Copper repletion enhances apical iron uptake and transepithelial iron transport by Caco-2 cells

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Han, Okhee, and Marianne Wessling-Resnick. Copper repletion enhances apical iron uptake and transepithelial iron transport by Caco-2 cells. Am J Physiol Gastrointest Liver Physiol 282: G527–G533, 2002; 10.1152/ajpgi.00414.2001.—The influence of copper status on Caco-2 cell apical iron uptake and transepithelial transport was examined. Cells grown for 7–8 days in media supplemented with 1 μM CuCl2 had 10-fold higher cellular levels of copper compared with control. Copper supplementation did not affect the integrity of differentiated Caco-2 cell monolayers grown on microporous membranes. Copper-repleted cells displayed increased uptake of iron as well as increased transport of iron across the cell monolayer. Northern blot analysis revealed that expression of the apical iron transporter divalent metal transporter-1 (DMT1), the basolateral transporter ferroportin-1 (Fpn1), and the putative ferroxidase hephaestin (Heph) was upregulated by copper supplementation, whereas the recently identified ferrireductase duodenal cytochrome b (Dcytb) was not. These results suggest that DMT1, Fpn1, and Heph are involved in the iron uptake process modulated by copper status. Although a clear role for Dcytb was not identified, an apical surface ferrireductase was modulated by copper status, suggesting that its function also contributes to the enhanced iron uptake by copper-repleted cells. A model is proposed wherein copper promotes iron depletion of intestinal Caco-2 cells, creating a deficiency state that induces upregulation of iron transport factors.

Iron is essential for numerous cellular metabolic functions but can also be toxic to cells when present in excess. Thus mammalian iron homeostasis is tightly maintained primarily through the regulation of iron absorption in the proximal intestine (recently reviewed in Refs. 32 and 36). The absorptive process of iron through the enterocyte entails transport of the metal across three formidable cellular barriers: the brush-border membrane, intracellular transport across the cytosol, and release of iron across the basolateral membrane and then into the circulation. In the past few years, several new factors have been identified to play a role in intestinal iron absorption. Divalent metal transporter-1 (DMT1), also known as divalent cation transporter-1 (DCT1) (13) and Nramp2 (11), is localized to the intestinal brush border and thought to mediate iron uptake; it is regulated in response to dietary iron deficiency (6, 13, 24, 38). Consistent with a function in dietary iron absorption, defects in the DMT1 gene promote microcytic anemia in the mk mouse (11) and Belgrade rat (10). Hephaestin (Heph) is a ceruloplasmin homolog and putative multicopper oxidase thought to be required for the iron exit across the basolateral membrane of enterocytes (35). This idea is supported by the fact that this gene is mutant in sla mice, which have a block in mucosal iron transfer (28, 29). A candidate basolateral iron exporter gene has also been identified in three different laboratories as ferroportin-1 (Fpn1) (8), iron-regulated protein 1 (Ireg-1) (22), and metal transporter-1 (MTP1) (1); the human gene is more recently defined as SLC11A3 (23). It has been proposed that this membrane protein is responsible for the upregulation of iron absorption under iron deficiency. Finally, the more recently identified ferrireductase duodenal cytochrome b (Dcytb) is thought to reduce ferric iron on the brush-border membrane of the enterocyte for dietary iron import, and its expression is also found to be upregulated in iron-deficiency conditions (21).

Identification of these novel factors points to a pathway mediating dietary iron absorption across the brush border via DMT1-mediated iron uptake that potentially involves Dcytb, and subsequent export of iron across the basolateral membrane of the enterocyte by Fpn1, possibly in conjunction with Heph. However, precisely how assimilation of this essential nutrient is regulated still remains unclear. The fact that Heph is a ceruloplasmin homolog suggests that copper may contribute to the regulation of its activity and the coordination of iron assimilation. A role for copper in iron absorption has been clearly demonstrated in animal models (7, 12, 19, 26). Copper deficiency causes iron-deficiency anemia due to decreased iron absorption: iron enters the intestinal epithelium normally but cannot exit into the circulation (19). The administration of copper, but not iron, to copper-deficient animals alleviates the anemia and facilitates the exit of iron from the tissues, including intestine (19). A role for copper has also been suggested for iron uptake in vitro, particularly in the reduction of ferric to ferrous iron, a step...
that is thought to be prerequisite for membrane translocation of the cation (40).

This investigation further examines the influence of copper status on iron absorption by utilizing the human intestinal Caco-2 cell line as a model system to study this process. On confluence, these cells spontaneously differentiate to exhibit many of the morphological and functional features of mature small intestinal enterocytes and have been widely used as a model of normal human intestinal epithelium (18, 27, 28, 31). In particular, fully differentiated Caco-2 cells are an excellent in vitro model of human intestinal iron absorption (2, 14, 15). As in the human intestine, Caco-2 cell transepithelial transport of iron (3, 34) as well as the expression of iron-responsive proteins, such as the transferrin receptor (TfR) and ferritin (16), are regulated in response to iron. Furthermore, Caco-2 cells are characterized to have an apical ferrireductase activity (9), and ferreduction by this enzyme is essential for iron uptake in the absence of exogenous reducing agents (15, 24). In this study, the influence of copper status on Caco-2 cell apical iron uptake and transepithelial transport, as well as the expression of the newly identified intestinal iron transport genes, were characterized.

MATERIALS AND METHODS

Reagents. DMEM, penicillin G-streptomycin, and trypsin-EDTA were purchased from Gibco-BRL (Grand Island, NY). FBS was obtained from Gemini Bio-Products (Woodland, CA). Ferric chloride (FeCl3·6H2O), copper chloride (CuCl2), ferricyanide [K3Fe(CN)6], ferrocyanide [K4Fe(CN)6], and desferrioxamine (DFO) were purchased from Sigma (St. Louis, MO). Ascorbic acid (C6H8O6) was from Fisher (Springfield, NJ). All restriction enzymes were purchased from New England Biolabs (Boston, MA). Radioisotopes ([55FeCl3] and [32P]dCTP) were purchased from NEN (Boston, MA). Unless otherwise noted, all other chemicals were reagent grade and obtained from Fisher or Sigma.

Caco-2 cell culture. The HTB-37 human Caco-2 cell line was purchased from American Type Culture Collection (ATCC) (Rockville, MD). Stock cultures were maintained in complete medium in a humidified atmosphere of 95% air-5% CO2 at 37°C and employed for experiments within 15 serial passages. The culture medium contained DMEM supplemented with 25 mM glucose, 2 mM glutamine, 1 mM pyruvate, 100 U/ml penicillin G, 100 mg/ml streptomycin, and 10% FBS. Stock cultures were seeded at 7,000 cells/cm2, and at ~80% confluence they were split by treatment with 0.5 g/l trypsin-0.5 mM EDTA in 25 mM Tris, 137 mM NaCl, 5 mM KCl, and 0.7 mM Na2HPO4, pH 7.4. For experiments, 50,000 cells/cm2 in a volume of 1.5 ml supplemented DMEM were seeded on 3-μm microporous membrane inserts (4.9 cm2 Falcon cell culture inserts; BD Biosciences, Bedford, MA). The bottom or basolateral chamber contained 2.5 ml supplemented DMEM. Culture medium was changed every other day, and cells were used at 17 days postconfluence for most experiments. For studies investigating the effects of copper, Caco-2 cells were grown on microporous membrane inserts under these same conditions, except that 1 μM CuCl2 was added to the culture medium 10 days postconfluence and their culture was continued in its presence for the next 7–8 days. The Caco-2 cell monolayer routinely formed tight gap junctions at 17 days postconfluence with transepithelial electrical resistance (TEER) > 250 Ω/cm2, thus these cells are fully differentiated under these cell culture conditions (15, 20).

55Fe transport and uptake. Transepithelial iron transfer from the apical chamber to the basolateral chamber and cellular iron assimilation were determined as described previously (15). Briefly, fully differentiated Caco-2 cells grown on microporous membrane inserts were incubated at 37°C with 1.5 ml of 10 μM [55Fe(NTA)2] in iron-uptake buffer (in mM: 130 NaCl, 10 KCl, 1 MgSO4, 5 glucose, and 50 HEPES, pH 7.0) in the apical compartment and 2.5 ml DMEM in the basolateral compartment. Aliquots (0.2 ml) were removed from the basolateral chamber every hour and replaced with an equivalent volume of DMEM; time course data were corrected to account for this sample replacement. The rate of 55Fe transfer across the cell monolayer was found to be linear between 1 and 3 h, and transport rates (pmol·h−1·mg protein−1) were calculated by linear regression analysis (r > 0.97). The integrity of tight junctions between cells was monitored by measuring TEER and phenol red transport; any loss of cell monolayers was discarded. To measure the cellular level of 55Fe, the monolayers were washed three times with ice-cold 150 mM NaCl and 10 mM HEPES, pH 7.0, containing 1 mM EDTA to remove any nonspecifically bound radioisotope. Cells were homogenized in PBS containing 1 mM EDTA and 0.2% Triton X-100, and 55Fe was quantified by liquid scintillation counting in glass vials.

Northern blot analysis. Total RNA was isolated from Caco-2 cells using RNAzol B (Tel-Test, Friendswood, TX) according to the manufacturer’s instructions. RNA samples (20–30 μg) were electrophoresed on 1.2% denaturing formaldehyde-agarose gels, transferred to Nytran-N membranes using the Turboblotter transfer system (Schleicher & Schuell, Keene, NH), and immobilized by ultraviolet crosslinking. The blots were prehybridized at 42°C in 50°C deionized formamide, 10× Denhardt’s solution, 10 mM EDTA, 0.1% SDS, and 100 μg/ml denatured salmon sperm DNA for 4–5 h, then hybridized overnight at 42°C in the same buffer containing 10% dextran sulfate and randomly primed 32P-labeled probe. After three 20-min washes with 0.1× standard sodium chloride-sodium citrate containing 0.1% sodium deoxycholate at room temperature, radioactivity was detected by phosphorimaging and quantified using Quantity One software (Bio-Rad, Hercules, CA). The following plasmids were digested with restriction enzymes as indicated, and the fragments produced were used as templates to synthesize randomly primed probes for Northern blot analyses: pBluescript II SK−hephaestin (kindly provided by Dr. T. Nagase, Kazusa DNA Research Institute) was digested with AluI to produce a 1,000-bp HepH fragment; pTT73D-pac-Fpn1 (IMAGE Clone I.D. 767706; ATCC) was cut with EcoRI/NcoI to generate a 1,300-bp fragment containing 5′ UTR, and pT7T3D-pac-DMT1 (IMAGE Clone I.D. 322022; ATCC) was cut with EcoRI/NcoI to generate a 1,100-bp DMT1 fragment; pME18S-FLJ23462 (kindly provided by Dr. S. Sugano, University of Tokyo) was digested with BglII to produce a 2,200-bp TfR fragment. The blots were rehybridized with a randomly primed 32P-labeled β-actin probe (Ambion) to normalize transcription levels. Control experiments confirmed that β-actin mRNA levels do not change during Caco-2 cell differentiation or on treatment with either DFO or copper relative to glyceraldehyde 3-phosphate dehydrogenase or ribosomal protein subunit 36B4 (data not shown).

Ferrerireductase assay. Cell-mediated ferrireductase activity was assessed by the production of membrane-impermeant ferrocyanide from ferricyanide (5, 17). Fully differentiated

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Caco-2 cell monolayers grown on microporous membrane inserts were incubated at 37°C for 1 h with 1.5 ml of 10 μM K₂Fe(CN)₆ in Hank’s buffered saline solution (137 mM NaCl, 5.36 mM KCl, 1.3 mM CaCl₂, 410 μM MgSO₄, 490 μM MgCl₂, 337 μM Na₂HPO₄, 440 μM KH₂PO₄, 4.17 μM NaHCO₃, and 5.55 mM dextrose) added to the upper chamber and with 2.5 ml supplemented DMEM placed in the lower chamber. Aliquots (700 μl) of the cell-conditioned medium from the apical chamber were then mixed with, in order: 100 μl of 3-M sodium acetate (pH 6.4), 100 μl of 0.2-M citric acid, 50 μl bathophenanthroline sulfate, and 50 μl of 3.3-M FeCl₃ in 0.1 M acetic acid. After 15–20 min incubation at room temperature, the absorbance at 535 nm was measured. The reaction assay time and concentration of substrate were both within linear range for membrane-bound ferrireductase activity (15, 17).

RESULTS

Copper status of differentiated Caco-2 cells. To investigate the potential effects of cellular copper status on the iron uptake and transport properties of intestinal enterocytes, human intestinal Caco-2 cell monolayers were grown on microporous membranes until fully differentiated (17 days postconfluence) as described in MATERIALS AND METHODS. Preliminary studies revealed that addition of CuCl₂ to the culture medium improved the rate and extent of iron transport, with maximal effects observed at copper supplementation of ~1 μM. With supplementation of 1 μM CuCl₂ for 1 wk, Caco-2 cells contained 10-fold higher levels of copper (Fig. 1). Copper treatment did not alter TEER (data not shown), confirming the integrity of the differentiated monolayer for copper-supplemented cells.

55Fe transport and uptake. Iron transport was determined by monitoring the transfer of apically presented 55Fe across the cell monolayer into the basolateral compartment as described previously (15). Supplementation with 1 μM copper enhanced iron transfer across the Caco-2 cell monolayer into the basolateral chamber by 59% after a 3-h incubation in the experiment shown in Fig. 2A. Consistent with its stimulatory effect on transepithelial 55Fe transport by Caco-2 cells, copper repletion also significantly enhanced the uptake of iron across the brush-border membrane and its assimilation by Caco-2 cells with 32% more iron taken up during the 3-h assay (Fig. 2B). Combined, these data suggest that both the uptake of apical iron and exit of iron from the basolateral surface of the enterocyte are stimulated by copper repletion. It is important to note that these effects are comparable to the influence of iron depletion on Caco-2 cell iron uptake and transport, since both are enhanced under iron-deficiency conditions (3, 34).

Effects of cellular differentiation on Heph, Fpn1, and Dcytb gene expression. Recently, several factors have been identified to play a role in the uptake and transport of iron across the intestinal enterocyte, including the apical iron transporter DMT1 (11, 13), the basolateral iron exporter Fpn1 (1, 8, 22), the ferrireductase Dcytb (21), and the putative multicopper oxidase Heph (36). Previous studies have shown that DMT1 is expressed by Caco-2 cells in a differentiation-dependent manner (16). This observation is consistent with the
proposed functional role of DMT1 in iron uptake across the brush-border membrane because, on reaching confluence, these cells differentiate to display morphological and functional changes similar to mature enterocytes of the small intestine (18, 27, 28, 31). To determine whether Heph, Fpn1, and Dcytb mRNA levels are also expressed during this cellular differentiation process, total RNA was isolated from Caco-2 cells grown on microporous membranes at 1–18 days postconfluence. Northern blot analysis (Fig. 3) demonstrates that Heph mRNA levels progressively increase during Caco-2 cell differentiation, with a maximal 5-fold induction observed. In contrast, although Fpn1 mRNA levels were also increased during Caco-2 cell differentiation, the size of this transcript pool does not change significantly until 11 days postconfluence, subsequently rising to threefold greater levels at day 18 relative to day 1. Unlike Heph and Fpn1, Dcytb mRNA remained unchanged during the entire time course of Caco-2 cell differentiation (data not shown; see Fig. 4). These data therefore confirm that the known iron transport factors are expressed in fully differentiated Caco-2 cells, implying that their activities function in iron uptake and transfer across the epithelial cell monolayer.

Influence of cellular iron and copper status on the expression of genes involved in intestinal iron absorption. To determine whether iron depletion modulates Heph, Fpn1, DMT1, and Dcytb gene expression, fully differentiated Caco-2 cells (17 days postconfluence) grown on microporous membrane inserts were treated for 20 h with 100 μM DFO, a specific iron chelator. This treatment effectively depleted cellular iron, as confirmed by the upregulation of iron-responsive TfR mRNA level (Fig. 4), consistent with previous observations (4, 16). Under these conditions, increased levels of Heph, Fpn1, and DMT1 but not Dcytb mRNA were observed. The modulation of gene expression in copper-repleted Caco-2 cells was also investigated. Similar to DFO treatment, increased levels of Heph, Fpn1, and DMT1 mRNA but not Dcytb mRNA were detected in copper-treated cells (Fig. 4). Interestingly, copper repletion also enhanced TfR mRNA level by 70% relative to control, suggesting that increases in iron uptake and transport in copper-treated cells (Fig. 4) result from reduced iron status, as has been previously reported (3, 34). The increased expression of Heph, Fpn1, and DMT1 correlate with the enhanced iron uptake and transport that is observed. Fpn1 (1, 22) and DMT1 (13) are both known to be upregulated by iron deficiency, although it remains to be determined if protein levels of these transporters are altered in copper-treated cells.

Influence of copper repletion on Caco-2 cell brush border surface ferrireductase activity. Unlike Heph, Fpn1, and DMT1, the duodenal ferrireductase Dcytb does not appear to be copper- or iron-regulated in Caco-2 cells. Because ferrireduction has been implicated as a limiting factor for the uptake of iron at the brush border surface of enterocytes (15), the influence of copper supplementation on cell-mediated ferrireductase activity was further examined. The reduction of ferri- to ferrocyanide on the apical surface of Caco-2 cells was monitored as previously described (17), and the ferrireductase activities measured for

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**Fig. 3.** Hephaestin (Heph) and ferroportin-1 (Fpn1) mRNA levels of Caco-2 cells during cellular differentiation. Total RNA was isolated from 1–18-days-postconfluent Caco-2 cells as indicated in MATERIALS AND METHODS. Samples (25–30 μg) were electrophoresed and transferred to Nytran N membrane as described in MATERIALS AND METHODS. The Northern blot was hybridized with randomly primed 32P-labeled probe for Heph or Fpn1 and exposed to phosphor screen for 20 h for image analysis. To confirm equal loading of RNA in each lane, each blot was rehybridized with 32P-labeled probe for β-actin. Shown are band intensities for ~5-kb and 3.4-kb transcripts for Heph and Fpn1, respectively.

**Fig. 4.** Copper treatment upregulates the expression of genes involved in intestinal iron absorption in Caco-2 cells. Total cellular RNA was isolated from fully differentiated Caco-2 cells treated with or without 1 μM CuCl2 for 8 days or with 100 μM desferrioxamine (DFO) for 20 h. A: 25 μg total RNA was analyzed for Heph, Fpn1, ferrireductase duodenal cytochrome b (Dcytb), divalent metal transporter-1 (DMT1), transferrin receptor (TfR), or β-actin by Northern blot analysis as described for Fig. 3. B: radioactivity in bands for Heph (~5 kb), Fpn1 (3.4 kb), Dcytb (4 kb), DMT1 (4.5 kb), or TfR (5 kb) was quantified using Quantity-One software (Bio-Rad) and normalized to values obtained for β-actin. Data are means ± SD as %control from 2 independent experiments. *P < 0.05 vs. control.
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**DISCUSSION**

A role for copper in iron transport and metabolism is well established (38), but the mechanistic details of the relationship between these two essential minerals is not fully understood. This investigation focused on copper-iron relationships in the human intestinal Caco-2 cell line. Supplementation of culture medium with 1 μM CuCl₂ raised Caco-2 cell copper levels ~10-fold. Several observations indicate that, as a consequence of copper depletion, Caco-2 cells have lower cellular iron levels: 1) copper-treated cells displayed enhanced apical iron uptake and transepithelial iron transport, both of which are known to increase under iron-depletion conditions (3, 34); 2) levels of TfR mRNA, which also increase on iron depletion (16), were enhanced on copper depletion of Caco-2 cells; and 3) consistent with previous reports that intestinal expression of Fpn1 (1, 22) and DMT1 (13) are increased by iron deficiency, copper supplementation also induced an upregulation in Caco-2 cell transcript levels of these genes. The view that an inverse relationship exists between copper and iron status in Caco-2 cells is consistent with a generalized function of copper in mobilization (i.e., depletion of cellular iron) from intestinal cells (26); however, alternative interpretations of these data include the ideas that copper and iron independently regulate the factors involved in intestinal iron uptake and transport in a reciprocal manner and/or that modulation of these factors represent a response to copper toxicity. Regardless of the precise regulatory mechanism(s), it is clear that DMT1, Fpn1, and HepH are coordinately expressed to support enhanced apical iron uptake and transfer across copper-treated Caco-2 cells.

A regulatory model for iron uptake and absorption based on an inverse relationship between Caco-2 cell copper and iron status is particularly attractive because of the postulated requirement for copper to support the function of HepH. This putative mult copper oxidase has striking homology with serum ceruloplasmin, a ferroxidase implicated to also play a role in iron mobilization from the intestine (26). HepH was identified because of a defect in its gene in sla mice (36), which suffer from anemia while accruing excessive iron within enterocytes (29, 30). This phenotype is consistent with a mucosal block at the point of iron release across the basolateral membrane. Without sufficient cellular copper, it is likely that HepH activity would be impaired, resulting in diminished iron export from Caco-2 cells. On copper repletion, restoration of HepH activity would be expected to accelerate the release of iron from the enterocytes, thus reducing intracellular iron levels. The observation that HepH mRNA levels are also increased by copper treatment further supports this view. However, this is the first report of the regulation of HepH expression by either iron or copper, and how this transcript pool is regulated has yet to be determined. Unlike mRNAs for DMT1 and Fpn1, the message for HepH does not harbor an iron-responsive element (IRE). IREs are posttranscriptional regulators of gene expression and are modulated via the iron-dependent RNA binding activity of iron-responsive proteins (reviewed in Ref. 35). Although direct evidence is lacking, the 3’ IRE of DMT1 (13) and the 5’ IRE of Fpn1 (1, 22) are believed to be responsible for the iron-dependent regulation of these factors. Thus further work is necessary to understand how HepH ex-

![Graph](image.png)

**Fig. 5.** Copper repletion stimulates brush border ferrireductase activity in Caco-2 cells. Hank’s buffered saline solution containing 10 μM K₃[Fe(CN)]₆ was added in the upper chamber of fully differentiated Caco-2 cells grown on microporous membrane inserts. After a 1-h incubation at 37°C, the cell-conditioned medium was collected and centrifuged at 800 g for 10 min at 4°C to remove debris. The concentration of Fe(III)-bathophenanthroline sulfate (BPS) complex was measured as described in MATERIALS AND METHODS to determine the reduction of ferricyanide to ferrocyanide. In the absence of cells, HBSS incubated alone under these conditions did not effectively reduce ferricyanide. Caco-2 cells were treated with or without copper as described in legend to Fig. 1, and to iron-replete copper-treated cells, 50 and 10 μM Fe(NTA)₂ were added to the apical and basolateral compartments, respectively, for 40 h. To deplete cellular iron, control Caco-2 cells were treated with 100 μM DFO for 40 h. Bars are means ± SE for triplicate samples from a representative experiment; *P < 0.05 compared with control not treated with copper.

Fully differentiated Caco-2 cells cultured in the absence or presence of supplemental 1 μM CuCl₂ are presented in Fig. 5. These results reveal that copper-repleted cells display fourfold greater apical surface reductase activity, an effect that could potentially contribute to the enhanced ferric iron uptake observed for these cells (Fig. 2). It has been reported that intestinal brush border membrane ferrireductase activity is also modulated by duodenal iron status (32); therefore, the effects of iron depletion on the Caco-2 cell reductase activity were investigated. The iron chelator DFO enhanced ferrireductase activity, confirming that this model system also upregulates brush border ferrireduction on iron depletion. Moreover, this observation suggests that increased ferrireductase activity in copper-repleted Caco-2 cells might also be due to an indirect effect of iron depletion. To further test this hypothesis, copper-repleted cells were incubated with excess Fe(NTA)₂ to load the cells with iron. This treatment significantly reduced the magnitude of ferrireduction in copper-treated cells. This result is again consistent with the idea that copper supplementation induces iron depletion, since iron loading with Fe(NTA)₂ apparently reversed copper’s stimulatory effects on ferrireduction.
expression is regulated in response to cellular iron and copper levels.

An unexpected finding in this study was that the duodenal ferrireductase Dcytb mRNA does not appear to be regulated in Caco-2 cells in response to iron status. This factor was initially identified based on the upregulation of its expression under severe iron-deficiency conditions induced by the chronic anemia of hpx mice (21). It is possible that DFO treatment for 20 h was not sufficient to induce iron-dependent Dcytb regulation in Caco-2 cells. For example, changes in the level of duodenal Dcytb mRNA in mice fed iron-deficient vs. normal diets were more modest than those observed in hpx animals (21). Because an iron-responsive apical ferrireductase was detected in Caco-2 cells in this investigation, similar to previous reports (9, 15), it is likely that such activity does contribute to the absorption of ferric iron (15). Previous studies of HeLa cell ferric iron uptake also suggested that copper status can modulate a cell surface ferrireductase activity important for this process (40), and therefore it is possible that multiple reductases other than Dcytb may exist to fulfill this transport function. Future research on Dcytb and other potential ferrireductases, as well as DMT1, Fpn1, and Heph, should provide further insight into the process of iron uptake, the mechanisms regulating dietary iron absorption, and the role for copper in modulating these activities.

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