Histol Histopathol (2007) 22: 791-804

**http://www.hh.um.es**

**Histology and Histopathology**

*Cellular and Molecular Biology*

## *Review*

# The role of hepcidin and ferroportin in iron absorption

**P.S. Oates**

Physiology M311, School of Biomedical, Biomolecular and Chemical Sciences, University of Western Australia, Nedlands, Western Australia

**Summary.** The field of iron metabolism is moving rapidly. There have been significant advances in our understanding of how proteins carry out the process of iron absorption. The three main tissues involved in iron exchange are the enterocyte which contributes new iron to the system, the hepatocyte which stores and releases iron and the macrophages which recycles iron from effete red blood cells to the plasma. This review examines recent evidence into the function of the iron transporters divalent metal transporter and ferroportin in enterocytes. Evidence is also provided from the author's laboratory which presents an alternative hypothesis into how hepcidin a key regulator molecule might interact with ferroportin in enterocytes. It is proposed that ferroportin operates differently in enterocytes compared with macrophages. Specifically in enterocytes ferroportin appears to function in the uptake stage of iron absorption.

**Key words:** Absorption, DMT1, Regulation, Uptake, Release, Inflammation, Ferroportin, Macrophages

## Introduction

Iron is essential for life. It is incorporated into enzymes and proteins required for important cellular processes such as oxygen transport, proliferation and energy production. If there is a sustained negative iron balance less iron is available for incorporation into haemoglobin, leading to anaemia, and in children, to central nervous system dysfunction and impaired growth. On the other hand, excess cellular iron is extremely harmful to cells by facilitating the production of reactive oxygen species which results in damage to cellular proteins, lipids and DNA. Consequences of such damage are organ failure and predisposition to cancer. Since mammals have a limited capacity to excrete iron, the iron content of the body must be tightly regulated at the stage of intake into the body namely, via intestinal absorption.

Internal daily iron balance is maintained by the efficient destruction of senescent red blood cells by macrophages and the return of the freed iron to the circulation where it is available for uptake by the bone marrow for erythropoiesis. In addition hepatocytes buffer changes in plasma iron by uptake and release. In the event of reduced iron stores or increased erythropoiesis such as might occur after a haemorrhage there is increased requirement for iron by the body. This results in reduced release of hepcidin by hepatocytes that signals to increase iron absorption by the enterocyte and iron release from macrophages via the iron efflux transport protein, ferroportin.

This review is divided into four parts, firstly it examines the recent literature into the expression of hepcidin normally and during inflammation and the evidence that ferroportin is the receptor for hepcidin. Secondly it examines the physiological significance of mutations to ferroportin that result in the ferroportin disease, where it is suggested that ferroportin combines with cell specific proteins to modulate iron absorption by enterocytes. Thirdly, evidence is provided from the author's laboratory which presents an alternative hypothesis on how hepcidin operates with ferroportin in enterocytes. The review then focuses on how ferroportin may be linked to heme breakdown in macrophages and this is compared with enterocytes. Finally a discussion of major unresolved issues regarding the structure and function of ferroportin is presented.

## Molecular explanations of primary iron overload

A persuasive hypothesis has recently been proposed which suggests that the liver regulates iron flux and iron absorption via the expression of at least four proteins namely, the hemochromatosis (HFE) protein (Feder et al., 1996), hemojuvelin (HJV) (Papanikolaou et al., 2004; Gehrke et al., 2005), transferrin receptor 2 (TfR2)

*Offprint requests to:* P.S. Oates, Physiology M311, School of Biomedical, Biomolecular and Chemical Sciences, University of Western Australia, 35 Stirling Highway, Nedlands 6009, Western Australia. e-mail: poates@cyllene.uwa.edu.au

(Camaschella et al., 2000) and hepcidin (Nicolas et al., 2001; Roetto et al., 2003; Gehrke et al., 2005). The roles of HFE, HJV and TfR2 are to regulate the appropriate expression and release of hepcidin which in turn acts as a humoral mediator determining the amounts of iron released by enterocytes and macrophages. The importance of these proteins is evidenced by mutations to their genes that manifest in various forms of hemochromatosis (Cazzola, 2003).

## Hepcidin 1

### *Identification of hepcidin and its importance in iron metabolism*

Hepcidin (aka, LEAP-liver-expressed antimicrobial peptide, HAMP- hepatocyte antimicrobial peptide) was discovered when an inadvertent hepatic iron overload phenotype was observed in the *Upstream Stimulatory Factor 2 (USF2)* knockout mice (Nicolas et al., 2001; Pigeon et al., 2001). The nature of this iron overload resembled that of genetic hemochromatosis (GH) where the hepatic iron distribution was in the periportal parenchyma while the reticuloendothelial system was spared from iron loading. It was shown that the iron overload in *USF2* knockout mice is due to the absence of the duplicated hepcidin gene in the liver of these mice (Nicolas et al., 2001). Supporting this, the phenotype reported in combined Hamp/USF2$^{+}$ (Nicolas et al., 2001) was similar to *Hamp*$^{-/-}$ mice (Lesbordes-Brion et al., 2006).

### *Increased hepcidin expression is linked to iron deficiency and anaemia*

Pigeon and co-workers demonstrated that liver iron accumulation increased hepcidin mRNA while reduced hepcidin mRNA expression produced progressive liver iron loading (Pigeon et al., 2001; Nicolas et al., 2002). Newborn animals in which hepcidin was overproduced resulted in severe iron deficiency and they died within a few hours of birth (Nicolas et al., 2002). Similarly hepcidin expressing tumours also result in iron deficiency anaemia due to reduced iron accumulation and availability (Roy et al., 2003; Rivera et al., 2005). Recently mutations in the hepcidin gene have been implicated in a new form of juvenile hemochromatosis which maps to chromosome 19 in humans (Roetto et al., 2003).

### *Intracellular hepcidin processing*

Hepcidin has been purified from human plasma ultrafiltrate and from urine (Park et al., 2001). It was shown to be a disulfide-bonded peptide exhibiting antimicrobial activity (Krause et al., 2000; Nicolas et al., 2001; Hunter et al., 2002). Hepcidin is synthesised in the liver in the form of a propeptide that contains 84 amino acids, including a putative 24 amino acid leader peptide. It is proteolytically cleaved into the bioactive peptide of 25 amino acids during cellular processing (Krause et al., 2000; Nicolas et al., 2001; Park et al., 2001; Nemeth et al., 2006). Hepcidin is also found as -22 and -20 peptide fragments but these are not biologically active (Nemeth et al., 2004). The differences between the active and inactive forms of hepcidin reside at the N-terminal (Nemeth et al., 2006).

### *Hepcidin is a hormone*

The physiological role of hepcidin as an effector of iron absorption was shown when changes in iron absorption coincided inversely with changes in the amount of hepcidin expressed. This was the case in response to alterations in erythropoiesis, body iron loading, pregnancy and hypoxia (Anderson et al., 2002; Frazer et al., 2002, 2004; Millard et al., 2004). Direct evidence was provided when recombinant hepcidin was injected into rodents or applied to intestinal cell lines and iron absorption fell around 24 hrs later (Laftah et al., 2004; Yamaji et al., 2004; Yeh et al., 2004).

The response time to changes in hepcidin expression on plasma iron levels was demonstrated in a transgenic mouse model with an inducible promoter linked to the hepcidin gene (Viatte et al., 2006). Activation of hepcidin expression reduced plasma iron levels within 6hrs. A quicker response was seen after parenteral administration of recombinant hepcidin-25 to mice (Rivera et al., 2005). The different response time represents the time required to activate transcription, translation and post-translational modifications of the peptide and its secretion before an effect is produced. These events are entirely consistent with the processing of a peptide hormone and its target action. Notwithstanding these data, direct measurements of plasma hepcidin are presently not available because of the lack of a suitable antibody directed against hepcidin-25, presumably because of the limited number of epitopes that can be generated from the 5 amino acids that comprise the bioactive form.

### *Structure of hepcidin*

The structures of Hepcidin-20 and -25 have been solved (Hunter et al., 2002). Both structures appear to be distorted ß-sheets with six disulphide bonds from cysteine residues maintaining interconnectivity between the sheets. At the N-terminal hepcidin-25 has several modifications around a proline residue near the N-terminal and a disulfide hairpin bridge that may confer functionality. The peptides are amphipathic in structure and that may confer antimicrobial activity. In solution hepcidin-20 maintains a monomeric state, whereas hepcidin-25 aggregates and appears to form an interface for peptide interactions. These variations may account for the difference in activities of the two peptides.

## Stimuli of hepcidin expression

### *Body iron stores*

It is known that iron absorption is inversely related to body iron stores (Bezwoda et al., 1979; Millard et al., 2004). In humans and mice under normal circumstances there is a strong correlation between hepcidin levels and hepatic iron stores (Simpson et al., 2003; Detivaud et al., 2005). How body iron stores produce appropriate hepcidin expression is unclear but it is probably relayed in part by plasma transferrin saturation.

### *Erythropoiesis*

Under normal circumstances there is a strong correlation between hepcidin expression and hemoglobin levels (Simpson et al., 2003; Detivaud et al., 2005), suggesting that hepcidin is responsive to erythropoiesis (Nicolas et al., 2001, Nicolas, Chauvet et al., 2002; Frazer et al., 2004; Ezeh et al., 2005; Leung et al., 2005; Kattamis et al., 2006; Latunde-Dada et al., 2006; Yoon et al., 2006).

### *Erythroid regulator versus the stores regulator*

The erythropoietic regulator has a greater capacity to increase iron absorption than the stores regulator. Supporting this, low hepcidin expression is found in the presence of high iron stores when there is ineffective erythropoiesis (Ajioka et al., 2002; Papanikolaou et al., 2005; Latunde-Dada et al., 2006; Weizer-Stern et al., 2006). This results in increased iron absorption and exacerbates the iron loading.

### *Anaemia of chronic disease (ACD)*

Inflammation either acute or chronic results in hypoferremia due to iron retention by macrophages (Noyes et al., 1960; Quastel and Ross, 1966; Cartwright, 1966, Lee, 1983) and reduced intestinal iron absorption (Gubler et al., 1950; Cortell and Conrad, 1967; Schade, 1972). The anaemia is the result of reduced iron availability because of impaired release from macrophages, reduced life span of the erythrocyte, reduced activity of erythropoietin affecting erythropoiesis (Epo) (Theurl et al., 2006) and a decreased responsiveness of erythroid precursors to EPO (Cartwright, 1966; Lee, 1983).

The hypoferremia associated with inflammation is most likely mediated by hepcidin in reducing iron efflux and absorption by macrophages and enterocytes, respectively. Injection of lipopolysaccharide (LPS) to humans increased urinary hepcidin by 6 hours and was followed by a fall in plasma iron (Kemna et al., 2005). LPS derived from the wall of bacterial cell membranes is known to initiate an inflammatory response by interacting with Toll-Like Receptors (TLR) on the cell membrane of hepatocytes (Schwabe et al., 2006) and macrophages (Hoshino et al., 1999). Since LPS stimulates hepcidin release from isolated hepatocytes (Pigeon et al., 2001, Lee et al., 2005), this suggests that TLR are linked to hepcidin expression (Lee et al., 2005; Constante et al., 2006).

### *Cytokines stimulate hepcidin expression that cause cellular iron sequestration*

The hypoferremia associated with inflammation is also mediated by increased release of hepcidin in response to pro-inflammatory cytokines (Weinstein et al., 2002; Nemeth et al., 2003, 2004; Lee et al., 2004, 2005; Andriopoulos and Pantopoulos, 2006). In response to LPS and/or specific cytokines hepcidin expression increased and hypoferremia followed (Nemeth et al., 2003, 2004, Kemna et al., 2005). In addition to IL-6 others have found that IL-1$\alpha$ and IL-1ß increased hepcidin expression (Lee et al., 2004, 2005). Conversely, producing an inflammatory response with turpentine in $HAMP^{-/-}$ mice did not produce hypoferremia (Nicolas et al., 2002). This loss of iron from the plasma is likely to be due to inhibition of iron release caused by reduced expression of ferroportin in macrophages. Whether enterocytes retain iron in the presence of increased hepcidin is discussed below.

### *Hepcidin-independent iron sequestration*

A hepcidin independent role in iron efflux has been suggested in macrophages (Yang et al., 2002; Liu et al., 2005) and enterocytes (Johnson et al., 2004; Sharma et al., 2005), whereby ferroportin expression is reduced or the protein is rendered non-functional. Thus there appear to be at least three ways in which hypoferremia can be produced, two hepcidin dependent processes involving TLR and cytokine receptors on hepatocytes and a third hepcidin -independent mechanism.

### *Hepcidin 2*

In mice a second gene encoding hepcidin is expressed in the pancreas (Hepcidin 2) (Ilyin et al., 2003). This appears to be a recent evolutionary development because rat and human pancreas do not express Hepcidin 2. Hepcidin 1 and 2 are both responsive to dietary iron loading (Mazur et al., 2003), but only Hepcidin 1 responds to lipopolysaccharide induced inflammation (Krijt et al., 2004). Furthermore, over-expression of Hepcidin 1 but not Hepcidin 2 produces an iron deficient phenotype (Lou et al., 2004), suggesting that Hepcidin 1 is the effector molecule regulating iron efflux and Hepcidin 2 in this role is dispensable. Supporting this, targeted disruption of Hepcidin 1 produced iron overload even in the presence of the USF2 gene (Lesbordes-Brion et al., 2006). Since the iron overload phenotype can be fully explained by Hepcidin 1 the function of Hepcidin 2 is unknown but it shares homology to fish Hepcidin-like peptides that may

function like a ß-defensin in innate immunity (Bao et al., 2006). Interestingly, in TfR2$^{-/-}$ knockout mice Hepcidin 2 but not Hepcidin 1 expression increased with iron loading (Wallace et al., 2005), suggesting that input via a TfR2 pathway is important for regulating hepcidin 2. The role of Hepcidin 2 in mice is presently unknown.

## Ferroportin is an iron exporter and the hepcidin 1 receptor

Ferroportin is a transmembrane protein (Abboud and Hailey, 2000; McKie et al., 2000; Donovan et al., 2005) expressed in tissues involved in maintaining body iron stores and turnover including enterocytes, macrophages, placenta and hepatocytes suggesting ferroportin is an iron efflux transporter and the receptor to hepcidin.

Evidence implicating ferroportin in the efflux of non-heme iron is provided by six observations. [1] Over-expression of ferroportin results in a cellular iron deficient phenotype indicating that ferroportin caused iron efflux from the cells (Abboud and Haile, 2000). [2] Ferroportin expression in Xenopus oocytes along with DMT1 resulted in iron efflux (Donovan et al., 2000; McKie et al., 2000). [3] Over-expression of ferroportin in cells exposed to hepcidin-25 resulted in internalisation of ferroportin and its targeting to lysosomes for degradation and was linked with increased cellular iron stores. Hepcidin-20 had no effect (Nemeth et al., 2004). [4] Mutations to ferroportin have been described which result in a hemochromatosis phenotype, suggesting that iron release from the stores is impaired or that ferroportin is un-responsive to hepcidin-25 (De Domenico et al., 2005, 2006). [5] Over-expression of ferroportin in macrophages during erythrophagocytosis increased the release of non-heme iron after its release from heme (Knutson et al., 2005). [6] *Ferroportin*$^{-/-}$ mice resulted in non-heme iron accumulation within enterocytes (Donovan et al., 2005). These data support the hypothesis that ferroportin is the receptor for hepcidin, is involved in non-heme iron release and that hepcidin is a major regulator of iron absorption and body iron turnover.

## The ferroportin disease

The ferroportin disease otherwise described as hereditary hemochromatosis type 4 is inherited as an autosomal dominant trait. There are numerous different mutations to the ferroportin gene that result in iron overload (Njajou et al., 2001; Pietrangelo, 2004; Sham et al., 2005). Affected individuals fall into two phenotypes, those that manifest with an early increased plasma ferritin, relatively low to normal transferrin saturation and iron overload of macrophages (Montosi et al., 2001; Cazzola et al., 2002; Devalia et al., 2002; Roetto et al., 2002; Wallace et al., 2002, 2004; Beutler, 2003; Jouanolle et al., 2003), the other with hyperferritinemia, high transferrin saturation and iron loading of the parenchyma (Rivard et al., 2003; Wallace et al., 2004, Sham et al., 2005).

At the molecular level the various mutations to ferroportin fall into three categories. Mutations to ferroportin (A77D, V162Δ, G490D, N174I) that result in the retention of ferroportin within the endoplasmic reticulum (De Domenico et al., 2005, 2006; Drakesmith et al., 2005; Liu et al., 2005), preventing its targeting to the cell surface. Lack of ferroportin on the membrane of macrophages produces hepcidin resistance (De Domenico et al., 2006) and manifests as an iron overload phenotype of the macrophages, presumably because heme is catabolised after entry but the iron cannot be released (see below). Nonetheless, in a Xenopus oocyte functional assay system A77D and V162Δ were able to target the membrane and transport iron albeit at reduced rate (McGregor et al., 2005). These data suggest the function of ferroportin needs to be considered in a cell context manner.

The second group of mutations present with iron overload of the parenchyma and increased plasma saturation that is interpreted as a gain of ferroportin function. Mutations to ferroportin (N144H, N144D, Q182H, Y64N, and C326Y) indicate appropriate targeting of ferroportin to the cell surface where it appears to bind hepcidin but fails to be internalised. This is proposed to lead to constitutive entry of iron from the intestinal tract into the body and iron loading of hepatocytes and increased transferrin saturation (Montosi et al., 2001).

### *Hypotheses accounting for dominant transmission*

Two hypotheses are proposed to account for the autosomal dominant transmission of the disease namely, dominant negative (De Domenico et al., 2005) and haploinsufficiency hypotheses (Schimanski et al., 2005), respectively. In the dominant negative hypothesis ferroportin wild type and mutant proteins oligomerise to produce a protein complex that combines functionally to produce a loss of iron transport capacity (De Domenico et al., 2005). McGregor and coworkers showed that adding equimolar amounts of wild type and mutant ferroportin mRNA to Xenopus oocytes reduced iron efflux, a finding that supports the dimer model of dominant negative action (McGregor et al., 2005). However, other groups have failed to observe dimerization of ferroportin both in vitro and in vivo (Schimanski et al., 2005; Canonne-Hergaux et al., 2006; Goncalves et al., 2006; Pignatti et al., 2006) and therefore suggest the haploinsufficiency hypothesis (Liu et al., 2005; Schimanski et al., 2005). This hypothesis suggests that reduced production of wild type protein is rate limiting when required at high transport capacity such as in macrophages, but not at low capacity such as in the enterocyte. However there is some doubt to the validity of this hypothesis.

### *Testing the haploinsufficiency hypothesis*

On a daily basis, macrophages recycle about 30mg of iron to the plasma iron pool derived from effete

erythrocytes whereas enterocytes contribute about 1mg of iron (Knutson and Wessling-Resnick, 2003). It is thought that insufficient production of ferroportin could be rate limiting (haploinsufficient) in the release of iron by macrophages but not from enterocytes. This assumes that the mass of macrophages and enterocytes engaged in iron efflux are similar. However previous studies using rats allow an estimate of efflux of iron by a single enterocyte and macrophage. If it is assumed that a macrophage breaks down one erythrocyte containing about 20 pg of haemoglobin per day or releases $10^9$ atoms of iron per day (Kondo et al., 1988; Knutson and Wessling-Resnick, 2003) approximately 100fg of iron will be returned to the circulation per macrophage. In the rat about 10 $\mu$g of iron is absorbed per day. However, since the conditions for absorption are only optimal in the duodenum the calculation for enterocyte iron absorption should be limited to this region. It was estimated that for the entire length of intestine, villus enterocytes contain 6.8 mg of DNA (Oates and Morgan, 1996b), however, since the duodenum represents 10% of this length there is 0.68 mg of DNA from enterocytes and based on the fact that a rat diploid nucleus contains 10pg of DNA there will be approximately 68 million enterocytes present in the duodenum. Therefore each enterocyte will absorb about 150 fg of iron per day. If these estimations apply to humans then the amount of iron released from an enterocyte and by macrophages per day will be similar. It is clear that enterocytes are exposed to sufficient dietary iron to produce non-heme iron loading and *ferroportin*$^{-/-}$ mice clearly demonstrate iron accumulation in enterocytes (Donovan et al., 2005). Thus these data do not support the halpoinsufficiency hypothesis. An alternative hypothesis points to a more fundamental difference in the functions of ferroportin in these two tissues. This review will now examine the recent literature on iron absorption in the enterocyte and in particular the function of ferroportin.

## Iron absorption by enterocytes

Iron absorption involves the movement of iron from the intestinal lumen across the apical membrane of the enterocyte (uptake), movement through the cell (intracellular processing) and then movement across the basal membrane (efflux) into the bloodstream. This occurs by several steps. A large number of proteins are known to be involved in non-heme iron absorption and these are tabled along with their known and expected sites of function in table 1.

## Uptake of iron

In the enterocyte divalent metal transporter 1 (DMT1) is essential for the uptake of Fe(II) across the apical membrane (Fleming et al., 1997; Gunshin et al., 1997), as evidenced by homozygous mk mice and b rats that carry a G185R mutation to DMT1. In mk/mk and b/b rats there is markedly impaired iron absorption that leads to a severe iron deficient phenotype (Oates and Morgan, 1996a). There are 4 possible transcripts for DMT1 two containing iron responsive elements (Hubert and Hentze, 2002), although most studies suggest that one isoform transcript of DMT1-IRE dominants expression.

In enterocytes during severe iron deficiency DMT1 operates at the apical membrane (Trinder et al., 2000; Canonne-Hergaux et al., 2001), however normally DMT1 is found at intracellular sites (West et al., 2006), suggesting that dietary iron and DMT1 can interact inside the cell (Thomas and Oates 2004a,b; Oates and Thomas, 2005b). Like most integral proteins expressed at the apical membrane, DMT1 is glycosylated, presumably to protect against digestion from luminal secretions and acidity (Thomas and Oates, 2002). DMT1 operates by a symport action in which equivalent amounts of ferrous iron (Fe(II)) and $H^+$ are co-

**Table 1.** Proteins both known and putatively involved in the uptake, movement and release of iron by enterocytes. Their intracellular function and location are also listed.

| PROTEIN | FUNCTION | REFERENCE |
|---|---|---|
| DMT1 membrane/cytosol | Transports Fe(II) to cytoplasm | (Gunshin et al., 1997) |
| ß3 integrin | Transports Fe(III) | (Conrad et al., 1993b) |
| Citrate binding | Bind Fe(III) at cell surface | (Thomas and Oates, 2004b) |
| Dcytb | Ferrireductase | (McKie et al., 2001) |
| Mobilferrin/Calreticum | Ferrireductase/Chaperone/Protein folding | (Conrad et al., 1993a) |
| Steap1 | Ferrireductase | (Ohgami et al., 2005) |
| HFE protein | Binds TfR/functions in iron transport | (Feder et al., 1998; West et al., 2006) |
| TfR | Binds HFE and diferric transferrin and modulates iron uptake from transferrin | (Trinder et al., 2002) |
| Transferrin | Binds Fe(III) in plasma | (Oates and Morgan, 1997) |
| Ferritin | Store Fe(III) | (Gerard et al., 1996) |
| Ferroportin | Exports Fe(II) | (Donovan et al., 2000; McKie et al., 2000) |
| Hephaestin | Ferroxidase + ? | (Vulpe et al., 1999) |

DMT: divalent metal transporter; HFE: Haemochrmatosis protein; TFR; Transferrin receptor; ?: putative.

transported into the enterocyte (Gunshin et al., 1997). Since most dietary non-heme iron is in the ferric (Fe(III)) form that cannot be transported by DMT1 the Fe(III) must undergo ferrireduction before transport by DMT1.

### *The role of Dcytb in iron absorption*

Dcytb is a ferrireductase expressed predominately on the cell surface (McKie et al., 2000). Reduction of dietary Fe(III) may occur by the ferrireductase Dcytb (McKie et al., 2000), although *Dcytb/Cryb1*$^{-/-}$ mice were not iron deficient, indicating that iron absorption was normal (Gunshin et al., 2005). This suggests redundancy in ferrireductase activity and therefore the existence of other ferrireductases (Conrad et al., 1993a,b; Ohgami et al., 2005) (Table 1).

### *Ferroportin is expressed on the apical membrane of enterocytes*

In the author's laboratory ferroportin is detectable on the apical membrane of enterocytes by immunofluorescence microscopy (Thomas and Oates 2004a), but not when prepared from wax sections as also suggested by others (Abboud and Haile, 2000; Oates and Thomas, 2005a; Canonne-Hergaux et al., 2006). These observations suggest that the low levels of expression of ferroportin at the apical membrane is lost by extensive fixation, or in the preparation for wax embedding as previously suggested for intestinal transferrin receptor (TfR) (Oates et al., 2000). Importantly these data are supported by Western blot analysis showing enrichment of ferroportin expression in apical membrane preparations compared with the starting homogenate. (Thomas and Oates, 2004a) Interestingly, others have shown in polarised epithelial cells that ferroportin is expressed on the apical membrane of airway epithelia (Yang et al., 2005).

### *DMT1 and Fe(II) interact with ferroportin in the uptake of iron*

Functional data supports a role for ferroportin in the uptake of Fe(II) by enterocytes and enterocyte-like cells (Oates and Thomas, 2005b). Using the predicted sequence of ferroportin (McKie et al., 2000) when a blocking antibody directed against the largest extracellular domain of ferroportin was incubated with rat Intestinal Epithelial Cells (IEC-6) and Caco-2 cells, uptake of Fe(II) fell. In IEC-6 cells this was due to internalisation and targeting of the ferroportin to lysosomes (Oates and Thomas, 2005b). Since the antibody had no effect on the release of iron it suggests that the binding site on ferroportin at the basal surface is unavailable or that other key proteins are masking the antibody binding site at the basal surface but not at the apical membrane (see below). It is unlikely that ferroportin transports Fe(II) into the cell as there is clear evidence that DMT1 is the transporter involved in iron uptake. Therefore DMT1 is a logical candidate to interact with ferroportin. Taken together with the study of Nemeth and co-workers it suggests that ferroportin has at least two binding sites, one where hepcidin binds to inhibit efflux (Nemeth et al., 2004) and a second identified by the ferroportin antibody that impairs uptake.

### *Citrate and Fe(III) interact with ferroportin in the uptake of iron*

Another interaction that has been observed in the author's laboratory is that between ferroportin, citrate and Fe(III) (2004). Citrate binding sites exist on the apical membrane of enterocytes (Muir et al., 1984) and the ferroportin antibody completely blocks the binding of Fe(III) to citrate on this membrane (2004). In IEC-6 cells the uptake of Fe(III) involves initial binding of Fe(III) to citrate binding sites on the cell membrane followed by internalisation of Fe(III). Since there is no evidence of ferrireduction on the cell surface the Fe(III) can enter the cell perhaps by a ß-integrin pathway (Conrad et al., 1993b). Once inside the cell the iron is reduced possibly by Dcytb, mobilferrin (Conrad et al., 1993) or Steap (Ohgami et al., 2005) and then transported by DMT1 to a distal site possibly involving lysosomes (Thomas and Oates, 2004a,b). This pathway supports the idea of redundancy in several steps in iron absorption, namely ferrireductase activity and uptake but non-redundancy for transport by DMT1 (Gunshin et al., 2005). The physiological significance of this pathway is still unclear but it does indicate that ferroportin functions in the uptake of Fe(III) into enterocytes.

### *Hepcidin reduces iron absorption by affecting uptake*

The effect of recombinant hepcidin-25 on the uptake and efflux steps of iron absorption in the human intestinal cell line (Caco-2) and mice has been studied (Laftah et al., 2004; Yamaji et al., 2004). In polarised Caco-2 cells and in mice hepcidin firstly decreased expression of the DMT1 which then decreased iron uptake and therefore overall iron absorption. In mice ferroportin expression remained unchanged as did the efflux of iron expressed as a percentage of iron internalised (Yamaji et al., 2004). Importantly when hepcidin was added to the apical membrane iron uptake was not affected indicating that the hepcidin binding site on ferroportin is not available possibly because it is masked by proteins expressed on the apical surfaces only or by mucous (see below for other possibilities) (Oates and Thomas, 2005b). This observation confirms the humoral nature of the action of hepcidin in its interaction with ferroportin at the basal membrane and indicates that the uptake step is affected by an interaction occurring at the basal membrane.

*Endotoxin induced inflammation reduced iron absorption by impairing uptake*

Inflammation is known to reduce iron absorption in humans and rodents (Cartwright, 1966; Cortell and Conrad, 1967; Haurani and Green, 1967; Beresford et al., 1971). Endotoxin-reduced iron absorption initially occurs at the uptake step and presumably was hepcidin mediated (Cortell and Conrad, 1967). Importantly this study revealed that the enterocyte is iron deficient suggesting that the reduction in iron uptake was not secondary to an initial loss of ferroportin function which is expected to increase cell iron stores as described above. This supports the idea that hepcidin operates indirectly at the apical step in iron absorption.

*Hypothesis: Ferroportin on the basal membrane interacts with ferroportin on the apical membrane to impair uptake*

A hypothesis has been proposed that takes the above observations into account and is summarised in figure 1. This hypothesis states that circulating hepcidin-25 in the

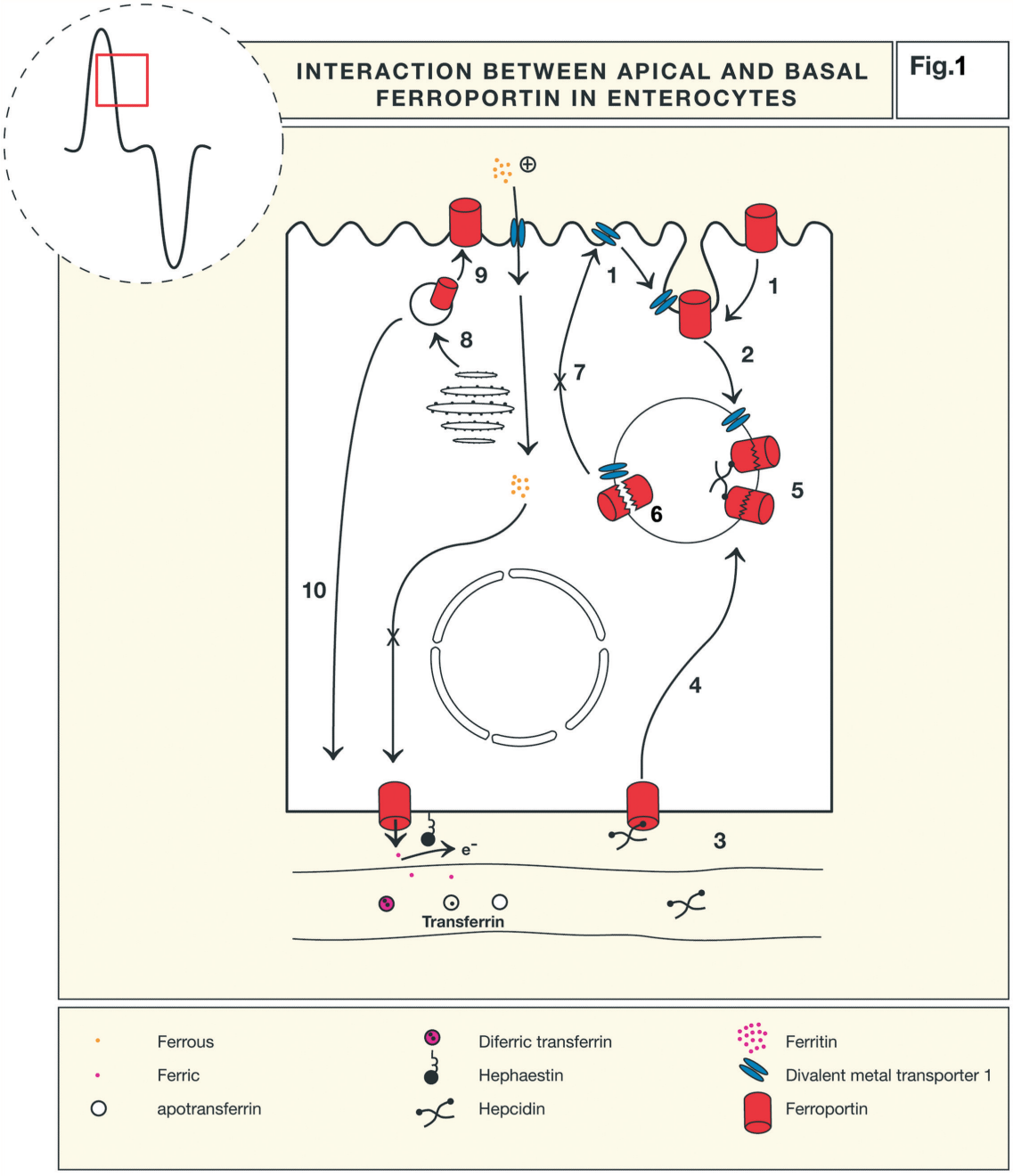


**Fig. 1.** Proposed mechanism involved in hepcidin induced decreased iron uptake. (1) Under normal circumstances ferroportin and DMT1 at the apical membrane constitutively undergoes endocytosis. (2) The endosomes fuse with late endosomes. (3) Bioactive hepcidin forms oligomers which bind to ferroportin on the basal membrane. (4) This initiates internalisation to the same compartment as apical localised ferroportin. (5) Other strands of hepcidin cross-link with ferroportin from the apical membrane. (6) Ferroportin is sorted to lysosomes for destruction. (7) Recycling of DMT1 is prevented from returning to the cell membrane resulting in a fall in iron uptake. (8) New ferroportin is synthesised by rough endoplasmic reticulum and targets to the apical (9) or basolateral (10) surface. Approximately 50% of the DMT1 remains on the apical membrane and is not linked to ferroportin induced internalisation (Oates and Thomas, 2005b). Iron transport across the basal membrane also falls.

aggregated form binds ferroportin at the basal membrane and is targeted to lysosomes via late endosomes. Ferroportin on the apical membrane also constitutively enters the cell along with DMT1. The two pathways converge in or near a late endosomes. At this site hepcidin cross links basal ferroportin with apical membrane derived ferroportin causing the complex to be targeted to the lysosomes along with DMT1. This leads to reduced apical membrane expression of DMT1 and in turn iron absorption. Normally DMT1 and ferroportin leave the apical membrane in the same endosome. In the absence of basal derived hepcidin/ferroportin DMT1 and ferroportin return to the apical membrane via a recycling compartment. The recent development of transgenic mouse models makes the testing of this hypothesis possible (Viatte et al., 2006).

## Intracellular handling of iron

There is considerable evidence to show that following uptake at the apical membrane the movement of iron through the enterocyte involves interactions with various organelles before it exits at the basal membrane. Organelles situated in a centrally located supranuclear region appear to be an important staging post for the movement of iron through the enterocyte. High resolution autoradiography have shown that following an intragastric dose of radiolabelled iron, the iron passes into the enterocyte via the microvilli and terminal web and links with the rough endoplasmic reticulum (Bedard et al., 1971a; Humphrys et al., 1977). In enterocytes HFE localises to the terminal web and may play a direct role in iron absorption (Levy et al., 2000; Fergelot et al., 2002; Laftah et al., 2004; West et al., 2006).

In *sla*$^{-/-}$ mice in which the ferroxidase hephaestin is mutated iron accumulates in ferritin adjacent to the endoplasmic reticulum (Bedard et al., 1971b). This is a similar site to where the majority of hephaestin is situated and suggests that hephaestin may have an intracellular role in iron movement (Frazer et al., 2001). Interestingly, this is a similar site to where iron accumulates in enterocytes of *ferroportin*$^{-/-}$ mice (Donovan et al., 2005), suggesting that ferroportin also may operate at this site. What leads to the accumulation of iron at this site when hephaestin and ferroportin loose function is unclear but one possibility is that failure of hephaestin and ferroportin to operate properly leads to sequestration by ferritin as evidenced by Perl's staining (Bedard et al., 1971b; Donovan et al., 2005). Nonetheless, these observations are in part consistent with the hypothesis from the author's laboratory that DMT1 and ferroportin operate within lysosomes (Thomas and Oates, 2004b; Oates and Thomas, 2005b) since these organelles are mainly concentrated in the apical cytoplasm (West et al., 2006). It also suggests that hephaestin and ferroportin may operate in at least two site during the movement of iron. Further studies are required to resolve these hypotheses.

Other proteins that are present within the enterocyte are transferrin and TfR. As stated above TfR haploinsufficiency results in mild iron deficiency perhaps suggesting a direct role in iron absorption. If this is the case then it is unlikely by binding to transferrin bound iron and providing a sense of transferrin saturation (Pollack et al., 1963). However, TfR may be involved in the regulation of iron absorption either by the enterocyte or in the crypt region (Trinder et al., 2002).

### *Release of iron*

The release component of iron absorption involves two recently cloned proteins, a copper-oxidase termed hephaestin (Vulpe et al., 1999) and ferroportin. Ferroxidation of released iron by hephaestin is thought necessary to enable binding to plasma transferrin (Osaki and Johnson, 1969) (Fig. 1).

## Protein interaction with ferroportin may be different in macrophages and enterocytes

Corradini and co-workers showed by histochemical means that mutations to ferroportin increased iron accumulation in macrophages but not in enterocytes (Corradini et al., 2005). Furthermore *ferroportin*$^{+/-}$ mice have iron loading of macrophages and mild iron-restricted erythropoiesis, however they do not show signs of liver iron loading. This suggests that iron absorption by enterocytes may be normal and the difference between macrophage and enterocyte may lie in the interaction of ferroportin with other cell specific proteins (Donovan et al., 2005).

This review has dealt with the handling of non-heme iron by the enterocyte. Since the major form of iron handled by macrophages is heme following erythrophagocytosis and in the case of enterocytes heme derived from animal sources this review will now compare and contrast possible ferroportin-protein interactions in macrophage and enterocytes with respect to heme-iron.

### *Uptake of heme occurs by different pathways in macrophages compared with enterocytes*

Unlike macrophages which process heme from phagocytosed red blood cells, enterocytes acquire dietary heme by the activity of a heme carrier protein (Latunde-Dada et al., 2006) or a receptor mediated endocytotic process (Oates and West, 2006). This is likely to result in partitioning of heme to different compartments during the breakdown of heme in the respective cell types.

### *Enterocytes and macrophages breakdown heme by different routes*

In macrophages, a key interaction is likely to be that of HO-1 and ferroportin in the recycling of heme from

senescent erythrocytes. In macrophages this requires HO-1 as evidenced by *HO1*$^{-/-}$ mice which manifest with iron overload particularly within macrophages (Poss and Tonegawa, 1997). There appear two separate fates for catabolized heme-iron in macrophages, a HO-1 dependent pathway, where iron from heme is efficiently effluxed. A second HO-1 independent pathway results in the freed iron remaining within the cell probably stored within ferritin (Bissell and Guzelian, 1980). This suggests that in macrophages for optimal release of Fe(II) from heme, HO-1 and ferroportin needs to interact (Knutson et al., 2005) but not in the HO-1 independent pathway where iron is retained within ferritin. Supporting this, knockdown of ferroportin in human macrophages produces iron accumulation within ferritin (Galli et al., 2004).

In enterocytes, heme is broken down by the activity of HO, although it is still unclear whether enterocytes use HO-1 or HO-2 to breakdown heme (Oates and West, 2006).

### *Regulation of ferroportin in macrophages versus enterocytes*

A co-ordinated up-regulation in ferroportin expression during iron-restricted erythropoiesis was observed in macrophages and enterocytes (Canonne-Hergaux et al., 2006), but this report is at variance with another study (Abboud and Haile, 2000). Interestingly, in *IRP2*$^{-/-}$ mice the expression of ferroportin in macrophages was reduced compared with the intestine. It was also independent of hepcidin since this was similar to wild type animals (Galy et al., 2005), and possibly modulated by nitric oxide (Liu et al., 2002). In contrast deletion of hepcidin resulted in upregulation of ferroportin in macrophages and enterocytes (Viatte et al., 2005). Thus ferroportin appears to be independently regulated by both hepcidin-dependent and hepcidin - independent pathways in macrophages and enterocytes.

### *Iron loading increases ferroportin expression in macrophages and down regulates it in enterocytes*

Iron loading in macrophages *in vitro* results in up-regulation of ferroportin expression (Knutson et al., 2005) and its displacement from intracellular vesicles to the cell membrane (Delaby et al., 2005). In the case of intestinal cell lines iron loading reduced ferroportin expression presumably by transcriptional means (Zoller et al., 2001; Martini et al., 2002).

### *Differences in the mass of ferroportin*

Ferroportin is glycosylated in both macrophage and enterocyte, although other yet to be determined modifications are likely since following glycosidase treatment the mass of ferroportin in enterocytes is greater than in macrophages (Canonne-Hergaux et al., 2006).

### *Macrophages require ceruloplasmin while enterocytes require hephaestin for the release of iron*

The released iron from tissues requires ferroportin coupled with ferroxidase activity probably to convert ferrous to ferric iron in order to bind transferrin (Osaki and Johnson, 1969). The ferroxidase used by macrophages is ceruloplasmin, while enterocytes require hephaestin (Harris et al., 1999; Vulpe et al., 1999). It is likely that ceruloplasmin operates extracellularly, while hephaestin either operates within vesicles or on the basal membrane.

### *Macrophages respond quicker to hepcidin than do enterocyte*

Exposure to hepcidin produced hypoferremia within 3 hrs (Rivera et al., 2005) while iron absorption fell after 24 hrs (Laftah et al., 2004). This suggests that the hepcidin-induced hypoferremia is primarily the result of sequestration of iron by macrophages rather than the lost contribution of iron from the intestine. Further studies are required to confirm this hypothesis.

In view of the preceding discussion it is likely that ferroportin functions in a distinctly cell context manner in macrophages compared with enterocytes. This review will now consider additional key unresolved questions regarding ferroportin.

## Additional unresolved issues

### *The topology of ferroportin requires clarification*

There is considerable debate about the membrane topology of ferroportin. It has been suggested that ferroportin has 10- (Donovan et al., 2000; McKie et al., 2000), 9- (Devalia et al., 2002; Goncalves et al., 2006), or 12- transmembrane domains (Liu et al., 2005). Some groups predict that the largest loop between transmembrane domains 5 and 6 is extracellular, while others suggest it is intracellular (Devalia et al., 2002; Liu et al., 2005; De Domenico et al., 2006). In addition, antibodies raised against sequences within this loop and used in immunofluorescent studies on viable cells transfected with ferroportin failed to produce a signal indicating the protein was intracellular (Liu et al., 2005; Goncalves et al., 2006). In marked contrast when an antibody raised against a similar region of ferroportin was incubated with viable enterocytes (Caco-2 and IEC-6 cells) the largest loop in ferroportin was extracellular (McKie et al., 2000; Thomas and Oates, 2002, 2004a,b ; Oates and Thomas, 2005b). This may suggest that ferroportin exists in two states, right side out and inside out. This might be achieved during synthesis, function, or that the structure differs between cells types, including enterocytes. If ferroportin can exists in two

states with respect to the membrane then this may be one explanation as to why hepcidin only interacts with ferroportin on the basal membrane and not the apical membrane (Yamaji et al., 2004) and conversely why the antibody used in the author's laboratory only operates on the apical and not the basal membrane (Thomas and Oates, 2004a). It may explain why under some circumstances the large loop in ferroportin is intracellular in some cases while extracellular in other circumstances. Finally it may also explain variations in ferroportin function with respect to the ferroportin disease if one state is favoured over another.

### *Full characterization of ferroportin as a transporter is missing*

The evidence for ferroportin as an iron efflux transporter is persuasive but still indirect and based on changes in iron responsive proteins, changes in cellular ferritin levels, efflux of iron from transferrin and iron from xenopus oocytes (McGregor et al., 2005). Two studies demonstrated release of iron from Xenopus oocytes by over expressing both DMT1 and ferroportin. Fe(II) was facilitated by DMT1 under acidic conditions and the iron released under neutral pH was measured and attributed to ferroportin. Similarly in vitro studies using various mutant forms of ferroportin were less effective in releasing iron than wild type ferroportin indicating that ferroportin is clearly involved in the release of iron (McGregor et al., 2005; Schimanski et al., 2005). However, it cannot be excluded that under some circumstances ferroportin modulates the activity of other proteins such as DMT1 in addition to being an iron exporter.

## Conclusion

It is likely that hepcidin interacts with ferroportin differently in enterocytes and macrophages. From the author's laboratory and from available evidence hepcidin/ferroportin plays a key role in the uptake of iron in enterocytes by an interaction between basal and apical localised ferroportin. In contrast in macrophages hepcidin/ferroportin impairs the release of iron resulting in cellular iron sequestration. In view of the fact that the enterocyte is primarily responsible for body iron loading, understanding the regulatory process and mechanisms that carry out iron absorption is vital to a more complete understanding of the molecular basis of the various forms of hereditary hemochromatosis. This will be aided by studies looking at protein-protein interaction in the context of the enterocyte.

*Acknowledgements.* The National Health and Medical Research Council of Australia for previous funding of this work (Grant#211940). The author thanks Carla Thomas for assistance and Umbreen Ahmed and Denise Tomizzi for constructive comments during the writing of this review. The artistic contribution by Joanna Lamb is also gratefully appreciated.

## References

Abboud S. and Haile D.J. (2000). A novel mammalian iron-regulated protein involved in intracellular iron metabolism. J. Biol. Chem. 275, 19906-19912.

Ajioka R.S., Levy J.E., Andrews N.C. and Kushner J.P. (2002). Regulation of iron absorption in Hfe mutant mice. Blood 100, 1465-1469.

Anderson G.J., Frazer D.M., Wilkins S.J., Becker E.M., Millard K.N., Murphy T.L., McKie A.T. and Vulpe C.D. (2002). Relationship between intestinal iron-transporter expression, hepatic hepcidin levels and the control of iron absorption. Biochem. Soc. Trans. 30, 724-726.

Andriopoulos B. and Pantopoulos K. (2006). Hepcidin generated by hepatoma cells inhibits iron export from co-cultured THP1 monocytes. J. Hepatol. 44, 1125-1131.

Bao B., Peatman E., Xu P., Li P., Zeng H., He C. and Liu Z. (2006). The catfish liver-expressed antimicrobial peptide 2 (LEAP-2) gene is expressed in a wide range of tissues and developmentally regulated. Mol. Immunol. 43, 367-377.

Bayele H.K., McArdle H. and Srai S.K. (2006). Cis and trans regulation of hepcidin expression by Upstream Stimulatory Factor. Blood 108, 4237-4245.

Bedard Y.C., Pinkerton P.H. and Simon G.T. (1971a). Radioautographic observations on iron absorption by the normal mouse duodenum. Blood 38, 232-245.

Bedard Y.C., Pinkerton P.H. and Simon G.T. (1971b). Ultrastructure of the duodenal mucosa of mice with a hereditary defect in iron absorption. J. Pathol. 104, 45-51.

Beresford C.H., Neale R.J. and Brooks O.G. (1971). Iron absorption and pyrexia. Lancet 1, 568-572.

Beutler E. (2003). The HFE Cys282Tyr mutation as a necessary but not sufficient cause of clinical hereditary hemochromatosis. Blood 101, 3347-3350.

Bezwoda W.R., Bothwell T.H., Torrance J.D., MacPhail A.P., Charlton R.W., Kay G. and Levin J. (1979). The relationship between marrow iron stores, plasma ferritin concentrations and iron absorption. Scand. J. Haematol. 22, 113-120.

Bissell D.M. and Guzelian P.S. (1980). Degradation of endogenous hepatic heme by pathways not yielding carbon monoxide. Studies in normal rat liver and in primary hepatocyte culture. J. Clin. Invest. 65, 1135-1140.

Camaschella C., Roetto A., Cali A., De Gobbi M., Garozzo G., Carella M., Majorano N., Totaro A. and Gasparini P. (2000). The gene TFR2 is mutated in a new type of haemochromatosis mapping to 7q22. Nat. Genet. 25, 14-15.

Canonne-Hergaux F., Donovan A., Delaby C., Wang H.J. and Gros P. (2006). Comparative studies of duodenal and macrophage ferroportin proteins. Am. J. Physiol. Gastrointest. Liver Physiol. 290, G156-163.

Canonne-Hergaux F., Levy J.E., Fleming M.D., Montross L.K., Andrews N.C. and Gros P. (2001). Expression of the DMT1 (NRAMP2/DCT1) iron transporter in mice with genetic iron overload disorders. Blood 97, 1138-1140.

Cartwright G.E. (1966). The anemia of chronic disorders. Semin. Hematol. 3, 351-375.

Cazzola M. (2003). Genetic disorders of iron overload and the novel "ferroportin disease". Haematologica 88, 721-724.

Cazzola M., Cremonesi L., Papaioannou M., Soriani N., Kioumi A.,

Charalambidou A., Paroni R., Romtsou K., Levi S., Ferrari M., Arosio P. and Christakis J. (2002). Genetic hyperferritinaemia and reticuloendothelial iron overload associated with a three base pair deletion in the coding region of the ferroportin gene (SLC11A3). Br. J. Haematol. 119, 539-546.

Conrad M.E., Umbreit J.N. and Moore E.G. (1993a). Rat duodenal iron-binding protein mobilferrin is a homologue of calreticulin. Gastroenterology 104, 1700-1704.

Conrad M.E., Umbreit J.N., Peterson R.D., Moore E.G. and Harper K.P. (1993b). Function of integrin in duodenal mucosal uptake of iron. Blood 81, 517-521.

Constante M., Jiang W., Wang D., Raymond V.A., Bilodeau M. and Santos M.M. (2006). Distinct requirements for Hfe in basal and induced hepcidin levels in iron overload and inflammation. Am. J. Physiol. Gastrointest. Liver Physiol. 291, G229-237.

Corradini E., Montosi G., Ferrara F., Caleffi A., Pignatti E., Barelli S., Garuti C. and Pietrangelo A. (2005). Lack of enterocyte iron accumulation in the ferroportin disease. Blood Cells Mol. Dis. 35, 315-318.

Cortell S. and Conrad M.E. (1967). Effect of endotoxin on iron absorption. Am. J. Physiol. 213, 43-47.

De Domenico I., Ward D.M., Musci G. and Kaplan J. (2006). Iron overload due to mutations in ferroportin. Haematologica 91, 92-95.

De Domenico I., Ward D.M., Nemeth E., Vaughn M.B., Musci G., Ganz T. and Kaplan J. (2005). The molecular basis of ferroportin-linked hemochromatosis. Proc. Natl. Acad. Sci. USA 102, 8955-8960.

Delaby C., Pilard N., Goncalves A.S., Beaumont C. and Canonne-Hergaux F. (2005). Presence of the iron exporter ferroportin at the plasma membrane of macrophages is enhanced by iron loading and down-regulated by hepcidin. Blood 106, 3979-3984.

Detivaud L., Nemeth E., Boudjema K., Turlin B., Troadec M.B., Leroyer P., Ropert M., Jacquelinet S., Courselaud B., Ganz T., Brissot P. and Loreal O. (2005). Hepcidin levels in humans are correlated with hepatic iron stores, hemoglobin levels, and hepatic function. Blood 106, 746-748.

Devalia V., Carter K., Walker A.P., Perkins S.J., Worwood M., May A. and Dooley J.S. (2002). Autosomal dominant reticuloendothelial iron overload associated with a 3-base pair deletion in the ferroportin 1 gene (SLC11A3). Blood 100, 695-697.

Donovan A., Brownlie A., Zhou Y., Shepard J., Pratt S.J., Moynihan J., Paw B.H., Drejer A., Barut B., Zapata A., Law T.C., Brugnara C., Lux S.E., Pinkus G.S., Pinkus J.L., Kingsley P.D., Palis J., Fleming M.D., Andrews N.C. and Zon L.I. (2000). Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. Nature 403, 776-781.

Donovan A., Lima C.A., Pinkus J.L., Pinkus G.S., Zon L.I., Robine S. and Andrews N.C. (2005). The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. Cell Metab. 1, 191-200.

Drakesmith H., Schimanski L.M., Ormerod E., Merryweather-Clarke A.T., Viprakasit V., Edwards J.P., Sweetland E., Bastin J.M., Cowley D., Chinthammitr Y., Robson K.J. and Townsend A.R. (2005). Resistance to hepcidin is conferred by hemochromatosis-associated mutations of ferroportin. Blood 106, 1092-1097.

Ezeh C., Ugochukwu C.C., Weinstein J. and Okpala I. (2005). Hepcidin, haemoglobin and ferritin levels in sickle cell anaemia. Eur. J. Haematol. 74, 86-88.

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., Prass C.E., Quintana L., Starnes S.M., Schatzman R.C., Brunke K.J., Drayna D.T., Risch N.J., Bacon B.R. 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. USA 95, 1472-1477.

Fergelot P., Ropert-Bouchet M., Abgueguen E., Orhant M., Radosavljevic M., Grimber G., Jouan H., Le Gall J.Y., Mosser J., Gilfillan S. and Bahram S. (2002). Iron overload in mice expressing HFE exclusively in the intestinal villi provides evidence that HFE regulates a functional cross-talk between crypt and villi enterocytes. Blood Cells Mol. Dis 28, 348-360.

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, 383-386.

Frazer D.M., Inglis H.R., Wilkins S.J., Millard K.N., Steele T.M., McLaren G.D., McKie A.T., Vulpe C.D. and Anderson G.J. (2004). Delayed hepcidin response explains the lag period in iron absorption following a stimulus to increase erythropoiesis. Gut 53, 1509-1515.

Frazer D.M., Vulpe C.D., McKie A.T., Wilkins S.J., Trinder D., Cleghorn G.J. and Anderson G.J. (2001). Cloning and gastrointestinal expression of rat hephaestin: relationship to other iron transport proteins. Am .J .Physiol. Gastrointest. Liver Physiol. 281, G931-939.

Frazer D.M., Wilkins S.J., Becker E.M., Vulpe C.D., McKie A.T., Trinder D. and Anderson G.J. (2002). Hepcidin expression inversely correlates with the expression of duodenal iron transporters and iron absorption in rats. Gastroenterology 123, 835-844.

Galli A., Bergamaschi G., Recalde H., Biasiotto G., Santambrogio P., Boggi S., Levi S., Arosio P. and Cazzola M. (2004). Ferroportin gene silencing induces iron retention and enhances ferritin synthesis in human macrophages. Br. J. Haematol. 127, 598-603.

Galy B., Ferring D., Minana B., Bell O., Janser H.G., Muckenthaler M., Schumann K. and Hentze M.W. (2005). Altered body iron distribution and microcytosis in mice deficient in iron regulatory protein 2 (IRP2). Blood 106, 2580-2589.

Gehrke S.G., Pietrangelo A., Kascak M., Braner A., Eisold M., Kulaksiz H., Herrmann T., Hebling U., Bents K., Gugler R. and Stremmel W. (2005). HJV gene mutations in European patients with juvenile hemochromatosis. Clin. Genet. 67, 425-428.

Gehrke S.G., Riedel H.D., Herrmann T., Hadaschik B., Bents K., Veltkamp C. and Stremmel W. (2003). UbcH5A, a member of human E2 ubiquitin-conjugating enzymes, is closely related to SFT, a stimulator of iron transport, and is up-regulated in hereditary hemochromatosis. Blood 101, 3288-3293.

Gerard B., Farman N., Raja K.B., Eugene E., Grandchamp B. and Beaumont C. (1996). Expression of H and L ferritin mRNAs in mouse small intestine. Exp. Cell Res. 228, 8-13.

Goncalves A.S., Muzeau F., Blaybel R., Hetet G., Driss F., Delaby C., Canonne-Hergaux F. and Beaumont C. (2006). Wild-type and mutant ferroportins do not form oligomers in transfected cells. Biochem. J. 396, 265-275.

Gubler C.J., Cartwright G.E. and Wintrobe M.M. (1950). The anemis of infection. X. The effect of infection on the absorption and storage of iron by the rat. J. Biol. Chem. 184, 563-574.

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, 482-488.

Gunshin H., Starr C.N., Direnzo C., Fleming M.D., Jin J., Greer E.L., Sellers V.M., Galica S.M. and Andrews N.C. (2005). Cybrd1 (duodenal cytochrome b) is not necessary for dietary iron absorption in mice. Blood 106, 2879-2883.

Harris Z.L., Durley A.P., Man T.K. and Gitlin J.D. (1999). Targeted gene disruption reveals an essential role for ceruloplasmin in cellular iron efflux. Proc. Natl. Acad. Sci. USA 96, 10812-10817.

Haurani F.I. and Green D. (1967). Primary defective iron reutilization. Response to testosterone therapy. Am. J. Med. 42, 151-158.

Hoshino K., Takeuchi O., Kawai T., Sanjo H., Ogawa T., Takeda Y., Takeda K. and Akira S. (1999). Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. J. Immunol. 162, 3749-3752.

Hubert N. and Hentze M.W. (2002). Previously uncharacterized isoforms of divalent metal transporter (DMT)-1: implications for regulation and cellular function. Proc. Natl. Acad. Sci. USA 99, 12345-12350.

Humphrys J., Walpole B. and Worwood M. (1977). Intracellular iron transport in rat intestinal epithelium: biochemical and ultrastructural observations. Br. J. Haematol. 36, 209-217.

Hunter H.N., Fulton D.B., Ganz T. and Vogel H.J. (2002). The solution structure of human hepcidin, a peptide hormone with antimicrobial activity that is involved in iron uptake and hereditary hemochromatosis. J. Biol. Chem. 277, 37597-37603.

Ilyin G., Courselaud B., Troadec M.B., Pigeon C., Alizadeh M., Leroyer P., Brissot P. and Loreal O. (2003). Comparative analysis of mouse hepcidin 1 and 2 genes: evidence for different patterns of expression and co-inducibility during iron overload. FEBS Lett. 542, 22-26.

Johnson D., Bayele H., Johnston K., Tennant J., Srai S.K. and Sharp P. (2004). Tumour necrosis factor alpha regulates iron transport and transporter expression in human intestinal epithelial cells. FEBS Lett. 573, 195-201.

Jouanolle A.M., Douabin-Gicquel V., Halimi C., Loreal O., Fergelot P., Delacour T., de Lajarte-Thirouard A.S., Turlin B., Le Gall J.Y., Cadet E., Rochette J., David V. and Brissot P. (2003). Novel mutation in ferroportin 1 gene is associated with autosomal dominant iron overload. J. Hepatol. 39, 286-289.

Kattamis A., Papassotiriou I., Palaiologou D., Apostolakou F., Galani A., Ladis V., Sakellaropoulos N. and Papanikolaou G. (2006). The effects of erythropoetic activity and iron burden on hepcidin expression in patients with thalassemia major. Haematologica 91, 809-812.

Kemna E., Pickkers P., Nemeth E., van der Hoeven H. and Swinkels D. (2005). Time-course analysis of hepcidin, serum iron, and plasma cytokine levels in humans injected with LPS. Blood 106, 1864-1866.

Knutson M. and Wessling-Resnick M. (2003). Iron metabolism in the reticuloendothelial system. Crit. Rev. Biochem. Mol. Biol. 38, 61-88.

Knutson M.D., Oukka M., Koss L.M., Aydemir F. and Wessling-Resnick M. (2005). Iron release from macrophages after erythrophagocytosis is up-regulated by ferroportin 1 overexpression and down-regulated by hepcidin. Proc. Natl. Acad. Sci. USA 102, 1324-1328.

Kondo H., Saito K., Grasso J.P. and Aisen P. (1988). Iron metabolism in the erythrophagocytosing Kupffer cell. Hepatology 8, 32-38.

Krause A., Neitz S., Magert H.J., Schulz A., Forssmann W.G., Schulz-Knappe P. and Adermann K. (2000). LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. FEBS Lett. 480, 147-150.

Krijt J., Cmejla R., Sykora V., Vokurka M., Vyoral D. and Necas E. (2004). Different expression pattern of hepcidin genes in the liver and pancreas of C57BL/6N and DBA/2N mice. J. Hepatol. 40, 891-896.

Laftah A.H., Ramesh B., Simpson R.J., Solanky N., Bahram S., Schumann K., Debnam E.S. and Srai S.K. (2004). Effect of hepcidin on intestinal iron absorption in mice. Blood 103, 3940-3944.

Latunde-Dada G.O., McKie A.T. and Simpson R.J. (2006). Animal models with enhanced erythropoiesis and iron absorption. Biochim. Biophys. Acta 1762, 414-423.

Latunde-Dada G.O., Simpson R.J. and McKie A.T. (2006). Recent advances in mammalian haem transport. Trends Biochem. Sci. 31, 182-188.

Lee G.R. (1983). The anemia of chronic disease. Semin. Hematol. 20, 61-80.

Lee P., Peng H., Gelbart T. and Beutler E. (2004). The IL-6- and lipopolysaccharide-induced transcription of hepcidin in HFE-, transferrin receptor 2-, and beta 2-microglobulin-deficient hepatocytes. Proc. Natl. Acad. Sci. USA 101, 9263-9265.

Lee P., Peng H., Gelbart T., Wang L. and Beutler E. (2005). Regulation of hepcidin transcription by interleukin-1 and interleukin-6. Proc. Natl. Acad. Sci. USA 102, 1906-1910.

Lesbordes-Brion J.C., Viatte L., Bennoun M., Lou D.Q., Ramey G., Houbron C., Hamard G., Kahn A. and Vaulont S. (2006). Targeted disruption of the hepcidin 1 gene results in severe hemochromatosis. Blood 108, 1402-1405.

Leung P.S., Srai S.K., Mascarenhas M., Churchill L.J. and Debnam E.S. (2005). Increased duodenal iron uptake and transfer in a rat model of chronic hypoxia is accompanied by reduced hepcidin expression. Gut 54, 1391-1395.

Levy J.E., Montross L.K. and Andrews N.C. (2000). Genes that modify the hemochromatosis phenotype in mice. J. Clin. Invest. 105, 1209-1216.

Liu X.B., Hill P. and Haile D.J. (2002). Role of the ferroportin iron-responsive element in iron and nitric oxide dependent gene regulation. Blood Cells Mol. Dis. 29, 315-326.

Liu X.B., Nguyen N.B., Marquess K.D., Yang F. and Haile D.J. (2005). Regulation of hepcidin and ferroportin expression by lipopolysaccharide in splenic macrophages. Blood Cells Mol. Dis. 35, 47-56.

Liu X.B., Yang F. and Haile D.J. (2005). Functional consequences of ferroportin 1 mutations. Blood Cells Mol. Dis. 35, 33-46.

Lou D.Q., Nicolas G., Lesbordes J.C., Viatte L., Grimber G., Szajnert M.F., Kahn A. and Vaulont S. (2004). Functional differences between hepcidin 1 and 2 in transgenic mice. Blood 103, 2816-2821.

Martini L.A., Tchack L. and Wood R.J. (2002). Iron treatment downregulates DMT1 and IREG1 mRNA expression in Caco-2 cells. J . Nutr. 132, 693-696.

Mazur A., Feillet-Coudray C., Romier B., Bayle D., Gueux E., Ruivard M., Coudray C. and Rayssiguier Y. (2003). Dietary iron regulates hepatic hepcidin 1 and 2 mRNAs in mice. Metabolism 52, 1229-1231.

McGregor J.A., Shayeghi M., Vulpe C.D., Anderson G.J., Pietrangelo A., Simpson R.J. and McKie A.T. (2005). Impaired iron transport activity of ferroportin 1 in hereditary iron overload. J. Membr. Biol. 206, 3-7.

McKie A.T., Barrow D., Latunde-Dada G.O., Rolfs A., Sager G., Mudaly E., Mudaly M., Richardson C., Barlow D., Bomford A., Peters T.J., Raja K.B., Shirali S., Hediger M.A., Farzaneh F. and Simpson R.J.

(2001). An iron-regulated ferric reductase associated with the absorption of dietary iron. Science 291, 1755-1759.

McKie A.T., Marciani P., Rolfs A., Brennan K., Wehr K., Barrow D., Miret S., Bomford A., Peters T.J., Farzaneh F., Hediger M.A., Hentze M.W. and Simpson R.J. (2000). A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. Mol. Cell 5, 299-309.

Millard K.N., Frazer D.M., Wilkins S.J. and Anderson G.J. (2004). Changes in the expression of intestinal iron transport and hepatic regulatory molecules explain the enhanced iron absorption associated with pregnancy in the rat. Gut 53, 655-660.

Montosi G., Donovan A., Totaro A., Garuti C., Pignatti E., Cassanelli S., Trenor C.C., Gasparini P., Andrews N.C. and Pietrangelo A. (2001). Autosomal-dominant hemochromatosis is associated with a mutation in the ferroportin (SLC11A3) gene. J. Clin. Invest. 108, 619-623.

Muir W.A., Hopfer U. and King M. (1984). Iron transport across brush-border membranes from normal and iron-deficient mouse upper small intestine. J. Biol. Chem. 259, 4896-4903.

Nemeth E., Preza G.C., Jung C.L., Kaplan J., Waring A.J. and Ganz T. (2006). The N-terminus of hepcidin is essential for its interaction with ferroportin: structure-function study. Blood 107, 328-333.

Nemeth E., Rivera S., Gabayan V., Keller C., Taudorf S., Pedersen B.K. and Ganz T. (2004). IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J. Clin. Invest. 113, 1271-1276.

Nemeth E., Tuttle M.S., Powelson J., Vaughn M.B., Donovan A., Ward D.M., Ganz T. and Kaplan J. (2004). Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 306, 2090-2093.

Nemeth E., Valore E.V., Territo M., Schiller G., Lichtenstein A. and Ganz T. (2003). Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. Blood 101, 2461-2463.

Nicolas G., Bennoun M., Devaux I., Beaumont C., Grandchamp B., Kahn A. and Vaulont S. (2001). Lack of hepcidin gene expression and severe tissue iron overload in upstream stimulatory factor 2 (USF2) knockout mice. Proc. Natl. Acad. Sci. USA 98, 8780-8785.

Nicolas G., Bennoun M., Porteu A., Mativet S., Beaumont C., Grandchamp B., Sirito M., Sawadogo M., Kahn A. and Vaulont S. (2002). Severe iron deficiency anemia in transgenic mice expressing liver hepcidin. Proc. Natl. Acad. Sci. USA 99, 4596-4601.

Nicolas G., Chauvet C., Viatte L., Danan J.L., Bigard X., Devaux I., Beaumont C., Kahn A. and Vaulont S. (2002). The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. J. Clin. Invest. 110, 1037-1044.

Njajou O.T., Vaessen N., Joosse M., Berghuis B., van Dongen J.W., Breuning M.H., Snijders P.J., Rutten W.P., Sandkuijl L.A., Oostra B.A., van Duijn C.M. and Heutink P. (2001). A mutation in SLC11A3 is associated with autosomal dominant hemochromatosis. Nat. Genet. 28, 213-214.

Noyes W.D., Bothwell T.H. and Finch C.A. (1960). The role of the reticulo-endothelial cell in iron metabolism. Br. J. Haematol. 6, 43-55.

Oates P.S. and Morgan E.H. (1996a). Defective iron uptake by the duodenum of Belgrade rats fed diets of different iron contents. Am. J. Physiol. 270, G826-832.

Oates P.S. and Morgan E.H. (1996b). Effects of dietary iron loading with carbonyl iron and of iron depletion on intestinal growth, morphology, and expression of transferrin receptor in the rat. Anat. Rec. 246, 364-371.

Oates P.S. and Thomas C. (2005a). Ferroportin is expressed on the mucous granule membrane of a subpopulation of goblet cells in the duodenum of the rat. Histol. Histopathol. 20, 681-687.

Oates P.S. and Thomas C. (2005b). Augmented internalisation of ferroportin to late endosomes impairs iron uptake by enterocyte-like IEC-6 cells. Pflugers Arch. 450, 317-325.

Oates P.S. and West A.R. (2006). Heme in intestinal epithelial cell turnover, differentiation, detoxification, inflammation, carcinogenesis, absorption and motility. World J. Gastroenterol. 12, 4281-4295.

Oates P.S., Thomas C. and Morgan E.H. (2000). Transferrin receptor activity and localisation in the rat duodenum. Pflugers Arch. 440, 116-124.

Ohgami R.S., Campagna D.R., Greer E.L., Antiochos B., McDonald A., Chen J., Sharp J.J., Fujiwara Y., Barker J.E. and Fleming M.D. (2005). Identification of a ferrireductase required for efficient transferrin-dependent iron uptake in erythroid cells. Nat. Genet. 37, 1264-1269.

Osaki S. and Johnson D.A. (1969). Mobilization of liver iron by ferroxidase (ceruloplasmin). J. Biol. Chem. 244, 5757-5758.

Papanikolaou G., Samuels M.E., Ludwig E.H., MacDonald M.L., Franchini P.L., Dube M.P., Andres L., MacFarlane J., Sakellaropoulos N., Politou M., Nemeth E., Thompson J., Risler J.K., Zaborowska C., Babakaiff R., Radomski C.C., Pape T.D., Davidas O., Christakis J., Brissot P., Lockitch G., Ganz T., Hayden M.R. and Goldberg Y.P. (2004). Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. Nat. Genet. 36, 77-82.

Papanikolaou G., Tzilianos M., Christakis J.I., Bogdanos D., Tsimirika K., MacFarlane J., Goldberg Y.P., Sakellaropoulos N., Ganz T. and Nemeth E. (2005). Hepcidin in iron overload disorders. Blood 105, 4103-4105.

Park C.H., Valore E.V., Waring A.J. and Ganz T. (2001). Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J. Biol. Chem. 276, 7806-7810.

Pietrangelo A. (2004). The ferroportin disease. Blood Cells Mol. Dis. 32, 131-138.

Pigeon C., Ilyin G., Courselaud B., Leroyer P., Turlin B., Brissot P. and Loreal O. (2001). A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. J. Biol. Chem. 276, 7811-7819.

Pignatti E., Mascheroni L., Sabelli M., Barelli S., Biffo S. and Pietrangelo A. (2006). Ferroportin is a monomer in vivo in mice. Blood Cells Mol. Dis. 36, 26-32.

Pollack S., Balcerzak S.P. and Crosby W.H. (1963). Transferrin and the absorption of iron. Blood 21, 33-38.

Poss K.D. and Tonegawa S. (1997). Heme oxygenase 1 is required for mammalian iron reutilization. Proc. Natl. Acad. Sci. USA 94, 10919-10924.

Quastel M.R. and Ross J.F. (1966). The effect of acute inflammation on the utilization and distribution of transferrin-bound and erythrocyte radioiron. Blood 28, 738-757.

Rivard S.R., Lanzara C., Grimard D., Carella M., Simard H., Ficarella R., Simard R., D'Adamo A.P., De Braekeleer M. and Gasparini P. (2003). Autosomal dominant reticuloendothelial iron overload (HFE type 4) due to a new missense mutation in the FERROPORTIN 1 gene (SLC11A3) in a large French-Canadian family. Haematologica 88, 824-826.

Rivera S., Liu L., Nemeth E., Gabayan V., Sorensen O.E. and Ganz T. (2005). Hepcidin excess induces the sequestration of iron and

exacerbates tumor-associated anemia. Blood 105, 1797-1802.

Rivera S., Nemeth E., Gabayan V., Lopez M.A., Farshidi D. and Ganz T. (2005). Synthetic hepcidin causes rapid dose-dependent hypoferremia and is concentrated in ferroportin-containing organs. Blood 106, 2196-2199.

Roetto A., Merryweather-Clarke A.T., Daraio F., Livesey K., Pointon J.J., Barbabietola G., Piga A., Mackie P.H., Robson K.J. and Camaschella C. (2002). A valine deletion of ferroportin 1: a common mutation in hemochromastosis type 4. Blood 100, 733-734.

Roetto A., Papanikolaou G., Politou M., Alberti F., Girelli D., Christakis J., Loukopoulos D. and Camaschella C. (2003). Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis. Nat. Genet. 33, 21-22.

Roy C.N., Weinstein D.A. and Andrews N.C. (2003). 2002 E. Mead Johnson Award for Research in Pediatrics Lecture: the molecular biology of the anemia of chronic disease: a hypothesis. Pediatr. Res. 53, 507-512.

Schade S.G. (1972). Normal incorporation of oral iron into intestinal ferritin in inflammation. Proc. Soc. Exp. Biol. Med. 139, 620-622.

Schimanski L.M., Drakesmith H., Merryweather-Clarke A.T., Viprakasit V., Edwards J.P., Sweetland E., Bastin J.M., Cowley D., Chinthammitr Y., Robson K.J. and Townsend A.R. (2005). In vitro functional analysis of human ferroportin (FPN) and hemochromatosis-associated FPN mutations. Blood 105, 4096-4102.

Schwabe R.F., Seki E. and Brenner D.A. (2006). Toll-like receptor signaling in the liver. Gastroenterology 130, 1886-1900.

Sham R.L., Phatak P.D., West C., Lee P., Andrews C. and Beutler E. (2005). Autosomal dominant hereditary hemochromatosis associated with a novel ferroportin mutation and unique clinical features. Blood Cells Mol. Dis. 34, 157-161.

Sharma N., Laftah A.H., Brookes M.J., Cooper B., Iqbal T. and Tselepis C. (2005). A role for tumour necrosis factor alpha in human small bowel iron transport. Biochem. J. 390, 437-446.

Simpson R.J., Debnam E.S., Laftah A.H., Solanky N., Beaumont N., Bahram S., Schumann K. and Srai S.K. (2003). Duodenal nonheme iron content correlates with iron stores in mice, but the relationship is altered by Hfe gene knock-out. Blood 101, 3316-3318.

Theurl I., Mattle V., Seifert M., Mariani M., Marth C. and Weiss G. (2006). Dysregulated monocyte iron homeostasis and erythropoietin formation in patients with anemia of chronic disease. Blood 107, 4142-4148.

Thomas C. and Oates P.S. (2002). IEC-6 cells are an appropriate model of intestinal iron absorption in rats. J. Nutr. 132, 680-687.

Thomas C. and Oates P.S. (2004a). Ferroportin/IREG-1/MTP-1/SLC40A1 modulates the uptake of iron at the apical membrane of enterocytes. Gut 53, 44-49.

Thomas C. and Oates P.S. (2004b). Differences in the uptake of iron from Fe(II) ascorbate and Fe(III) citrate by IEC-6 cells and the involvement of ferroportin/IREG-1/MTP-1/SLC40A1. Pflugers Arch. 448, 431-437.

Trinder D., Oates P.S., Thomas C., Sadleir J. and Morgan E.H. (2000). Localisation of divalent metal transporter 1 (DMT1) to the microvillus membrane of rat duodenal enterocytes in iron deficiency, but to hepatocytes in iron overload. Gut 46, 270-276.

Trinder D., Olynyk J.K., Sly W.S. and Morgan E.H. (2002). Iron uptake from plasma transferrin by the duodenum is impaired in the Hfe knockout mouse. Proc. Natl. Acad. Sci. USA 99, 5622-5626.

Viatte L., Lesbordes-Brion J.C., Lou D.Q., Bennoun M., Nicolas G., Kahn A., Canonne-Hergaux F. and Vaulont S. (2005). Deregulation of proteins involved in iron metabolism in hepcidin-deficient mice. Blood 105, 4861-4864.

Viatte L., Nicolas G., Lou D.Q., Bennoun M., Lesbordes-Brion J.C., Canonne-Hergaux F., Schonig K., Bujard H., Kahn A., Andrews N.C. and Vaulont S. (2006). Chronic hepcidin induction causes hyposideremia and alters the pattern of cellular iron accumulation in hemochromatotic mice. Blood 107, 2952-2958.

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, 195-199.

Wallace D.F., Clark R.M., Harley H.A. and Subramaniam V.N. (2004). Autosomal dominant iron overload due to a novel mutation of ferroportin1 associated with parenchymal iron loading and cirrhosis. J . Hepatol. 40, 710-713.

Wallace D.F., Pedersen P., Dixon J.L., Stephenson P., Searle J.W., Powell L.W. and Subramaniam V.N. (2002). Novel mutation in ferroportin1 is associated with autosomal dominant hemochromatosis. Blood 100, 692-694.

Wallace D.F., Summerville L., Lusby P.E. and Subramaniam V.N. (2005). First phenotypic description of transferrin receptor 2 knockout mouse, and the role of hepcidin. Gut 54, 980-986.

Weinstein D.A., Roy C.N., Fleming M.D., Loda M.F., Wolfsdorf J.I. and Andrews N.C. (2002). Inappropriate expression of hepcidin is associated with iron refractory anemia: implications for the anemia of chronic disease. Blood 100, 3776-3781.

Weizer-Stern O., Adamsky K., Amariglio N., Rachmilewitz E., Breda L., Rivella S. and Rechavi G. (2006). mRNA expression of iron regulatory genes in beta-thalassemia intermedia and beta-thalassemia major mouse models. Am. J. Hematol. 81, 479-483.

West A.R., Thomas C., Sadlier J. and Oates P.S. (2006). Haemochromatosis protein is expressed on the terminal web of enterocytes in proximal small intestine of the rat. Histochem. Cell Biol. 125, 283-292.

Yamaji S., Sharp P., Ramesh B. and Srai S.K. (2004). Inhibition of iron transport across human intestinal epithelial cells by hepcidin. Blood 104, 2178-2180.

Yang F., Haile D.J., Wang X., Dailey L.A., Stonehuerner J.G. and Ghio A.J. (2005). Apical location of ferroportin 1 in airway epithelia and its role in iron detoxification in the lung. Am. J. Physiol. Lung Cell. Mol. Physiol. 289, L14-23.

Yang F., Liu X.B., Quinones M., Melby P.C., Ghio A. and Haile D.J. (2002). Regulation of reticuloendothelial iron transporter MTP1 (Slc11a3) by inflammation. J. Biol. Chem. 277, 39786-39791.

Yeh K.Y., Yeh M. and Glass J. (2004). Hepcidin regulation of ferroportin 1 expression in the liver and intestine of the rat. Am. J. Physiol. Gastrointest. Liver Physiol .286, G385-394.

Yoon D., Pastore Y.D., Divoky V., Liu E., Mlodnicka A.E., Rainey K., Ponka P., Semenza G.L., Schumacher A. and Prchal J.T. (2006). Hypoxia-inducible Factor-1 Deficiency Results in Dysregulated Erythropoiesis Signaling and Iron Homeostasis in Mouse Development. J. Biol. Chem. 281, 25703-25711.

Zoller H., Koch R.O., Theurl I., Obrist P., Pietrangelo A., Montosi G., Haile D.J., Vogel W. and Weiss G. (2001). Expression of the duodenal iron transporters divalent-metal transporter 1 and ferroportin 1 in iron deficiency and iron overload. Gastroenterology 120, 1412-1419.

Accepted December 18, 2006