Critical Reviews in Biochemistry and Molecular Biology, 34(5):285–314 (1999)

Biochemistry of Iron Uptake

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ABSTRACT: Recent information gained from genetic and biochemical studies of iron transport in yeast, coupled with the identification of specific mutations causing iron uptake disorders in mice and man, has provided new clues about the mechanisms involved in iron uptake. This article summarizes these discoveries and discusses their impact on our current understanding of the biochemistry of iron uptake.

KEY WORDS: iron transport, ferrireductases, ferroxidases, transferrin, transferrin receptors.

I. INTRODUCTION

Iron contributes a central and essential role in the metabolism of nearly all organisms, from microbes to man. Indeed, only members of the *Lactobacillus* family appear capable of sustaining life without iron (Archibald, 1983). The biological importance of iron results from its chemistry, which involves a reversible one electron oxidation-reduction reaction that exchanges ferrous and ferric iron between its two common oxidation states. This chemistry is harnessed to enable vital physiological activities such as the oxygen-carrying capacity of hemoglobin and the production of ATP supported by iron-dependent enzymes involved in electron transport. Unfortunately, the same oxidation-reduction reaction contributes to Fenton chemistry converting superoxide and its dismutation product, hydrogen peroxide, to the potent oxidizing hydroxyl radical (·OH):

FENTON REACTION

$$Fe(III) + O_2^{\cdot -} \rightarrow Fe(II) + O_2$$
$$Fe(II) + H_2O_2 \rightarrow Fe(III) + {\cdot}OH + OH^-$$

To enable survival, organisms must not only guard against the capacity of iron to unleash this highly reactive species, but they must also devise the means to manage iron in the face of the extreme insolubility of hydrolyzed ferric iron; the solubility product of $Fe(OH)_3$ is 10^{-38} M^4, and it is estimated that the free iron concentration in neutral solutions is 10^{-18} M. Thus, molecules involved in iron transport need to acquire and then maintain iron in a soluble and nontoxic form that is bioavailable. Therefore, it is not sur-

prising that multicomponent mechanisms for iron uptake have evolved to include a complex array of ferrireductases and ferroxidases as well as permeases. In fact, most organisms display multiple uptake systems under tight regulatory control. For example, *E. coli* utilizes six siderophore-mediated Fe(III) transport systems and one Fe(II) transport mechanism (reviewed by Braun et al., 1998). In response to environmental levels of iron, the Fur repressor tightly coordinates expression of these factors by the bacteria. Such regulation is a common but critical regulatory feature of iron uptake by all organisms in that *iron homeostasis is maintained by restricting its import rather than modulating export in states of iron overload*. This review focuses on the mechanism and regulation of iron uptake by eukaryotic cells and the recent discoveries that have identified the molecules involved.

II. MULTIFACTORIAL YEAST SYSTEMS

Studies of iron uptake by the yeast *Saccharomyces cerevisiae* have provided major contributions to our understanding of cellular iron transport through combined biochemical and genetic approaches. These investigations have revealed the complexity of this process through the identification of multifactorial iron uptake systems involving both reductases and oxidases that are tightly coupled to membrane permeases.

A. Ferrireduction and Iron Uptake

The first problem encountered in the acquisition of iron is the limited bio-availability of the ferric form due to the propensity for insoluble ferric hydroxides to form under aerobic conditions at neutral pH. Early biochemical evidence indicated that import of iron by yeast involved the reduction of ferric chelates to generate Fe(II) (Lesuisse and Crichton, 1987), and subsequent genetic analysis led to the identification of the ferrireductase genes FRE1 (Dancis et al., 1990, 1992) and FRE2 (Georgatsou and Alexandraki, 1994). Characterization of the products of these genes revealed that the expression of their activity was under transcriptional control in response to cellular iron levels such that ferrireductase activity is induced under iron-deficient conditions. Such studies supported the essential role for Fre1 and Fre2 in iron uptake and uncovered the tight homeostatic regulation of iron import by yeast. The iron-responsive transactivator Aft1 was later found not only to modulate FRE1 and FRE2 gene expression (Yamaguchi-Iwai et al., 1995), but also to control the synthesis of other yeast iron transport factors (Yamaguchi-Iwai et al., 1995; Martins et al., 1998). Klausner and colleagues have proposed a yeast "iron regulon" that becomes induced under conditions of iron deprivation (Yamaguchi-Iwai et al., 1996), and indeed the dominant mutant $AFT1^{up}$ allele supports increased yeast ferrireductase activity and iron.

The Fre1 protein has a predicted mass of 79 kDa and appears to be a polytopic integral membrane protein based on hydrophobicity analysis (Dancis et al., 1992). Sequence comparison reveals similarity with the large subunit $gp91^{phox}$ of cytochrome b_{558} especially in domains thought to contribute to the binding of FAD and NADPH cofactors by NADPH oxidoreductase (Shatwell et al., 1996). The FRE2 gene product displays similarity with $gp91^{phox}$ as well as FRE1 (Georgatsou and Alexandraki, 1994), and all three resemble the fission

yeast *Saccharomyces pombe* ferrireductase Frp1 (Roman et al., 1993). Coupled with $p22^{phox}$, $gp91^{phox}$ forms the membrane-associated b_{558} flavocytochrome component of the NADPH oxidoreductase complex in phagocytes. When phagocytes are stimulated, this complex transfers electrons from cytosolic NADPH across membranes to reduce molecular oxygen, thus generating superoxide anions to be utilized in host defense against microbial invasion. The homology with $gp91^{phox}$ led Shatwell et al. (1996) to speculate that Fre1, Fre2, and Frp1 are members of a membrane-associated flavocytochrome family capable of moving electrons across the bilayer. Indeed, these investigators have presented strong spectroscopic evidence demonstrating that Fre1 is a *b*-type cytochrome (Shatwell et al., 1996), consistent with the fact that heme-deficient yeast lack ferrireductase (Lesuisse and Labbe, 1989; Amillet et al., 1996). Mutation of any one of four critical histidine residues of Fre1 eliminates the entire heme spectrum associated with its expression (Finegold et al., 1996), revealing an intramembrane bis-heme motif that appears to be conserved in $gp91^{phox}$ of the NADPH oxidase complex as well.

Whether Fre1 incorporates a flavin group and is capable of oxidizing NADPH is somewhat equivocal, but yeast overexpressing Fre1 do have increased levels of membrane-associated FAD as well as enhanced diaphorase activity (Shatwell et al., 1996). However, it is significant that unlike the NADPH oxidoreductase, which effectively reduces molecular oxygen, Fre1 does not (Shatwell et al., 1996; Lesuisse et al., 1996). The b_{558} flavocytochrome of the NADPH oxidase complex contains not only the larger β subunit of $gp91^{phox}$ with the NADPH and FAD binding sites, but also the smaller α subunit of $gp22^{phox}$, which may play a regulatory role; the flavocytochrome is coupled with three additional cytosolic components in response to a Rac-activated signalling pathway to generate and transport electrons producing superoxide. These observations raise the question of whether Fre1 acts as a member of a multicomponent electron transport chain with other yeast factors (Lesuisse et al., 1996). Although some evidence suggests that levels of NADPH dehydrogenase activity in isolated yeast plasma membranes do not correlate with Fre1 expression, measurements of this diaphorase activity appear to reflect contributions from membrane-associated cytochrome P-450 (Lesuisse et al., 1997), and therefore the precise relationship between Fre1 and NADPH dehydrogenase activity remains to be fully explored. Shatwell et al. (1996) point out that expression of FRE1 from a high copy number plasmid is sufficient to increase cell surface iron reduction, concluding that any additional factors, if required, are not limiting for this activity. These authors also report that searches for additional ferrireductase-deficient mutants have failed to yield mutant alleles other than FRE1, although a mutant in UTR1 does modify reductase activity (Anderson et al., 1994). Lesuisse et al. (1996) also report that while expression of UTR1 from a multicopy plasmid only sightly increased ferrireductase activity, co-transformation with FET1 reveals strong synergistic effects. Unlike FRE1, UTR1 gene expression does not appear to be iron-responsive. The exact function of its product, which is predicted to be a cytosolic factor, remains to be rigorously determined. Because yeast ferrireductase activity is also modulated by pleiotropic factors such as cAMP (Lesuisse et al., 1991), one speculation is that ferrireduction may be post-transcriptionally regulated by a protein kinase A-dependent pathway potentially involving phosphorylation of Utr1 (Lesuisse et al., 1996). This notion arises because ferrire-

ductase activity persists in iron-rich media during late exponential growth, conditions under which FRE1/FRE2 transcription should be repressed (LeSuisse et al., 1990).

B. The Role of FET3 Ferroxidase in Fe(II) Transport

While the identification and characterization of the yeast ferrireductase Fre1 contributed a great deal of information regarding the mechanisms of iron uptake, the membrane transport molecules remained unknown. Biochemical studies revealed that yeast import iron through at least two distinct pathways: a high affinity system with a Km = 0.15 μ*M* and a lower-affinity system with a Km > 5 μ*M* (Lesuisse and Labbe, 1989; Dancis et al., 1990; Eide et al., 1992). What factors were involved in either of these process remained unknown, however, until two different genetic selections converged to identify an unexpected and surprising role for copper in high-affinity iron uptake.

Askwith et al. (1994) selected *S. cerevisiae* mutants defective in iron transport using the aminoquinone antibiotic streptonigrin, which is toxic in the present of iron. Cells grown in low iron have induced expression of Fe(II) uptake and therefore increased sensitivity to streptonigrin when challenged with iron-rich medium. One mutant that emerged from this screen, *fet3*, was found to have defective Fe(II) uptake despite normal levels of ferrireductase activity. However, although FET3 gene transcripts are regulated by iron as expected based on the selection of *fet3* yeast, its product was found to encode a single transmembrane-spanning domain factor that had similarity to multi-copper oxidases. Using a different approach, Dancis et al. (1994) provided evidence that also implicated important role for copper in yeast iron uptake. To select for yeast mutants defective in Fe(II) transport, this group of investigators utilized the FRE1 gene promoter that confers iron-dependent expression (Dancis et al., 1992) fused to HIS3 to produce a strain that was auxotrophic for histidine under iron-dependent conditions. Selection was performed on iron-rich plates without histidine such that mutants suppressed for the conditional auxotrophy could be identified. Complementation cloning identified a gene responsible for suppression in one such mutant given the designation CTR1. The CTR1 gene product is predicted to be a polytopic membrane protein with 11 repeats of the consensus M-X-X-M, an amino acid motif found in copper-binding proteins (Dancis et al., 1994). Most significantly, CTR1 deletion mutants were found to be not only defective in copper transport, but in iron uptake as well.

The relationship between the copper transporter Ctr1 and Fe(II) import was actually made evident by the multi-copper oxidase Fet3 implicated in iron uptake. Studies on the role of copper in Fe(II) uptake indeed revealed that copper-deficient cells are defective in iron uptake (Askwith et al., 1994). Moreover, while the Fe(II) uptake defect in Δ*ctr1* yeast could be suppressed by extremely high levels of copper (500 μ*M*), transport by Δ*ctr1*Δ*fet3* yeast could not be restored (Dancis et al., 1992). Thus, a model emerged that the function of Ctr1 in copper transport was required to support Fet3 activity, an essential component of a copper-dependent high-affinity iron transport system. Later studies were to reveal the role of a second transporter, Ccc2, that provides copper to Fet3 within a cytosolic compartment, indicating that the latter's oxidase activity is lumenal or extracellular (Yuan et al., 1995).

The identification of Fet3 enabled the ultimate discovery of its associated per-

mease, Ftr1. Using the same selection strategy described above that led to the discovery of Ctr1, Stearman et al. (1996) identified and characterized a second yeast strain defective in high-affinity iron transport mutated in a gene designated as FTR1. FTR1 mutants are phenotypically similar to FET3 mutants in the loss of high-affinity uptake that cannot be corrected by manipulating media copper concentrations. The FTR1 gene product appears to be a hydrophobic protein predicted to span the membrane bilayer six times, implicating this factor as the potential permease component of the copper-dependent Fet3-mediated iron uptake system. Like FET3, FTR1 gene expression is induced by iron starvation; as both are under control of the iron-regulated activator Aft1, they appear to be key components associated with the yeast iron regulon (Yamaguchi-Iwai et al., 1996; Stearman et al., 1996). Using high copy number plasmids to express these factors independently, the interactions between Fet3 and Ftr1 were confirmed; when either one is expressed alone, iron uptake is not enhanced, but transport is increased above wild-type levels when both are expressed (Stearman et al., 1996). Importantly, *ftr1* strains display poor Fet3 oxidase activity due to an apparent defect in its synthesis, glycosylation, and cellular localization; conversely, Ftr1 does not appear at the cell surface in *fet3* deletion strains. These observations led Stearman et al. (1996) to theorize that Ftr1 and Fet3 assemble into an iron transport complex in the secretory pathway wherein Fet3 acquires copper delivered by Ccc2 (Yuan et al., 1995). Arrival of both proteins at the cell surface together enables high-affinity iron uptake presumably through the Fet3-mediated oxidation of ferrous iron reduced by the yeast Fre1/Fre2 ferrireductases and the subsequent transfer of the cation across the bilayer by Ftr1 (Stearman et al., 1996).

Fet3 is tethered to the membrane via a single transmembrane spanning domain as a type 1 membrane glycoprotein. DeSilva et al. (1995) correlated iron-dependent O_2 consumption with Fet3 expression; trypsinized spheroplasts lost this iron-dependent activity confirming the topological disposition of the membrane protein's functional activity as being extracellular. These authors also confirmed the expected ratio for the ferroxidase reaction of 4Fe: $1O_2$:

$$4Fe(II) + O_2 + 4H^+ \rightarrow 4Fe(III) + 2H_2O$$

Only the human serum protein ceruloplasmin has been shown to catalyze this same reaction, and characterization of Fet3 has revealed its close structural similarities to this multi-copper oxidase. The properties of a soluble form of recombinant Fet3 lacking the C-terminal membrane-spanning domain were investigated by Kosman and colleagues (Hassett et al., 1998). The purified protein's spectral characteristics show that Fet3 has one of each of the three types of Cu(II) sites found in multinuclear copper oxidases like ceruloplasmin. Kinetic studies of the ferroxidase activity associated with soluble Fet3 also confirms the reaction stoichiometry of 4Fe: $1O_2$ with Km values determined to be 4.8 and 1.3 μM, respectively; this analysis is consistent with results reported for the membrane-bound form of the enzyme (De Silva et al., 1997). Despite the structural similarities with ceruloplasmin, Hassett et al. (1998) estimate that the k_{cat} value for Fe(II) turnover of the latter is some 10 to 50 times greater than that of the soluble Fet3. These authors speculate that association of the membrane-bound Fet3 with Ftr1 may enhance its capacity to oxidize Fe(II) this idea is supported by the fact that the Km for the ferroxidase reaction is much higher than the Km value

of 0.15 μ*M* measured for high-affinity iron uptake of Fe(II).

Initial studies of the Fet3/Ftr1 complex had indicated that both partners required each other for their assembly and trafficking to the cell surface (Stearman et al., 1996) in fact, only when both *S. pombe* homologs Fio1 (ferrous iron oxidase) and Fip1 (ferriferous permease) are co-expressed is high-affinity iron uptake restored to *S. cerevisiae fet3* yeast (Askwith and Kaplan, 1997). Thus, the questions of whether the membrane-bound multicopper oxidase Fet3 was only necessary for cell surface localization of the permease Ftr1 and to what extent, if any, its ferroxidase activity was required to support the iron transport reaction of Ftr1 were raised. To address these issues, Askwith and Kaplan (1998) performed site-directed mutagenesis studies. They found that mutations of Fet3 that inactivate its ferroxidase function did not interfere with its ability to co-assemble and deliver Ftr1 to the cell surface but did disrupt high-affinity uptake. Thus, these important studies demonstrated that not only does Fet3 play an important role in the membrane targetting and delivery of Ftr1 to the cell surface, but that its oxidase activity must also be intrinsic to the mechanism of Ftr1-mediated iron import.

Why ferrireduction that generates Fe(II) from Fe(III) is required for high-affinity uptake involving Fet3-mediated ferroxidation to reproduce Fe(III) from Fe(II) is still ambiguous. Kaplan and colleagues have proposed that Fet3 activity imparts specificity and selectivity of high-affinity Fe(III) uptake in combination with Ftr1 (Askwith and Kaplan, 1998; Askwith et al., 1996). Ferrireduction most likely plays an important role in extracting iron from ferric chelates to provide Fe(II), which is more soluble than the ferric form at physiologic pH; to prevent insoluble ferric hydroxides from forming, the ferroxidase activity must not only oxidize Fe(II) but also then capture the Fe(III) produced, maintaining its bioavailability for uptake presumably by providing the cation directly to Ftr1. However, the precise function of Ftr1 as the putative iron permease remains poorly understood because its activity has not been fully characterized. By sequence analysis, Ftr1 appears to be an integral membrane protein with multiple membrane-spanning domains, but its true topology has yet to be verified by biochemical experiments. Ftr1 does display the amino acid sequence REGLE resembling an iron binding motif in L-ferritin and mutations within this domain disrupt high-affinity transport activity even though Fet3 oxidase activity is unaffected (Stearman et al., 1996). Because co-expression of both of the *S. pombe* Fio1 and Fip1 homologs confers high-affinity uptake to *S. cerevisiae fet3* strains (Askwith and Kaplan, 1997), these two factors appear to be both necessary and sufficient for this activity and together they most likely represent the minimal transport machinery. However, exactly how Ftr1 acts as a permease, what its energy requirements might be, or whether Fe(III) is the actual cationic form of iron translocated across the bilayer by this protein are among the unanswered questions that remain. A second gene with homology to FTR1 has been identified as FTH1 (Stearman et al., 1996), and the Fet5 gene product that encodes a multicopper oxidase similar to Fet3 is also suggested to play a role in yeast iron uptake (Spizzo et al., 1997), but these activities and how they may relate to FET3/FTR1 function need to be more fully explored as well.

C. FET4 and other Yeast Iron Transport Mechanisms

The discovery and characterization of the FET3 gene in *Saccharomyces cerevisiae*

enabled genetic screens to identify the genes responsible for low-affinity iron uptake. Biochemical studies indicated that low-affinity iron transport was unaffected in *fet3* mutant strains, and this activity was found to prefer Fe(II) over Fe(III) for uptake (Dix et al., 1994). Using *fet3* mutants to select for genes that increase growth on iron-deficient plates when overexpressed, FET4 was identified. Like Ftr1, Fet4 is predicted to have six transmembrane-spanning domains, although it is slightly larger (~65 kDa vs. ~ 45 kDa). Uptake of Fe(II) correlates with expression levels of Fet4 and site-directed mutagenesis of selected transmembrane domain residues alters kinetic characteristics of Fet4-mediated uptake, strongly supporting the idea that this transporter interacts directly with iron as a substrate and therefore is intrinsic to its transbilayer passage.

Because *fet3fet4* mutant strains still survive, low-affinity Fe(II) uptake by Fet4 and high-affinity Fe(III) uptake mediated by Fet3/Ftr1 are not the only mechanisms by which yeast can acquire iron, and at least two other systems have been reported. One involves members of the Smf family, which are thought to be involved in transport of divalent metals; expression of a mouse protein called Nramp2 complements *smf1smf2* null mutants, and this factor is implicated to play a role in mammalian iron transport (Pinner et al., 1997). Another potential source of iron for the yeast *Saccharomyces cerevisiae* comes from its ability to scavenge iron-binding bacterial siderophores. In particular, Lesuisse et al. (1998) have characterized the ability of *fet3fet4* yeast strains to take up ferrioxamine B and ferricrocin via high-affinity transport mechanism(s). Mutants that have impaired ferrioxamine B uptake were identified to harbor defects in a gene designated SIT1 (siderophore iron transport) that is thought to encode a permease of the major facilitator superfamily. Moreover, because ferricrocin uptake is unaffected in SIT1 mutants, additional siderophore permeases are predicted to enable iron acquisition by *Saccharomyces cerevisiae* (Lesuisse et al., 1998).

D. From Yeast to Plants

It is worthwhile to note that advances in our understanding of yeast iron uptake have had a significant impact on our molecular understanding of iron transport in plants. Using the yeast *fet3fet4* double mutant, Eide and colleagues (Eide et al., 1996) were able to clone an iron-regulated metal transport protein called Irt1 from *Arabidopsis thaliana* by functional expression. Their approach was based on evidence indicating that very much like yeast, many plants utilize a similar strategy (called strategy I) to induce a surface reductase under iron-poor conditions to reduce Fe(III) into its more bioavailable form (Fett et al., 1998). Although it lacks significant sequence homology with either Fet3, Ftr1, or Fet4, the discovery of Irt1 has been complemented recently by the identification of a ferrireductase gene FRO2 (Robinson et al., 1999). Using degenerate primers common to the FAD binding site of $gp91^{phox}$ and Fre1/2, a PCR fragment was produced to screen DNA libraries in order to identify plant members of the flavocytochrome family, one of which (FRO2) was found to be allelic with *Arabidopsis frd1* mutations that disrupt ferric reductase activity. Like other family members discussed above, the protein encoded by FRO2 is predicted to transport electrons across the membranes and to bind heme and nucleotide cofactors, although there is rather little homology between this factor and $gp91^{phox}$ or Fre1/2 based on sequence comparison (Robinson et al., 1999). Nonetheless, the coupling of ferrireduction to the import of

iron appears to be a common mechanistic paradigm utilized by different organisms because the molecules involved appear to be relatively conserved in function if not in sequence.

E. Coordinate Control of Iron Uptake

As discussed earlier, iron homeostasis is maintained by regulating its import: under times of deficiency, uptake must be stimulated, while under iron-rich conditions transport should be suppressed to prevent toxicity due to iron overload. The identification of the iron-responsive Aft1 as a key transcriptional regulator of FET3, FTR1, FRE1/FRE2, and the copper transporter CCC2 led to the idea of the yeast iron regulon coordinating control of iron uptake (Yamaguchi-Iwai et al., 1995; Yamaguchi-Iwai et al., 1996). Aft1 also has been shown to regulate the expression of the copper chaperone Atx1, which is responsible for the delivery of copper to Fet3 (Lin et al., 1997). Additional studies revealed that the yeast ferrireductase Fre1 was also involved in Cu(II) reduction and uptake (Hassett and Kosman, 1995; Georgatsou et al., 1997) and that FRE1 and other genes responsible for yeast copper uptake are under the control of a copper-responsive transcription factor Mac1 (Graden and Winge, 1997; Labbe et al., 1997; Yamaguchi-Iwai et al., 1997). Although additional FRE homologs have been identified in the yeast genome, unlike FRE1 they appear to be exclusively regulated by iron (FRE2–FRE6) or copper alone (FRE7; Finegold et al., 1996; Martins et al., 1998). Thus, the intricate cross-talk between copper and iron metabolism mediated through Mac1 and Aft1 enables yeast to sense when copper-limiting conditions might restrict iron transport by upregulating FRE1 to guard against iron deficiency and to ensure under iron-limiting conditions that the necessary cadre of gene products (e.g., Ccc2 and Atx1) are available to fulfill the copper requirements for FET3 function.

Although FET4 gene expression is regulated by iron, low-affinity iron uptake is not under control of Aft1-mediated transcriptional activation (Dix et al., 1997). This is a subtle but important point because of questions surrounding how Aft1 itself senses cellular iron levels to regulate high-affinity import. Aft1 binds DNA in an iron-dependent manner, and it has been proposed that Fe(II) is the species that represses the function of this trans-activator (Yamaguchi-Iwai et al., 1995; Yamaguchi-Iwai et al., 1996). However, this model presents an apparent paradox because Fe(III) would be the predicted form of iron imported by the yeast high-affinity Fet3/Ftr1 system. Because O_2 also plays a role in the regulation of FET3/FTR1 genes, Kosman and colleagues have proposed that dioxygen modulates the ratio of Fe(III)/Fe(II) in a labile pool of cellular iron responsible for Aft1 regulation and that Fet4 is a likely source for this intracellular Fe(II) pool (Hassett et al., 1998). How Fet4 expression itself is regulated by iron still remains unknown. There is evidence to suggest that the activity of the Nramp family member Smf1 is controlled by posttranslational degradation in response to heavy metals (Liu and Culotta, 1999), suggesting that posttranscriptional regulatory mechanisms exist that may regulate the import of toxic metals by yeast.

III. UPTAKE OF TRANSFERRIN- AND NONTRANSFERRIN-BOUND IRON BY MAMMALIAN CELLS

Like yeast, mammalian cells have evolved multiple pathways of iron import

that are tightly regulated to guard against deficiency and overload. Despite the rapid progress in our molecular understanding of the components involved in yeast iron uptake, surprisingly little information about the mechanisms of mammalian cell transport has been garnered from these studies. Instead, insights have been generated from recent advances in our understanding of the molecular basis of genetic disorders found in mice and humans.

A. TRANSFERRIN-MEDIATED IRON UPTAKE

To handle the complications of safe and effective iron delivery between organs and tissues, mammalian systems have evolved a serum iron-binding protein, transferrin, to maintain Fe(III) in a soluble, circulating form that is bioavailable yet non-toxic. This ~80-kDa glycoprotein has two homologous domains, each of which binds an atom of Fe with extremely high affinity (Kd ~ 10^{-23} *M*) and in a reversible manner. It is synthesized predominantly in the liver and in a manner that responds to iron stores, that is, higher rates of synthesis occur under conditions of iron deficiency or anemia and are lower when iron stores are excessive. Transferrin may acquire newly absorbed dietary iron as well as iron that has been liberated from sensecent red cells phagocytosed by macrophages of the reticuloendothelial system or other non-erythroid sources. Indeed, there is a high degree of conservation of iron within the body such that its turnover is about 30 mg/day, with about 80% of this pool directed toward heme synthesis in erythroid cells (for detailed recent reviews see Ponka et al., 1998; Richardson and Ponka, 1997).

How these conserved iron pools are rapidly turned over and released to circulating transferrin is rather poorly understood. However, it is well known that copper-deficient animals have deranged iron metabolism, exhibiting what appears to be an iron-deficiency anemia despite adequate levels of iron. Instead, iron accumulates in duodenal enterocytes and reticuloendothelial cells of these animals, leading to the idea that copper is required for the release of iron to the circulation (Lee et al., 1968). These observations led to the proposal that loss of the serum ferroxidase ceruloplasmin activity due to copper deficiency results in failure to mobilize iron because the oxidation of Fe(II) to Fe(III) is required for this process to occur. In fact, restoration of ceruloplasmin activity promotes the release of iron to the circulation in the copper-deficient animals (Lee et al., 1968) and in studies of perfused liver, Osaki et al. (1971) were able to confirm a direct role for the serum ferroxidase in iron release. More recent genetic evidence that strengthens this model has arisen from molecular characterization of the disease aceruloplasminemia; patients with this disorder lack ceruloplasmin and display iron-loading in a number of tissues (Harris et al., 1995; Yoshida et al., 1995). Thus, unlike yeast, which require the ceruloplasmin homolog Fet3 for high-affinity iron uptake, mammalian cells appear to utilize the serum ferroxidase to liberate stored iron instead.

Circulating transferrin binds to specific cell surface receptors that are constitutively endocytosed. Much of our knowledge of the endocytic pathway has arisen from detailed studies of the transferrin receptor trafficking pathway and its entry and transit through acidic intracellular compartments called endosomes (Mukherjee et al., 1997). Transferrin binds iron tightly at neutral pH but after entering the acidic milieu of the endosomal compartment, it discharges iron (Klausner et al., 1983; Dautry-Varsat et al., 1983) most likely by a mechanism that is facilitated by its binding to the transferrin

receptor (Bali and Aisen, 1992; Bali and Aisen, 1991; Bali et al., 1991; Sipe and Murphy, 1991). The transferrin receptor is a type II membrane glycoprotein with a single transmembrane domain orienting its C-terminus to the outside of the cell. It functions as a homodimer with each ~90-kDa subunit capable of binding one molecule of transferrin. The crystal structures of both transferrin (Bailey et al., 1988) and the ectodomain of the transferrin receptor have been solved* such that detailed molecular information regarding functionally important residues may now emerge for both proteins.

Transferrin receptor synthesis is post-transcriptionally controlled in response to cellular iron levels such that when the demand for iron is high, the pool of receptors available to bind transferrin is increased. This effect is mediated through iron-responsive elements (IREs) in the 3′ untranslated region of the receptor message to which iron regulatory proteins (IRPs) can bind and stabilize the mRNA against degradation (Kuhn, 1998). Recently, a second isoform has been identified with close structural resemblance and functional properties related to the well-characterized transferrin receptor; however, its transcript appears to lack the necessary IREs to promote iron-dependent regulation by this mechanism (Kawabata et al., 1999).

While the transferrin/transferrin receptor cycle has been studied extensively, the relative physiological importance of this mechanism remained undetermined until the recent generation of transferrin receptor knockout mice (*Trfr*$^{-/-}$) (Levy et al., 1999) . Previous studies of mice with hypotransferrinemia (*hpx*) demonstrated that these animals could survive with little or no circulating transferrin, although they suffer from severe anemia (Bernstein, 1987). Of great interest is the fact that *hpx* mice actually absorb iron in an excessive manner (Buys et al., 1991) and become iron-loaded (Bernstein, 1987; Craven et al., 1987), suggesting that transferrin was not requisite for delivery to tissues other than erythroid cells. However, studies of *Trfr*$^{-/-}$ mice confirm the central role of transferrin-mediated iron uptake as homozygous animals die *in utero* with impaired erythropoiesis and defective neurological development. Levy et al. (1999) hypothesize that the *Trfr*$^{-/-}$ defects are more severe than those observed in homozygous *hpx* mice because in the latter situation insufficient amounts of transferrin result in an increased pool of nontransferrin-bound iron available for uptake by alternative mechanisms (see below). In the homozygous *Trfr*$^{-/-}$ animals, transferrin is present to chelate free iron, but its ligand cannot be made bioavailable to cells in the absence of the transferrin receptor.

A second remarkable advance in our understanding of the transferrin/transferrin receptor cycle was uncovered by the molecular identification of the gene responsible for hereditary hemochromatosis (Feder et al., 1996). This common autosomal-recessive disease induces progressive iron-loading of tissues, most notably the liver, leading to cirrhosis and hepatoma as well as cardiomyopathies, endocrinophathies, and arthritis. A single point mutation (C282Y) that disrupts the function of an MHC class I-like molecule called HFE is found in ~80% of patients. The fact that defects in this gene promote iron overload has been confirmed in studies of HFE knockout animals that are found to recapitulate the human disease (Zhou et al., 1998; Levy et al., 1999). Like other MHC class I molecules, HFE associates with β2-microglobulin, and this interaction is disrupted by the C282Y mutation such that the protein fails to be expressed at the cell surface (Waheed et al., 1997; Feder et al., 1997). These observations help to explain the iron overload observed in

* C. M. Lawrence and S. C. Harrison, *in press*.

β2-microglobulin knockout animals because they would also fail to produce functional HFE (De Sousa et al., 1994; Rothenberg and Voland, 1996). Characterization of HFE revealed that not only does it associate with β2-microglobulin, but immunoprecipitation (Feder et al., 1998) and *in vitro* binding experiments (Parkkila et al., 1997; Lebron et al., 1998) also demonstrated its ability to bind to transferrin receptors. Thus, models of transferrin/transferrin receptor trafficking must now incorporate the additional complexity of interactions with this MHC class I-like complex.

The precise functional role of HFE and its regulation of transferrin receptor activity remain obscure. HFE interaction with the receptor is known to lower its affinity for transferrin ~10–fold (Lebron et al., 1998; Feder et al., 1998). However, the physiological impact of this effect would appear to be insignificant because circulating levels of transferrin are micromolar compared with the nanomolar Kd for the receptor, that is, receptor binding sites would be occupied with ligand at all times despite the observed increase in Kd. Using inducible expression to overproduce HFE in HeLa cells, it has been shown that HFE rapidly associates with and is stabilized by its association with transferrin receptors during biosynthesis in the secretory pathway (Gross et al., 1998; Salter-Cid et al., 1999). However, equivocal results have been reported regarding its subsequent interactions with the receptor in the endocytic pathway. Enns and colleagues have demonstrated that HFE and transferrin receptors co-traffic into endosomes (Gross et al., 1998) and that HFE overexpression does not alter receptor endocytosis or recycling (Roy et al., 1999). In contrast, Salter-Cid et al. (Salter-Cid et al., 1999) report that overexpression of HFE reduces the number of functional cell surface transferrin binding sites and impairs receptor internalization. Nonetheless, overexpression of HFE has the net effect of diminishing transferrin-mediated iron uptake in HeLa cells (Roy et al., 1999). Roy et al. (1999) propose that HFE interacts to block release of iron from the transferrin/transferrin receptor complex within the endosome, although it is not clear whether this action is direct or indirect because HFE dissociates from the receptor at low pH (Lebron et al., 1998). Alternatively, these authors suggest that HFE might modulate transporters responsible for the passage of iron across the endosomal membrane in subsequent stages of iron delivery.

The idea that the endosome is the functional site of HFE action is in keeping with histochemical studies that show its predominantly intracellular localization and association with transferrin receptors of intestinal enterocytes (Parkkila et al., 1997; Waheed et al., 1999). Increased intestinal iron absorption in hemochromatosis patients results in the massive overload in other tissues (McLaren et al., 1991), suggesting that HFE-transferrin receptor interactions must modulate iron homeostasis at the level of dietary iron uptake by the enterocyte. However, enterocytes of hemochromatosis patients actually appear to be iron deficient relative to the overload status created by this disease (Pietrangelo et al., 1995). Thus, while the functional studies in HeLa cells suggest that the loss of HFE function in hemochromatosis promotes iron assimilation from the transferrin/transferrin receptor pathway, how the opposite effect may be elicited in intestinal cells to regulate dietary absorption awaits determination (Waheed et al., 1999).

B. Nontransferrin-Bound Iron Uptake

The prediction that specific mechanisms exist to import nontransferrin bound iron

(NTBI) is bolstered by studies of the *hpx* mouse, wherein tissue iron overload occurs despite the animal's hypotransferrinemia (Bernstein, 1987; Craven et al., 1987), and pathophysiologic states of human iron overload such as hemochromatosis, wherein circulating nontransferrin iron levels can reach micromolar levels (Batey et al., 1980; Gutteridge et al., 1985). Indeed, this prediction is borne out to the extent that NTBI uptake has been identified and characterized in many different cell types, including hepatocytes (Brissot et al., 1985; Wright et al., 1986; Wright et al., 1988; Wright and Lake, 1990; Barisani et al., 1995; Baker et al., 1998), reticulocytes and erythrocytes (Egyed, 1988; Morgan, 1988; Qian and Morgan, 1991; Quail and Morgan, 1994; Hodgson et al., 1995), fibroblasts (Sturrock et al., 1990; Kaplan et al., 1991; Oshiro et al., 1993), HeLa cells (Sturrock et al., 1990; Yu and Wessling-Resnick, 1998), CHO cells (Sturrock et al., 1990; Kaplan et al., 1991; Chan et al., 1992), K562 cells (Inman and Wessling-Resnick, 1993; Inman et al., 1994; Conrad et al., 1994; Attieh et al., 1999), cardiomyocytes (Parkes et al., 1997), monocyte-derived macrophages (Olakanmi et al., 1994), and HepG2 cells (Parkes and Templeton, 1994; Randell et al., 1994; Mukhopadhyay et al., 1998). The import of dietary iron across the microvillous membrane of duodenal enterocytes represents a unique and physiologically significant form of nontransferrin-bound iron uptake and is discussed further below.

Both high- and low-affinity uptake mechanisms for NTBI uptake have been characterized with Km values ranging from 0.5 to 20 μM. These different uptake mechanisms can be distinguished not only by their apparent affinity for substrate iron, but also by their sensitivity to various other transition metals (Sturrock et al., 1990; Kaplan et al., 1991; Inman and Wessling-Resnick, 1993; Attieh et al., 1999; Savigni and Morgan, 1998). These properties suggest that like yeast, mammalian cells have multiple transport systems to mediate iron uptake, each with different mechanistic properties. For example, low-affinity iron uptake can be mediated the Na^{+}/Mg^{+2} antiport system in erythroid cells (Stonell et al., 1996). In general, NTBI uptake appears to require energy (Qian and Morgan, 1991; Quail and Morgan, 1994; Gutierrez et al., 1998), but how this is coupled to transbilayer movement of iron remains to be rigorously determined and is most likely dependent on the exact transport system utilized. Evidence to support the regulation of NTBI transport in response to iron loading has been mixed; exposure of some cells to iron in the form of ferric ammonium citrate (FAC) appears to enhance the Vmax of transport and thereby induce uptake of iron (Kaplan et al., 1991; Parkes et al., 1993; Randell et al., 1994). However, Richardson and Ponka (1995) have reported that addition of free radical scavengers suppresses FAC-induced uptake, suggesting that this process may occur via FAC-stimulated production of hydroxyl radicals rather than through up-regulation of transporter synthesis and/or recruitment to the cell surface. Other trivalent metals also have been reported to influence NTBI uptake, but their mechanism of action remains undetermined (Olakanmi et al., 1997; Chitambar and Wereley, 1997).

Interestingly, despite the intense study of iron import, iron export or release from cells remains rather obscure. A prediction is that the copper-binding ferroxidase ceruloplasmin would stimulate this process, but contradictory results have been reported (Young et al., 1997; Mukhopadhyay et al., 1998). However, iron chelators can promote the efflux of iron from cells and this process appears to be time-, temperature-, and energy-dependent (Baker et al., 1981;

Richardson, 1997). These properties suggest that specific export systems exist for the release of iron from cells, although whether this occurs via NTBI import mechanisms pumping in reverse is unclear.

C. Melanotransferrin (p97) and other Iron Transport Systems

In 1982, Brown et al. (1982) reported the discovery of a membrane protein structurally related to transferrin that is associated with human melanomas. Melanotransferrin (p97) has one intact transferrin-like Fe(III)-binding site (Baker et al., 1992), but unlike transferrin, it is linked to the surface of cells via a glycosylphosphatidylinositol anchor (Food et al., 1994). Because enhanced iron delivery could potentially aid melanoma cell proliferation, it has been speculated that melanotransferrin may represent an iron import pathway for these rapidly growing cells (Brown et al., 1982). Overexpression of melanotransferrin by transfected CHO cell lines has revealed its capacity to be internalized and to deliver iron (Kennard et al., 1995), thereby defining a novel transferrin receptor-independent pathway for iron uptake (Jefferies et al., 1996). The physiological relevance of this uptake system remains to fully ascertained; although an iron-binding surface protein that corresponds to melanotransferrin has been characterized for the SK-MEL-28 melanoma cell line (Richardson and Baker, 1990), this factor does not appear to mediate significant levels of iron uptake (Richardson and Baker, 1991; Richardson and Baker, 1991).

A second transferrin homolog implicated in an alternate pathway for iron uptake is lactoferrin. This iron-binding protein is found not only in milk, as its name implies, but it is also secreted by neutrophils. Despite the fact that its role in immune function serves to scavenge iron, like transferrin it can bind and deliver iron to hepatocytes that clear circulating lactoferrin by endocytosis (McAbee, 1995). Lactoferrin also appears to bind to the intestinal brush border membrane where it may mediate Fe(III) delivery as well, although the relative significance of its contribution to dietary iron absorption remains to be fully determined (for detailed review see Lonnerdal and Iyer, 1995).

Finally, it is important to note that a transferrin receptor-independent pathway also exists for iron delivery from transferrin. This process is best characterized for hepatocytes (Page et al., 1984; Trinder et al., 1988; Trinder et al., 1996; Trinder and Morgan, 1997), although studies in other cells have implicated a similar low-affinity nonreceptor-mediated absorptive process (Richardson and Baker, 1992; Chan et al., 1992). The lack of receptor involvement in this process is confirmed by the fact that when hepatocytes are incubated with a blocking antibody against its receptor, transferrin-mediated iron delivery continues (Trinder et al., 1988). Furthermore, an N-terminal "half-transferrin" that binds poorly to the transferrin receptor still delivers iron to hepatocytes (Thorstensen et al., 1995), and suppression of transferrin receptor synthesis using antisense approaches reduces but does not completely block transferrin-mediated iron uptake (Sasaki et al., 1993; Trinder et al., 1996; Trinder and Morgan, 1997). The mechanism by which cells acquire transferrin-bound iron in the absence of transferrin receptors appears to share elements in common with pathway(s) for NTBI uptake (Trinder and Morgan, 1997; Graham et al., 1998). Although iron delivery by the receptor-independent mechanism can proceed via the endocytic pathway (Trinder et al., 1996), inhibition studies suggest that some uptake occurs at the cell surface (Trinder and Morgan, 1997). The

fact that transferrin coupled to Sepharose beads can still provide iron to cells without being internalized further suggests that plasma membrane components are available to mediate this process (Oshiro et al., 1993). Whether this involves the reduction and release of iron from transferrin at the cell surface has been debated (Low et al., 1996; Thorstensen and Romslo, 1988; Thorstensen and Aisen, 1990), but it does appear that hepatocytes can effectively mobilize iron from chelates to extracellular acceptor molecules (Schieber and Goldenberg, 1998).

D. Biochemistry of Mammalian Transmembrane Iron Uptake

One of the most intensely studied and perhaps the most important physiological system that has been investigated to define biochemical mechanisms associated with mammalian iron transport is the uptake and export of iron by enterocytes. Because iron is so highly conserved by the body, dietary iron absorption must be tightly regulated to prevent overload or deficiency. Thus, the intestinal mucosa must respond to the body's iron levels in an appropriate manner, not only modulating the uptake of dietary iron across the brush border, but also controlling release of absorbed iron across the basolateral membrane to the blood. Furthermore, the delivery of iron to fulfill the cellular and metabolic demands of the enterocyte must be carried out by internalization of transferrin-transferrin receptor complexes into endosomal compartments with the subsequent release of iron and its transmembrane movement into the cytoplasm or via the direct uptake of circulating NTBI at the enterocyte basal surface. Hence, the polarized enterocyte must manage iron import from two cellular poles as well as its entry across intracellular endosomal membranes, while regulating the uptake, transfer, and efflux of dietary iron across the intestinal cell to maintain the body's iron homeostasis.

The numerous biochemical studies of intestinal iron transport have led to a three-component model for iron uptake involving (1) iron-binding moieties, (2) ferrireductase activity, and (3) specific transport molecules. The molecular contributions of various ligand-binding sites to iron transport remains ambiguous, but a number of brush border iron-binding proteins have been described (Teichmann and Stremmel, 1990; Conrad et al., 1993; Conrad et al., 1990; Danielsen and van Deurs, 1995; Nichols et al., 1992; O'Donnell and Cox, 1982). In particular, a 520-kDa membrane complex called paraferritin that contains integrin, mobilferrin (a calreticulin homolog), and flavin monooxygenase is suggested by Conrad and co-workers to participate in uptake of dietary iron bound in the gut lumen by mucin (Umbreit et al., 1998). While the relative contribution to the dietary absorptive process of these various iron-binding moities has yet to be rigorously assessed, the notion that iron binding is an initial step in the import process is consistent with characteristics associated with NTBI uptake and ligand sites reported for other cell types (Akompong et al., 1995; Barisani and Wessling-Resnick, 1996; Musilkova et al., 1998; Graham et al., 1998; Schieber and Goldenberg, 1998).

The contribution of a mucosal ferrireductase activity in iron uptake has been studied in isolated duodenal fragments (Raja et al., 1992; Riedel et al., 1995), and preliminary characterization of this enzyme has been performed using the model intestinal cell lines Caco-2 (Ekmekcioglu et al., 1996) and HuTu-80 (Riedel et al., 1995). Its activity is trypsin-sensitive and heat-labile (Riedel

et al., 1995), and values for Km (4 μ*M*) and Vmax (3 nmol/min/mg protein) have been reported with NADH as electron donor (Ekmekcioglu et al., 1996). The rate of mucosal ferrireduction is quantitatively higher than the rate of iron uptake, but increases in the Vmax of this activity parallel changes in iron uptake induced by iron deficiency (Raja et al., 1992). Moreover, duodenal mucosal biopsies from patients with hemochromatosis display higher ferrieductase activity compared with normal controls, a finding that is also consistent with higher iron uptake measured for these specimens (Raja et al., 1996). Finally, the functional importance of Fe(III) reduction in brush border iron import has been confirmed by studies in Caco-2 cells wherein inhibition of ferrireductase activity was found to reduce brush border iron uptake (Han et al., 1995; Nunez et al., 1994).

The model that reduction of Fe(III) precedes uptake of Fe(II) across the microvillous membrane of the intestine not only agrees with the defined role of ferrireduction in iron uptake by yeast and plants described above, but this mechanism is also compatible with properties found for NTBI uptake by other mammalian cells. For example, K562 cells display a surface ferrireductase activity (Goldenberg et al., 1990; Berczi et al., 1991; Schweinzer and Goldenberg, 1993) that has been shown to be involved in NTBI transport (Inman et al., 1994), and cell-mediated ferrireduction also appears to be important for uptake by HepG2 cell (Randell et al., 1994) as well as HeLa cells and fibroblasts (Jordan and Kaplan, 1994). The fact that Fe(II) chelators block transport when Fe(III) is presented to cells is consistent with a mechanism involving a ferrireduction (Inman and Wessling-Resnick, 1993; Randell et al., 1994), and further evidence is provided by the fact that ferricyanide not only inhibits ferrireductase activity, but iron uptake as well (Inman et al., 1994). Finally, cadmium has been found to inhibit K562 cell ferrireductase and iron transport activities with nearly the same IC50, suggesting that these two processes are coupled (Inman et al., 1994).

While the molecule(s) responsible for the ferrireductase activity implicated to play a role in intestinal iron uptake have yet to be identified, a candidate transporter has been discovered. Using a functional expression cloning approach in *Xenopus* oocytes to isolate factors mediating Fe(II) uptake, Gunshin et al. (1997) identified a homolog of Nramp1, a protein that had been previously identified to function in host resistance to pathogens (Vidal et al., 1993; Gruenheid et al., 1995). The characterization of this factor's activity elucidated its ability to mediate import of iron, among other divalent metals, and defined its iron-regulated expression in rat intestine (Gunshin et al., 1997). Using a genetic approach, Fleming et al. (1997) found that *mk* mice, which suffer from microcytic anemia due to defects in intestinal iron absorption (Edwards and Hoke, 1972), have a mutation in the gene for this same factor. Thus, these two independent lines of evidence converged to reveal the role for DMT1 (divalent metal transporter 1) in intestinal iron absorption. This factor is often referred to also as Nramp2 or DCT1 (divalent cation transporter 1).

DMT1 transport activity is proton coupled, and it is thought that the stoichiometry of symport is 1 Fe^{+2}: 1 H^{+} (Gunshin et al., 1997). The apparent affinities for Fe^{+2} and H^{+} are 6 μ*M* and 1 to 2 μ*M*, respectively. With 77% similarity with Nramp1, DMT1 is a member of the Nramp family of polytopic membrane proteins having 12 predicted transmembrane spanning domains with a conserved hydrophobic core of 10 transmembrane segments and a consensus "transport" signature (Cellier et al., 1995).

Nramp family members have been identified in yeast as Smf1 and Smf2. Functionally, DMT1 can complement *smf1/smf2* yeast (Pinner et al., 1997), supporting the idea that this transporter mediates manganese as well as iron uptake because overexpression of Smf1 by yeast results in increased Mn^{+2} uptake (Supek et al., 1996). In fact, studies of DMT1 activity in *Xenopus* oocytes indicate a rather broad substrate range for transport in the order: Fe^{+2}, Zn^{+2}, Mn^{+2}, Co^{+2}, Cd^{+2}, Cu^{+2}, Ni^{+2}, Pb^{+2} (Gunshin et al., 1997). It is known that Mn^{+2}, Zn^{+2}, Cu^{+2}, and Cd^{+2} can inhibit intestinal iron absorption (Rossander-Hulten et al., 1991; Iturri and Nunez, 1998), reinforcing the role of a multi-metal uptake system in this transport process.

The DMT1 protein is heavily glycosylated and migrates on SDS-polyacrylamide gels with an apparent $M_r \sim 90$ to 100 kDa (Gruenheid et al., 1999). The *mk* mutation (G185R) in DMT1 (which affects iron uptake activity) corresponds to an adjacent residue when aligned with the *bcg* mutation (G169D) in Nramp1 (which confers host susceptibility to infection), prompting Su et al. (1998) to speculate that the fourth transmembrane domain may represent an important functional element for transport. Unlike Nramp1, however, which appears to be exclusively expressed in reticuloendothelial cells, DMT1 mRNA is found in many different tissues (Gruenheid et al., 1995; Gunshin et al., 1997). DMT1 protein and mRNA are most abundant in the proximal duodenum and decrease along the distal axis, consistent with functional pattern of iron absorption by the intestine (Gunshin et al., 1997; Canonne-Hergaux et al., 1999).

Transfection experiments in HEK293T cells, CHO cells, and RAW macrophages revealed that in addition to the plasma membrane, DMT1 is localized to endocytic compartments involved in the transferrin-transferrin receptor internalization pathway (Su et al., 1998; Gruenheid et al., 1999). These findings suggest that in addition to its apparent role in intestinal iron absorption, DMT1 may function in iron delivery to peripheral tissues via transferrin-mediated uptake and indeed defects in erythroid iron assimilation by *mk* animals have been reported (Bannerman et al., 1972; Harrison, 1972). The link between DMT1 function and transferrin-mediated iron uptake was confirmed by the remarkable finding that another animal model of iron deficiency anemia, the Belgrade (*b*) rat, harbors the exact same mutation in DMT1 as found for the *mk* mouse (Fleming et al., 1998). While defects in intestinal iron absorption have been reported for the *b* rat (Oates and Morgan, 1996), perturbations in iron uptake from transferrin by Belgrade reticulocytes have been characterized more extensively (Bowen and Morgan, 1987; Edwards et al., 1986; Edwards et al., 1980). Transferrin is endocytosed by these cells, but iron is not captured and is instead recycled with transferrin and the transferrin receptor back to the cell surface (Farcich and Morgan, 1992; Garrick et al., 1999). Because functional defects are observed in NTBI uptake by Belgrade reticulocytes as well, the lack of iron assimilation from transferrin cannot be simply assigned by failure to release Fe(III) within endosomes; rather, this effect appear to be associated with a loss in transmembrane transport activity (Farcich and Morgan, 1992) Savigni and Morgan, 1998; Garrick et al., 1999). Studies on the uptake of other transition metals further support the idea that DMT1 is involved in NTBI uptake: (1) erythroid Fe^{+2} transport can be blocked by several divalent cations (Mn^{+2}, Zn^{+2}, Co^{+2}, and Ni^{+2}) (Morgan, 1988; Stonell et al., 1996; Savigni and Morgan, 1996), (2) the uptake of Fe^{+2} as well as Mn^{+2}, Co^{+2}, Zn^{+2}, Cd^{+2}, and Ni^{+2} is reduced in Belgrade

reticulocytes (Savigni and Morgan, 1998) and (3) Belgrade animals have defective manganese metabolism (Chua and Morgan, 1997). These characteristics identify a common pathway for divalent metal transport involving DMT1 that is most likely involved in both cell surface NTBI uptake and efflux of iron delivered by transferrin from the endosome. One caveat to this conclusion, however, is that deficiencies in other metals caused by loss of DMT1 function could contribute to impairments in iron uptake observed for *mk* and *b* animals; for example, copper depletion has been found to reduce both NTBI uptake and transferrin-mediated iron assimilation in HeLa cells (Yu and Wessling-Resnick, 1998).

While both the *mk* mouse and *b* rat have severe iron deficiency anemias, iron delivery must persist at some level to enable survival, and therefore additional pathways for iron import are predicted. Using a different functional expression strategy in *Xenopus* oocytes, a factor called SFT (stimulator of Fe transport) was identified to enhance uptake of both NTBI and transferrin-bound iron (Gutierrez et al., 1997). While the molecular basis for SFT's effects is still under study, the characteristics of iron uptake stimulated by SFT are quite different than those associated with DMT1 expression. SFT stimulates uptake of either ferric or ferrous iron at pH 7.4 (Yu and Wessling-Resnick, 1998) in contrast, DMT1 prefers Fe(II) and its activity is pH dependent, with optimal function at pH < 6 (Gunshin et al., 1997). Furthermore, the role for a surface reductase activity has been linked to SFT-stimulated iron uptake (Yu and Wessling-Resnick, 1998), while exogenous reduction of Fe(III) by ascorbate has been utilized to measure DMT1 function (Gunshin et al., 1997; Su et al., 1998). The idea that nonDMT1-mediated iron transport occurs is also consistent with the findings of Savigni and Morgan (1998). These investigators have characterized both high- and low-affinity Fe(II) uptake systems in erythroid cells but find that while the high-affinity process is reduced in Belgrade reticulocytes, the low-affinity mechanism is unchanged. Finally, a ceruloplasmin-stimulated iron uptake mechanism also has been characterized recently that appears to be specific for trivalent metals (Attieh et al., 1999). The functional properties of all of these alternative mechanisms indicate that like yeast, mammalian cells have multiple pathways for iron acquisition, although the molecules involved remain to be more fully defined.

While DMT1-mediated absorption of iron appears to be a principle route for dietary assimilation, the fact that this transporter resides exclusively at the apical surface of enterocytes (Canonne-Hergaux et al., 1999) is an indication that it is unlikely to be involved in the transfer and exit of iron across the intestinal epithelium. One theory that has been advanced is that circulating transferrin, either in the holo- or apoform, is internalized by enterocytes from the basolateral surface with its receptor to acquire iron imported from the apical brush border (Huebers et al., 1983; Alvarez-Hernandez et al., 1994; Nunez et al., 1997; Alvarez-Hernandez et al., 1998; Nunez and Tapia, 1999). However, the relative significance of transferrin in intestinal iron absorption is unclear because it does not appear to regulate iron uptake by the duodenum (Simpson et al., 1986). For example, the *hpx* mouse accumulates iron in parenchymal tissues despite the loss of transferrin (Buys et al., 1991). The recent discovery of the gene responsible for sex-linked anemia (*sla*) in mice may provide new clues about this exsorption process. This inherited iron disorder is characterized by the failure to release iron from the intestine. As perhaps predicted from earlier studies on the role of

the copper-binding ferroxidase ceruloplasmin on iron efflux discussed above, the *sla* defect has been found to reside in a ceruloplasmin homology called hephaestin (Vulpe et al., 1999). Hephaestin mRNA is abundant in the small intestine and colon with lower levels in other tissues. The protein itself is predicted to contain a single transmembrane spanning domain and to have ferroxidase activity, but its structure and function await further biochemical characterization. Vulpe et al. (1999) suggest that their discovery of hephaestin helps to explain the paradox that while copper deficiency results in anemia, anemia is not typically found in Menkes disease, an inherited disorder of copper transport in man. The latter disease results from defects in a copper-transporting ATPase (Vulpe et al., 1993), which is homologous to the yeast Ccc2 transporter (Yuan et al., 1995). Because this defect serves to trap copper within intestine, hephaestin could still bind copper to enable its function in mobilizing dietary iron, thereby preventing anemia. Thus, unlike yeast, which couple Fet3 ferroxidase activity to the cellular import of iron by Ftr1, intestinal cells appear to utilize ferroxidation in the export or release of iron.

E. Regulation of Mammalian Iron Transport

To maintain iron homeostasis, dietary iron absorption must be tightly regulated, but how the intestine senses body iron demands remains a critical aspect of transport that is poorly understood. Like transferrin receptors, DMT1 transcripts appear to harbor an iron-responsive element (IRE) in the 3′ untranslated region present in one of two differentially spliced isoforms (Gunshin et al., 1997; Lee et al., 1998). It is clear that iron depletion induces a dramatic increase in DMT1 mRNA in the intestine (Canonne-Hergaux et al., 1999; Canonne-Hergaux et al., 1999), leading to the prediction that binding of iron regulatory proteins (IRPs) to the IRE stabilize DMT1 message to enhance transporter synthesis in times of iron deprivation. In fact, Han et al. (1999) have found that DMT1 message is reciprocally regulated in Caco-2 cells in response to cellular iron status, consistent with this hypothesis. However, while the transferrin receptor message possesses five 3′ IREs, DMT1 transcripts only contain one. Moreover, an instability determinant such as that found for the transferrin receptor mRNA has not yet been identified for DMT1 and while the loop of its IRE contains the consensus 5′-CAGUGN-3′, the bulge in its stem differs from other IRE structures. Wardrop and Richardson (1999) have confirmed that DMT1 mRNA binds IRPs present in LMTK$^-$ cell lysates, but these cells fail to display iron-dependent regulation of DMT1 transcript levels. Moreover, NTBI uptake by the LMTK$^-$ cells does not appear to be regulated by the IRE/IRP system, although transferrin-mediated import is appropriately controlled (Wardrop and Richardson, 1999). Other studies predict that IRPs would act to sense iron status in duodenal enterocytes (Oates and Morgan, 1997; Schumann et al., 1999) and the intestinal Caco-2 cell line (Arredondo et al., 1997). Thus, whether DMT1 expression is modulated by the IRE/IRP system in a manner that reflects the body's iron status still awaits verification. DMT1 also appears to import other divalent metals, suggesting that additional elements must be involved in its regulation. Recent characterization of the human DMT1 gene has revealed five potential MREs (metal-responsive elements) in the 5′ regulatory

region, but the functionality of these elements remains to be explored (Lee et al., 1998).

While modulation of DMT1 synthesis might be predicted to control absorption of iron from the gut lumen, independent mechanism(s) also exist that regulate its release to the blood. For example, while the uptake of inorganic iron species can be modulated in response to iron status, the absorption of heme iron is not. Although this represents a relatively small pool of dietary iron, its efficient entry across the brush border provides a source of iron released to intestinal cells by the action of heme oxygenase (reviewed by Uzel and Conrad, 1998). The subsequent transfer of this pool in combination with iron entering enterocytes from inorganic sources through various transport mechanisms must be coordinately regulated at the basolateral face of the intestinal epithelium. It is this point of entry that appears to be affected in the genetic disorder hemochromatosis such that excessive amounts of dietary iron become assimilated (McLaren et al., 1991). Nonetheless, DMT1 expression levels are reported to be higher in HFE knockout animals (Fleming et al., 1999) and in duodenal biopsies from hemochromatosis patients (Zoller et al., 1999), suggesting that its activity may contribute to the iron overload disorder. What remains to be uncovered by future investigations is a comprehensive understanding of how multiple pathways of iron transport are coordinately regulated to maintain homeostasis and how factors like hephaestin contribute to this process. Based on the identification of iron-responsive transcription factors like Aft1 in yeast (Yamaguchi-Iwai et al., 1995) and the elucidation of posttranscriptional control of the Nramp homolog Smf1 by protein degradation (Liu and Culotta, 1999), one prediction is that an array of transcriptional, posttranscriptional, and posttranslational regulatory mechanisms may potentially contribute to the control of mammalian iron transport.

ACKNOWLEDGMENT

I express my gratitude to colleagues who shared information for this review and apologize for insufficient space to acknowledge all of the contributions made to this field.

REFERENCES

Akompong, T., Inman, R. S., and Wessling-Resnick, M. 1995. Phorbol esters stimulate non-transferrin iron uptake by K562 cells. *J. Biol. Chem.* **270**: 20937–41.

Alvarez-Hernandez, X., Smith, M., and Glass, J. 1998. The effect of apotransferrin on iron release from Caco-2 cells, an intestinal epithelial cell line. *Blood* **91**: 3974–9.

Alvarez-Hernandez, X., Smith, M., and Glass, J. 1994. Regulation of iron uptake and transport by transferrin in Caco-2 cells, an intestinal cell line. *Biochim. Biophys. Acta* **1192**: 215–22.

Amillet, J. M., Galiazzo, F., and Labbe-Bois, R. 1996. Effect of heme and vacuole deficiency on FRE1 gene expression and ferrireductase activity in *Saccharomyces cerevisiae*. *FEMS Microbiol. Lett.* **137**: 25–9.

Anderson, G. J., Dancis, A., Roman, D. G., and Klausner, R. D. 1994. Ferric iron reduction and iron uptake in eucaryotes: studies with the yeasts *Saccharomyces cerevisiae* and *Saccharomyces pombe*. *Adv. Exp. Med. Biol.* **356**: 81–89.

Archibald, F. 1983. *Lactobacillus plantarum*, an organism not requiring iron. *FEMS Microbiol. Lett.* **19**: 29–32.

Arredondo, M., Orellana, A., Garate, M. A., and Nunez, M. T. 1997. Intracellular iron regulates iron absorption and IRP activity in intestinal epithelial (Caco-2) cells. *Am. J. Physiol.* **273**: G275–80.

Askwith, C., Eide, D., Ho, A. V., Bernard, P. S., Li, L., Davis-Kaplan, S., Sipe, D. M., and Kaplan, J. 1994. The FET3 gene of *S. cerevisiae* encodes a multicopper oxidase required for ferrous iron uptake. *Cell* **76**: 403–410.

Askwith, C. and Kaplan, J. 1998. Iron and copper transport in yeast and its relevance to human disease. *Trends Biochem. Sci.* **23**: 135–138.

Askwith, C. and Kaplan, J. 1997. An oxidase-permease-based iron transport system in *Schizosaccharomyces pombe* and its expression in *Saccharomyces cerevisiae. J. Biol. Chem.* **272**: 401–5.

Askwith, C. C., de Silva, D., and Kaplan, J. 1996. Molecular biology of iron acquisition in *Saccharomyces cerevisiae. Mol. Microbiol.* **20**: 27–34.

Askwith, C. C. and Kaplan, J. 1998. Site-directed mutagenesis of the yeast multicopper oxidase Fet3p. *J. Biol. Chem.* **273**: 22415–9.

Attieh, Z. K., Mukhopadhyay, C. K., Seshadri, V., Tripoulas, N. A., and Fox, P. L. 1999. Ceruloplasmin ferroxidase activity stimulates cellular iron uptake by a trivalent cation-specific transport mechanism. *J. Biol. Chem.* **274**: 1116–23.

Bailey, S., Evans, R. W., Garratt, R. C., Gorinsky, B., Hasnain, S., Horsburgh, C., Jhoti, H., Lindley, P. F., Mydin, A., Sarra, R., and Watson, J. L. 1988. Molecular structure of serum transferrin at 3.3 Å resolution. *Biochemistry* **27**: 5804–5812.

Baker, E., Baker, S. M., and Morgan, E. H. 1998. Characterisation of non-transferrin-bound iron (ferric citrate) uptake by rat hepatocytes in culture. *Biochim. Biophys. Acta* **1380**: 21–30.

Baker, E., Vicary, F. R., and Huehns, E. R. 1981. Iron release from isolated hepatocytes. *Br. J. Haematol.* **47**: 493–504.

Baker, E. N., Baker, H. M., Smith, C. A., Stebbins, M. R., Kahn, M., Hellstrom, K. E., and Hellstrom, I. 1992. Human melanotransferrin (p97) has only one functional iron-binding site. *FEBS Lett.* **298**: 215–218.

Bali, P. K. and Aisen, P. 1992. Receptor-induced switch in site-site cooperativity during iron release by transferrin. *Biochemistry* **31**: 3963–7.

Bali, P. K. and Aisen, P. 1991. Receptor-modulated iron release from transferrin: differential effects on N- and C-terminal sites. *Biochemistry* **30**: 9947–52.

Bali, P. K., Zak, O., and Aisen, P. 1991. A new role for the transferrin receptor in the release of iron from transferrin. *Biochemistry* **30**: 324–8.

Bannerman, R. M., Edwards, J. A., Kreimer-Birnbaum, M., McFarland, E., and Russell, E. S. 1972. Hereditary microcytic anaemia in the mouse; studies in iron distribution and metabolism. *Br. J. Haematol.* **23**: 235–245.

Barisani, D., Berg, C. L., Wessling-Resnick, M., and Gollan, J. L. 1995. Evidence for a low Km transporter for non-transferrin-bound iron in isolated rat hepatocytes. *Am. J. Physiol.* **269**: G570–6.

Barisani, D. and Wessling-Resnick, M. 1996. Fe-nitrilotriacetic acid-binding proteins associated with rat liver plasma membranes. *Hepatology* **24**: 934–8.

Batey, R. G., Fong, P. L. C., Shamir, S., and Sherlock, S. 1980. A non-transferrin-bound serum iron in idiopathic hemochromatosis. *Dig. Dis. Sci.* **25**: 340–346.

Berczi, A., Sizensky, J. A., Crane, F. L., and Faulk, W. P. 1991. Diferric transferrin reduction by K562 cells: a critical study. *Biochim. Biophys. Acta* **1073**: 562–570.

Bernstein, S. E. 1987. Hereditary hypotransferrinemia with hemosiderosis, a murine disorder resembling human atransferrinemia. *J. Lab. Clin. Med.* **110**: 690–705.

Bowen, B. J. and Morgan, E. H. 1987. Anemia of the Belgrade rat: evidence for defective membrane transport of iron. *Blood* **70**: 38–44.

Braun, V., Hantke, K., and Koster, W. 1998. Bacterial iron transport: mechanisms, genetics, and regulation. *Met. Ions Biol. Syst.* **35**: 67–145.

Brissot, P., Wright, T. L., Ma, W. L., and Weisiger, R. A. 1985. Efficient clearance of non-transferrin-bound iron by rat liver. *J. Clin. Invest.* **76**: 1463–1470.

Brown, J. P., Hewick, R. M., Hellstrom, I., Hellstrom, K. E., Doolittle, R. F., and Dreyer, W. J. 1982. Human melanoma-associated antigen p97 is structurally and functionally related to transferrin. *Nature* **296**: 171–173.

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

Canonne-Hergaux, F., Gruenheid, S., Ponka, P., and Gros, P. 1999. Cellular and subcellular localization of the Nramp2 iron transporter in the intestinal brush border and regulation by dietary iron. *Blood* **93**: 4406–17.

Cellier, M., Prive, G., Belouchi, A., Kwan, T., Rodrigues, V., Chia, W., and Gros, P. 1995. Nramp defines a family of membrane proteins. *Proc. Natl. Acad. Sci. U.S.A.* **92**: 10089–10093.

Chan, R. Y. Y., Ponka, P., and Schulman, H. M. 1992. Transferrin-receptor-independent but iron-dependent proliferation of variant Chinese hamster ovary cells. *Exp. Cell. Res.* **202**: 326–336.

Chitambar, C. R. and Wereley, J. P. 1997. Resistance to the antitumor agent gallium nitrate in human leukemic cells is associated with decreased gallium/iron uptake, increased activity of iron regulatory protein-1, and decreased ferritin production. *J. Biol. Chem.* **272**: 12151–7.

Chua, A. C. and Morgan, E. H. 1997. Manganese metabolism is impaired in the Belgrade laboratory rat. *J. Comp. Physiol.* **167**: 361–9.

Conrad, M. E., Umbreit, J. N., Moore, E. G., Peterson, R. D., and Jones, M. B. 1990. A newly identified iron binding protein in duodenal mucosa of rats. Purification and characterization of mobilferrin. *J. Biol. Chem.* **265**: 5273–9.

Conrad, M. E., Umbreit, J. N., Moore, E. G., Uzel, C., and Berry, M. R. 1994. Alternate iron transport pathway. Mobilferrin and integrin in K562 cells. *J. Biol. Chem.* **269**: 7169–73.

Conrad, M. E., Umbreit, J. N., Peterson, R. D., Moore, E. G., and Harper, K. P. 1993. Function of integrin in duodenal mucosal uptake of iron. *Blood* **81**: 517–21.

Craven, C. M., Alexander, J., Eldridge, M., Kushner, J. P., Bernstein, S., and Kaplan, J. 1987. Tissue distribution and clearance kinetics of non-transferrin-bound iron in the hypotransferrinemic mouse: a rodent model for hemochromatosis. *Proc. Natl. Acad. Sci. U.S.A.* **84**: 3457–3461.

Dancis, A., Klausner, R. D., Hinnebusch, A. G., and Barriocanal, J. G. 1990. Genetic evidence that ferric reductase is required for iron uptake in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **10**: 2294–2301.

Dancis, A., Roman, D. G., Anderson, G. J., Hinnebusch, A. G., and Klausner, R. D. 1992. Ferric reductase of *Saccharomyces cerevisiae*: molecular characterization, role in iron uptake and transcriptional control by iron. *Proc. Natl. Acad. Sci. U.S.A.* **89**: 3869–3873.

Dancis, A., Yuan, D. S., Haile, D., Askwith, C., Eide, D., Moehle, C., Kaplan, J., and Klausner, R. D. 1994. Molecular characterization of a copper transport protein in *S. cerevisiae*: an unexpected role for copper in iron transport. *Cell* **76**: 393–402.

Danielsen, E. M. and van Deurs, B. 1995. A transferrin-like GPI-linked iron-binding protein in detergent-isolated noncaveolar microdomains at the apical surface of fetal intestinal epitheliar cells. *J. Cell. Biol.* **131**: 939–950.

Dautry-Varsat, A., Ciechanover, A., and Lodish, H. F. 1983. pH and the recycling of transferrin during receptor-mediated endocytosis. *Proc. Natl. Acad. Sci. U.S.A.* **80**: 2258–2262.

De Silva, D., Davis-Kaplan, S., Fergestad, J., and Kaplan, J. 1997. Purification and characterization of Fet3 protein, a yeast homologue of ceruloplasmin. *J. Biol. Chem.* **272**: 14208–13.

De Sousa, M., Reimao, R., Lacerdo, R., Hugo, P., Kaufmann, S. H. E., and Porto, G. 1994. Iron overload in beta2-microglobulin-deficient mice. *Immunol. Lett.* **39**: 105–111.

DeSilva, D. M., Askwith, C. C., Eide, E., and Kaplan, J. 1995. The FET3 gene product required for high-affinity iron transport in yeast is a cell surface ferroxidase. *J. Biol. Chem.* **270**: 1098–1101.

Dix, D., Bridgham, J., Broderius, M., and Eide, D. 1997. Characterization of the FET4 protein of yeast. Evidence for a direct role in the transport of iron. *J. Biol. Chem.* **272**: 11770–7.

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

Edwards, J. A., and Hoke, J. E. 1972. Defect of intestinal mucosal iron uptake in mice with hereditary microcytic anemia. *Proc. Soc. Exp. Biol. Med.* **141**: 81–84.

Edwards, J. A., Huebers, H., Kunzler, C., and Finch, C. 1986. Iron metabolism in the Belgrade rat. *Blood* **67**: 623–628.

Edwards, J. A., Sullivan, A. L., and Hoke, J. E. 1980. Defective delivery of iron to the developing red cell of the Belgrade laboratory rat. *Blood* **51**: 645–648.

Egyed, A. 1988. Carrier-mediated iron transport through erythroid cell membrane. *Br. J. Haematol.* **68**: 483–486.

Eide, D., Broderius, M., Fett, J., and Guerinot, M. L. 1996. A novel iron-regulated metal transporter from plants identified by functional expression in yeast. *Proc. Natl. Acad. Sci. U.S.A.* **93**: 5624–5628.

Eide, D., Davis-Kaplan, S., Jordan, I., Sipe, D., and Kaplan, J. 1992. Regulation of iron uptake in saccharomyces cerevisiae: the ferrireductase and Fe(II) transporter are regulated independently. *J. Biol. Chem.* **267**: 20774–20781.

Ekmekcioglu, C., Feyertag, J., and Marktl, W. 1996. A ferric reductase activity is found in brush border membrane vesicles isolated from Caco-2 cells. *J. Nutr.* **126**: 2209–2217.

Farcich, E. A. and Morgan, E. H. 1992. Uptake of transferrin-bound and nontransferrin-bound iron by reticulocytes from the Belgrade laboratory rat: comparison with Wistar rat transferrin and reticulocytes. *Am. J. Hematol.* **39**: 9–14.

Feder, J. N., Gnirke, A., Thomas, W., Tsuchihashi, Z., Ruddy, D. A., Basava, A., Dormishian, F., Domingo, R., Jr., Ellis, M. C., Fullan, A., Hinton, L. M., Jones, N. L., Kimmel, B. E., Kronmal, G. S., Lauer, P., Lee, V. K., Loeb, D. B., Mapa, F. A., McClelland, E., Meyer, N. C., Mintier, G. A., Moeller, N., Moore, T., Morikang, E., and Wolff, R. K. 1996. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat. Genet.* **13**: 399–408.

Feder, J. N., Penny, D. M., Irrinki, A., Lee, V. K., Lebron, J. A., Watson, N., Tsuchihashi, Z., Sigal, E., Bjorkman, P. J., and Schatzman, R. C. 1998. The hemochromatosis gene product complexes with the transferrin receptor and lowers its affinity for ligand binding. *Proc. Natl. Acad. Sci. U.S.A.* **95**: 1472–7.

Feder, J. N., Tsuchihashi, Z., Irrinki, A., Lee, V. K., Mapa, F. A., Morikang, E., Prass, C. E., Starnes, S. M., Wolff, R. K., Parkkila, S., Sly, W. S., and Schatzman, R. C. 1997. The hemochromatosis founder mutation in

HLA-H disrupts beta2– microglobulin interaction and cell surface expression. *J. Biol. Chem.* **272**: 14025–8.

Fett, J. P., LeVier, K., and Guerinot, M. L. 1998. Soil microorganisms and iron uptake by higher plants. *Met. Ions Biol. Syst.* **35**: 187–214.

Finegold, A. A., Shatwell, K. P., Segal, A. W., Klausner, R. D., and Dancis, A. 1996. Intramembrane bis-heme motif for transmembrane electron transport conserved in a yeast iron reductase and the human NADPH oxidase. *J. Biol. Chem.* **271**: 31021–4.

Fleming, M. D., Romano, M. A., Su, M. A., Garrick, L. M., Garrick, M. D., and Andrews, N. C. 1998. Nramp2 is mutated in the anemic Belgrade (b) rat: evidence of a role for Nramp2 in endosomal iron transport. *Proc. Natl. Acad. Sci. U.S.A.* **95**: 1148–53.

Fleming, M. D., Trenor, C. C., 3rd, Su, M. A., Foernzler, D., Beier, D. R., Dietrich, W. F., and Andrews, N. C. 1997. Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. *Nat. Genet.* **16**: 383–6.

Fleming, R. E., Migas, M. C., Zhou, X. Y., Jiang, J., Britton, R. S., Brunt, E. M., Tomatsu, S., Waheed, A., Bacon, B. R., and Sly, W. S. 1999. Mechanism of increased iron absorption in murine model of hereditary hemochromatosis: increased duodenal expression of the iron transporter DMT1. *Proc. Natl. Acad. Sci. U.S.A.* **96**: 3143–3148.

Food, M. R., Rothenberger, S., Gabathuler, R., Haidl, I. D., Reid, G., and Jefferies, W. A. 1994. Transport and expression in human melanomas of a transferrin-like glycosylphosphatidylinositol-anchored protein. *J. Biol. Chem.* **269**: 3034–40.

Garrick, L. M., Dolan, K. G., Romano, M. A., and Garrick, M. D. 1999. Non-transferrin-bound iron uptake in Belgrade and normal rat erythroid cells. *J. Cell. Physiol.* **178**: 349–58.

Georgatsou, E. and Alexandraki, D. 1994. Two distinctly regulated genes are required for ferric reduction, the first step of iron uptake in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **14**: 3065–3073.

Georgatsou, E., Mavrogiannis, L. A., Fragiadakis, G. S., and Alexandraki, D. 1997. The yeast Fre1p/Fre2p cupric reductases facilitate copper uptake and are regulated by the copper-modulated Mac1p activator. *J. Biol. Chem.* **272**: 13786–92.

Goldenberg, H., Dodel, B., and Seidl, D. 1990. Plasma membrane Fe2–transferrin reductase and iron uptake in K562 cells are not directly related. *Eur. J. Biochem.* **192**: 475–480.

Graden, J. A., and Winge, D. R. 1997. Copper-mediated repression of the activation domain in the yeast Mac1p transcription factor. *Proc. Natl. Acad. Sci. U.S.A.* **94**: 5550–5.

Graham, R. M., Morgan, E. H., and Baker, E. 1998. Ferric citrate uptake by cultured rat hepatocytes is inhibited in the presence of transferrin. *Eur. J. Biochem.* **253**: 139–45.

Gross, C. N., Irrinki, A., Feder, J. N., and 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. *J. Biol. Chem.* **273**: 22068–74.

Gruenheid, S., Canonne-Hergaux, F., Gauthier, S., Hackam, D. J., Grinstein, S., and Gros, P. 1999. The iron transport protein NRAMP2 is an integral membrane glycoprotein that colocalizes with transferrin in recycling endosomes. *J. Exp. Med.* **189**: 831–41.

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

Gunshin, H., Mackenzie, B., Berger, U. V., Gunshin, Y., Romero, M. F., Boron, W. F., Nussberger, S., Gollan, J. L., and Hediger, M. A. 1997. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* **388**: 482–8.

Gutierrez, J. A., Inman, R. S., Akompong, T., Yu, J., and Wessling-Resnick, M. 1998. Metabolic depletion inhibits the uptake of nontransferrin-bound iron by K562 cells. *J. Cell. Physiol.* **177**: 585–92.

Gutierrez, J. A., Yu, J., Rivera, S., and Wessling-Resnick, M. 1997. Functional expression cloning and characterization of SFT, a stimulator of Fe transport. *J. Cell. Biol.* **139**: 895–905.

Gutteridge, J. M. C., Rowley, D. A., Griffiths, E., and Halliwell, B. 1985. Low-molecular-weight iron complexes and oxygen radical reactions in idiopathic hemochromatosis. *Clin. Sci.* **68**: 463–467.

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

Han, O., Fleet, J.C., and Wood, R.J. 1999. Reciprocal regulation of HFE and Nramp2 gene expression in human intestinal cells. *J. Nutr.* **129**: 98–104.

Harris, Z. L., Takahashi, Y., Miyajima, H., Serizawa, M., MacGillivray, R. T., and Gitlin, J. D. 1995. Aceruloplasminemia: molecular characterization of this disorder of iron metabolism. *Proc. Natl. Acad. Sci. U.S.A.* **92**: 2539–43.

Harrison, D. E. 1972. Marrow transplantation and iron therapy in mouse hereditary microcytic anemia. *Blood* **40**: 893–901.

Hassett, R. and Kosman, D. J. 1995. Evidence for Cu(II) reduction as a component of copper uptake by *Saccharomyces cerevisiae*. *J. Biol. Chem.* **270**: 128–134.

Hassett, R. F., Romeo, A. M., and Kosman, D. J. 1998. Regulation of high-affinity iron uptake in the yeast *Saccharomyces cerevisiae*. Role of dioxygen and Fe. *J. Biol. Chem.* **273**: 7628–36.

Hassett, R. F., Yuan, D. S., and Kosman, D. J. 1998. Spectral and kinetic properties of the Fet3 protein from *Saccharomyces cerevisiae*, a multinuclear copper ferroxidase enzyme. *J. Biol. Chem.* **273**: 23274–23282.

Hodgson, L. L., Quail, E. A., and Morgan, E. H. 1995. Iron transport mechanisms in reticulocytes and mature erythrocytes. *J. Cell. Physiol.* **162**: 181–90.

Huebers, H. A., Huebers, E., Csiba, E., Rummel, W., and Finch, C. A. 1983. The significance of transferrin for intestinal iron absorption. *Blood* **61**: 283–290.

Inman, R. S., Coughlan, M. M., and Wessling-Resnick, M. 1994. Extracellular ferrireductase activity of K562 cells is coupled to transferrin-independent iron transport. *Biochemistry* **33**: 11850–7.

Inman, R. S. and Wessling-Resnick, M. 1993. Characterization of transferrin-independent iron transport in K562 cells. Unique properties provide evidence for multiple pathways of iron uptake. *J. Biol. Chem.* **268**: 8521–8.

Iturri, S. and Nunez, M. T. 1998. Effect of copper, cadmium, mercury, manganese and lead on Fe^{2+} and Fe^{3+} absorption in perfused mouse intestine. *Digestion* **59**: 671–5.

Jefferies, W. A., Gabathuler, R., Rothenberger, S., Food, M., and Kennard, M. L. 1996. Pumping iron in the '90s. *Trends Cell Biol.* **6**: 223–228.

Jordan, I. and Kaplan, J. 1994. The mammalian transferrin-independent iron transport system may involve a surface ferrireductase activity. *Biochem. J.* **302**: 875–879.

Kaplan, J., Jordan, I., and Sturrock, A. 1991. Regulation of the transferrin-independent iron transport system in cultured cells. *J. Biol. Chem.* **266**: 2997–3004.

Kawabata, H., Yang, R., Hirama, T., Vuong, P. T., Kawano, S., Gombart, A. F., and Koeffler, H. P. 1999. Molecular cloning of transferrin receptor 2. A new member of the transferrin receptor-like family. *J. Biol. Chem.* **274**: 20826–32.

Kennard, M. L., Richardson, D. R., Gabathuler, R., Ponka, P., and Jefferies, W. A. 1995. A

novel iron uptake mechanism mediated by GPI-anchored human p97. *Embo. J.* **14**: 4178–86.

Klausner, R. D., Ashwell, G., Van Renswoude, J., Harford, J. B., and Bridges, K. R. 1983. Binding of apotransferrin to K562 cells: explanation of the transferrin cycle. *Proc. Natl. Acad. Sci. U.S.A.* **80**: 2263–2266.

Kuhn, L. C. 1998. Iron and gene expression: molecular mechanisms regulating cellular iron homeostasis. *Nutr. Rev.* **56**: s11–9.

Labbe, S., Zhu, Z., and Thiele, D. J. 1997. Copper-specific transcriptional repression of yeast genes encoding critical components in the copper transport pathway. *J. Biol. Chem.* **272**: 15951–8.

Lebron, J. A., Bennett, M. J., Vaughn, D. E., Chirino, A. J., Snow, P. M., Mintier, G. A., Feder, J. N., and Bjorkman, P. J. 1998. Crystal structure of the hemochromatosis protein HFE and characterization of its interaction with transferrin receptor. *Cell* **93**: 111–23.

Lee, G. R., Nacht, S., Lukens, J. N., and Cartwright, G. E. 1968. Iron metabolism in copper-deficient swine. *J. Clin. Invest.* **47**: 2058–2069.

Lee, P. L., Gelbart, T., West, C., Halloran, C., and Beutler, E. 1998. The human Nramp2 gene: characterization of the gene structure, alternative splicing, promoter region and polymorphisms. *Blood Cells Mol. Dis.* **24**: 199–215.

Lesuisse, E., Casteras-Simon, M., and Labbe, P. 1997. Cytochrome P-450 reductase is responsible for the ferrireductase activity associated with isolated plasma membranes of *Saccharomyces cerevisiae*. *FEMS Microbiol. Lett.* **156**: 147–52.

Lesuisse, E., Casteras-Simon, M., and Labbe, P. 1996. Evidence for the *Saccharomyces cerevisiae* ferrireductase system being a multicomponent electron transport chain. *J. Biol. Chem.* **271**: 13578–83.

LeSuisse, E., Crichton, R., and Labbe, P. 1990. *Biochem. Biophys. Acta* **1038**: 253–259.

Lesuisse, E., Horion, B., Labbe, P., and Hilger, F. 1991. The plasma membrane ferrireductase activity of *Saccharomyces cerevisiae* is partially controlled by cyclic AMP. *Biochem. J.* **290**: 545–548.

Lesuisse, E. and Labbe, P. 1989. Reductive and non-reductive mechanisms of iron assimilation by the yeast *Saccharomyces cerevisiae*. *J. Gen. Microbiol.* **135**: 257–263.

Lesuisse, E., Simon-Casteras, M., and Labbe, P. 1998. Siderophore-mediated iron uptake in *Saccharomyces cerevisiae*: the SIT1 gene encodes a ferrioxamine B permease that belongs to the major facilitator superfamily. *Microbiology* **144**: 3455–62.

Lesuisse E. R. F. and Crichton, R. R. 1987. Iron uptake by the yeast *Saccharomyces cerevisiae*: involvement of a reduction step. *J. Gen. Microbiol.* **133**: 3229–3236.

Levy, J. E., Jin, O., Fujiwara, Y., Kuo, F., and Andrews, N. C. 1999. Transferrin receptor is necessary for development of erythrocytes and the nervous system. *Nat. Genet.* **21**: 396–9.

Levy, J. E., Montross, L. K., Cohen, D. E., Fleming, M. D., and Andrews, N. C. 1999. The C282Y mutation causing hereditary hemochromatosis does not produce a null allele. *Blood* **94**: 9–11.

Lin, S. J., Pufahl, R. A., Dancis, A., O'Halloran, T. V., and Culotta, V. C. 1997. A role for the *Saccharomyces cerevisiae* ATX1 gene in copper trafficking and iron transport. *J. Biol. Chem.* **272**: 9215–20.

Liu, X. F., and Culotta, V. C. 1999. Posttranslation control of Nramp metal transport in yeast. Role of metal ions and the BSD2 gene. *J. Biol. Chem.* **274**: 4863–8.

Lonnerdal, B. and Iyer, S. 1995. Lactoferrin: molecular structure and biological function. *Annu. Rev. Nutr.* **15**: 93–110.

Low, H., Sun, I.L., Navas, P., Grebing, C., Crane, F.L., and Morre, D.J. 1986. Transplasmalemma electron transport from cells is part of a diferric transferrin reductase system. *Biochim. Biophys. Acta* **139**: 1117–1123.

Martins, L. J., Jensen, L. T., Simon, J. R., Keller, G. L., Winge, D. R., and Simons, J. R. 1998. Metalloregulation of FRE1 and FRE2 homologs in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **273**: 23716–21.

McAbee, D. D. 1995. Isolated rat hepatocytes acquire iron from lactoferrin by endocytosis. *Biochem. J.* **311**: 603–9.

McLaren, G. D., Nathanson, M. H., Jacobs, A., Trevett, D., and Thomson, W. 1991. Regulation of intestinal iron absorption and mucosal iron kinetics in hereditary hemochromatosis. *J. Lab. Clin. Med.* **117**: 390–401.

Morgan, E. H. 1988. Membrane transport of non-transferrin-bound iron by reticulocytes. *Biochim. Biophys. Acta.* **943**: 428–39.

Mukherjee, S., Ghosh, R. N., and Maxfield, F. R. 1997. Endocytosis. *Physiol. Rev.* **77**: 759–803.

Mukhopadhyay, C. K., Attieh, Z. K., and Fox, P. L. 1998. Role of ceruloplasmin in cellular iron uptake. *Science* **279**: 714–7.

Musilkova, J., Kriegerbeckova, K., Krusek, J., and Kovar, J. 1998. Specific binding to plasma membrane is the first step in the uptake of non-transferrin iron by cultured cells. *Biochim. Biophys. Acta.* **1369**: 103–8.

Nichols, G. M., Pearce, A. R., Alverez, X., Bibb, N. K., Nichols, K. Y., Alfred, C. B., and Glass, J. 1992. The mechanisms of nonheme iron uptake determined in IEC-6 rat intestinal cells. *J. Nutr.* **122**: 945–952.

Nunez, M. T., Alvarez, X., Smith, M., Tapia, V., and Glass, J. 1994. Role of redox systems on Fe3+ uptake by transformed human intestinal epithelial (Caco-2) cells. *Am. J. Physiol.* **267**: C1582–8.

Nunez, M. T., Nunez-Millacura, C., Beltran, M., Tapia, V., and Alvarez-Hernandez, X. 1997. Apotransferrin and holotransferrin undergo different endocytic cycles in intestinal epithelia (Caco-2) cells. *J. Biol. Chem.* **272**: 19425–19428.

Nunez, M. T., and Tapia, V. 1999. Transferrin stimulates iron absorption, exocytosis, and secretion in cultured intestinal cells. *Am. J. Physiol.* **276**: C1085–90.

O'Donnell, M., and Cox, T. M. 1982. Microvillar iron-binding glycoproteins isolated from the rabbit small intestine. *Biochem. J.* **202**: 107–115.

Oates, P. S. and Morgan, E. H. 1996. Defective iron uptake by the duodenum of Belgrade rats fed diets of different iron contents. *Am. J. Physiol.* **270**: G826–32.

Oates, P. S. and Morgan, E. H. 1997. Ferritin gene expression and transferrin receptor activity in intestine of rats with varying iron stores. *Am. J. Physiol.* **273**: G636–46.

Olakanmi, O., Stokes, J. B., and Britigan, B. E. 1994. Acquisition of iron bound to low molecular weight chelates by human monocyte-derived macrophages. *J. Immunol.* **153**: 2691–2703.

Olakanmi, O., Stokes, J. B., Pathan, S., and Britigan, B. E. 1997. Polyvalent cationic metals induce the rate of transferrin-independent iron acquisition by HL-60 cells. *J. Biol. Chem.* **272**: 2599–606.

Osaki, S., Johnson, D. A., and Frieden, E. 1971. The mobilization of iron from the perfused mammalian liver by a serum copper enzyme, ferroxidase I. *J. Biol. Chem.* **246**: 3018–3023.

Oshiro, S., Nakajima, H., Markello, T., Krasnewick, D., Bernardini, I., and Gahl, W. A. 1993. Redox, transferrin-independent, and receptor-mediated endocytosis iron uptake systems in cultured human fibroblasts. *J. Biol. Chem.* **268**: 21586–21591.

Page, M. A., Morgan, E. H., and Baker, E. 1984. Transferrin binding and iron uptake by rat hepatocytes in culture. *Am. J. Physiol.* **246**: G26–G33.

Parkes, J. G., Hussain, R. A., Olivieri, N. F., and Templeton, D. M. 1993. Effects of iron loading on uptake, speciation, and chelation of iron in cultured mycardial cells. *J. Lab. Clin. Med.* **122**: 36–47.

Parkes, J. G., Olivieri, N. F., and Templeton, D. M. 1997. Characterization of Fe^{2+} and Fe3+ transport by iron-loaded cardiac myocytes. *Toxicology* **117**: 141–51.

Parkes, J. G. and Templeton, D. M. 1994. Iron transport and subcellular distribution in HepG2 hepatocarcinoma cells. *Ann. Clin. Lab. Sci.* **24**: 509–520.

Parkkila, S., Waheed, A., Britton, R. S., Bacon, B. R., Zhou, X. Y., Tomatsu, S., Fleming, R. E., and Sly, W. S. 1997. Association of the transferrin receptor in human placenta with HFE, the protein defective in hereditary hemochromatosis. *Proc. Natl. Acad. Sci. U.S.A.* **94**: 13198–202.

Parkkila, S., Waheed, A., Britton, R. S., Feder, J. N., Tsuchihashi, Z., Schatzman, R. C., Bacon, B. R., and Sly, W. S. 1997. Immunohistochemistry of HLA-H, the protein defective in patients with hereditary hemochromatosis, reveals unique pattern of expression in gastrointestinal tract. *Proc. Natl. Acad. Sci. U.S.A.* **94**: 2534–9.

Pietrangelo, A., Casalgrandi, G., Quaglino, D., Gualdi, R., Conte, D., Milani, S., Montosi, G., Cesarini, L., Ventura, E., and Cairo, G. 1995. Duodenal ferritin synthesis in genetic hemochromatosis. *Gastroenterology* **108**: 208–17.

Pinner, E., Gruenheid, S., Raymond, M., and Gros, P. 1997. Functional complementation of the yeast divalent cation transporter family SMF by NRAMP2, a member of the mammalian natural resistance-associated macrophage protein family. *J. Biol. Chem.* **272**: 28933–28938.

Ponka, P., Beaumont, C., and Richardson, D. R. 1998. Function and regulation of transferrin and ferritin. *Semin. Hematol.* **35**: 35–54.

Qian, Z. M. and Morgan, E. H. 1991. Effect of metabolic inhibitors on uptake of non-transferrin-bound iron by reticulocytes. *Biochim. Biophys. Acta* **1073**: 456–62.

Quail, E. A. and Morgan, E. H. 1994. Role of membrane surface potential and other factors in the uptake of non-transferrin-bound iron by reticulocytes. *J. Cell. Physiol.* **159**: 238–44.

Raja, K. B., Pountney, D., Bomford, A., Przemioslo, R., Sherman, D., Simpson, R. J., Williams, R., and Peters, T. J. 1996. A duodenal mucosal abnormality in the reduction of Fe(III)in patients with genetic hemochromatosis. *Gut* **38**: 765–769.

Raja, K. B., Simpson, R. J., and Peters, T. J. 1992. Investigation of a role for reduction in ferric iron uptake by mouse duodenum. *Biochim. Biophys. Acta* **1135**: 141–146.

Randell, E. W., Parkes, J. G., Olivieri, N. F., and Templeton, D. M. 1994. Uptake of non-transferrin-bound iron by both reductive and nonreductive processes is modulated by intracellular iron. *J. Biol. Chem.* **269**: 16046–16053.

Richardson, D. and Baker, E. 1992. Two mechanisms of iron uptake from transferrin by melanoma cells. The effects of desferrioxamine and ferric ammonium citrate. *J. Biol. Chem.* **267**: 13972–13979.

Richardson, D. and Baker, E. 1991. The uptake of inorganic iron complexes by human melanoma cells. *Biochim. Biophys. Acta* **1093**: 20–28.

Richardson, D. R. 1997. Mobilization of iron from neoplastic cells by some iron chelators is an energy-dependent process. *Biochim. Biophys. Acta* **1320**: 45–57.

Richardson, D. R. and Baker, E. 1991. The release of iron and transferrin from the human melanoma cell. *Biochim. Biophys. Acta* **1091**: 294–302.

Richardson, D. R. and Baker, E. 1990. The uptake of iron and transferrin by the human malignant melanoma cell. *Biochim. Biophys. Acta* **1053**: 1–12.

Richardson, D. R. and Ponka, P. 1995. Identification of a mechanism of iron uptake by

cells which is stimulated by hydroxyl radicals generated via the iron-catalyzed Haber-Weiss reaction. *Biochim. Biophys. Acta* **1269**: 105–114.

Richardson, D. R. and Ponka, P. 1997. The molecular mechanisms of the metabolism and transport of iron in normal and neoplastic cells. *Biochim. Biophys. Acta* **1331**: 1–40.

Riedel, H.-D., Remus, A. J., Fitscher, B. A., and Stremmel, W. 1995. Characterization and partial purification of a ferrireductase from human duodenal microvillous membrane. *Biochem. J.* **309**: 745–748.

Robinson, N. J., Procter, C. M., Connolly, E. L., and Guerinot, M. L. 1999. A ferric-chelate reductase for iron uptake from soils. *Nature* **397**: 694–7.

Roman, D. G., Dancis, A., Anderson, G. J., and Klausner, R. D. 1993. The fission yeast ferric reductase gene frp1+ is required for ferric iron uptake and encodes a protein that is homologous to the gp91–phox subunit of the human NADPH phagocyte oxidoreductase. *Mol. Cell. Biol.* **13**: 4342–4350.

Rossander-Hulten, L., Brune, M., Sandstrom, B., Lonnerdal, B., and Hallberg, L. 1991. Competitive inhibition of iron absorption by manganese and zinc in humans. *Am. J. Clin. Nutr.* **54**: 152–156.

Rothenberg, B. E. and Voland, J. R. 1996. Beta 2 knockout mice develop parenchymal iron overload: a putative role for class I genes of the major histocompatibility complex in iron metabolism. *Proc. Natl. Acad. Sci. U.S.A.* **93**: 1529–1534.

Roy, C. N., Penny, D. M., Feder, J. N., and Enns, C. A. 1999. The hereditary hemochromatosis protein, HFE, specifically regulates transferrin-mediated iron uptake in HeLa cells. *J. Biol. Chem.* **274**: 9022–8.

Salter-Cid, L., Brunmark, A., Li, Y., Leturcq, D., Peterson, P. A., Jackson, M. R., and Yang, Y. 1999. Transferrin receptor is negatively modulated by the hemochromatosis protein HFE: implications for cellular iron homeostasis. *Proc. Natl. Acad. Sci. U.S.A.* **96**: 5434–9.

Sasaki, K., Zak, O., and Aisen, P. 1993. Antisense suppression of transferrin receptor gene expression in a human hepatoma cell (HuH-7) line. *Am. J. Hematol.* **42**: 74–80.

Savigni, D. L. and Morgan, E. H. 1998. Transport mechanisms for iron and other transition metals in rat and rabbit erythroid cells. *J. Physiol. (London)* **508**: 837–50.

Savigni, D. L. and Morgan, E. H. 1996. Use of inhibitors of ion transport to differentiate iron transporters in erythroid cells. *Biochem. Pharmacol.* **52**: 371–7.

Schieber, B. and Goldenberg, H. 1998. The surface of rat hepatocytes can transfer iron from stable chelates to external acceptors. *Hepatology* **27**: 1075–1080.

Schumann, K., Moret, R., Kunzle, H., and Kuhn, L. C. 1999. Iron regulatory protein as an endogenous sensor of iron in rat intestinal mucosa. Possible implications for the regulation of iron absorption. *Eur. J. Biochem.* **260**: 362–72.

Schweinzer, E. and Goldenberg, H. 1993. Monodehydroascorbate reductase activity in the surface membrane of leukemic cells, characterization by a ferricyanide-driven redox cycle. *Eur. J. Biochem.* **218**: 1057–1062.

Shatwell, K. P., Dancis, A., Cross, A. R., Klausner, R. D., and Segal, A. W. 1996. The FRE1 ferric reductase of *Saccharomyces cerevisiae* is a cytochrome b similar to that of NADPH oxidase. *J. Biol. Chem.* **271**: 14240–14244.

Simpson, R. J., Lombard, M., Raja, K. B., Snape, S. D., and Peters, T. J. 1986. Studies on the role of transferrin and endocytosis in the uptake of Fe^{3+} from Fe-nitriloacetate by mouse duodenum. *Biochim. Biophys. Acta* **884**: 166–171.

Sipe, D. M. and Murphy, R. F. 1991. Binding to cellular receptor results in increased iron

release from transferrin at mildly acidic pH. *J. Biol. Chem*. **266**: 8002–8007.

Spizzo, T., Byersdorfer, C., Duesterhoeft, S., and Eide, D. 1997. The yeast FET5 gene encodes a FET3–related multicopper oxidase implicated in iron transport. *Mol. Gen. Genet.* **256**: 547–56.

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

Stonell, L. M., Savigni, D. L., and Morgan, E. H. 1996. Iron transport into erythroid cells by the Na+/Mg2+ antiport. *Biochim. Biophys. Acta* **1282**: 163–70.

Sturrock, A., Alexander, J., Lamb, J., Craven, C. M., and Kaplan, J. 1990. Characterization of a transferrin-independent uptake system for iron in HeLa cells. *J. Biol. Chem.* **265**: 3139–3145.

Su, M. A., Trenor, C. C., Fleming, J. C., Fleming, M. D., and Andrews, N. C. 1998. The G185R mutation disrupts function of the iron transporter Nramp2. *Blood* **92**: 2157–63.

Supek, F., Supekova, L., Nelson, H., and Nelson, N. 1996. A yeast manganese transporter related to the macrophage protein involved in conferring resistance to mycobacteria. *Proc. Natl. Acad. Sci. U.S.A.* **93**: 5105–5110.

Teichmann, R. and Stremmel, W. 1990. Iron uptake by human upper small intestine microvillous membrane vesicles: indication for a facilitated transport mechanism mediated by a membrane iron-binding protein. *J. Clin. Invest.* **86**: 2145–2153.

Thorstensen, K. and Aisen, P. 1990. Release of iron from diferric transferrin in the presence of rat liver plasma membranes: no evidence of a plasma membrane diferric transferrin reductase. *Biochim. Biophys. Acta* **1052**: 29–55.

Thorstensen, K. and Romslo, I. 1988. Uptake of iron from transferrin by isolated rat hepatocytes: a redox-mediated plasma membrane process. *J. Biol. Chem.* **263**: 8844–8850.

Thorstensen, K., Trinder, D., Zak, O., and Aisen, P. 1995. Uptake of iron from N-terminal half-transferrin by isolated rat hepatocytes: evidence of transferrin receptor-independent iron uptake. *Eur. J. Biochem.* **232**: 129–133.

Trinder, D. and Morgan, E. 1997. Inhibition of uptake of transferrin-bound iron by human hepatoma cells by nontransferrin-bound iron. *Hepatology* **26**: 691–8.

Trinder, D., Morgan, E. H., and Baker, E. 1988. The effects of an antibody to the rat transferrin receptor and of rat serum albumin on the uptake of diferric transferrin by rat hepatocytes. *Biochim. Biophys. Acta* **943**: 440–6.

Trinder, D., Zak, O., and Aisen, P. 1996. Transferrin receptor independent uptake of diferric transferrin by human hepatoma cells with antisense inhibition. *Hepatology* **23**: 1512–1520.

Umbreit, J. N., Conrad, M. E., Moore, E. G., and Latour, L. F. 1998. Iron absorption and cellular trxansport: the mobilferrin/paraferritin paradigm. *Semin. Hematol.* **35**: 13–26.

Uzel, C. and Conrad, M. E. 1998. Absorption of heme iron. *Semin. Hematol.* **35**: 27–34.

Vidal, S. M., Malo, D., Vogan, K., Skamene, E., and 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., and Gitschier, J. 1993. Isolation of a candidate gene for Menkes disease and evidence that it encodes a copper-transporting ATPase. *Nat. Genet.* **3**: 7–13.

Vulpe, C. D., Kuo, Y. M., Murphy, T. L., Cowley, L., Askwith, C., Libina, N., Gitschier, J., and Anderson, G. J. 1999. Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is

defective in the sla mouse. *Nat. Genet.* **21**: 195–9.

Waheed, A., Parkkila, S., Saarnio, J., Fleming, R. E., Zhou, X. Y., Tomatsu, S., Britton, R. S., Bacon, B. R., and Sly, W. S. 1999. Association of HFE protein with transferrin receptor in crypt enterocytes of human duodenum. *Proc. Natl. Acad. Sci. U.S.A.* **96**: 1579–84.

Waheed, A., Parkkila, S., Zhou, X. Y., Tomatsu, S., Tsuchihashi, Z., Feder, J. N., Schatzman, R. C., Britton, R. S., Bacon, B. R., and Sly, W. S. 1997. Hereditary hemochromatosis: effects of C282Y and H63D mutations on association with beta2-microglobulin, intracellular processing, and cell surface expression of the HFE protein in COS-7 cells. *Proc. Natl. Acad. Sci. U.S.A.* **94**: 12384–9.

Wardrop, S. L. and Richardson, D. R. 1999. The effect of intracellular iron concentration and nitrogen monoxide on nramp2 expression and non-transferrin-bound iron uptake. *Eur. J. Biochem.* **263**: 41–50.

Wright, T. L., Brissot, P., Ma, M. L., and Weisiger, R. A. 1986. Characterization of non-transferrin-bound iron clearance by rat liver. *J. Biol. Chem.* **261**: 10909–10914.

Wright, T. L., Fitz, J. G., and Weisiger, R. A. 1988. Non-transferrin-bound iron uptake by rat liver: role of membrane potential difference. *J. Biol. Chem.* **263**: 1842–1847.

Wright, T. L. and Lake, J. R. 1990. Mechanisms of transport of nontransferrin-bound iron in basolateral and canalicular rat liver plasma membrane vesicles. *Hepatology* **12**: 498–504.

Yamaguchi-Iwai, Y., Dancis, A., and Klausner, R. D. 1995. AFT1: a mediator of iron regulated transcriptional control in *Saccharomyces cerevisiae*. *Embo. J.* **14**: 1231–9.

Yamaguchi-Iwai, Y., Serpe, M., Haile, D., Yang, W., Kosman, D. J., Klausner, R. D., and Dancis, A. 1997. Homeostatic regulation of copper uptake in yeast via direct binding of MAC1 protein to upstream regulatory sequences of FRE1 and CTR1. *J. Biol. Chem.* **272**: 17711–8.

Yamaguchi-Iwai, Y., Stearman, R., Dancis, A., and Klausner, R. D. 1996. Iron-regulated DNA binding by the AFT1 protein controls the iron regulon in yeast. *Embo J.* **15**: 3377–84.

Yoshida, K., Furihata, K., Takeda, S., Nakamura, A., Yamamoto, K., Morita, H., Hiyamuta, S., Ikeda, S., Shimizu, N., and Yanagisawa, N. 1995. A mutation in the ceruloplasmin gene is associated with systemic hemosiderosis in humans. *Nat. Genet.* **9**: 267–72.

Young, S. P., Fahmy, M., and Golding, S. 1997. Ceruloplasmin, transferrin and apotransferrin facilitate iron release from human liver cells. *FEBS Lett.* **411**: 93–96.

Yu, J. and Wessling-Resnick, M. 1998. Influence of copper depletion on iron uptake mediated by SFT, a stimulator of Fe transport. *J. Biol. Chem.* **273**: 6909–15.

Yuan, D. S., Stearman, R., Dancis, A., Dunn, T., Beeler, T., and Klausner, R. D. 1995. The Menkes/Wilson disease gene homologue in yeast provides copper to a ceruloplasmin-like oxidase required for iron uptake. *Proc. Natl. Acad. Sci. U.S.A.* **92**: 2632–2636.

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., and Sly, W. S. 1998. HFE gene knockout produces mouse model of hereditary hemochromatosis. *Proc. Natl. Acad. Sci. U.S.A.* **95**: 2492–7.

Zoller, H., Pietrangelo, A., Vogel, W., and Weiss, G. 1999. Duodenal metal-transporter (DMT1, NRAMP2) expression in patients with hereditary haemochromatosis. *Lancet* **353**: 2120–2123.