IEC-6 Cells Are an Appropriate Model of Intestinal Iron Absorption in Rats

Carla Thomas and Phillip S. Oates

Department of Physiology, The University of Western Australia, Nedlands 6907, Western Australia

ABSTRACT Regulation of iron absorption, which is the primary mechanism for maintaining body iron stores, occurs primarily in the proximal small intestine. Recent identification of proteins that are involved in iron absorption such as the uptake transporter-divalent metal transporter (DMT1), the basolateral transporter, IREG1, and the ferroxidase-hephaestin provide new opportunities to study this process. We evaluated the rat intestinal cell line, IEC-6, as a model of intestinal iron transport. This involved measuring the expression of DMT1 and IREG1 by Western blot analysis and confocal microscopy, and hephaestin by protein-dependent copper oxidase activity. DMT1 and IREG1 were expressed in IEC-6 cells. The uptake of 1 μmol/L ferrous iron [Fe(II)]ascorbate and its efflux also was associated with the expression of DMT1 under different levels of iron loading. The expression of DMT1 changed inversely with iron levels as did the uptake of Fe(II). However, with different levels of cellular iron, IREG1 expression remained constant, as did the release of iron from the cells, suggesting that they could be related. Ceruloplasmin and apotransferrin did not enhance the rate or extent of iron release. Copper oxidase activity, considered to indicate hephaestin activity, was detected only intracellularly. Confocal microscopy showed DMT1 and IREG1 on the cell membrane of IEC-6 cells at 4°C but at intracellular locations at 37°C, indicating that these proteins can function at the cell membrane and intracellularly. In terms of iron absorption, IEC-6 cells have a villous enterocyte phenotype and are regulated by iron stores as occurs in vivo; therefore, they represent an appropriate cell model with which to study this process.


KEY WORDS: • iron uptake • efflux • DMT1 • IREG1 • hephaestin

Iron is a trace element that is required for numerous cellular functions (1). However, because it reacts with oxygen to cause oxidative damage, body iron stores must be maintained within strict limits (2). This is controlled at the level of iron absorption by duodenal enterocytes. Despite extensive study, details on the mechanism of iron absorption remain unclear. Recent identification of proteins involved in this process such as the uptake transporter IREG1/ferroportin (12,13), IREG1/ferroportin/metals transporter protein 1 (MTP1) (5–7) and hephaestin (8) provide new opportunities to clarify this mechanism.

DMT1 is expressed on the apical membrane of the enterocyte; in the presence of a proton gradient provided by gastric secretions, it cotransports ferrous iron [Fe(II)] from the membrane into the cell (3,9,10). Supporting evidence for the role of DMT1 in the uptake of iron is found in homozygous sex-linked anemic mice impairs iron release (14).

The recent cloning and characterization of IREG1/ferroportin/MTP1 suggests that it is the efflux carrier of the enteroocyte as shown in expression systems in which IREG1 exported iron in the presence of ceruloplasmin (Cp) (5–7). However, the efflux of iron from the enterocyte probably requires the Cp homologue hephaestin (8) because its mutation as seen in sex-linked anemic mice impairs iron release (14).

Understanding how these proteins coordinate iron absorption is assisted by studies using cell lines of human origin (15,16). However, it would be advantageous to have a cell line derived from the same species used in studies of whole animals, such as the rat, so that additional mechanistic questions can be studied in conjunction with those obtained in vivo and in turn compared with those found in humans. These cells must be of intestinal origin, express the above genes, have functional uptake and efflux transporters, and be able to be transfected for the further study of genes of interest. In view of this we evaluated the nontransformed rat small intestinal epithelial cell line IEC-6 as a model for the study of nonheme iron absorption. This involved comparing the expression and cellular localization of DMT1 and IREG1 in IEC-6 cells and rat duodenum. Also, the existence of uptake and efflux iron transport processes along with changes in the expression of the above genes with variations in iron loading was investigated in IEC-6 cells.

MATERIALS AND METHODS

Cell culture. IEC-6 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and routinely main-
tained in the presence of Dulbecco’s modified Eagle’s medium (DMEM) containing 5% fetal calf serum (FCS), 100 U insulin, 50 mg/L penicillin, 50 mg/L streptomycin and 2.5 mg/L fungizone (In-vitrogen, Groningen, The Netherlands). Cells were grown at 37°C in a humidified atmosphere of 5%CO₂/95% air and viability was moni-
tored using phase contrast microscopy and trypan blue staining. All experiments were done on cells between passages 17 and 30.

Variations in iron loading were performed as described below (see ferrous uptake). Total cellular iron concentration was measured by atomic absorbance spectrometry. This involved washing the cells with PBS and removing them from a 25 cm² culture flask. The cells were centrifuged (700 × g for 5 min) and resuspended in 2.8 mol/L nitric acid and left at 90°C for 60 min. The solution was centrifuged (12,000 × g for 5 min) to remove denatured protein and appropriately diluted supernatant measured for Fe concentration using an atomic absorbance spectrometer (Varian, Victoria, Australia).

The results presented in this study have been normalized to the protein content of the cells. The protein content of IEC-6 cells was determined using the BCA protein assay kit (Pierce, Rockford, IL).

Animals. The use of animals in this study has been approved by the Animal Welfare Committee of the University of Western Aus-
tralia. Outbred male Wistar rats (6 wk old) were obtained from the Animal Resource Center (Animal Resource Centre, Murdoch, Western Australia). Rats were fed a semipuri-
fied diet either normal or loaded by the addition of 15 mol/L Fe(II) as above, except after the 1-h incubation, the cells were washed with BSS and then incubated with a MEM solution deficient in the tracer iron solution with or without 500 nmol/L Cp or 200 μmol/L apotransferrin. The cells were incubated for 0, 5, 10, 15, 30 and 60 min at 37°C in an atmosphere of 5%CO₂/95% air. The efflux medium was removed and counted for radioactivity. The cells were washed and then incubated with 1 μg/ml pronase to remove membrane-bound proteins. After centrifugation (700 × g for 5 min), the cells were washed and lysed in 0.1 mol/L NaOH, 1% Triton-
X100. Cells were counted for radioactivity in a gamma counter.

Copper oxidase activity. The intracellular and extracellular copper oxidase activity of IEC-6 cells was measured by the rate of oxidation of p-phenylenediamine (18). A sodium acetate solution (200 mmol/L) containing 1.5 g/L p-phenylenediamine was incubated with viable cells in a 6-well plate, fresh and freshly boiled homoge-
nates for 15–60 min at 37°C. The reaction was stopped by the addition of 1.5 mol/L sodium azide. Parallel reactions with sodium
azide present throughout the incubation were carried out to account for spontaneous oxidation of p-phenylenediamine. The color change was quantified by measuring its absorbance at 530 nm. Activity is expressed in units as follows: 1 U = 1 absorbance unit change/30 min.

Western blot analysis. Protein concentrations of IEC-6 cells, glycologase F-treated and –untreated intestinal mucosal scrapings were determined; 50 μg of each preparation was heated at 100°C for 5 min in standard Laemmli buffer and separated on a 12% SDS-
polyacrylamide gel. Cells were electroblotted onto Immobilon-P transfer membrane (Millipore, Bedford, MA). Similar loading and transfer of proteins to the membranes was veri-
died. The membranes were incubated with polyclonal antibodies (Serotech, Oxford, UK) and an anti-mouse
antibody (1:1500) (see below for veri-
ymen). The resulting bands were developed with ECL chemiluminensecent assay (Amer-
sham, Bucks, UK).

Production and validation of IREG1–1 antibody. A synthetic peptide corresponding to amino acids 247–264 of rat IREG1 was conjugated to keyhole limpet hemocyanin and then used to immu-
nize a rabbit for production of anti-IREG1 antiserum as described previously (9). Several weeks after sensitization, antisem was obtained and subjected to an ELISA using immobilized immunizing peptide as antigen to determine antibody titer.

IREG1 cDNA construction. A polymerase chain reaction (PCR) fragment representing nucleotides 269-2012 of the published rat IREG1 sequence (Accession #L76714) and encompassing the open reading frame of the gene was generated by a one-step reverse transcriptase (RT) PCR (Invitrogen) using purified total RNA from rat duodenum. Primer used for the RT step was also the 3’ primer, i.e., CAGCTTATTTAGGTGATC; the 5’ primer was CTAG-
CATCCGGACACAAACAG. The IREG1 cDNA was unidirectional-
ally cloned in the sense direction into the Not1 and BamH1 sites of pcDNA3.1/V5/His-TOPO eucaryotic expression vector (Invitrogen). The orientation and sequence of the cDNA clone were confirmed by sequencing.

Oeoverexpression and detection of IREG1 in Cos-7 cells and dud-
testinal tissue. Cos-7 cells were transfected by Lipofectamine treat-

Downloaded from https://academic.oup.com/jn/article/132/4/680/4687346 by guest on 12 October 2018
ment (Invitrogen). One day before transfection, $3 \times 10^5$ cells were seeded per $25\text{cm}^2$ culture flask, so that on the day of transfection, the culture was 70% confluent. No antibiotics were used throughout the transfection period. Cos-7 cells were transfected with either pcDNA3.1-IREG1 or pcDNA3.1 plasmids. Lipofectamine at $5 \text{mg}$ was used for the transfections. The DNA/lipofectamine mixture was removed after 5 h of incubation at $37^\circ\text{C}$ and the cells were incubated for 24 h in DMEM with 5% FCS before they were lysed in lysis buffer as described above. The lysate was incubated with 5 $\mu$L of anti-IREG1 antibody for 1 h at 4°C. Immunoprecipitates were collected by incubation with 100 $\mu$L of protein A-agarose for 2 h at 4°C, washed three times with PBS, heated at $100^\circ\text{C}$ for 5 min in standard Laemmli buffer and separated on a 12% SDS-polyacrylamide gel. Western blot analysis was carried out as above. As an additional control, the tissue sections were incubated with the IREG1 antibody in the presence or absence of immunizing peptide (see below).

**Validation of the isolation of villous mucosa from crypt mucosa.** Protein ($50 \text{mg}$) isolated from mid-villus, crypt cells and IEC-6 cells in log-phase were subjected to Western blot analysis using PCNA to detect cells in proliferation.

**Immunofluorescence microscopy.** IEC-6 cells were grown on glass coverslips in $35\text{mm}$ dishes. Cells were seeded at $1.5 \times 10^5$ cells/dish $2 \text{d}$ before the experiment and were in the exponential phase of cell growth for the experiment. Cells were prepared for immunofluorescence microscopy either while viable or after fixation in $12\%$ formalin. Primary antibodies were used as follows: monoclonal anti-sucrase-isomaltase (SI) (1:100) (20), affinity purified rabbit polyclonal anti-rat DMT1 (1:300) and rabbit polyclonal anti-rat IREG1 (1:100). Rabbit preimmune serum was used as a control. Secondary antibodies were anti-rabbit Texas-red conjugated (Molecular Probes, Eugene, OR) or anti-mouse FITC-conjugated (Serotech) diluted 1:100 with BSS. Viable and fixed cells were incubated with primary and secondary antibodies together for 1 h at $37^\circ\text{C}$ or $4^\circ\text{C}$ and then washed 5 times with PBS. The viable cells were then fixed. Co-incubation of both primary and secondary antibodies with viable cells enabled the detection of these proteins after movement from the cell surface if this was a temperature-dependent process. The proximal 0.5-cm segment of the duodenum from an iron-deficient and a normal rat were frozen in liquid nitrogen-cooled isopentane. This tissue was then stored at $-80^\circ\text{C}$ until sectioned. At the time of sectioning, the tissue was glued to a chuck with Tissue Tek O.C.T. compound (Miles Scientific, Naperville, IL). Sections (7 $\mu\text{m}$) were then cut at $-13^\circ\text{C}$ and adhered to a 0.5 $\text{g} / \text{L}$ gelatin-coated microscope slides. The sections were dried for 10 min at room temperature and fixed in $12\%$ formalin. The tissue was then used immediately for immunofluorescent microscopy for the localization of SI, DMT1 and IREG1 as described above. In addition, tissue was incubated with labeled iron within 60 min. Cells in the stationary or exponential growth phase. When iron uptake was measured over the $105$ min, it was $20.1 \pm 3$, $11.2 \pm 2$ and $7.7 \pm 2 \text{fmol/g protein} \cdot \text{min}$ in iron-deficient, control and iron-loaded cells, respectively, all of which differed (Fig. 1).

**Iron Release by IEC-6 cells.** Iron was released from IEC-6 cells over time (Fig. 2). The rate of release was constant for all iron conditions, resulting in the release of 50% of the radiolabeled iron within 60 min. Cells in the stationary or exponential growth phase did not differ in rate of release of iron. Addition of Cp or apotransferrin to the medium had no effect on the release of Fe (data not shown).

**Validation of IREG1 antibody.** To determine the presence of IREG1 antisera we used ELISA to show that there was immunoreactivity when the antisera were reacted with immunizing antigen. To rigorously test the specificity of the antisera, we overexpressed recombinant rat IREG1 in Cos 7 cells.
When the protein was immunoprecipitated and subjected to Western blot analysis using the antisera, a single band migrating at ~60 kDa was observed. The signal was markedly greater than in untransfected or vector alone transfected Cos-7 cells in which the signal was equally low (Fig. 3). In addition, the immunofluorescent signal seen with the IREG1 antibody on sections of tissue was identical to that previously reported (see below). This signal can be eliminated if the IREG1 antibody is incubated overnight with the immunizing peptide used to generate it, which indicates that the antibody is specific for rat IREG1 protein (Fig. 4C).

**Validation of the isolation of villous mucosa from crypt mucosa.** Protein obtained from crypt mucosa and IEC-6 cells reacted positively for PCNA. Mid-villous cells were negative for PCNA (Fig. 5).

**Protein expression in IEC-6 cells and duodenal mucosa.** DMT1 migrated predominantly as a band of ~66 kDa in IEC-6 cells although there were an additional two bands (iron-deficient cells) and one smaller sized band (control cells) (Fig. 6A). In villous mucosa, DMT1 migrated as a band at ~90 kDa (Fig. 6A). In addition, DMT1 expression in IEC-6 cells was inversely related to cellular iron loading (Fig. 6A). Ferritin was demonstrated as a ~20-kDa protein in both IEC-6 cells and rat intestinal scrapings (Fig. 6B). Ferritin levels were directly related to cellular iron loading (Fig. 6B). IREG1—1 migrated as a ~60-kDa protein in IEC-6 cells and duodenal mucosa scrapings (Fig. 6C). In IEC-6 cells, IREG1 expression was unaffected by cellular iron loading (Fig. 6C).

**Glycosylation of DMT1 in vivo.** Digesting duodenal mucosal protein with glycosidase F resulted in a reduction of the 90-kDa band to ~66 kDa (Fig. 7), suggesting that the heavier 90-kDa band observed in vivo is due to glycosylation of the DMT1.

**Copper oxidase activity of IEC-6 cells.** Copper oxidase activity of 0.16 U was detected in the homogenate of IEC-6 cells, but no activity could be detected extracellularly. In addition, this activity was lost when the IEC-6 homogenate was boiled (data not shown).

**Localization of sucrase isomaltase, DMT1 and IREG1.** SI was not detected in IEC-6 cells. In whole mounts of duodenum, SI was not detected in the crypt region but its expression commenced at the crypt-villus junction where it localized strongly along the brush border throughout the length of the villus, but attained the highest concentrations in the mid-villous region (Fig. 8).

DMT1 was detected on the IEC-6 cell membrane only when immunofluorescent preparation was carried out at 4°C (Fig. 9A). However, when the reaction was carried out at 37°C, DMT1 was also seen at intracellular locations, where it localized to vesicles resembling endosomes (Fig. 9B). IEC-6 cells fixed before immunofluorescent preparation showed the same pattern as that at 37°C (Fig. 9C).

In whole mounts of duodenum, DMT1 expression was studied in iron-deficient rats (Fig. 9D). Here, DMT1 was seen predominantly above the crypt-villus junction and reaching
highest levels in the mid-villous region; it fell, however, in more distal cells, but was still present in enterocytes of the villous-tip (Fig. 9D). In these cells, it was detected along the brush border as well as in the apical third of the cytoplasm. Villous-tip fluorescence was seen when preimmune serum was substituted for the primary antibody (Fig. 9E).

In IEC-6 cells, IREG1 was detected only on the cell membrane when immunofluorescence preparation was carried out on viable cells at 4°C (Fig. 10A). However, when the reaction was carried out at 37°C, the protein was also seen in intracellular locations, where it localized to vesicles resembling endosomes (Fig. 10B). When cells were fixed before processing for immunofluorescence, the pattern of staining was similar to that at 37°C (Fig. 10C).

In duodenum, IREG1 expression was seen predominantly above the crypt-villus junction and was seen in enterocytes throughout the length of the villus. In these cells it was seen along the lateral and basal membranes as well as in the basal cytoplasm (Fig. 4A and B). IREG1 was not detected along the apical membrane (Fig. 4B).

Unfortunately, because the DMT1 and IREG1 antibodies were both produced in rabbits, it was not possible to do colocalization studies to determine whether these proteins colocalized to the same organelles in IEC-6 cells and from tissue.

**DISCUSSION**

We evaluated the use of IEC-6 cells as a model with which to study the mechanism of intestinal iron transport. These cells are derived from the rat small intestine and were produced from a single clone. Therefore they are homogeneous, contain the normal rat somatic karyotype and because they are nontransformed, they have a limited lifespan in culture (up to 50 passages). Due to the lack of staining for the villous enterocyte marker, SI, which was confirmed in this study, these cells are described as having an immature, crypt-like phenotype (21). Nonetheless, by Western blot analysis, we found that DMT1, IREG1 and Fn are expressed by IEC-6 cells. Because these proteins are reported to be expressed in villous enterocytes and not crypt epithelium as assessed morphologically here and elsewhere, it suggests that IEC-6 cells have a mature phenotype at least as far as iron absorption is concerned (22–25).

Because of the recognized role of DMT1 in the uptake of divalent metals and IREG1 in Fe release, we next examined whether these transport processes are present in IEC-6 cells. From preliminary studies, the addition of Fe(II) at a concentration known to saturate the transporter resulted in the uptake of Fe(II). Furthermore, our results are consistent with the requirement of a proton gradient for DMT1 to transport Fe(II) in the uptake step by showing this was highest at pH 5.5 and least effective at 7.5 (10). Analysis of the data for the uptake of iron over time showed that this was curvilinear, suggesting the coexistence of an efflux transporter. Indeed we found that cells preloaded with radiolabeled iron released 50% of this within 60 min, revealing an efflux transporter. Interestingly, the release of iron from these cells does not require the presence of Cp or apotransferrin, nor was iron efflux increased when these were added to the medium. This differs from hepatocytes and macrophages that require the ferroxidase activity of Cp for most efficient release of iron as evidenced by its retention in the absence of Cp (26). These results therefore appear consistent with the functioning of the Cp homologue hephaestin in the release of iron from the enterocyte (8). Hephastin is predicted to contain a carboxy terminal transmembrane domain, suggesting that it operates extracellularly (8). We tested this possibility by determining protein-dependent copper oxidase activity both within and outside the cell and found it present only within IEC-6 cells. This suggests that hephaestin functions intracellularly and supports the observation that it occupies a perinuclear location in villous enterocytes (27).

The expression of DMT1, IREG1 and the likely presence of hephaestin in IEC-6 cells are consistent with these proteins functioning in the uptake and efflux transport pathways. These findings differ from the only other study assessing Fe(II) uptake in IEC-6 cells performed 10 years ago (28). They showed an uptake carrier in confluent cells, but there was no evidence of an efflux transporter. Here we show that these cells have both uptake and efflux transporters and have similar efficiencies in proliferating and stationary cells. Apart from the use of these cells in different states of proliferation, there appear to be no other major differences in the methodology of the two studies.

An important property of iron absorption is that it is regulated. In vivo iron absorption is increased by anemia, hypoxia, erythropoiesis and feeding diets low in iron; conversely it is inhibited by high iron stores (29–32). Variations in uptake of Fe(II) are mediated by alterations in the expression of DMT1, whereas IREG1 appears to respond only to...
severe iron deficiency (5,7,33). In view of this, we tested whether expression and function of these transport proteins in IEC-6 cells changed with variation in iron loading as is seen in vivo. DMT1 increased in proportion to uptake and this in turn was inversely related to cellular iron stores, a finding that supports studies using a human cell line (15,16). In addition to the 66-kDa product that can be predicted from the cDNA of DMT1, we also found two smaller products in iron-deficient cells and only one in control cells. Previously, it was suggested that DMT1 is produced de novo and is subject to rapid degradation if iron stores rise (34). It is therefore possible that these smaller bands represent degradation of DMT1 as a result of activity associated with the uptake of iron that would be greatest with iron deficiency. Interestingly, the larger mass of DMT1 obtained from in vivo preparations compared with IEC-6 cells can be explained by glycosylation of DMT1 in mucosal enterocytes. This is based on the reduction in size of DMT1 to that of IEC-6 cells after glycosidase F treatment.

Ferritin expression increased with cellular iron levels and is consistent with its role in iron sequestration to prevent oxidative damage. The expressions of DMT1 and Fn conform to the known molecular mechanisms regulating their expression. In the case of DMT1 and Fn, there is an IRE within the 3′ and 5′ untranslated regions (UTR) of the mRNAs encoding these genes, respectively. With a decrease in cytoplasmic iron levels, there is increased activity of iron responsive proteins (IRP) I

FIGURE 9 Confocal immunofluorescence microscopy of IEC-6 cells (A-C) and rat duodenum (D and E) detecting the expression of rat divalent metal transporter 1 (DMT1). In IEC-6 cells, staining was seen only at the cell membrane at 4°C (A) as well as intracellularly at 37°C (B). In cells fixed before preparation, staining was identical to that with viable cells were studied at 37°C (C). Low (D) and high (E) power photomicrographs detecting the expression of DMT1 in a frozen section of duodenum from a rat fed the iron-deficient diet. Staining was predominantly found along the apical membrane in villous enterocytes. Arrows in D demonstrates crypt-villus junction. Arrows in E demonstrate apical (right arrow) and basolateral (left arrow) membranes. Preimmune serum was substituted for the primary antibodies to test the specificity of the reaction and showed no reaction (F).

FIGURE 10 Confocal immunofluorescence microscopy of IEC-6 cells prepared for the expression of rat basolateral transporter (IREG1). Staining was only seen at the cell membrane at 4°C (A) as well as intracellularly at 37°C (B) and with fixed cells (C).
and II which leads to increased binding to IRE. This stabilizes the DMT1 transcript, enhancing the translation of DMT1 but reducing that of Fn. Thus, with cellular iron deficiency, increased expression of DMT1 leads to greater uptake of Fe(II) and because there is less Fn to sequester the iron, it is exported from the cell. Conversely, increased iron stores result in the degradation of DMT1 mRNA and inhibition of translation. The lack of detection of DMT1 in iron-loaded IEC-6 cells by Western blotting in this study supports this interpretation. Furthermore, because the DMT1 antibody used here recognizes both IRE and non-IRE forms of the protein, the non-IRE form would be expressed under iron-loaded conditions. Because no DMT1 was detected under these conditions, it suggests that DMT1 non-IRE is expressed at levels below detection or is absent from IEC-6 cells. In contrast to DMT1, IREG1 has a putative IRE in its 5′ UTR, and its expression did not correspond to alterations in iron loading, suggesting that under these circumstances, it is not regulated by an IRE/IRP mechanism. This is further supported by the similar rates of iron release seen with IEC-6 cells with different iron loads.

It is clear from confocal microscopy of intact tissue of villous enterocytes that DMT1 is localized to the cell membrane but can also be seen in the apical cytoplasm, suggesting that it can cycle between these sites as was suggested previously (9,35). We investigated this hypothesis using viable IEC-6 cells and showed that at 4°C, DMT1 was localized only to the cell membrane; however, when warmed to 37°C, DMT1 was also found inside the cell in structures resembling endosomes, suggesting that it can also function within the cell. The finding that the localization of DMT1 at 37°C and in cells fixed before immunofluorescence preparation was similar suggests that surface-bound DMT1 is in contact with all DMT1 localized intracellularly.

With respect to IREG1, we along with others have found that in intact tissue, it is localized to the basal and lateral membranes and lower one third of the cytoplasm (5–7). This suggests that it may also cycle. This was tested in viable IEC-6 cells; at 4°C, IREG1 was localized to the cell membrane; when warmed to 37°C, however, the protein was internalized, revealing IREG1 in the cytoplasm similar to that in intact tissue. Collectively, the findings taken from intact tissue and IEC-6 cells show for the first time that IREG1 functions by moving from the basolateral membrane and basal cytoplasm of the enterocyte as part of the uptake of iron, whereas DMT1 operates between the microvillous cell membrane and apical cytoplasm. Clearly, it will be of interest to define the signaling and structures involved in this cycling process and to determine whether DMT1 and IREG1 interact directly.

This study has validated the use of the IEC-6 cells as a model of intestinal iron transport by demonstrating the expression of intestinal transport proteins that function in the uptake and efflux of iron and that these processes are regulated by variation in cellular iron. Thus, this cell model is appropriate to use in conjunction with in vivo studies on rats, mice and other intestinal cell lines such as Caco-2 cells (15,16) to study the mechanism of iron transport. Studies such as the cycling of DMT1 and IREG1 from cell membrane to intracellular sites as shown in this study and further studies will provide new insights into this mechanism. Clearly understanding the molecular mechanisms of IREG1 will be important because this is reported to be up-regulated in genetic hemochromatosis and therefore is likely to contribute to the inappropriately high level of iron absorption seen in these individuals.

ACKNOWLEDGMENT

The authors extend their appreciation to A. Quaroni and D. Trinder for supplying the SI and DMT1 antibodies, respectively. The constructive comments provided by Evan Morgan during the writing of this manuscript are gratefully appreciated.

LITERATURE CITED