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Ironing out ferroportin

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Abstract

Maintaining physiologic iron concentrations in cells and tissues is critical for oxygen carriage, metabolism, growth and immunity. Iron is acquired from the diet by duodenal enterocytes, then transported in blood plasma to every cell in the body, with most iron utilized for erythrocyte production. Senescent erythrocytes' iron is recycled into plasma by splenic red pulp macrophages and liver Kupffer cells. Reserve iron is stored in macrophages and hepatocytes. There is only one route for the transfer of non-heme iron out of enterocytes, macrophages and hepatocytes to plasma: through the dedicated iron exporter ferroportin. The concentration of functional membrane-associated ferroportin is tightly controlled by its ligand, the iron-regulatory hormone hepcidin, and fine-tuned by additional tissue-specific regulatory mechanisms serving iron homeostasis, oxygen sensing, defense against infection and the requirements of increased erythropoiesis. Although much has been learned, fundamental questions about the structure and biology of this intriguing protein remain to be answered.

Introduction

A major destination for iron in the body is the heme of hemoglobin in red blood cells. Humans normally complete the synthesis of at least 2 million erythrocytes per second; each mature red blood cell contains about 280 million molecules of hemoglobin, and each of the four globin subunits contains one iron atom in heme, so that the total iron flux required to maintain erythropoiesis is about 2-3 x 10^{15} atoms of iron per second in an adult human. The erythropoietic use of iron governs the lion's share of iron trafficking, but iron is also required for mitochondrial function, DNA synthesis and other basic activities in all cells in the body; and in pregnancy the growing fetus places a further large demand on the maternal iron transport system. In humans, >90% of iron demand (or about 20-25 mg/day) is met by recycling existing iron, with uptake of 'new' iron from the diet accounting for about 1 mg of iron per day. Macrophages in the red pulp of the spleen specialise in the uptake and digestion of senescent red blood cells, can liberate the iron from heme via the enzyme heme-oxygenase-1, and release iron back into plasma. Dietary iron uptake is mediated by duodenal enterocytes that capture iron from the diet at their apical surface via the ferric reductase DcytB and the importer DMT1, and export iron into plasma through their basolateral membranes (Mackenzie and Garrick, 2005). Dietary heme represents an important source of iron, especially in meat eaters, but the mechanism of its absorption is not yet understood, and it is not known to what extent heme is converted to inorganic iron within enterocytes or transferred intact to plasma. Iron transfer from duodenal enterocytes to plasma (referred to as "mucosal transfer" in older literature on intestinal iron absorption) has long been known to be subject to regulation by systemic influences including iron deficiency, anemia and hypoxia (Finch, 1994). How iron entered plasma from either recycling macrophages or absorptive enterocytes was unknown until the year 2000, when it became apparent that despite arising from distinct lineages, and despite their sources of iron being very different, both macrophages and enterocytes use the same protein to mediate iron export: ferroportin (SLC40A1, also referred to in the literature as FPN1, MTP1 and IREG1). Moreover, ferroportin is also used to export stored iron from hepatocytes and to release maternal iron from the placenta to the fetal circulation. Three groups (Abboud and Haile, 2000; Donovan et al., 2000; McKie et al., 2000) independently discovered ferroportin, a multitransmembrane protein that is well conserved in mammals, appears to be ancient with orthologs present in plants and worms, and intriguingly is not closely related to any other known mammalian transporter proteins (although there may be some distant homology to bacterial transport proteins).

Function and importance in physiology

Early work confirmed that ferroportin is highly expressed by cells and tissues associated with iron transport: duodenal enterocytes, liver Kupffer cells and splenic red pulp macrophages, periportal hepatocytes, and the placental syncytiotrophoblast (**Figure 1)**. The ability of ferroportin to mediate iron export was shown using radioactive iron isotopes in *Xenopus laevis* oocytes injected with ferroportin cRNA and human cell lines transfected with ferroportin-expressing constructs (Donovan et al., 2000; McKie et al., 2000).

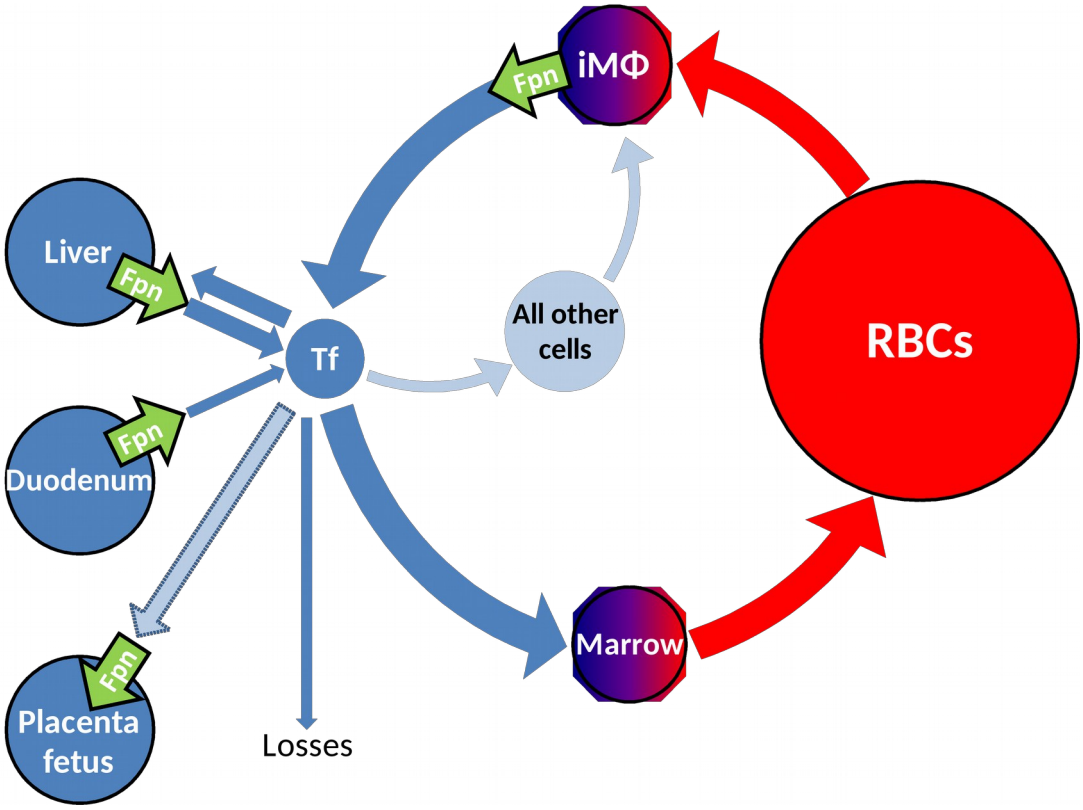

Figure 1: Systemic flows of elemental iron. The release of iron into blood plasma is mediated by the iron transporter ferroportin which also serves as the control point for the regulation of iron flows. Red blood cells (RBCs) contain the majority of the body iron, and iron fluxes in humans are dominated by the utilization of iron by erythroblasts in the marrow, and recycling of iron from the hemoglobin of senescent erythrocytes by erythrophagocytosing macrophages (iMΦ=iron-recycling macrophages, mostly in the spleen). Other cells in the body utilize smaller amounts of iron, and this too is recycled by macrophages after cells die. The liver is the major storage organ for iron from which iron can be mobilized in times of need, and where it is deposited when there is a surplus of iron in the body. Duodenal absorption of iron is the sole external source of iron, and compensates for the losses of iron from the body which are normally relatively small. In pregnant women, iron is transferred to fetal blood through ferroportin in the placenta.

The key role of ferroportin in iron export *in vivo* was established using a series of transgenic mice (Donovan et al., 2005). Global $Fpn^{null/null}$ mice do not complete embryonic development, likely because ferroportin is required for transfer of iron to the embryo through epithelial cells of the extraembryonic visceral endoderm (exVE). When ferroportin was deleted only in embryo proper but spared in exVE and placenta, mice survived to birth but rapidly became anemic and runted after birth and showed marked iron accumulation in enterocytes, splenic macrophages and Kupffer cells, and in hepatocytes. Adult mice in which ferroportin was inducibly and exclusively deleted in intestinal cells also rapidly became anemic, demonstrating that ferroportin is essential for intestinal absorption of iron. Further work inactivating ferroportin in macrophages and in hepatocytes showed that ferroportin is required in these cells for efficient mobilisation of stored iron; mice lacking ferroportin in these sites become anemic more rapidly than controls when fed iron-deficient diets (Zhang et al., 2011b; Zhang et al., 2012). However the same mice on standard diet maintained intact erythropoiesis, indicating that duodenal ferroportin upregulation and increased intestinal absorption can compensate for the decreased activity of ferroportin in recycling and storage compartments, especially in mice which compared to humans derive a larger proportion of their iron flows from intestinal absorption. Overall, it appears that ferroportin is the only way elemental iron can exit cells to enter plasma.

Multilevel regulation of ferroportin

The regulation of ferroportin expression is complex, with important layers of control at transcriptional, post-transcriptional, post-translational and cell-lineage levels. These different phases of control allow multiple and diverse physiological inputs to influence ferroportin activity at its various sites of expression. Notably, ferroportin regulation varies among different cell types, allowing added flexibility in controlling systemic iron flow under different conditions (**Figure 2**).

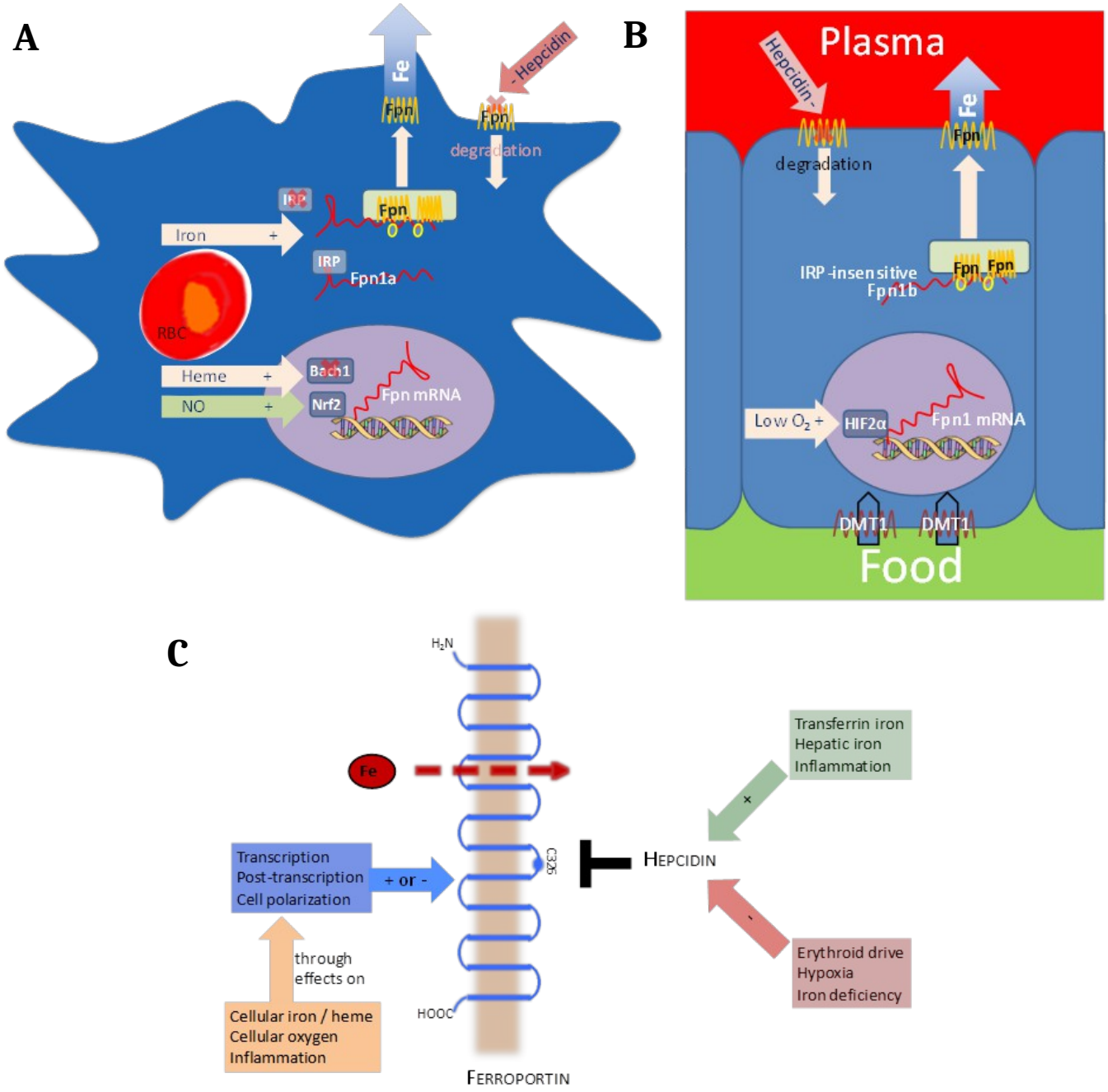

Figure 2: Ferroportin regulation. Cellular iron efflux is proportional to the concentration of ferroportin molecules on the membrane, and multiple levels of ferroportin regulation exist in different cell types. Panel A: In iron-recycling macrophages, following erythrophagocytosis both heme and iron increase ferroportin production. Heme regulates ferroportin transcriptionally via the Bach1/Nrf2 complex (heme causes degradation of repressor Bach1, resulting in the stimulation of Fpn transcription by Nrf2). Iron regulates ferroportin translationally through modulating the interaction of IRPs with IREs in the 5' UTR of ferroportin (iron prevents IRPs from binding to Fpn mRNA, allowing translation to proceed). During infections with intracellular pathogens, increased NO production leads to the activation of Nrf2 and increased ferroportin transcription. Ferroportin is also post-translationally regulated by hepcidin-mediated endocytosis and proteolysis. Panel B: In duodenal enterocytes, hypoxia induces ferroportin transcription via HIF2alpha. Retention of iron in enterocytes during iron deficiency is avoided by the presence of ferroportin mRNAs that lack 5'IRE. Hepcidin likely controls iron efflux from enterocytes posttranslationally by inducing the endocytosis and proteolysis of ferroportin. Panel C: Integration of diverse local and systemic influences on cellular iron efflux through ferroportin regulation at the transcriptional, translational and posttranslational level.

As noted above, macrophages are critical to iron homeostasis because they take up senescent red blood cells (by a process referred to as erythrophagocytosis), liberate heme from hemoglobin, liberate iron from heme, and release iron back into plasma via ferroportin. Although the overall rate of iron release is subject to systemic control, the cellular response to erythrophagocytosis is cell-autonomously regulated: in the terminal step of this coordinated sequence ferroportin expression in macrophages is increased by heme and iron

released from digested erythrocytes (Delaby et al., 2008). Ferroportin contains in its promoter sequence an antioxidant response element (ARE) 7kb upstream of the transcription start site (Marro et al., 2010); AREs can be bound either by Bach1 (leading to gene repression) or Nrf2 (leading to gene activation). Heme causes Bach1 degradation, which may allow Nrf2 to facilitate transcriptional activation of ferroportin (with a similar effect on heme oxygenase and ferritin, in a coordinated cellular response). Pharmacological activators of Nrf2 increase *Fpn1* mRNA in macrophages in an Nrf2-dependent manner, and can counteract the inflammation-induced suppression of *Fpn1* mRNA caused by LPS (Harada et al., 2011). Iron itself also enhances ferroportin expression at the translational level. Ferroportin mRNA (isoform 1a, which is abundant in macrophages) contains a 5' iron response element (IRE), which under conditions of iron deficiency binds IRP proteins, causing translational repression (Lymboussaki et al., 2003). In erythrophagocytosing macrophages, iron liberated from heme by heme oxygenase-1 (that is also activated by Nrf2) inactivates IRPs, resulting in derepression of *Fpn1a* mRNA translation. Moreover, the 3' untranslated region of ferroportin is targeted by miR-485-3p microRNA which is induced by cellular iron deficiency (Sangokoya et al., 2013). As iron cellular iron levels rise, the downregulation of this miRNA may also contribute to increased ferroportin expression. The sequential heme- and iron-mediated induction of ferroportin expression facilitates efficient recycling or iron from macrophages that have internalised and degraded senescent erythrocytes (Delaby et al., 2008; Marro et al., 2010) (**Figure 2a**).

In duodenal enterocytes, ferroportin production is strongly regulated by iron and hypoxia, all geared toward increasing absorption of dietary iron during iron deficiency and anemia (**Figure 2b**). The molecular basis for these effects is becoming clearer. Hypoxia acts by stabilisation of hypoxia inducible factors (HIFs) that transcriptionally activate genes in order to respond to a low cellular oxygen state. Iron deficiency also stabilises HIFs because their degradation is triggered by iron-dependent hydroxylation of proline residues and therefore HIFs may also function as sensors of iron scarcity. HIF activation in enterocytes was shown to result in the upregulation of both the apical iron import machinery, DcytB and DMT1 (Shah et al., 2009) and the basolateral iron exporter ferroportin (Taylor et al., 2011). The promoter of the ferroportin gene contains HIF response elements (HREs) that are targets for HIF2alpha binding, and mice lacking HIF2alpha, but not HIF1alpha, fail to upregulate duodenal ferroportin mRNA in response to iron deficiency (Taylor et al., 2011). Thus HIF2alpha has a demonstrable role in increasing ferroportin transcription during hypoxia and iron deficiency. Interestingly, HIF2alpha is also the predominant factor in the regulation of erythropoietin transcription, hinting at its possible role as a coordinator between iron supply and erythropoiesis during the response to hypoxia.

At first glance, the IRP/IRE system in iron-deficient enterocytes would be expected to impair ferroportin expression through translational repression by the 5'IRE of ferroportin. The presence of a 5' IRE in Hif2alpha mRNA (Sanchez et al., 2007) also raises the question why .the translation of Hif2alpha mRNA is not repressed during enterocyte iron depletion. Although such responses would protect enterocytes from cellular iron deficiency, they would be detrimental to an iron-deficient organism as it would retain iron in enterocytes and decrease the transfer of iron to plasma. In fact, iron deficiency clearly increases both ferroportin protein and mRNA expression. The inability of the IRE/IRP system to repress ferroportin translation in duodenal enterocytes is explained only in part by the presence of a splicing variant of ferroportin mRNA, termed *Fpn1b*, which lacks the 5' IRE but produces an identical protein (Zhang et al., 2009). It appears that other, perhaps hypoxia-dependent, mechanisms predominate to limit IRP1-mediated translational repression (Anderson et al., 2013) and increase the concentrations of HIF2alpha and the transcription and translation of ferroportin. Therefore under conditions of iron deficiency, increased production of ferroportin, combined with its decreased hepcidin-mediated endocytosis and proteolysis, result in higher transfer of iron from the diet into plasma at the expense of iron depletion of enterocytes. Enterocytes are short-lived cells which are shed into the intestinal lumen after a few days, so the organism may tolerate and even benefit from their iron depletion. The relative timing and amplitude of these complex iron– and hypoxia–sensitive elements in the ferroportin regulatory circuits has not yet been reported.

Interestingly, ferroportin lacking a 5' IRE (*Fpn1b* mRNA isoform) is also expressed by erythrocyte progenitor cells (Cianetti et al., 2005; Zhang et al., 2009; Zhang et al., 2011a). Under conditions of iron limitation, Fpn1b would be continually translated, and this may allow erythropoiesis to be 'altruistic' and give up some of its iron in order to increase supply to other tissues that require it.

In contrast to hypoxia and iron deficiency, inflammation decreases the expression of ferroportin, through effects on transcription. This effect was initially observed *in vivo* for liver and splenic *Fpn1* mRNA as a consequence of inflammation caused by administration of bacterial lipopolysaccharide (LPS), which is a ligand for the innate immune sensor Toll-like receptor 4 (TLR4), (Liu et al., 2005a; Yang et al., 2002). Human monocytic cells were also shown to decrease *FPN* mRNA in response to LPS and to interferon-gamma (Ludwiczek et al., 2003). The mechanism of decreased *Fpn* mRNA caused by inflammation is only partially understood. Suppression of *Fpn1* mRNA by the bacteria *Pseudomonas aeruginosa* requires intact TLR4 (Huang et al., 2012; Peyssonnaux et al., 2006), whereas the *Mycoplasma*-derived molecule FSL1 decreases *Fpn1* in a

TLR2-dependent fashion (Guida et al., 2015); however the decrease of *Fpn1* mRNA by LPS and the TLR3 ligand poly(I:C) do not require the intracellular adaptors of TLR signalling, *MyD88* or *TRIF* (Huang et al., 2012). Even in the absence of hepcidin, these mechanisms are sufficient to cause downregulation of duodenal ferroportin (and DMT-1) by LPS (Deschemin and Vaulont, 2013) and downregulation of splenic and liver ferroportin by FSL1 (Guida et al., 2015) leading to acute (3 – 6 hours after injection) hypoferremia in mice. These results suggest that transcriptional downregulation of ferroportin could contribute to the hypoferremia of inflammation that can be protective against infection with extracellular pathogens. However, the degree and duration of hypoferremia caused by this inflammatory suppression of Fpn1 mRNA are relatively minor in the absence of hepcidin, as evidenced by the small effects on circulating iron after infection with extracellular bacteria in hepcidin knockout mice (Arezes et al., 2015).

Another layer of regulation of ferroportin activity arises from macrophage differentiation and polarization. Macrophages are markedly phenotypically and functionally diverse depending on their source (yolk sac-derived precursors or circulating bone-marrow derived monocytes), tissue location (resident in various organs, or recruited from the blood), and exposure to a variety of signals originating from other cells. Iron-recycling macrophages in the red pulp of the spleen, which express high levels of ferroportin, depend on the transcription factor SpiC for their development (Kohyama et al., 2009). SpiC activity is normally repressed by Bach1; heme causes Bach1 degradation and derepresses SpiC in monocytes, leading to their differentiation into red pulp macrophages expressing ferroportin and heme-oxygenase-1 (Haldar et al., 2014). Transcription of ferroportin and heme-oxygenase-1 increases after heme-mediated Bach1 degradation and Nrf2 activation, as mentioned before. Thus increased levels of potentially toxic heme, for instance derived from hemolytic events, drive both the development of the cell type and the specific molecular processes that are equipped to detoxify heme and recycle heme iron. However, macrophages can also be differentiated in multiple other directions, simplified as ranging from the M1 pro-inflammatory phenotype induced by LPS or interferon-gamma to the M2 alternative specializations characterised by anti-inflammatory and tissue remodelling function (Mosser and Edwards, 2008). In line with the transcriptional stimulation of hepcidin by inflammatory signals, ferroportin expression by M1 macrophages is low, and this coupled with increased ferritin levels confers an iron sequestration phenotype (Recalcati et al., 2010). However, macrophages exposed to M-CSF, IL-4 or glucocorticoids assume an iron recycling / exporting phenotype expressing hemoglobin processing machinery, and high levels of ferroportin (Corna et al., 2010; Recalcati et al., 2010; Sierra-Filardi et al., 2010; Vallelian et al., 2010).

As described in this section, ferroportin gene transcription and translation is modulated by a number of multi-layered signals. However, the activity of the functional transporter on cell membranes is predominantly governed posttranslationally by a systemically acting peptide hormone, hepcidin (e.g. (Laftah et al., 2004; Rivera et al., 2005). Because hepcidin expression is itself regulated by a complex integration of many physiological signals including those arising from iron status, inflammation and erythroid drive (Ganz, 2013), hepcidin adds a crucial final layer to the control of ferroportin activity (**Figure 2c**).

Ferroportin and hepcidin

Hepcidin is a 25 amino acid peptide secreted into blood plasma predominantly by hepatocytes. The sole well-documented physiologic activity of hepcidin is its binding to ferroportin which results in ferroportin endocytosis and proteolysis, predominantly in lysosomes (Nemeth et al., 2004). Accordingly, low hepcidin states are characterized by high levels of ferroportin in iron-transporting tissues and brisk iron export to extracellular fluid and plasma. In contrast, high circulating hepcidin concentrations cause ferroportin to disappear from cell membranes and iron export to plasma to cease. The central importance of the hepcidin-ferroportin interaction for iron homeostasis is highlighted by the profound consequences of hepcidin deficiency or excess. Complete genetic hepcidin deficiency causes the most severe form of iron overload in mice (Lesbordes-Brion et al., 2006; Nicolas et al., 2001) and humans (Roetto et al., 2003), and mutations in ferroportin that prevent hepcidin binding (Altamura et al., 2014) largely phenocopy hepcidin deficiency, with the exception of iron-induced pancreatic injury which for unclear reasons causes premature mortality in the hepcidin-resistant homozygous C326S ferroportin mouse. At the opposite extreme, transgenic overexpression of hepcidin results in lethal perinatal iron deficiency (Nicolas et al., 2002) and the administration of synthetic hepcidin or its analogs to mice causes dose-dependent hypoferremia (Rivera et al., 2005) and inhibits iron absorption (Laftah et al., 2004), again indicating the dominant effect of hepcidin on the regulation of ferroportin function.

It has been reported that macrophage ferroportin may be much more sensitive to the effect of hepcidin than ferroportin on enterocytes (Chaston et al., 2008; Chung et al., 2009). In principle, such organ-specific ferroportin responses to hepcidin could result from differences in hepcidin binding, perhaps due to organ-specific differences in ferroportin glycosylation (Canonne-Hergaux et al., 2006), possible differences in cellular

endocytic machinery (not specifically analysed) or from differential rates of ferroportin synthesis which strongly affects the net expression of ferroportin on cell membranes. Definitive determination of differences in ferroportin responses in the two organs may be difficult. In vivo studies of radiolabelled hepcidin binding found substantial binding both in the duodenum (proximal more than distal) and in the spleen where iron-recycling macrophages are abundant (Rivera et al., 2005) but this technique cannot detect small differences in receptor affinities. Despite any possible quantitative differences, the phenotypes of major iron disorders and of their animal models indicate that hepcidin exerts a controlling influence on both duodenal enterocytes and recycling macrophages: high hepcidin concentrations suppress both intestinal iron absorption and macrophage iron recycling, and conversely, hepcidin deficiency leads to both excessive iron absorption and iron depletion of macrophages.

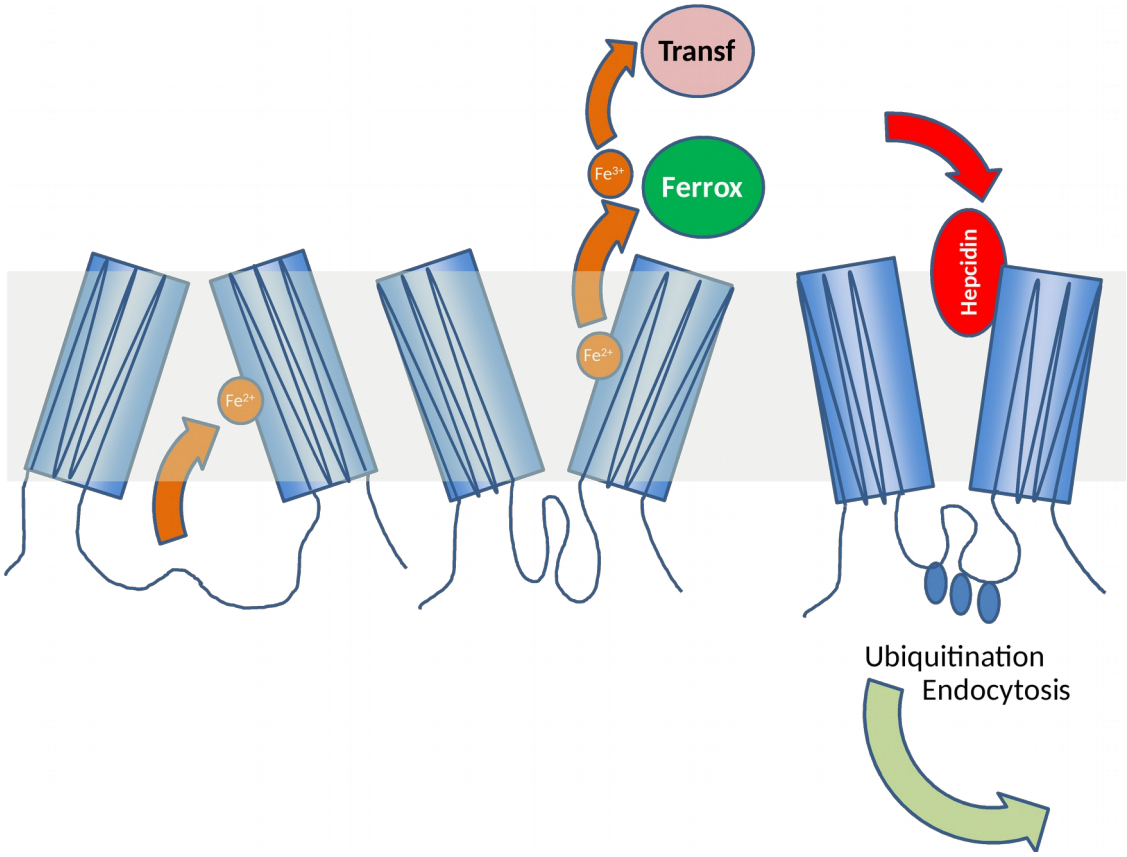

Figure 3: Alternating access model of iron transport by ferroportin. Ferroportin is a member of the major facilitator superfamily of transporters which share a structural feature known as the MFS fold: 12 transmembrane segments (TM) organized into two 6-helix halves, connected by a large cytoplasmic loop between TM-6 and -7. Transport across the membrane likely occurs via the alternate-access (rocker-switch) mechanism whereby the two halves of the molecule cycle through inward-facing, occluded and outward-facing conformations to facilitate substrate transport. We hypothesize that i) ferrous iron binds to ferroportin in the open-in conformation, ferroportin flips to the open-out conformation and releases ferrous iron to be oxidized by a ferroxidase (Ferrox) and delivered to transferrin (Transf) in the ferric form; and ii) that hepcidin binds to the open out conformation inducing a conformational change that results in ubiquitination of the connecting loop and endocytosis of ferroportin.

The structural determinants of the hepcidin-ferroportin interaction are partially understood. Ferroportin is a 12-transmembrane domain protein (Liu et al., 2005b) **(Figure 3)** belonging to the major facilitator superfamily of transporters of small molecules. Although technical obstacles caused some early disagreement on this point, there is now consensus in the field that both ferroportin termini are located on the cytoplasmic side of the membrane (Liu et al., 2005b; Rice et al., 2009). The thiol cysteine C326 is exposed to the extracellular milieu and is essential for hepcidin binding as demonstrated by the lack of radiolabeled hepcidin binding by mutant ferroportins C326S and C326T (Fernandes et al., 2009). The lack of hepcidin binding to C326S ferroportin confers a gain of function phenotype in humans and in mice, resulting in early and severe iron overload as a consequence of hyperabsorption of dietary iron (Altamura et al., 2014; Sham et al., 2009). Extensive mutagenesis and molecular modelling identified the N-terminal 9 amino acids of the 25 amino acid mature hepcidin interacting with the C326-containing exofacial ferroportin segment, mainly through pi-stacking interactions of aromatic side chains (Nemeth et al., 2006; Preza et al., 2011a). The confidence in this

model was reinforced by the rational design of 9-amino acid minihepcidins that were as potent as and more drug-like than hepcidin itself (Preza et al., 2011b; Ramos et al., 2012).

It was reported that an unstructured 19 amino acid peptide based on the C326-containing ferroportin loop acted as a specific binder of hepcidin and could be used for measurements of hepcidin in plasma or urine (De Domenico et al., 2008). However, this claim could not be replicated in other laboratories, and the publication was retracted as it was found to have been affected by research misconduct (paper #10 in the report by (McCormack et al., 2013)).

As with other receptors that undergo ligand-induced endocytosis, the interaction of hepcidin with ferroportin could be expected to trigger a conformational change in ferroportin followed by covalent modifications of one or more of its cytoplasmic segments to initiate endocytosis. Mutations that interfere with these mechanisms would confer resistance to hepcidin. Several ferroportin mutations associated with partial resistance to hepcidin (Drakesmith et al., 2005) affect amino acids located within the cell membrane and do not prevent hepcidin binding (Fernandes et al., 2009) but likely interfere with the conformational change that is required to initiate endocytosis. Although the structural details have not yet been elucidated, ferroportin endocytosis is now known to depend on the hepcidin-induced ubiquitination of multiple cytoplasmic lysines on the extended cytoplasmic loop connecting the two halves of the ferroportin molecule (**Figure 3**) (Qiao et al., 2012; Ross et al., 2012). The specific ubiquitin ligases involved in the endocytosis of ferroportin remain to be identified.

An earlier proposed mechanism claimed that hepcidin-induced ferroportin endocytosis depended on JAK2-mediated phosphorylation of ferroportin on a pair of cytoplasmic tyrosines (Y302 and Y303)(De Domenico et al., 2009; De Domenico et al., 2007b). However, these studies were subsequently contradicted (Ross et al., 2012) and the articles were found to be affected by scientific misconduct (papers #2 and #8 in the report by (McCormack et al., 2013)). Contrary to the original claim that Y302F/Y303F mutations confer resistance to hepcidin (De Domenico et al., 2007b), these mutations were found to prevent ferroportin trafficking to the membrane (Ross et al., 2012). Morover, Y302F/Y303F homozygosity in mice caused an embryonic lethal phenotype due to inadequate iron transfer across placenta (Altamura et al., 2011), an opposite phenotype from that expected of hepcidin resistance. Concerns were also raised about the reported key role of a single lysine residue K253 and the Nedd4-2 ligase system in hepcidin-dependent and hepcidin-independent endocytosis of ferroportin (De Domenico et al., 2011). The article was confounded by misconduct (paper #1 in report by (McCormack et al., 2013)) and was retracted.

Compared to most other cell types, macrophages are particularly ferroportin-rich, especially in the spleen and the liver where they recycle iron from senescent erythrocytes. Macrophages also have an important role as orchestrators of innate immunity, in part by producing cytokines that modify the activity of cells participating in host resistance to infection. Both functions of macrophages, in iron homeostasis and in host defense, influence each other. Understanding the effects of iron on host resistance has important implications for the treatment of iron deficiency in settings where serious infections are endemic. However, understanding of the effect of iron on macrophage cytokine production has been complicated by the contradictory findings in different studies. In mouse models, iron deficiency and the resulting iron depletion of macrophages has been associated with a heightened inflammatory response to LPS, which could be prevented by hepcidin administration which promotes macrophage iron retention (Pagani et al., 2011). However, in HFE-/- mice, where macrophages are also iron-depleted and hepcidin concentrations are also low, there was no difference compared to WT mice in TNFα and IL-6 plasma levels after LPS stimulation (Roy et al., 2004). Furthermore, studies from isolated macrophages demonstrated an opposite association between macrophage iron and cytokine production: iron depletion via chelation reduced TNFα and IL-6 secretion (Wang et al., 2008) and iron accumulation in ferroportin-deficient macrophages potentiated TNFα and IL-6 release compared to intact macrophages (Zhang et al., 2011b). These apparently conflicting conclusions from in vivo and in vitro experiments may be caused by the heterogeneity of macrophage types, their differing exposure to iron sources and varying levels of ferroportin expression.

In principle, hepcidin could modulate cytokine secretion by macrophages either through ferroportin-generated signalling or by causing cytoplasmic iron retention and altering iron-dependent intracellular signalling, including the hypoxia pathways and the iron-regulatory protein pathways. One study proposed that hepcidin triggered ferroportin signalling via JAK2-STAT3 pathway, causing anti-inflammatory transcriptional changes (De Domenico et al., 2010). The study, however, was affected by research misconduct (paper #3 in the report by (McCormack et al., 2013)) and the involvement of JAK2-STAT3 was not confirmed in subsequent studies (Ross et al., 2012). Thus both the pathophysiology of iron effects on inflammation and the potential mechanisms by which iron, hepcidin and ferroportin modulate inflammatory function of macrophages remain uncertain (Table 1).

Mechanism of iron transport by ferroportin and the role of ferroxidases

Surprisingly little is known about how ferroportin transports iron. Recent studies of the efflux of microinjected iron radioisotopes from *Xenopus* oocytes established that this system should prove useful for detailed analysis of iron transport by ferroportin (Mitchell et al., 2014). Oocytes microinjected with ferroportin-GFP mRNA express ferroportin on their membranes, export up to 300-times more iron than control oocytes, and iron efflux is strongly inhibited by the addition of hepcidin. The two common iron forms Fe^{2+} and Fe^{3+} are readily interconverted so definitive determination of which species is transported by ferroportin is challenging. Support for the transport of Fe^{2+} is provided by the observation that the very similar cation Co^{2+} that is more stable to oxidation under the usual experimental conditions is exported from *Xenopus* oocytes with a similar rate constant as iron. Moreover, multiple experiments with the *Xenopus* oocyte system found that iron efflux was potentiated by apotransferrin (Donovan et al., 2000) (Mitchell et al., 2014). and ceruloplasmin (McKie et al., 2000; Mitchell et al., 2014)., the latter known as the principal ferroxidase in blood plasma that catalyzes the conversion of Fe^{2+} to Fe^{3+}. This potentiating effect is ascribed to the increased concentration gradient for the release of Fe^{2+} from ferroportin facilitating the movement of iron through the transporter.

All three known mammalian multicopper-containing ferroxidases - ceruloplasmin, hephaestin and zyklopen - oxidize Fe^{2+} to Fe^{3+}, the form that is loaded onto plasma transferrin for distribution to tissues. Ceruloplasmin and hephaestin have been shown to facilitate cellular iron efflux in vivo, especially in situations of increased demand for iron. In the duodenum, hephaestin is a transmembrane protein on enterocytes with an extracellular domain that is highly homologous to the plasma protein ceruloplasmin. Ablation of hephaestin in mice results in mild systemic iron deficiency and mild anemia both of which improve with age as iron demand for growth declines (Fuqua et al., 2014). Systemic iron deficiency was caused by decreased intestinal iron absorption but was associated with excess iron retention within enterocytes, indicative of impaired basolateral transport of iron to plasma. The decrement in iron absorption occured despite an increased expression of ferroportin, demonstrating impaired iron transport by ferroportin. Hephaestin is also found in the brain where its ablation leads to age-dependent regional iron accumulation (Jiang et al., 2015).

Ceruloplasmin is known in two forms, a blood plasma protein and a GPI-linked membrane-associated protein, encoded by splicing variants of the same gene. The GPI-linked ceruloplasmin has been reported to be particularly abundant in the brain where it has a different distribution than hephaestin (Jiang et al., 2015). Patients and mouse models that lack ceruloplasmin accumulate iron in the liver, the brain and the retina with resulting damage to these organs. Ceruloplasmin-deficient humans and mice also show signs of impaired iron mobilization from macrophages and hepatocytes resulting in anemia (Harris et al., 1999; Osaki and Johnson, 1969). During erythropoietic stress after bleeding, ceruloplasmin may also facilitate augmented dietary iron transfer to plasma (Cherukuri et al., 2005). Combined hephaestin-ceruloplasmin deficiency has particularly severe consequences in the retina where both proteins are expressed, causing a severe form of macular degeneration (Hadziahmetovic et al., 2008). A third ferroxidase, zyklopen, was described as a membrane protein highly expressed in the placenta (Chen et al., 2010) but its physiological function has not yet been reported.

In view of the well-documented ability of the multicopper ferroxidases to influence ferroportin function, various modes by which ferroxidases and ferroportin could interact have been explored. In one model, GPI-linked ceruloplasmin (or an equivalent sink for Fe^{2+}) is required to remove Fe^{2+} from ferroportin in astrocytes. In the absence of ceruloplasmin, Fe^{2+} becomes “stuck” in ferroportin and this conformational state causes astrocyte ferroportin to be ubiquitinated on K523 and to undergo endocytosis and proteolysis (De Domenico et al., 2007a). Although the hypothesis has some attractive features, including the explanation of why aceruloplasminemia has such severe effects in the brain where soluble ceruloplasmin concentrations are very low, the evidence for it is again confounded by misconduct (paper #7 in the report by (McCormack et al., 2013)) and has not yet been independently verified. Others have documented by coimmunoprecipitation that hephaestin or GPI-ceruloplasmin physically interact with ferroportin (Jeong and David, 2003; Yeh et al., 2009). Physical contact with a ferroxidase would assure very low concentrations of Fe^{2+} on the exoface of ferroportin and could facilitate iron efflux.

Not much is known about the mechanism by which intracellular iron is delivered to ferroportin for export. A recent study (Yanatori et al., 2014) reported that the iron chaperone protein PCBP2 (but not its paralogue PCBP1) interacts with both DMT1 and ferroportin. The results suggested that ferrous iron imported into the cytoplasm by DMT1 may be shuttled to ferroportin via PCBP2.

Fundamental questions about ferroportin, including its structure, the molecular mechanism of iron transport, the nature of any symported or antiported species and the mechanism by which iron is delivered to ferroportin for export, remain open for investigation (Table 1).

Genetic disorders of ferroportin and their effects on systemic iron homeostasis

In the absence of definitive structural studies, important insights into ferroportin structure emerged from studies of informative patients with iron disorders. Clinically detectable ferroportin mutations are very heterogeneous, with a systematic review reporting on 161 patients with 31 distinct mutations (Mayr et al., 2010). They manifest a dominant pattern of inheritance and fall into two broad phenotypic categories with some overlap. One group of mutations causes a ferroportin gain-of-function owing to partial or complete resistance to hepcidin-induced ferroportin endocytosis, as discussed in previous sections. The affected patients hyperabsorb iron and present, often at a young age, with high transferrin saturation, high plasma ferritin, and hepatic and other organ iron overload with evidence of toxic damage. The phenotype resembles that of patients with classical hemochromatosis from homozygous or compound heterozygous recessive mutations in hepcidin or its regulators (HFE, TfR2 or hemojuvelin). Another group of patients have dominant loss-of-function mutations that cause high plasma ferritin but not high transferrin saturation (a condition referred to as "ferroportin disease"(Pietrangelo, 2004)). These mutations appear to limit the rate of iron export from recycling macrophages so that these macrophages retain iron and as a consequence secrete high amounts of ferritin into plasma (Cohen et al., 2010). Any loss of transport capacity in the duodenum appears to be sufficiently compensated to prevent systemic iron deficiency, unless the patients are phlebotomized to remove accumulated iron. This disorder does not appear to cause clinically important organ damage, presumably because macrophages (unlike hepatocytes, cardiac myocytes or endocrine glandular tissue) are equipped to produce very large amounts of reactive oxygen species during respiratory burst and are therefore well defended against similar toxins generated when they become iron overloaded. In principle, some mutations could cause both decreased iron export and partial resistance to hepcidin and this could help explain the variable iron-loading phenotypes in patients with the same mutation.

The ferroportin variant Q248H is common (a few %) in populations of African ancestry and has been weakly associated with increased serum ferritin (Gordeuk et al., 2003; Kasvosve et al., 2015; Rivers et al., 2007). The polymorphism may confer a somewhat decreased sensitivity of ferroportin to hepcidin, perhaps because of its proximity to cytoplasmic ubiquitination sites involved in hepcidin-induced ferroportin endocytosis (Nekhai et al., 2013).

The puzzling feature of ferroportin disease is the structural basis of its mode of inheritance. Essentially all of the several dozen reported mutations are heterozygous missense mutations that act dominantly. This suggests that the ferroportin loss-of-function is not due to haploinsufficiency, otherwise heterozygous nonsense mutations or other gene defects that completely ablate the production of protein from one of the alleles would also be expected. At the functional level, the mutations cause either a trafficking defect so that insufficient ferroportin is displayed on the cell membrane (Liu et al., 2005b; Schimanski et al., 2005), or an iron transport defect that reduces the rate of iron efflux per ferroportin molecule on the cell membrane (Liu et al., 2005b; McGregor et al., 2005). There is evidence that some mutations known to impair trafficking may also impair iron efflux by membrane-associated ferroportin (McGregor et al., 2005). Compared to the mouse model of haploinsufficiency (heterozygous knockout) which had only a very mild form of macrophage iron retention (Donovan et al., 2005), mice with "ferroportin disease" (flatiron mouse, H32R ferroportin mutation) showed age-dependent accumulation of iron in hepatic macrophages and relatively severe iron restriction, manifested by low transferrin saturation and hypochromic erythrocytes on blood smears (Zohn et al., 2007). Although the comparison was not made side-by-side under the same conditions and identical strain backgrounds, these findings are consistent with a dominant negative effect of the mutation. If ferroportin is transported to the membrane in a multimeric form, a dominant negative effect could result from missense mutations that cause mistrafficking of the multimer. However, several dominant mutations (e.g. G323V, N174I, G80S, R88G,) were reported to cause deficient iron efflux rather than mistrafficking of ferroportin (Callebaut et al., 2014; De Domenico et al., 2006; Liu et al., 2005b), and here the dominant negative effect may imply cooperativity between multiple ferroportin molecules engaged in iron transport. In such a model, a transport-impaired ferroportin molecule would need to disrupt the function of neighbouring wild-type molecules.

Although the genetic evidence points to some kind of interaction between ferroportin molecules that would cause a dominant negative effect, it has not been definitively determined whether ferroportin is monomeric or multimeric during trafficking or in the cell membrane. This is in part because the main published evidence supporting the multimeric nature of ferroportin (De Domenico et al., 2007c) is clouded by scientific misconduct (paper #9 in the report by (McCormack et al., 2013)). Several groups reported evidence that ferroportin is a monomer, as determined by a variety of methods (Goncalves et al., 2006; Pignatti et al., 2006; Schimanski et al., 2008) but others detected a dimeric form complexed with hephaestin (Yeh et al., 2009). Detailed physicochemical analysis of recombinant ferroportin extracted from membranes (Rice et al., 2009) led to the conclusion that the monomeric form is stable and functional, and binds hepcidin, but that weak,

detergent-sensitive homophilic interactions favouring multimerization in the cell membrane could not definitively be ruled out.

The role of ferroportin in local tissue iron homeostasis and its disorders

In addition to its role as the sole cellular iron exporter in tissues that provide iron for systemic needs (duodenum, iron-recycling macrophages, hepatocytes and placenta), there is increasing evidence that ferroportin may also be involved in local iron homeostasis. This function of ferroportin is best revealed by the consequences of its tissue-specific ablation, as was done in cardiac myocytes (Lakhal-Littleton et al., 2015), with resulting progressive impairment of the function of cardiomyocytes and the premature death of the affected mice. These experiments raise questions about the function of ferroportin in cells that do not participate in systemic iron homeostasis. Is ferroportin functioning as a safety valve to release iron generated during subcellular remodelling (e.g. autophagy) or during uncontrolled uptake in the course of transient episodes of complete transferrin saturation with iron? Ferroportin in erythrocyte precursors (Zhang et al., 2011a) may serve as such a safety valve that also makes erythroblast iron available during periods of severe iron deficiency to sustain vital organs at the expense of erythropoiesis.

An important role for ferroportin in the brain is presaged by the adverse consequences of ceruloplasmin deficiency in mice and humans, further exacerbated in mice when both hephaestin and ceruloplasmin are ablated. It has also been suggested that □-amyloid precursor protein (APP) may function as a ferroxidase in neurons (Duce et al., 2010), implicating ferroportin in the pathogenesis of Alzheimer's disease; however the ferroxidase activity of APP turned out to be an artefact (Wong et al., 2014). This does not exclude a role for APP in neuronal iron metabolism as the same authors provided more evidence that APP can stabilize ferroportin on the cell membrane and increase iron efflux by an alternative and as yet undefined mechanism (Wong et al., 2014).

Cancer cells with their high metabolic rates and rapid multiplication have a particularly high requirement for iron. Recent studies suggest that as cancer cells become more malignant, they clonally evolve to retain iron by decreasing the expression of ferroportin and increasing the secretion of hepcidin (Torti and Torti, 2011). These characteristics are accompanied by increased pool of labile iron in cancer cells. Tumor-associated macrophages appear to have an iron-exporting phenotype which may provide iron to the cancer cells and further promote tumor growth (Torti and Torti, 2011).

Ferroportin and infection

Almost all infectious pathogens need to scavenge iron from the host in order to multiply. The ferroportin/hepcidin axis controls release of cellular iron from macrophages into plasma, and therefore may play an important role in infections with microbes that reside within macrophages or microbes that survive in the bloodstream or other extracellular spaces (Drakesmith and Prentice, 2012). Iron-dependent extracellular microbes are exemplified by the pathogen *Vibrio vulnificus* that causes rapid death in mice lacking hepcidin. Here unrestricted ferroportin activity provides ample extracellular iron (likely in the form of non-transferrin-bound iron), potentiating pathogen replication. Blockade of ferroportin-mediated iron release into plasma by administration of mini-hepcidin peptides restored the ability of these mice to resist *Vibrio vulnificus* and to survive the infection (Arezes et al., 2015).

Other work has concentrated on intracellular (macrophage-tropic) pathogens in which ferroportin plays a different role. In this context, iron release from infected cells into plasma could be expected to deprive microbes of the iron they need to grow. Consistent with this notion, increasing ferroportin expression by retroviral gene transfer into cell lines *in vitro* decreased multiplication of *Salmonella enterica* serovar Typhimurium, and this effect could be reversed by the addition of hepcidin (Chlosta et al., 2006). *In vivo,* during the first few days of murine infection with *Salmonella enterica* serovar Typhimurium, the stimulatory effect of IL-6 on hepcidin production may lead to loss of ferroportin from cell membranes, iron retention in macrophages and stimulation of intracellular growth (Kim et al., 2014). However, countervailing effects may predominate during chronic infection: mechanisms that increase ferroportin transcription appear as a part of a natural and protective iron-modulating innate immune response to *Salmonella* infection of macrophages, and this response can be enhanced by interferon-gamma (Nairz et al., 2008). This increased expression of ferroportin in macrophages is also observed *in vivo* following infection of mice, where it results in loss of spleen iron (Brown et al., 2015). The upregulation of ferroportin in *Salmonella*-infected macrophages is at least in part driven by nitric-oxide-mediated Nrf2 activation, which in turn increases transcription of the ferroportin gene (Nairz et al., 2013).

The interaction of other macrophage-tropic intracellular pathogens with ferroportin activity has been investigated. Ferroportin over-expression was found to suppress the initial growth of *Mycobacterium tuberculosis* in macrophage-like cell lines, although the microbicidal activity of these cells was also

compromised (Johnson et al., 2010). In the case of macrophage infection with *Leishmania monocytogenes*, wild-type bacteria that gain access to the cytosol cause an upregulation of ferroportin (perhaps via nitric oxide formation) which in turn limits iron availability to the pathogen; in contrast *L. monocytogenes* lacking listeriolysin O, which is required for bacteria to escape the phagolysosome and enter the cytoplasm, do not increase ferroportin expression, and are susceptible to iron-induced, ROS-mediated microbicidal activity (Haschka et al., 2015).

Some pathogens may have evolved mechanisms for assuring their intracellular iron supply. In addition to the early hypoferremic and macrophage-iron-sequestering effect of *Salmonella enterica* serovar Typhimurium infection (Kim et al., 2014), it has also been reported that macrophages infected with *Leishmania amazonensis* decreased ferroportin expression in a TLR4- and hepcidin-dependent manner (Ben-Othman et al., 2014). Furthermore, replication of the parasite was enhanced by iron retention caused by exogenously added hepcidin or by expression of inactive ferroportin, whereas *Leishmania* growth was inhibited by expression of a partially hepcidin-resistant ferroportin mutant (N144H). In summary, ferroportin-mediated iron release from macrophages can be a critical determinant for pathogen growth. Enhanced ferroportin expression following *Salmonella, Listeria*, *Mycobacteria* and *Leishmania* infection is host-protective in each case, but it would appear that *Salmonella* (in acute infection) and *Leishmania* may counteract this effect at least temporarily and ensure their own iron supply by downregulating ferroportin. Further work is required to elucidate the complex time-dependent iron redistribution during *in vivo* infection with various intracellular pathogens. Moreover, the specific role of ferroportin in other infections that are known to interact with iron homeostasis, for example the liver-stage of *Plasmodium* (Portugal et al., 2011), is not clear. Although in cell lines ferroportin-mediated iron release inhibits replication of HIV-1 (Xu et al., 2010), the interactions of hepcidin, ferroportin and iron with viral infections remain relatively unexplored.

Conclusions

Ferroportin is a unique iron exporter, essential for dietary iron absorption, recycling of iron from senescent erythrocytes, mobilization of stored iron, and iron transfer to the developing fetus. It is the only known receptor for the iron-regulatory hormone hepcidin that binds to ferroportin, induces its endocytosis and thereby controls its membrane concentration and iron-exporting activity. Especially in macrophages, ferroportin synthesis is regulated transcriptionally and translationally, presumably to meet the rapidly changing requirements for iron export after erythrophagocytosis and in response to macrophage infection by intracellular microbes. The role of ferroportin in cellular iron homeostasis, innate immunity and cancer is becoming increasingly recognized. Despite its importance, the structural basis of ferroportin function and the mechanism by which ferroportin transports iron are not understood.

Figures –
Figure 1: Systemic flows of elemental iron
Figure 2: Ferroportin regulation
Figure 3: Alternating access model of iron transport by ferroportin

Table 1: Some unanswered questions about ferroportin

What is the structure of ferroportin?
What is the molecular mechanism of iron transport?
How does hepcidin cause ferroportin endocytosis?
What is the molecular mechanism of ferroportin disease and its autosomal dominance?
How does ferroportin-mediated iron export affect macrophage immune functions?
What is the role of ferroportin in viral infection?
How does inflammation suppress ferroportin transcription?
What is the role of ferroportin in the heart, the erythroblast, the retina and in the brain?
Does ferroportin have other organ-specific roles?
Is ferroportin activity co-ordinated with fluxes of other nutrients?

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.

Altamura, S., Galy, B., Kessler, R., Hentze, M.W., and Muckenthaler, M.U. (2011). Mouse models for ferroportin with impaired hepcidin regulation. American Journal of Hematology *86*, E42.

Altamura, S., Kessler, R., Groene, H.J., Gretz, N., Hentze, M.W., Galy, B., and Muckenthaler, M.U. (2014). Resistance of Ferroportin to Hepcidin Binding causes Exocrine Pancreatic Failure and Fatal Iron Overload. Cell Metabolism *20*, 359-367.

Anderson, E.R., Taylor, M., Xue, X., Ramakrishnan, S.K., Martin, A., Xie, L., Bredell, B.X., Gardenghi, S., Rivella, S., and Shah, Y.M. (2013). Intestinal HIF2alpha promotes tissue-iron accumulation in disorders of iron overload with anemia. Proc.Natl.Acad.Sci.U.S.A *110*, E4922-E4930.

Arezes, J., Jung, G., Gabayan, V., Valore, E., Ruchala, P., Gulig, P.A., Ganz, T., Nemeth, E., and Bulut, Y. (2015). Hepcidin-induced hypoferremia is a critical host defense mechanism against the siderophilic bacterium Vibrio vulnificus. Cell host & microbe *17*, 47-57.

Ben-Othman, R., Flannery, A.R., Miguel, D.C., Ward, D.M., Kaplan, J., and Andrews, N.W. (2014). Leishmania-mediated inhibition of iron export promotes parasite replication in macrophages. PLoS pathogens *10*, e1003901.

Brown, D.E., Nick, H.J., McCoy, M.W., Moreland, S.M., Stepanek, A.M., Benik, R., O'Connell, K.E., Pilonieta, M.C., Nagy, T.A., and Detweiler, C.S. (2015). Increased Ferroportin-1 Expression and Rapid Splenic Iron Loss Occur with Anemia Caused by Salmonella enterica Serovar Typhimurium Infection in Mice. Infect Immun *83*, 2290-2299.

Callebaut, I., Joubrel, R., Pissard, S., Kannengiesser, C., Gérolami, V., Ged, C., Cadet, E., Cartault, F., Ka, C., Gourlaouen, I., et al. (2014). Comprehensive functional annotation of 18 missense mutations found in suspected hemochromatosis type 4 patients. Human Molecular Genetics *23*, 4479-4490.

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-G163.

Chaston, T., Chung, B., Mascarenhas, M., Marks, J., Patel, B., Srai, S.K., and Sharp, P. (2008). Evidence for differential effects of hepcidin in macrophages and intestinal epithelial cells. Gut *57*, 374-382.

Chen, H., Attieh, Z.K., Syed, B.A., Kuo, Y.M., Stevens, V., Fuqua, B.K., Andersen, H.S., Naylor, C.E., Evans, R.W., Gambling, L., et al. (2010). Identification of zyklopen, a new member of the vertebrate multicopper ferroxidase family, and characterization in rodents and human cells. J Nutr *140*, 1728-1735.

Cherukuri, S., Potla, R., Sarkar, J., Nurko, S., Harris, Z.L., and Fox, P.L. (2005). Unexpected role of ceruloplasmin in intestinal iron absorption. Cell Metab *2*, 309-319.

Chlosta, S., Fishman, D.S., Harrington, L., Johnson, E.E., Knutson, M.D., Wessling-Resnick, M., and Cherayil, B.J. (2006). The iron efflux protein ferroportin regulates the intracellular growth of Salmonella enterica. Infect.Immun. *74*, 3065-3067.

Chung, B., Chaston, T., Marks, J., Srai, S.K., and Sharp, P.A. (2009). Hepcidin decreases iron transporter expression in vivo in mouse duodenum and spleen and in vitro in THP-1 macrophages and intestinal Caco-2 cells. J Nutr. *139*, 1457-1462.

Cianetti, L., Segnalini, P., Calzolari, A., Morsilli, O., Felicetti, F., Ramoni, C., Gabbianelli, M., Testa, U., and Sposi, N.M. (2005). Expression of alternative transcripts of ferroportin-1 during human erythroid differentiation. Haematologica *90*, 1595-1606.

Cohen, L.A., Gutierrez, L., Weiss, A., Leichtmann-Bardoogo, Y., Zhang, D.L., Crooks, D.R., Sougrat, R., Morgenstern, A., Galy, B., Hentze, M.W., et al. (2010). Serum ferritin is derived primarily from macrophages through a nonclassical secretory pathway. Blood *116*, 1574-1584.

Corna, G., Campana, L., Pignatti, E., Castiglioni, A., Tagliafico, E., Bosurgi, L., Campanella, A., Brunelli, S., Manfredi, A.A., Apostoli, P., et al. (2010). Polarization dictates iron handling by inflammatory and alternatively activated macrophages. Haematologica *95*, 1814-1822.

De Domenico, I., Lo, E., Ward, D.M., and Kaplan, J. (2009). Hepcidin-induced internalization of ferroportin requires binding and cooperative interaction with Jak2. Proc.Natl.Acad.Sci.U.S.A *106*, 3800-3805.

De Domenico, I., Lo, E., Yang, B., Korolnek, T., Hamza, I., Ward, D.M., and Kaplan, J. (2011). The role of ubiquitination in hepcidin-independent and hepcidin-dependent degradation of ferroportin. Cell Metab *14*, 635-646.

De Domenico, I., McVey, W.D., Nemeth, E., Ganz, T., Corradini, E., Ferrara, F., Musci, G., Pietrangelo, A., and Kaplan, J. (2006). Molecular and clinical correlates in iron overload associated with mutations in ferroportin. Haematologica *91*, 1092-1095.

De Domenico, I., Nemeth, E., Nelson, J.M., Phillips, J.D., Ajioka, R.S., Kay, M.S., Kushner, J.P., Ganz, T., Ward, D.M., and Kaplan, J. (2008). The hepcidin-binding site on ferroportin is evolutionarily conserved. Cell Metab *8*, 146-156.

De Domenico, I., Ward, D.M., di Patti, M.C., Jeong, S.Y., David, S., Musci, G., and Kaplan, J. (2007a). Ferroxidase activity is required for the stability of cell surface ferroportin in cells expressing GPI-ceruloplasmin. EMBO J *26*, 2823-2831.

De Domenico, I., Ward, D.M., Langelier, C., Vaughn, M.B., Nemeth, E., Sundquist, W.I., Ganz, T., Musci, G., and Kaplan, J. (2007b). The Molecular Mechanism of Hepcidin-mediated Ferroportin Down-Regulation. Molecular Biology of the Cell *18*, 2569-2578.

De Domenico, I., Ward, D.M., Musci, G., and Kaplan, J. (2007c). Evidence for the multimeric structure of ferroportin. Blood *109*, 2205-2209.

De Domenico, I., Zhang, T.Y., Koening, C.L., Branch, R.W., London, N., Lo, E., Daynes, R.A., Kushner, J.P., Li, D., Ward, D.M., et al. (2010). Hepcidin mediates transcriptional changes that modulate acute cytokine-induced inflammatory responses in mice. J Clin.Invest *120*, 2395-2405.

Delaby, C., Pilard, N., Puy, H., and Canonne-Hergaux, F. (2008). Sequential regulation of ferroportin expression after erythrophagocytosis in murine macrophages: early mRNA induction by haem, followed by iron-dependent protein expression. Biochem.J *411*, 123-131.

Deschemin, J.C., and Vaulont, S. (2013). Role of hepcidin in the setting of hypoferremia during acute inflammation. PLoS One *8*, e61050.

Donovan, A., Brownlie, A., Zhou, Y., Shepard, J., Pratt, S.J., Moynihan, J., Paw, B.H., Drejer, A., Barut, B., Zapata, A., et al. (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., and Prentice, A.M. (2012). Hepcidin and the iron-infection axis. Science *338*, 768-772.

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., et al. (2005). Resistance to hepcidin is conferred by hemochromatosis-associated mutations of ferroportin. Blood *106*, 1092-1097.

Duce, J.A., Tsatsanis, A., Cater, M.A., James, S.A., Robb, E., Wikhe, K., Leong, S.L., Perez, K., Johanssen, T., Greenough, M.A., et al. (2010). Iron-export ferroxidase activity of beta-amyloid precursor protein is inhibited by zinc in Alzheimer's disease. Cell *142*, 857-867.

Fernandes, A., Preza, G.C., Phung, Y., De Domenico, I., Kaplan, J., Ganz, T., and Nemeth, E. (2009). The molecular basis of hepcidin-resistant hereditary hemochromatosis. Blood *114*, 437-443.

Finch, C. (1994). Regulators of iron balance in humans. Blood *84*, 1697-1702.

Fuqua, B.K., Lu, Y., Darshan, D., Frazer, D.M., Wilkins, S.J., Wolkow, N., Bell, A.G., Hsu, J., Yu, C.C., Chen, H., et al. (2014). The multicopper ferroxidase hephaestin enhances intestinal iron absorption in mice. PLoS One *9*, e98792.

Ganz, T. (2013). Systemic iron homeostasis. Physiol Rev. *93*, 1721-1741.

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.

Gordeuk, V.R., Caleffi, A., Corradini, E., Ferrara, F., Jones, R.A., Castro, O., Onyekwere, O., Kittles, R., Pignatti, E., Montosi, G., et al. (2003). Iron overload in Africans and African-Americans and a common mutation in the SCL40A1 (ferroportin 1) gene. Blood cells, molecules & diseases *31*, 299-304.

Guida, C., Altamura, S., Klein, F.A., Galy, B., Boutros, M., Ulmer, A.J., Hentze, M.W., and Muckenthaler, M.U. (2015). A novel inflammatory pathway mediating rapid hepcidin-independent hypoferremia. Blood *125*, 2265-2275.

Hadziahmetovic, M., Dentchev, T., Song, Y., Haddad, N., He, X., Hahn, P., Pratico, D., Wen, R., Harris, Z.L., Lambris, J.D., et al. (2008). Ceruloplasmin/hephaestin knockout mice model morphologic and molecular features of AMD. Investigative ophthalmology & visual science *49*, 2728-2736.

Haldar, M., Kohyama, M., So, A.Y., Kc, W., Wu, X., Briseno, C.G., Satpathy, A.T., Kretzer, N.M., Arase, H., Rajasekaran, N.S., et al. (2014). Heme-mediated SPI-C induction promotes monocyte differentiation into iron-recycling macrophages. Cell *156*, 1223-1234.

Harada, N., Kanayama, M., Maruyama, A., Yoshida, A., Tazumi, K., Hosoya, T., Mimura, J., Toki, T., Maher, J.M., Yamamoto, M., et al. (2011). Nrf2 regulates ferroportin 1-mediated iron efflux and counteracts lipopolysaccharide-induced ferroportin 1 mRNA suppression in macrophages. Arch.Biochem.Biophys. *508*, 101-109.

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. Proceedings of the National Academy of Sciences of the United States of America *96*, 10812-10817.

Haschka, D., Nairz, M., Demetz, E., Wienerroither, S., Decker, T., and Weiss, G. (2015). Contrasting regulation of macrophage iron homeostasis in response to infection with Listeria monocytogenes depending on localization of bacteria. Metallomics : integrated biometal science.

Huang, Y.H., Yang, Y.L., Tiao, M.M., Kuo, H.C., Huang, L.T., and Chuang, J.H. (2012). Hepcidin protects against lipopolysaccharide-induced liver injury in a mouse model of obstructive jaundice. Peptides *35*, 212-217.

Jeong, S.Y., and David, S. (2003). Glycosylphosphatidylinositol-anchored ceruloplasmin is required for iron efflux from cells in the central nervous system. J Biol Chem *278*, 27144-27148.

Jiang, R., Hua, C., Wan, Y., Jiang, B., Hu, H., Zheng, J., Fuqua, B.K., Dunaief, J.L., Anderson, G.J., David, S., et al. (2015). Hephaestin and ceruloplasmin play distinct but interrelated roles in iron homeostasis in mouse brain. J Nutr *145*, 1003-1009.

Johnson, E.E., Sandgren, A., Cherayil, B.J., Murray, M., and Wessling-Resnick, M. (2010). Role of ferroportin in macrophage-mediated immunity. Infect Immun *78*, 5099-5106.

Kasvosve, I., Tshwenyego, U., Phuthego, T., Koto, G., Zachariah, M., Nyepetsi, N.G., and Motswaledi, M.S. (2015). Serum ferritin concentration is affected by ferroportin Q248H mutation in Africans. Clinica chimica acta; international journal of clinical chemistry *444*, 257-259.

Kim, D.K., Jeong, J.H., Lee, J.M., Kim, K.S., Park, S.H., Kim, Y.D., Koh, M., Shin, M., Jung, Y.S., Kim, H.S., et al. (2014). Inverse agonist of estrogen-related receptor gamma controls Salmonella typhimurium infection by modulating host iron homeostasis. Nat.Med. *20*, 419-424.

Kohyama, M., Ise, W., Edelson, B.T., Wilker, P.R., Hildner, K., Mejia, C., Frazier, W.A., Murphy, T.L., and Murphy, K.M. (2009). Role for Spi-C in the development of red pulp macrophages and splenic iron homeostasis. Nature *457*, 318-321.

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.

Lakhal-Littleton, S., Wolna, M., Carr, C.A., Miller, J.J., Christian, H.C., Ball, V., Santos, A., Diaz, R., Biggs, D., Stillion, R., et al. (2015). Cardiac ferroportin regulates cellular iron homeostasis and is important for cardiac function. Proc Natl Acad Sci U S A *112*, 3164-3169.

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.

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

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

Ludwiczek, S., Aigner, E., Theurl, I., and Weiss, G. (2003). Cytokine-mediated regulation of iron transport in human monocytic cells. Blood *101*, 4148-4154.

Lymboussaki, A., Pignatti, E., Montosi, G., Garuti, C., Haile, D.J., and Pietrangelo, A. (2003). The role of the iron responsive element in the control of ferroportin1/IREG1/MTP1 gene expression. J Hepatol *39*, 710-715.

Mackenzie, B., and Garrick, M.D. (2005). Iron Imports. II. Iron uptake at the apical membrane in the intestine. AJP - Gastrointestinal and Liver Physiology *289*, G981-G986.

Marro, S., Chiabrando, D., Messana, E., Stolte, J., Turco, E., Tolosano, E., and Muckenthaler, M.U. (2010). Heme controls ferroportin1 (FPN1) transcription involving Bach1, Nrf2 and a MARE/ARE sequence motif at position -7007 of the FPN1 promoter. Haematologica *95*, 1261-1268.

Mayr, R., Janecke, A.R., Schranz, M., Griffiths, W.J., Vogel, W., Pietrangelo, A., and Zoller, H. (2010). Ferroportin disease: a systematic meta-analysis of clinical and molecular findings. J Hepatol. *53*, 941-949.

McCormack, W., Dorsky, R., Wright, S., Rainier, J., Davis, D., and Skliar, M. (2013). Consolidated Hearing Committee Report: Complaint--Research Misconduct. (University of Utah).

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. The Journal of membrane biology *206*, 3-7.

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

Mitchell, C.J., Shawki, A., Ganz, T., Nemeth, E., and Mackenzie, B. (2014). Functional properties of human ferroportin, a cellular iron exporter reactive also with cobalt and zinc. Am J Physiol Cell Physiol *306*, C450-459.

Mosser, D.M., and Edwards, J.P. (2008). Exploring the full spectrum of macrophage activation. Nat Rev Immunol *8*, 958-969.

Nairz, M., Fritsche, G., Brunner, P., Talasz, H., Hantke, K., and Weiss, G. (2008). Interferon-gamma limits the availability of iron for intramacrophage Salmonella typhimurium. Eur.J Immunol. *38*, 1923-1936.

Nairz, M., Schleicher, U., Schroll, A., Sonnweber, T., Theurl, I., Ludwiczek, S., Talasz, H., Brandacher, G., Moser, P.L., Muckenthaler, M.U., et al. (2013). Nitric oxide-mediated regulation of ferroportin-1 controls macrophage iron homeostasis and immune function in Salmonella infection. J Exp.Med. *210*, 855-873.

Nekhai, S., Xu, M., Foster, A., Kasvosve, I., Diaz, S., Machado, R.F., Castro, O.L., Kato, G.J., Taylor, J.G.t., and Gordeuk, V.R. (2013). Reduced sensitivity of the ferroportin Q248H mutant to physiological concentrations of hepcidin. Haematologica *98*, 455-463.

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., 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.

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.U.S.A *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.U.S.A *99*, 4596-4601.

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

Pagani, A., Nai, A., Corna, G., Bosurgi, L., Rovere-Querini, P., Camaschella, C., and Silvestri, L. (2011). Low hepcidin accounts for the proinflammatory status associated with iron deficiency. Blood *118*, 736-746.

Peyssonnaux, C., Zinkernagel, A.S., Datta, V., Lauth, X., Johnson, R.S., and Nizet, V. (2006). TLR4-dependent hepcidin expression by myeloid cells in response to bacterial pathogens. Blood *107*, 3727-3732.

Pietrangelo, A. (2004). The ferroportin disease. Blood Cells, Molecules, and Diseases *32*, 131-138.

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

Portugal, S., Carret, C., Recker, M., Armitage, A.E., Goncalves, L.A., Epiphanio, S., Sullivan, D., Roy, C., Newbold, C.I., Drakesmith, H., et al. (2011). Host-mediated regulation of superinfection in malaria. Nat.Med. *17*, 732-737.

Preza, G.C., Ruchala, P., Pinon, R., Qiao, B., Peralta, M., Sharma, S., Waring, A.J., Ganz, T., and Nemeth, E. (2011a). Analysis of the hepcidin-ferroportin interface yields minihepcidins, small peptides for the treatment of iron overload.

Preza, G.C., Ruchala, P., Pinon, R., Ramos, E., Qiao, B., Peralta, M.A., Sharma, S., Waring, A., Ganz, T., and Nemeth, E. (2011b). Minihepcidins are rationally designed small peptides that mimic hepcidin activity in mice and may be useful for the treatment of iron overload. J Clin.Invest *121*, 4880-4888.

Qiao, B., Sugianto, P., Fung, E., Del-Castillo-Rueda, A., Moran-Jimenez, M.J., Ganz, T., and Nemeth, E. (2012). Hepcidin-induced endocytosis of ferroportin is dependent on ferroportin ubiquitination. Cell Metab *15*, 918-924.

Ramos, E., Ruchala, P., Goodnough, J.B., Kautz, L., Preza, G.C., Nemeth, E., and Ganz, T. (2012). Minihepcidins prevent iron overload in a hepcidin-deficient mouse model of severe hemochromatosis. Blood *120*, 3829-3836.

Recalcati, S., Locati, M., Marini, A., Santambrogio, P., Zaninotto, F., De Pizzol, M., Zammataro, L., Girelli, D., and Cairo, G. (2010). Differential regulation of iron homeostasis during human macrophage polarized activation. European Journal of Immunology *40*, 824-835.

Rice, A.E., Mendez, M.J., Hokanson, C.A., Rees, D.C., and Bjorkman, P.J. (2009). Investigation of the biophysical and cell biological properties of ferroportin, a multipass integral membrane protein iron exporter. J Mol.Biol *386*, 717-732.

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.

Rivers, C.A., Barton, J.C., Gordeuk, V.R., Acton, R.T., Speechley, M.R., Snively, B.M., Leiendecker-Foster, C., Press, R.D., Adams, P.C., McLaren, G.D., et al. (2007). Association of ferroportin Q248H polymorphism with elevated levels of serum ferritin in African Americans in the Hemochromatosis and Iron Overload Screening (HEIRS) Study. Blood cells, molecules & diseases *38*, 247-252.

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.

Ross, S.L., Tran, L., Winters, A., Lee, K.J., Plewa, C., Foltz, I., King, C., Miranda, L.P., Allen, J., Beckman, H., et al. (2012). Molecular Mechanism of Hepcidin-Mediated Ferroportin Internalization Requires Ferroportin Lysines, Not Tyrosines or JAK-STAT. Cell Metab *15*, 905-917.

Roy, C.N., Custodio, A.O., de Graaf, J., Schneider, S., Akpan, I., Montross, L.K., Sanchez, M., Gaudino, A., Hentze, M.W., Andrews, N.C., et al. (2004). An Hfe-dependent pathway mediates hyposideremia in response to lipopolysaccharide-induced inflammation in mice. Nat.Genet. *36*, 481-485.

Sanchez, M., Galy, B., Muckenthaler, M.U., and Hentze, M.W. (2007). Iron-regulatory proteins limit hypoxia-inducible factor-2alpha expression in iron deficiency. Nat.Struct.Mol.Biol *14*, 420-426.

Sangokoya, C., Doss, J.F., and Chi, J.T. (2013). Iron-responsive miR-485-3p regulates cellular iron homeostasis by targeting ferroportin. PLoS Genet *9*, e1003408.

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., et al. (2005). In vitro functional analysis of human ferroportin (FPN) and hemochromatosis-associated FPN mutations. Blood *105*, 4096-4102.

Schimanski, L.M., Drakesmith, H., Talbott, C., Horne, K., James, J.R., Davis, S.J., Sweetland, E., Bastin, J., Cowley, D., and Townsend, A.R. (2008). Ferroportin: lack of evidence for multimers. Blood Cells.Mol.Dis *40*, 360-369.

Shah, Y.M., Matsubara, T., Ito, S., Yim, S.H., and Gonzalez, F.J. (2009). Intestinal hypoxia-inducible transcription factors are essential for iron absorption following iron deficiency. Cell Metab *9*, 152-164.

Sham, R.L., Phatak, P.D., Nemeth, E., and Ganz, T. (2009). Hereditary hemochromatosis due to resistance to hepcidin: high hepcidin concentrations in a family with C326S ferroportin mutation. Blood *114*, 493-494.

Sierra-Filardi, E., Vega, M.A., Sánchez-Mateos, P., Corbí, A.L., and Puig-Kröger, A. (2010). Heme Oxygenase-1 expression in M-CSF-polarized M2 macrophages contributes to LPS-induced IL-10 release. Immunobiology *215*, 788-795.

Taylor, M., Qu, A., Anderson, E.R., Matsubara, T., Martin, A., Gonzalez, F.J., and Shah, Y.M. (2011). Hypoxia-inducible factor-2alpha mediates the adaptive increase of intestinal ferroportin during iron deficiency in mice. Gastroenterology *140*, 2044-2055.

Torti, S.V., and Torti, F.M. (2011). Ironing out cancer. Cancer Res *71*, 1511-1514.

Vallelian, F., Schaer, C.A., Kaempfer, T., Gehrig, P., Duerst, E., Schoedon, G., and Schaer, D.J. (2010). Glucocorticoid treatment skews human monocyte differentiation into a hemoglobin-clearance phenotype with enhanced heme-iron recycling and antioxidant capacity. Blood *116*, 5347-5356.

Wang, L., Johnson, E.E., Shi, H.N., Walker, W.A., Wessling-Resnick, M., and Cherayil, B.J. (2008). Attenuated Inflammatory Responses in Hemochromatosis Reveal a Role for Iron in the Regulation of Macrophage Cytokine Translation. The Journal of Immunology *181*, 2723-2731.

Wong, B.X., Tsatsanis, A., Lim, L.Q., Adlard, P.A., Bush, A.I., and Duce, J.A. (2014). beta-Amyloid precursor protein does not possess ferroxidase activity but does stabilize the cell surface ferrous iron exporter ferroportin. PLoS One *9*, e114174.

Xu, M., Kashanchi, F., Foster, A., Rotimi, J., Turner, W., Gordeuk, V.R., and Nekhai, S. (2010). Hepcidin induces HIV-1 transcription inhibited by ferroportin. Retrovirology. *7*, 104.

Yanatori, I., Yasui, Y., Tabuchi, M., and Kishi, F. (2014). Chaperone protein involved in transmembrane transport of iron. The Biochemical journal *462*, 25-37.

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. Journal of Biological Chemistry *277*, 39786-39791.

Yeh, K.Y., Yeh, M., Mims, L., and Glass, J. (2009). Iron feeding induces ferroportin 1 and hephaestin migration and interaction in rat duodenal epithelium. Am J Physiol Gastrointest Liver Physiol *296*, G55-65.

Zhang, D.L., Hughes, R.M., Ollivierre-Wilson, H., Ghosh, M.C., and Rouault, T.A. (2009). A ferroportin transcript that lacks an iron-responsive element enables duodenal and erythroid precursor cells to evade translational repression. Cell Metab *9*, 461-473.

Zhang, D.L., Senecal, T., Ghosh, M.C., Ollivierre-Wilson, H., Tu, T., and Rouault, T.A. (2011a). Hepcidin regulates ferroportin expression and intracellular iron homeostasis of erythroblasts. Blood *118*, 2868-2877.

Zhang, Z., Zhang, F., An, P., Guo, X., Shen, Y., Tao, Y., Wu, Q., Zhang, Y., Yu, Y., Ning, B., et al. (2011b). Ferroportin1 deficiency in mouse macrophages impairs iron homeostasis and inflammatory responses. Blood *118*, 1912-1922.

Zhang, Z., Zhang, F., Guo, X., An, P., Tao, Y., and Wang, F. (2012). Ferroportin1 in hepatocytes and macrophages is required for the efficient mobilization of body iron stores in mice. Hepatology *56*, 961-971.

Zohn, I.E., De Domenico, I., Pollock, A., Ward, D.M., Goodman, J.F., Liang, X., Sanchez, A.J., Niswander, L., and Kaplan, J. (2007). The flatiron mutation in mouse ferroportin acts as a dominant negative to cause ferroportin disease. Blood *109*, 4174-4180.