Comparative studies of duodenal and macrophage ferroportin proteins

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Iron is crucial for mammalian physiology as a major constituent of hemoglobin and other cellular proteins, but it also promotes the formation of cytotoxic oxygen derivatives. Therefore, complex and tightly regulated homeostatic mechanisms have evolved to respond to increased erythropoiesis, and inflammation. A circulating peptide, hepcidin, appears to regulate iron export from both intestinal and phagocytic cells. Our data also confirm a predominant regulation of ferroportin through systemic regulator(s) likely including hepcidin.

Iron is efficiently recycled for reuse. Macrophages of the spleen, liver, and bone marrow play a critical role in this process because they are the primary sites for phagocytosis and destruction of effete red blood cells (22). The various mechanistic steps involved in iron recycling by macrophages, including possible export from phagosomes and efflux from cells, are poorly understood. Ferroportin is highly expressed in macrophages (1, 9, 41) and plays a critical role in heme iron recycling by macrophages (10, 23).

Therefore, although iron uptake mechanisms differ in intestinal epithelial cells and reticuloendothelial macrophages, both cell types appear to use the same transporter, ferroportin (Slc40a1), to export iron. The aim of this study was to compare the localization, expression, and regulation of ferroportin in both duodenal and macrophage cells. Using a high-affinity purified polyclonal antibody, we analyzed the localization and expression of ferroportin protein in the spleen, liver, and duodenum isolated from normal mice as well as from well-characterized mouse models of altered iron homeostasis. Ferroportin was found to be predominantly expressed in enterocytes of the duodenum, in splenic macrophages, and in liver Kupffer cells. Interestingly, the protein species detected in these cells migrated differently on SDS-PAGE. These differences in apparent molecular masses were partly explained by posttranslational complex N-linked glycosylations. In addition, in enterocytes, the transporter was mostly expressed at the basolateral membrane, whereas in bone marrow-derived macrophages, ferroportin was found predominantly localized in the intracellular vesicular compartment. However, some microdomains positive for ferroportin were also detected at the plasma membrane of macrophages. Despite these differences, we observed a parallel up-regulation of ferroportin expression in tissue macrophages and enterocytes in response to iron-restricted erythropoiesis, suggesting that iron homeostasis is likely maintained through coordinate expression of the iron exporter in both intestinal and phagocytic cells. Our data also confirm a predominant regulation of ferroportin through systemic regulator(s) likely including hepcidin.

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compared with iron overload obtained by iron-dextran injection, iron deficiency obtained through feeding of a low-iron diet was shown to induce ferroportin expression in duodenal enterocytes but to downregulate its expression in Kupffer cells of the liver (1). In addition, contrasting with previous immunohistological studies (9, 29), some authors recently reported the localization of ferroportin in the apical brush border of duodenal enterocytes, where it can modulate the uptake of iron, possibly by modulating the activity of DMT1 (36).

In view of this, we produced a high-affinity antibody against ferroportin to precisely reevaluate the expression and sublocalization of the protein in both duodenal and phagocytic cells. We analyzed by Western blot analysis and immunohistochemistry the expression of ferroportin in the mouse duodenum, liver, and spleen. In addition, using mouse models with characterized aberrations of iron homeostasis, we investigated whether ferroportin protein expression is reciprocally regulated in both cell types in response to iron demand. By immunofluorescence, we also looked at the subcellular localization of ferroportin in macrophage cell cultures.

MATERIALS AND METHODS

Animals and treatments. Mk−/−, mklink (129S6/SvEvTac background), and slay (C57BL/6J background) mice were derived from founders purchased from the Jackson Laboratory. Hpx+/− and hpx/hpx mice (BALB/cJ background) were derived from founders generously provided by Jerry Kaplan (Salt Lake City, UT). Homozygous hpx mice were maintained by weekly injection of human Tf during the first 3 wk of life, as previously described (37). All strains were maintained as inbred stocks and fed identical diets in the animal facility at Children’s Hospital (Boston, MA). For iron depletion studies, wild-type 129/SvEvFBrTac mice were fed either a low-iron diet (ICN; Costa Mesa, CA) for a period of 8 wk to produce iron deficiency or an identical diet supplemented with 3% ferric phosphate for controls (3). We have previously documented that hpx/hpx mutant mice have profound anemia with marked tissue iron overload, whereas animals fed a low-iron diet and mk or slay mutant mice are iron deficient and anemic (2, 37, 40). Human recombinant erythropoietin (EPO; Eprex, Janssen-Ortho; North York, Ontario, Canada; 4,000 IU/ml) and neutralized phosphyllyhdroxan (PHZ; 50 μg·kg−1·day−1) were used as previously described (4).

 Constructs, transfection, and cell culture. For inducible expression of a ferroportin-enhanced green fluorescent protein (EGFP) fusion protein [Fpn(A77D)-EGFP] in Madin-Darby Canine Kidney (MDCK) cells, the coding region of mouse ferroportin was cloned into the XhoI site of the EGFP expression vector pEGFP-N1 (Clontech). Because overexpression of wild-type ferroportin was shown to be highly toxic for MDCK cells, a mutation (A77D) was introduced into the ferroportin coding region by site-directed mutagenesis (Stratagene). The sequence encoding the Fpn(A77D)-EGFP fusion protein was then subcloned into the tetracycline inducible vector pTRE2-Hyg (Clontech). Tet-off MDCK cells (Clontech) were transfected with this sequence encoding the Fpn(A77D)-EGFP fusion protein and were grown in 2

Antibodies. For the production of the rabbit polyclonal anti-mouse ferroportin antibody, a fusion protein containing an 80-amino acid sequence length (residues 224–304) of mouse ferroportin protein fused in-frame to glutathione-S-transferase (GST) was produced, affinity purified on GST columns, and injected into a New Zealand White rabbit. Antiserum was then affinity purified against the same ferroportin peptide segment fused to dihydrofolate reductase using a preparative immunoblot procedure (3). The anti-Nramp1 and anti-DMT1/Nramp2 antisera have been previously described (3, 18). The anti-CD11b (Mac-1) antibody developed by T. A. Springer and the anti-Lamp1 antibody developed by J. T. August were obtained from the Developmental Studies Hybridoma bank developed under the auspices of the National Institute of Child Health and Human Development and maintained by the Department of Biological Science, University of Iowa, Iowa City, IA. Rat monoclonal anti-mouse TIR and mouse anti- rat Grp78/BiP antibodies were purchased from Bio-source and Stressgen, respectively.

Western blot analysis. Crude membrane fractions from cells or mouse tissues were prepared as previously described (3, 25). Protein concentrations were determined by the Bradford assay (Bio-Rad). Proteins from mouse tissues and MDCK, BMDM, J774A, and RAW264.7 cells were solubilized in 1× Laemmli buffer and incubated for 30 min at room temperature (RT) before SDS-PAGE and transfer onto polyvinylidene difluoride (PVDF) membranes. Ponceau red staining confirmed similar gel loading and similar transfer of proteins to the membranes. Immunoblots were preincubated with blocking solution [7% skim milk in 0.15% Tween 20 in Tris-buffered saline (TBS)] for 16 h at 4°C before incubation with primary antibodies for 2 h at RT. Antibodies were diluted in blocking solution as follows: anti-mouse ferroportin, 1/200 (intestine and liver) or 1/500 (spleen); and anti-mouse DMT1, 1/500. After being incubated with antibodies, membranes were washed six times (5 min each) in TBST and then incubated with goat peroxidase-labeled anti-rabbit immunoglobulin (1/10,000, Nordic Immunologic) for 1 h at RT. Signals were visualized by enhanced chemiluminescence (Amersham). Some PVDF membranes were stripped [100 mmol/l β-mercaptoethanol, 2% SDS, and 62.5 mmol/l Tris-HCl (pH 6.8); at 50°C for 30 min] and then reprobed with a different primary antibody.

Glycosidase treatments. Endoglycosidase H (endo H) and peptide N-glycosidase F (PNGase F) were obtained from New England Biolabs. Aliquots of membrane preparations from mouse tissues and BMDM cells were denatured before digestion in buffer containing 1% SDS and 2% β-mercaptoethanol for 30 min at RT. Endo H (1,500 units) and PNGase F (1,500 units) digestions were performed at room temperature for 75 min in 50 mM sodium citrate (pH 5.5) for Endo H or 50 mM sodium phosphate (pH 7.5) and 1% NP-40 for PNGase F. Reactions were stopped by adding an equivalent volume of Laemmli buffer (2.5×) to the samples. Samples were then processed for Western blot analysis. Controls were treated identically except that an equivalent volume of distilled water was added in place of Endo H or PNGase F.

Immunohistochemistry. Tissues were fixed in Bouin’s solution (9 g/l picric acid, 4% acetic acid, and 25% formaldehyde) for 72 h at 20°C and embedded in paraffin for sectioning. After being mounted onto glass slides, sections (5 μm) were processed for immunohistochemistry (3′-diaminobenzidine tetrahydrochloride staining) as previously described (3). Primary antibodies were diluted as follows: anti-mouse ferroportin and corresponding preimmune serum, 1/75 (for the intestine and liver), 1/100 (for the spleen); anti-mouse Nramp1,
1/100; and anti-mouse DMT1, 1/100. Sections were counterstained with 0.1% methylene blue in PBS and mounted in Permount.

**Immunofluorescence.** After being fixed with methanol (100% at −20°C for 15 min), cells were permeabilized with Triton X-100 (0.1% in PBS) for 10 min, washed twice with PBS, and then incubated in a blocking solution (1% BSA and 10% goat serum heat inactivated in PBS) for 45 min at RT. Incubation with primary antibodies was performed in a humid chamber at RT for 1 h using the following dilutions in blocking solution: anti-Nramp1, 1/200; anti-ferroportin, 1/100; anti-CD11b, 1/100; anti-BiP, 1/50; anti-Lamp1, 1/500; and anti-TIR, 1/100. After being washed three times (5 min each) with PBS-0.5% BSA, cells were incubated for 1 h at RT with secondary antibodies (goat anti-rabbit-alexa 488 or goat anti-rat Cy3, Molecular Probes) diluted at 1/200 in blocking solution. Slide coverslips were washed, mounted with anti-fading mounting reagent (Prolong Antifade kit P-7481, Molecular Probes), and processed for immunofluorescence. Cells were visualized using either an epifluorescence microscope (LEICA DM-IRM) with a ×40 and ×100 oil-immersion objective or a Zeiss confocal fluorescent microscope with a ×60 oil-immersion objective. Images were acquired using either ARCHIMED-PRO (Microvision Instruments) or Zeiss LSM Image Browser software.

**RESULTS**

**Expression and subcellular localization of ferroportin in the mouse duodenum, spleen, and liver.** Our polyclonal antiserum was raised against a GST fusion protein containing a portion of mouse ferroportin (amino acid positions 224–304). Antibody specificity was tested on membrane extracts from cells expressing a full-length ferroportin-EGFP fusion protein (Fig. 1A). To induced MDCK transfectant cells, ferroportin was detected as a major immunoreactive species that was absent from membrane preparations of uninduced cells. Using this antibody, we found that ferroportin was strongly detected in microsomal fractions isolated from the mouse spleen and intestine. A much lower level of ferroportin expression was detected in the mouse liver. In addition, by immunohistochemistry (Fig. 1B), we observed that ferroportin was highly expressed along the lateral membrane (see inset in Fig. 1B,a) and at the basal pole of villus enterocytes, in contrast with DMT1 staining present at the brush border of these cells (Fig. 1B,b). In the spleen, ferroportin expression was noted in clusters of cells from red pulp surrounding nodules of white pulp (Fig. 1B,c). In the liver, staining was limited to a few discrete cells interspersed among well-organized clusters of hepatocytes that appeared negative for ferroportin staining (Fig. 1B,e). In both tissues, the ferroportin staining pattern was similar to the one observed for the macrophage-specific marker Nramp1 (Fig. 1B,d and f), indicating that splenic macrophages and Kupffer cells are the predominant ferroportin-positive cells in these tissues, as previously observed (1, 8, 41). Altogether, these results attested for the high specificity of our anti-ferroportin antibodies and confirmed the duodenal- and macrophage-specific expression of ferroportin protein.

**Effects of iron status and erythropoietic drive on ferroportin and DMT1 expression in the mouse duodenum, spleen, and liver.** In the duodenums of mice fed a low-iron diet and sla hemizygous and mk homozygous (not shown) animals, we observed a net increase in ferroportin expression compared with control animals (Fig. 2A1). There was an anatomic gradient in ferroportin induction, with the largest increase in expression seen in the proximal duodenum and less expression in the distal duodenum, similar to that seen for DMT1 (Fig. 2A2). Hpx mice also showed a dramatic increase of ferroportin in the duodenum. In mice fed a low-iron diet, as well as in sla and hpx homozygous mice, we observed coordinate increases of both ferroportin and DMT1 protein expression compared with their respective control mice. In hemizygous sla/Y mice, expression of DMT1 was only weakly enhanced. Interestingly, the upregulation of ferroportin expression detected in the small intestine of the above-mentioned mouse models was associated with an increased detection of ferroportin in both the spleen (Fig. 2B) and liver (Fig. 2C).

Stimulation of erythropoiesis by EPO or PHZ treatment was found to cause a dramatic increase in the total level of ferro-
portin expression in the intestine (Fig. 3A) and in the liver (Fig. 3C) but not in the spleen (Fig. 3B). In the duodenum, ferroportin (Fig. 3A1) and DMT1 (Fig. 3A2) expression were concomitantly stimulated in treated animals compared with untreated control mice. We again observed a gradient of ferroportin expression (proximal to distal), as seen in normal and iron-deprived mice. The upregulation of ferroportin analyzed in the different mouse models by Western blot analysis was confirmed by immunohistochemistry and associated with increased staining in both enterocytes and tissues macrophages [data not shown and supplemental data 1 (see http://ajpgi.physiology.org/cgi/content/full/00227.2005/DC1)].

Ferroportin is an integral membrane glycoprotein. Interestingly, we noted that the major protein species detected in the liver, duodenum, and spleen migrated differently under our SDS-PAGE conditions (Figs. 1–3). These differences were

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**Fig. 2.** Effect of iron status on expression of ferroportin and DMT1 in mouse tissues. The proximal (Duo1), distal (Duo2), or total (Duo) duodenum (A), spleen (B), and liver (C) were harvested from mice maintained on either a low-iron diet (−Fe) or a normal diet (+Fe) and from control (+/Y, mk+/+, or hpx+/+) or mutant (sla/Y, mk/mk, or hpx/hpx) mice. Microsomal membranes were analyzed by SDS-PAGE (intestine: 70–80 μg; spleen: 20–40 μg; liver: 50μg) followed by immunoblotting with anti-ferroportin (A1, B, and C) and anti-DMT1 sera (A2). The positions and sizes (in kDa) of molecular mass markers are indicated on the right.

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**Fig. 3.** Effect of stimulation of erythropoiesis on expression of ferroportin and DMT1 in mouse tissues. Microsomal fractions were prepared from the proximal (Duo1), distal (Duo2), or total (Duo) duodenum (A), spleen (B), and liver (C) of mice treated with erythropoietin (EPO) or phenylhydrazine (PHZ). Immunoblotting was performed with either anti-ferroportin (A1, B, and C) or anti-DMT1 antisera (A2). The positions and sizes (in kDa) of molecular mass markers are indicated on the right.

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**Fig. 4.** Electrophoretic mobility and glycosylation of ferroportin proteins. Membrane extracts were prepared from tissues isolated from either untreated (spleen) or EPO-treated mice (duodenum and liver). Proteins were then fractionated on a 7% polyacrylamide gel and analyzed by immunoblotting with anti-ferroportin (A1 and C–E) or anti-DMT1 (B) antisera. *An unspecific band. C–E: effects of endoglycosidase H (Endo H) or peptide N-glycosidase F (PNGase F) on ferroportin expressed in the duodenum (50 μg; C), spleen (25 μg; D), and liver (50 μg; E), respectively. Open arrowheads indicate endogenous protein species before treatment, whereas solid arrowheads indicate major protein species detected after PNGase F treatment. The positions and sizes (in kDa) of molecular mass markers are indicated.
clearly observed when membrane protein samples were separated on a low percentage acrylamid gel (Fig. 4A). In these experiments, to facilitate the detection of ferroportin expressed in the duodenum and liver, we used samples isolated from EPO-injected animals because this treatment was shown to increase ferroportin levels in these organs with no changes in the apparent molecular mass of the protein species detected. Endogenous ferroportin species from the duodenum, spleen, and liver migrated around 70, 65, and 63 kDa, respectively (Fig. 2). These differences in electrophoretic mobility may reflect tissue-specific variations in posttranslational modification of the protein. To test the possibility that ferroportin is posttranslationally modified by glycosylation, membrane fractions from mouse tissues were treated with endoglycosidases followed by electrophoresis and immunoblotting. As a control, DMT1 expressed in the duodenum was shown to be highly modified by glycosylation, as illustrated by the conversion of the 80- to 90-kDa molecular species into a 50- to 55-kDa form (Fig. 4B) after PNGase F, which hydrolyzes high mannose and hybrid and complex oligosaccharides from N-linked glycoproteins. As previously observed (17), DMT1 was resistant to Endo H, which specifically cleaves high mannose and some hybrid oligosaccharides from N-linked glycoproteins. In all tissue samples tested, ferroportin species were also resistant to Endo H (Fig. 4, C–E). On the other hand, PNGase F converted ferroportin species into smaller forms of 61.5 kDa in the duodenum (Fig. 4C) and 58 kDa in the spleen and liver (Fig. 4, D and E).

**Detection of ferroportin in macrophages.** Ferroportin expression was studied by Western blot analysis in BMDM and in two macrophage cell lines, J774A and Raw264.7 (Fig. 5A).

In these experiments, membrane proteins from MDCK cells expressing a ferroportin-EGFP fusion protein were used to adjust the time of enhanced chemiluminescence detection to compare the level of expression of ferroportin in these different macrophage populations. In BMDM, ferroportin was detected as an abundantly expressed ~65-kDa polypeptide (Fig. 5A, left). Lower levels of ferroportin expression were also detected in the macrophage cell line J774A (Fig. 5A, right), whereas the macrophage line RAW264.7 appeared negative for ferroportin expression. Microsomal fractions from BMDM were also treated with endoglycosidases followed by electrophoresis and immunoblotting with anti-ferroportin (Fig. 5B). To facilitate the detection of ferroportin in these experiments, BMDM were pretreated overnight with 100 μM Fe nitrolotriacetate (NTA) solution (100 μM FeCl3-400 μM NTA) before membrane preparation. Indeed, iron treatment has been shown to considerably enhance the basal level of ferroportin expression in macrophages with no change in the apparent molecular mass of the protein species detected (Ref. 22 and personal observations). The ferroportin expressed in macrophage cell cultures was resistant to digestion with Endo H. In contrast, PNGase F converted the 65- to 70-kDa ferroportin species into smaller forms of ~60 kDa. These results confirm that the macrophage form of ferroportin expressed in primary cultures is posttranscriptionally modified by complex N-linked glycosylation.

The subcellular localization of ferroportin was next investigated in BMDM by immunofluorescence (Figs. 5C and 6). As a negative control, no staining was observed when the primary anti-ferroportin antibody was omitted in the immunofluorescence procedure (Fig. 5C,a). Ferroportin showed a punctuated intracellular staining pattern compatible with localization in an...
DISCUSSION

Using our specific antibody against ferroportin, we detected ferroportin along the entire basolateral membrane in fully mature enterocytes populating duodenal villi in agreement with earlier studies (9, 29). Ferroportin was detected in the same cells as DMT1 but at the basolateral, not apical, membrane (3). In contrast to one report (36), we did not detect any overlap between the expression of ferroportin and DMT1 proteins. As previously shown (1, 41), ferroportin was also detected at high levels in tissue macrophages, including Kupffer cells of the liver and splenic macrophages from the red pulp, consistent with a role in recycling iron from senescent erythrocytes. It is significant that we failed to detect ferroportin staining in hepatocytes, even in the context of iron deficit, which would be expected to maximize iron release from hepatic stores. However, we cannot exclude the possibility that hepatocytes do indeed express ferroportin at levels that could not be detected with our antibody or through our staining procedure. Indeed, some authors reported a plasma membrane staining of ferroportin in hepatocytes that was evident in paraffin-embedded sections only at high antibody concentrations (1).

In addition to tissue macrophages, ferroportin was also shown to be expressed in primary bone marrow cell cultures and in J774A cells. However, it appeared to be absent from RAW264.7 macrophages. Because the J774A cell line is known to have arisen by immortalization of a more mature and more differentiated myeloid precursor than the RAW264.7 cell line, including expression of more terminal markers of activation (such as Nramp1 and inducible nitric oxide synthase) (16), these results suggest that ferroportin protein expression is acquired at a fairly late stage during macrophage maturation and differentiation from myeloid precursors.

Interestingly, this study highlighted some differences between the ferroportin proteins expressed in enterocytes and macrophage cells. First, the apparent molecular masses on a SDS-PAGE gel of the ferroportin species detected in enterocytes and macrophage cells. First, the apparent molecular masses on a SDS-PAGE gel of the ferroportin species detected in enterocytes and macrophage cells were different, with the highest apparent molecular mass detected in enterocytes, splenic macrophages, and Kupffer cells seemed to be more differentiated myeloid precursor than the RAW264.7 cell line, including expression of more terminal markers of activation. Second, ferroportin was detected in hepatocytes that was evident in paraffin-embedded sections only at high antibody concentrations (1).

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tissues and macrophages in culture have apparent masses (from 63 to 70 kDa) higher than the calculated mass predicted by the primary amino sequence (62 kDa). Indeed, we have shown that duodenal and macrophage ferroportin proteins are both post-translationally modified by complex N-linked glycosylation. However, after glycosidase treatment, the ferroportin protein expressed in the duodenum migrated slower than the form expressed in the liver, spleen, or BMDM, suggesting the existence of other modifications in duodenal versus macrophage cells. Such modifications may be important for the proper localization and/or function of the protein in duodenal cells. Moreover, in contrast to the clear detection of ferroportin at the basolateral membrane of enterocytes, immunofluorescence studies with BMDM revealed a pronounced vesicular and mostly intracellular localization that differed strongly from the cell surface staining of CD11b. However, extensive studies using confocal analysis revealed the presence of some ferroportin microdomains at the plasma membrane, likely suggesting a vesicular trafficking of the protein between the cytosol and cell surface. Ferroportin may be stored within the cell until it is needed for iron export, at which point it may be recruited to the membrane, as previously reported for other metal transporters (7). Alternatively, ferroportin may mediate iron export through the use of an intracellular vesicular compartment, in which ferroportin would act as an iron “concentrator.” Such a vesicular compartment could then be recruited to the plasma membrane via exocytosis. Although this model is highly speculative, it is compatible with the known direction of ferroportin-mediated iron transport at the plasma membrane and the observed subcellular distribution of the protein. On the other hand, the presence of ferroportin at the plasma membrane may also indicate a direct cell surface export of iron during macrophage iron recycling. This point needs to be clarified.

Our results showed a coordinated upregulation of ferroportin in enterocytes and macrophages in response to iron-restricted erythropoiesis. Increased ferroportin was seen when wild-type mice were fed an iron-restricted diet and when both intestinal iron absorption and erythroid iron assimilation were impaired in mk mice with mutations in DMT1. Hpx mice, which have massive systemic iron overload yet iron-restricted erythropoiesis (37), also have elevated ferroportin expression, suggesting that it is the iron-restricted erythropoiesis rather than the amount of total body iron that signals these increases. Similarly, mice with accelerated erythropoiesis due to treatment with EPO or PHZ have normal amounts of total body iron but have a marked increase of ferroportin expression in the duodenum and liver. By Western blot analysis, we did not observe major changes in ferroportin expression in the spleens of EPO- and PHZ-treated animals. However, spleens from these mice are highly erythropoietic (4), and this may mask a change in ferroportin expression through dilution of the mononuclear phagocytes by expansion of the erythroid compartment [Ref. 9 and supplemental data 2 (http://ajpgi.physiology.org/cgi/content/full/00227.2005/DC1)]. Finally, mice with a mutation in hephaestin (sla) have iron-restricted erythropoiesis but marked iron deposition in enterocytes (27), and they too have increased ferroportin expression in enterocytes and macrophages. This indicates that the signal to increase ferroportin in response to iron-restricted erythropoiesis is not interrupted even when the enterocytes themselves are iron overloaded. Together, these observations suggest that ferroportin is predominantly (if not exclusively) regulated by systemic signals in response to iron-restricted erythropoiesis.

The coordinate upregulation of ferroportin in duodenal enterocytes and tissue macrophages in response to iron-restricted erythropoiesis contrasts with a previous report (1) documenting a reciprocal regulation of ferroportin in the liver and duodenum in response to iron status. Differences between the two studies may be due to different experimental systems. Our results strongly suggest that iron homeostasis is maintained through coordinate expression of the iron exporter in both intestinal and phagocytic cells. Such regulation would modulate both dietary iron absorption and iron mobilization from reticulendothelial cells. A possible common pathway for regulation of ferroportin in macrophages and in epithelial cells of the duodenum has been recently elucidated with the characterization of hepcidin (31, 32, 34). It is now well established that circulating levels of hepcidin negatively regulate intestinal iron absorption and macrophage iron release (14). In all mouse models with iron-restricted erythropoiesis, liver hepcidin mRNA expression was shown to be downregulated (33, 40). In tissue culture cells, hepcidin was shown to bind to ferroportin directly, causing its internalization and subsequent degradation in lysosomes (30). In addition, recently, hepcidin was shown to markedly decrease the ferroportin protein level in J774A macrophages (23). Therefore, hepcidin may interact with ferroportin at the cell surface of enterocytes and macrophages to induce its degradation and thereby downregulates subsequent iron export. On the other hand, decreased hepcidin protein observed in the setting of iron-restricted erythropoiesis is likely associated with less degradation and subsequent accumulation of ferroportin in these cells. This hypothesis is reinforced by the recent work of Viatte et al. (38), which clearly shows coordinate upregulation of ferroportin protein levels in the duodenum, liver, and spleen in hepcidin-deficient mice. However, other studies have documented changes in intestinal ferroportin at the mRNA level in response to iron deficiency, anemia, or hypoxia (6, 13, 29), suggesting the existence of other regulatory mechanisms.

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