiquitinated and degraded via the proteasome, but in the absence of iron, it is stabilized and is able to bind to target mRNAs.

Both IRPs are expressed quite widely in body tissues, although IRP2 has the more restricted expression pattern of the two. It proved somewhat surprising, therefore, that when Irp1 was deleted in mice the phenotype was very mild [157], whereas deletion of Irp2 led to much more severe pathology, including anemia and significant neurological disease [158, 159]. These observations imply that IRP2 is the most important of the IRPs under physiological conditions. A possible explanation for this apparent paradox has come from Rouault and colleagues who showed that, under the low tissue oxygen concentrations typically found in vivo, the Fe–S cluster of IRP1 is very stable and may not be able to respond effectively to changes in cellular iron levels [149, 160]. However, this is not the case for IRP2 which operates by an Fe–S-independent mechanism.

Transcriptional regulation

Not all the proteins involved in iron transport are regulated by the IRE/IRP system, and even those that are show some degree of transcriptional regulation in response to changes in cellular iron status. For example, the expression of the splice variant of DMT1 mRNA that does not contain an IRE is increased under iron-deficient conditions [161, 162], and, as noted above, the FPN gene is likely to be regulated transcriptionally, at least in the small intestine [112]. Similarly, expression of the enterocyte brush border iron reductase DCYTB is increased by iron deficiency, but its mRNA does not contain an IRE, so transcriptional regulation is likely [107]. Iron-dependent transriptional regulation has also been described for the ferritin [163] and TfR1 [164] genes, but the effects are small. The promoter elements required for these genes to respond to iron have not yet been identified.

Genes encoding proteins of iron homeostasis can also be regulated by a number of factors in addition to iron, and these likely exert their effects at the transcriptional level. Two of the most important of these regulatory stimuli are hypoxia and proinflammatory cytokines. Under hypoxic conditions, the expression of a number of iron homeostasis genes is increased [165], and this reflects the physiological requirement to move more iron to the bone marrow for hemoglobin synthesis. The hypoxia-inducible factors appear to be particularly important in modulating the expression of TfR1 [166, 167]. Similarly, inflammatory cytokines have been shown to affect various iron metabolism genes at the transcriptional level [168]. The response of iron homeostasis to inflammatory stimuli likely evolved as an adaptive response to limit iron availability for invading pathogens.

It is also important to recognize that there are significant tissue-dependent variations in the expression of DMT1 and FPN. Under most circumstances, DMT1 expression is increased with iron depletion and decreased as iron levels rise. However, in some tissues such as the kidney and liver, DMT1 has been reported either not to respond to changes in iron status or even to increase when iron levels increase [169, 170]. The mechanisms underlying these responses are not well understood. FPN mRNA levels are increased in the liver in response to iron loading, consistent with IRP-dependent regulation, but in the small intestine they are reduced under these conditions and evidence suggests that both transcriptional and non-transcriptional mechanisms are in operation here.

If iron-dependent transcriptional regulation is poorly characterized in mammals, this is not the case in unicellular eukaryotes. Most of the genes in the yeast *S. cerevisiae* that encode proteins of iron homeostasis are regulated transcriptionally under the control of the iron-dependent transcription factor Aftp1 [2]. Aftp1 is constitutively expressed, but moves from the cytoplasm to the nucleus when cellular iron levels decline. There, it activates a network of genes encoding proteins involved in iron acquisition, mobilization and utilization.

Alterations in subcellular localization

An important mechanism for altering the membrane transport of iron quickly is to change the location of the transport proteins. This is a much faster regulatory mechanism than altering gene expression, and usually serves as a ‘first line’ response to alterations in iron requirements. Often such changes are supported by longer-term regulation through translational or transcriptional mechanisms. Several examples serve to illustrate how changes in the location of the protein can affect iron transport. (1) DMT1 is expressed on the brush border membrane of enterocytes and remains there as long as the body requires iron. However, if iron transfer across the basolateral membrane decreases and enterocyte iron levels subsequently increase, then DMT1 can be internalized rapidly [171]. This prevents excess iron accumulating in the enterocytes when it is not required. (2) FPN can be rapidly removed from the plasma membrane following the binding of the iron regulatory peptide hepcidin and this prevents iron export [172]. The internalized complex is targeted to the proteasome for degradation. Details of hepcidin and its action are discussed below. (3) The proportion of TfR1 on the cell surface can vary depending on cellular iron status. When iron demand is high, more TfR1 is expressed on the cell surface, and vice versa [16]. And (4) a final example is the stabilization of TfR2 in the presence of diferric Tf and its redirection to recycling endosomes [173]. This may play a role in the regulation of body iron metabolism as TfR2 mediates important regulatory signals.

Despite the importance of processes such as these, we know very little about the mechanisms underlying the changes in subcellular localization and this is a fruitful area for future investigations. DMT1 has been shown to bind to several proteins likely to be involved in membrane protein trafficking and degradation. These include Ndflps1 and 2, proteins of the Nedd4 family that regulate DMT1 levels by acting as adaptors to recruit the ubiquitin ligase WWP2 [174]. In addition, the two major DMT1 isoforms have been shown to exhibit different trafficking patterns [175], consistent with their different physiological roles. The internalization of FPN in response to hepcidin binding involves phosphorylation at either of two adjacent tyrosine residues by Jak2 kinase [176, 177]. After internalization, the protein is dephosphorylated, ubiquitinated and targeted for degradation. Other studies have shown that the membrane-trafficking protein Mon1A is required for efficient transport of FPN to the plasma membrane of macrophages in an iron-dependent manner [178]. However, for all these processes, much remains to be learned.

Systemic iron metabolism

Plasma iron transport

Under normal physiological conditions, essentially all iron in the plasma is bound to transferrin. Only under pathological conditions, when iron entry into the circulation is excessive, do significant amounts of NTBI appear. Tf is one of the most abundant of all plasma proteins and, in a normal adult human, it is present at 25–50 μM (2–4 mg/ml). The great majority of Tf in the plasma is produced by hepatocytes, but locally it can also be synthesized by the CNS and testis (i.e., behind the blood-brain and blood-testis barriers, respectively) [151, 179, 180]. Its abundance means that Tf has substantial iron binding capacity, but under normal conditions it is only approximately 30% saturated with iron. Thus, Tf is able to buffer against a rapid increase in plasma iron and the prevent the accumulation of potentially toxic NTBI.

The structure of Tf has been extensively investigated [7, 181]. It consists of two globular lobes (the N and C terminal lobes), each of which can bind a single iron atom with exceptionally high affinity (K_d 10^{-23} M^{-1}) [78]. This explains why the concentration of plasma NTBI can be kept so low.

As noted above, the role of Tf in the body is not so much to deliver iron to the tissues (as NTBI can be very efficiently taken up by most cells) but to enable it to be delivered in a controlled or regulated way. Since it plays such an essential role, it is not surprising that Tf is synthesized at a constitutively high level that varies only over quite a limited range in response to various stimuli. Tf levels will increase slightly under iron-deficient conditions and can also be stimulated by hypoxia and estrogens [151, 182]. Most regulation of Tf appears to occur at the level of transcription, although there may be some regulation at the translational level [183].

Systemic regulation of iron homeostasis

No consideration of iron transport would be complete without mention of the recent major advances in our understanding of the coordinated regulation of body iron homeostasis. These advances have been driven by the demonstration that the liver-derived peptide hepcidin plays a critical role in modulating iron entry into the plasma from macrophages, intestinal enterocytes and other body cells [184–187] (Fig. 4). Hepcidin in its mature form is a 25 amino acid peptide that is secreted by hepatocytes [188]. It acts by binding to FPN and facilitating its internalization and degradation [172, 176], thus reducing iron export from the cell. It is the factors that regulate hepcidin synthesis that lead to an integrated system. Hepcidin levels are relatively high when the body is iron replete, but when iron requirements increase, hepcidin synthesis is reduced and iron entry into the plasma is stimulated. Iron deficiency, hypoxia, increased erythropoiesis and pregnancy are all situations where the body requires more iron and where hepcidin synthesis is reduced [188–190]. High iron levels and inflammation lead to increased hepcidin concentrations with the consequent partitioning of iron to storage sites and a reduction in iron absorption [188, 190].

Much effort in recent years has been directed at understanding how hepcidin is regulated. HFE, TfR2 and hemojuvelin (HJV) are all proteins of the hepatocyte plasma membrane that influence hepcidin expression. HJV exerts its effects by signaling through the BMP (bone morphogenetic protein)/SMAD pathway [191–193], and this pathway appears to play a central role in hepdcidin regulation. BMP6, which itself is regulated by iron, is emerging as a particularly important positive regulator of hepcidin [194, 195]. How HFE and TfR2 exert their effects is less clear. There is general agreement, however, that it is these proteins that communicate body iron needs to hepcidin. The nature of the extracellular signals that act on these proteins remains poorly understood, but there is solid evidence that the circulating level of diferric Tf is involved [196, 197]. There is also strong evidence that the hypoxia-inducible transcription factors (HIFs) play an important role in the iron response, and that hypoxia will decrease hepcidin expression [198]. The importance of hepcidin and its upstream regulators in maintaining body iron homeostasis is well demonstrated by the profound changes in iron balance (body iron loading) that are associated with

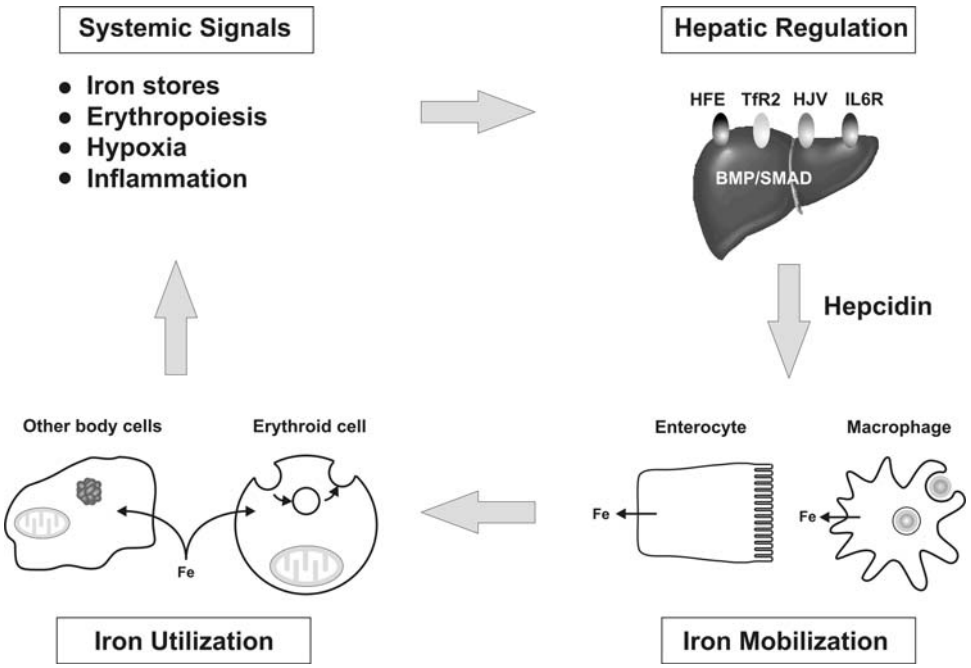

Fig. 4 The regulation of body iron metabolism. The amount of iron entering the plasma is controlled by body iron demand and this in turn reflects a range of stimuli. Low body iron levels, increased erythropoiesis and hypoxia are all stimuli for increased iron export into the circulation from the tissues. Inflammation has the opposite effect and leads to tissue iron withholding. These stimuli exert their effects by acting through a series of proteins on the hepatocyte plasma membrane, including *HFE*, *TfR2*, *HJV* and the *IL-6* receptor, to modulate the expression of the iron regulatory hormone hepcidin. Hepcidin in turn binds to FPN on the surface of target cells to influence how much iron they release. Enterocytes and macrophages are major targets, but it is likely that most body cells can respond to hepcidin. An increased hepcidin level leads to reduced FPN on the target membrane and reduced iron release. Iron that enters the plasma is used by all body cells for metabolic functions, but immature erythroid cells have particularly high iron requirements for hemoglobin synthesis. It is the iron requirements of these target cells which complete the cycle

mutations in these genes in humans [199–204]. The pathways involved in hepcidin regulation have been extensively reviewed (e.g., [205, 206]).

Iron transport in specialized mammalian cell types

There are some fundamental aspects of iron handling that are shared by most cells, but, superimposed upon this, each cell type handles iron in its own specific way that is appropriate for its function. Some cells turn over iron rapidly, others store it readily, and yet others, such as immature erythroid cells, utilize most of their iron for a single specific function, i.e., heme synthesis. Some brief summaries of the iron metabolism of some key cells involved in iron homeostasis are described below.

Intestinal enterocytes

In post-partum life, all iron entering the body must come from the diet, and the vast majority of this is absorbed across the mature enterocytes of the proximal small intestine [111]. These specialized absorptive cells can utilize both heme and non-heme iron. The enterocyte brush border membrane expresses high levels of DMT1 and DCYTB, particularly in times of high body iron demand. It also elaborates the protein(s) required for heme uptake, but whether HCP1 or some other protein is involved in this process is unclear. When iron is required by the body, it leaves the enterocyte through a pathway that requires FPN and hephaestin. The enterocyte is one of the most prominent sites of FPN expression and the main site of hephaestin expression. When iron demand is reduced, systemically produced hepcidin binds to FPN on the enterocyte basolateral membrane and causes its internalization and degradation, thus limiting iron efflux to the plasma and iron absorption overall. Iron subsequently accumulates in the enterocytes and this in turn leads to reduced DMT1 synthesis. The accumulated iron is stored within ferritin and is lost when the enterocytes migrate up the villus and are sloughed into the intestinal lumen. This enables the enterocyte to buffer against excessive iron absorption.

The immature enterocytes of the intestinal crypts handle iron quite differently from their mature counterparts. They are not specialized for vectorial iron transport and

act rather more like a generic cell. They are rapidly growing and dividing, so they absorb a large amount of Tf-bound iron from the serosal or 'blood' side, a characteristic not shown by mature enterocytes. Both TfR1-dependent and TfR1-independent diferric Tf uptake has been demonstrated in crypt enterocytes [37]. As the cells mature and differentiate, they acquire their absorption characteristics but lose the capacity to take up iron from the circulation.

Erythroid cells

Immature erythroid cells have the task of synthesizing hemoglobin and, consequently, they have the highest iron requirements of any cells [207]. They take up iron almost entirely through the Tf-TfR1 pathway, and non-TfR1-mediated iron utilization is negligible. Other proteins required for the delivery of Tf-bound iron, including DMT1 and STEAP3, are strongly expressed in these cells. They have little need to export iron and, consequently, FPN levels are low, although the capacity to export excess heme could be important [121]. Iron uptake in erythroid precursors is particularly prevalent in erythroblasts and reticulocytes [208], but as the cells mature they lose their ability to synthesize new proteins and their capacity to acquire iron. Since the erythroid compartment is quantitatively the most important sink for iron in the body, erythropoiesis-associated signals play a major role in controlling hepcidin expression and, consequently, how much iron is released into the plasma from other cells types.

Macrophages

The critical role of recycling iron in the body is largely carried out by macrophages [110]. They phagocytose senescent red cells, break them down, and recycle the iron. Heme released from degraded globin is the substrate for heme oxygenases 1 and 2 which cleave the ferri-protoporphyrin complex to release iron, carbon monoxide and biliverdin. The iron is then exported from the cells through FPN. Macrophages are perhaps the most prominent site of FPN synthesis of any cells in the body. Although a large amount of erythrocyte-derived iron passes through the macrophages, the great majority of this is not used for the cells' metabolic functions. Thus, macrophages also possess the capacity to take up Tf-bound iron. They also represent a major storage site for iron, and when iron is not required for hemoglobin synthesis or other functions, it is retained in macrophages in ferritin. Under pathological conditions, macrophages play an important role in scavenging hemoglobin/haptoglobin and heme/hemopexin complexes.

Hepatocytes

Iron homeostasis in hepatocytes is a microcosm of what happens in the body as a whole [209]. Most of the mechanisms of cellular iron uptake that have been described are operational in hepatocytes. They can readily utilize Tf-bound iron (via TfR1, TfR2 or TfR-independent processes) and NTBI. They can also scavenge various other forms of iron such as heme/hemopexin complexes and ferritin. Hepatocytes are the major site of iron storage and sequester iron within ferritin or its degradation product hemosiderin. In addition, hepatocytes are the major site of synthesis of key plasma proteins involved in iron homeostasis, namely Tf, Cp, hemopexin and haptoglobin.

The recognition that the hepatocyte plays the central role in orchestrating systemic iron homeostasis has come from the identification of hepcidin as the key iron metabolism regulator. The hepatocyte is the main site of synthesis of hepcidin, and it is secreted into the circulation and subsequently acts to control iron release from most body cells. The hepatocyte is also the main site of synthesis of the major hepcidin upstream regulators HFE and TfR2, and is a prominent site of HJV synthesis.

Concluding remarks

Over the last 10–15 years, our understanding of iron transport has advanced enormously. Before that time, we had a reasonable understanding of Tf endocytosis, but how iron traversed biological membranes was largely a mystery. Since that time, advances in molecular techniques, the availability of tractable experimental systems, such as knockout mice and yeast, and the analysis of inherited disorders of iron homeostasis have led to the identification of the important iron import and export proteins of the plasma membrane (DMT1 and FPN, respectively), as well as a range of organelle iron transporters and their supporting oxidoreductases. However, the molecular mechanisms by which these proteins transport 'free' and bound iron remain relatively poorly understood and represent important areas for ongoing research. This is also true of the cellular biology and, in many cases, the regulation of these proteins. The focus of much research in iron metabolism in recent years has been on how systemic signals for the regulation of body iron homeostasis exert their effects. The demonstration that the liver-derived peptide hepcidin can respond to body iron demand and regulate the release of iron into the plasma by binding to the iron exporter FPN has been a major breakthrough in iron physiology. It has also helped us to understand at the molecular level a range of iron-related pathologies. There is no indication that the rapid pace of research in this field

is going to slow anytime soon, and we can look forward to many exciting new discoveries.

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