Influence of Copper Depletion on Iron Uptake Mediated by SFT, a Stimulator of Fe Transport*

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We recently identified a novel factor involved in cellular iron assimilation called SFT or Stimulator of Fe Transport (Gutierrez, J. A., Yu, J., Rivera, S., and Wessling-Resnick, M. (1997) J. Cell Biol. 149, 985–905). When stably expressed in HeLa cells, SFT was found to stimulate the uptake of both transferrin- and nontransferrin-bound Fe (iron). Assimilation of nontransferrin-bound Fe by HeLa cells stably expressing SFT was time- and temperature-dependent; both the rate and extent of uptake was enhanced relative to the activity of control nontransfected cells. Although the apparent Kₘ for Fe uptake was unaffected by expression of SFT (5.6 μM measured for control), the Vₘₐₓ of transport was increased from 7.0 to 14.7 pmol/ml/min/mg protein. Transport mediated by SFT was inhibited by diethyleneetriaminepentaacetic acid and ferrozine, Fe⁴⁺, and Fe⁴⁺-specific chelators. Because cellular copper status is known to influence Fe assimilation, we investigated the effects of Cu (copper) depletion on SFT function. After 4 days of culture in Cu-deficient media, HeLa cell Cu,Zn superoxide dismutase activity was reduced by more than 60%. Both control cells and cells stably expressing SFT displayed reduced Fe uptake as well; levels of transferrin-mediated import fell by ~80%, whereas levels of nontransferrin-bound Fe uptake were ~50% that of Currepelle cells. The failure of SFT expression to stimulate Fe uptake above basal levels in Cu-depleted cells suggests a critical role for Cu in SFT function. A current model for both transferrin- and nontransferrin-bound Fe uptake involves the function of a ferrireductase that acts to reduce Fe³⁺ to Fe²⁺, with subsequent transport of the divalent cation across the membrane bilayer. SFT expression did not enhance levels of HeLa cell surface reductase activity; however, Cu depletion was found to reduce endogenous activity by 60%, suggesting impaired ferrireductase function may account for the influence of Cu depletion on SFT-mediated Fe uptake. To test this hypothesis, the ability of SFT to directly mediate Fe²⁺ import was examined. Although expression of SFT enhanced Fe²⁺ uptake by HeLa cells, Cu depletion did not significantly reduce this activity. Thus, we conclude that a ferrireductase activity is required for SFT function in Fe³⁺ transport and that Cu depletion reduces cellular iron assimilation by affecting this activity.

Mammalian cells can acquire iron either via the binding of Fe₂-transferrin (Tf)³ to high affinity surface receptors and its subsequent internalization into endosomal compartments or through Tf-independent pathways utilizing plasma membrane-bound transport systems (1, 2). Several lines of evidence suggest that Tf-dependent and -independent import involves at least two functional activities as follows: a ferrireductase that converts Fe³⁺ to Fe²⁺ and a carrier mechanism that subsequently translocates Fe²⁺ across membrane bilayers (3–8). Genetic studies in yeast have begun to identify a number of factors involved in membrane transport of Fe, and at least two uptake systems have been characterized: a high affinity transport mechanism involving FTR1 and FET3 (9, 10) and a low affinity activity provided by FET4 (11, 12). In addition, two ferrireductases, FRE1 and FRE2, are known to play a role in yeast iron transport (13, 14). Whereas FET4 can mediate the import of Fe²⁺ in the absence of ferrireductase activity (11), FTR1 is believed to be a transporter for iron that has been first reduced by FRE1 (or FRE2) and then oxidized by the multicopper oxidase FET3 (2, 10). However, although it is presumed that FET3-oxidized Fe³⁺ is then translocated across the yeast membrane, the exact valency of transported iron and the precise function of FTR1 remain poorly defined. Nonetheless, an interesting connection between Cu and Fe metabolism has been revealed by the observation that FTR1 biosynthesis is impaired in the absence of Cu-requiring FET3 (10). The relationship between Cu and Fe transport in yeast (2) is highly reminiscent of the role of copper for iron metabolism in mammals (15). Dietary Cu deficiency leads to microcytic hypochromic anemia (16–18); this apparent iron deficiency appears to be promoted by the loss of oxidase activity provided by circulating ceruloplasmin, a Cu-requiring enzyme (19).

Although progress in characterizing the proteins involved in yeast Fe transport has been made, relatively little is known about mammalian uptake systems. Recently, two factors were identified to be involved in this process. DCT1 (or nRAMP2) was defined to function in rat intestinal iron uptake by the functional expression cloning of Fe²⁺ transport activity in Xenopus oocytes (20). Moreover, the mck mouse, which results from microcytic anemia due to inefficient iron absorption (21), was found to have a defect in the nRAMP2 gene; this missense mutation is predicted to impair nRAMP2 transport function (22). DCT1/nRAMP2 appears not only to mediate uptake of Fe²⁺, but it is capable of facilitating the translocation of other divalent cations as well (20). In contrast, a second transport protein that we have identified, called SFT or Stimulator of Fe Transport, enables Xenopus oocytes to take up iron presented as Fe³⁺, and this activity is inhibited by Cd²⁺ but not other divalent cations (23). Our preliminary investigation of SFT

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1 The abbreviations used are: Tf, transferrin; SFT, stimulator of Fe transport; GFP, green fluorescent protein; DMEM, Dulbecco’s modified Eagle’s medium; PBS, phosphate-buffered saline; NTa, nitroprusside acid; SOD, superoxide dismutase; Fe, iron; Cu, copper; TRIEN, Triethylenetetramine™.
function in mammalian cells revealed its capacity to also stimulate the assimilation of Fe from the Tf-mediated pathway. Here, we report a more detailed functional characterization of the stimulation of SFT of non-Tf-bound Fe uptake in mammalian cells. These studies define the function of SFT in promoting the uptake of iron presented to cells as either Fe\(^{2+}\) or Fe\(^{3+}\). Furthermore, we explored the potential role of copper for the activity of SFT by depleting cells in culture with Cu-deficient media (24). Our results demonstrate that Cu depletion decreases both Tf- and non-Tf-bound Fe uptake by HeLa cells and that the function of SFT is impaired under these conditions. Cu depletion was also found to partially inhibit a cell surface ferrireductase, implicating a role for this activity in SFT-mediated import of Fe\(^{2+}\). This idea is strongly supported by the finding that SFT-stimulated Fe\(^{2+}\) uptake is unaffected in Cu-depleted cells.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Preparation for Experiments—**HeLa cells stably expressing a green fluorescent protein (GFP) chimera of SFT, referred to here as HeLa(SFT), were previously established (23). HeLa(SFT) and HeLa cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 300 g/liter l-glutamine, 10% fetal bovine serum, 50 units/ml penicillin G, and 50 μg/ml streptomycin. For some experiments, cells were cultured under the same conditions, except that serum was Cu-depleted as described below. For transport assays, cells were grown to near confluence in 6-well (35-mm) plates. In preparation for ferrireductase assays, HeLa cells were washed three times with ice-cold phosphate-buffered saline (PBS), followed by one additional wash in Hanks’ buffer (137 mM NaCl, 5.36 mM KCl, 1.3 mM CaCl\(_2\), 410 μM MgSO\(_4\), 490 μM MgCl\(_2\), 337 mM Na\(_2\)HPO\(_4\), 440 μM KH\(_2\)PO\(_4\), 4.17 mM NaHCO\(_3\), and 5.55 mM dextrose).

**Iron Uptake Measurements—**FeCl\(_3\) was purchased from NEN Life Science Products (≤3 μCi/mg). Fe-nitritotriacetate acid (NTA) was prepared by complexing \(^{55}\)Fe with a 4-fold molar excess of NTA in 20 mM HEPES-Tri, pH 6.0, 100 mM NaCl; this mixture was then adjusted to pH 7 with NaOH essentially as described by Teichmann and Stremmel (25). To start uptake assays, HeLa cells were incubated in serum-free DMEM for 1 h, and then fresh serum-free DMEM was added with specified concentrations of \(^{55}\)FeNTA. Transport assays were performed at 37 or 4 °C for times indicated in the figure legends. To quench uptake, cells were rapidly placed on ice. For previously published methods, cells were washed with ice-cold PBS, and then incubated on ice with 1000-fold molar excess unlabeled FeNTA for 20 min to remove nonspecifically bound iron (26). Six hundred μl of PBS containing 1 mM EDTA were added to each well to lift cells, and the amount of cell-associated radioactivity was measured by scintillation counting of duplicate 200-μl aliquots of this cell suspension. A 40-μl aliquot of the cell suspension was solubilized with 10 mM Tris-Cl, pH 8.0, 150 mM NaCl, 0.05% Tween 20 and then incubated in ice-cold PBS for 10 min to remove non-specifically bound iron. After a 3-min incubation at room temperature, the reaction products were measured spectrophotometrically at 340 nm. The activity of Cu,Zn-SOD was determined by subtracting the activity due to the managino form of SOD found in cytosol treated with 2.0 mM KCN. Enzyme activity was expressed as ΔA\(_{550}\)/min. 10 cells.

**RESULTS**

**SFt Stimulates Tf-Independent Iron Uptake—**SFT, a “stimulator of Fe transport,” was recently cloned by functional expression using Xenopus oocytes (23). Although it has been previously shown that SFT can stimulate Tf-mediated Fe transport by mammalian cells, the fact that SFT was identified by expression of Tf-independent transport activity indicates its involvement in non-Tf-bound Fe uptake across the plasma membrane. To characterize further the latter activity, iron uptake by HeLa cells stably expressing an SFT-GFP chimera, HeLa(SFT), was measured. The results of Fig. 1 show the time course of non-Tf-bound Fe uptake measured for both non-transformed HeLa cells (squares) and HeLa(SFT) (circles). \(^{55}\)Fe uptake by HeLa(SFT) cells is approximately 50% greater than the activity of control cells, demonstrating the stimulation of Tf-independent transport by SFT. As previously reported (26, 30), the transport of \(^{55}\)Fe is time- and temperature-dependent. At 37 °C, uptake is saturable, whereas at 4 °C, cell-associated radioactivity is less than 5% of the total cell-associated radiactivity measured at 37 °C. To better define properties of SFT-mediated Fe transport, a series of inhibition experiments were carried out (Table 1).
FeNH₄ citrate blocks uptake, indicating that the observed transport activity is specific for Fe rather than its chelating ligand (NTA). Excess diethylenetriaminepentaacetic acid (1 mM), a membrane-impermeant Fe³⁺-specific chelator, also completely blocks ⁵⁵Fe uptake by both HeLa(SFT) and control cells. Interestingly, an Fe²⁺-specific chelator, ferrozine, also suppresses ⁵⁵Fe accumulation by 60–70%, suggesting that SFT-mediated transport may not be specific for Fe⁵⁺. Treatment of cells with 50 μM chloroquine, a diffusible weak base that inhibits the dissociation of Fe from internalized Tf to block iron assimilation (31, 32), has no effect on SFT-mediated uptake of non-Tf bound Fe. This result suggests that SFT either functions at the plasma membrane or within endosomal compartments in a pH-independent manner, although the time course of uptake (Fig. 1) and the observed saturability of transport strongly argue for the involvement of a cell surface carrier in this process. Finally, it has been previously reported that Cd²⁺ inhibits Tf-independent Fe transport by HeLa (30) and K562 cells (26); the presence of 1 mM Cd²⁺ appears to partially antagonize SFT-mediated iron transport in a similar fashion.

To characterize further the kinetic properties of non-Tf-bound Fe uptake by HeLa(SFT) cells, initial rates of transport were measured as a function of FeNTA concentration. The results of these experiments (Fig. 2, panel A) reveal the typical Michaelis-Menten curve, indicating that the transport process is mediated by a limited number of carriers. A double-reciprocal plot of initial transport rates, ν₀, is shown in panel B (Fig. 2). HeLa(SFT) (circles) and control cells (squares) have nearly identical values for the apparent Kₘ of uptake: 5.1 ± 1.0 μM versus 5.6 ± 1.1 μM (n = 4). These values are consistent with previous studies of Tf-independent Fe uptake by HeLa cells (30). As discussed by Sturrock et al. (30), this kinetic parameter is an "apparent Kₘ" since it essentially reflects the property of iron accumulation by cells and not necessarily transport. Nevertheless, expression of SFT increases the Vₘ₉₉ of transport from 7.0 ± 1.2 to 14.7 ± 4.2 pmol/min/mg protein for HeLa(SFT); this increase would be expected if more transport sites were expressed in the stably transfected cells.

Western blot experiments reveal that the stably transfected cells do indeed express ~2-fold greater amounts of SFT/SFT-GFP. To raise antibodies against SFT, a glutathione S-transferase fusion peptide was prepared containing residues Glu¹⁴⁹ to Met²²⁴, representing the integral membrane protein's "loop 4" or L4 domain. This segment is predominantly hydrophilic and therefore predicted to be extramembranous (23). Fig. 3
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Fig. 3. Western blot analysis of SFT present in HeLa and HeLa(SFT) cells. Antiserum against the L4 domain of SFT was raised as detailed under “Experimental Procedures.” Membrane fractions (100 μg of protein) from HeLa(SFT) and nontransfected control cells were electrophoresed on a 6% SDS-polyacrylamide gel and transferred to nitrocellulose. After blocking, the Western blot was incubated with a 1:200 dilution of primary antibody overnight and then washed and incubated with secondary alkaline phosphate-conjugated anti-rabbit antibody (1:10,000) prior to color development. SFT and SFT-GFP are denoted by right arrows; standards (rabbit muscle phosphorylase b, 97 kDa, and bovine serum albumin, 68 kDa) are denoted by left arrows.

Fig. 4. SFT expression does not alter intrinsic activity of HeLa cell surface ferrireductase. Ferrireductase activity was measured as described under “Experimental Procedures.” Briefly, cells were incubated with 10 μM K3Fe(CN)6 at 37 or 4 °C in Hank’s buffer. At times shown, 700 μl from the cell assay medium was mixed with 100 μl of 3 mM sodium acetate, pH 6.4, 100 μl of 0.2 mM citric acid, 50 μl of bathophenanthroline sulfonate, and 50 μl of 3.3 mM FeCl3, prepared in 0.1 mM acetic acid to assay for ferrocyanide production. Results shown are the difference in absorption at 535 nm measured at 37 and 4 °C for duplicate samples (±S.E.). The time course of ferrireduction catalyzed by HeLa(SFT) (circles) and control cells (squares) is presented with similar results obtained in three separate experiments.

Expression of SFT Does Not Stimulate HeLa Cell Ferrireductase—Combined, the results discussed above indicate a strong correlation between the level of SFT expressed by HeLa cells and the Vmax of iron uptake, implicating a direct role for SFT as an Fe carrier. This idea is consistent with the characterized properties of SFT-mediated Fe uptake (Table I) that closely resemble features previously described for Tf-independent uptake (26). One cannot completely rule out the possibility, however, that SFT enhances Fe uptake through some rather indirect but stoichiometric interaction. A candidate role for SFT might be that of a ferrireductase to reduce Fe3+ to Fe2+ prior to translocation across the bilayer. Indeed, multiple studies have revealed the requisite function of such a reductase activity in the uptake of both Tf-bound (3, 4) and non-Tf-bound iron (5–8).

To investigate whether expression of SFT alters the intrinsic cell surface ferrireductase activity of HeLa cells (6), cell-mediated production of ferrocyanide from ferricyanide was measured (Fig. 4). Neither the rate nor the extent of ferrireduction is affected comparing the activity of HeLa(SFT) cells (circles) with the endogenous function of nontransfected cells (squares). This evidence excludes a functional role for SFT in stimulating cell surface ferrireductase activity. Our previous studies have shown that ferricyanide (the substrate used in the ferrireduc-

tase assay) is a competitive inhibitor of non-Tf-bound Fe3+ transport (5). Thus, these data suggest that SFT stimulates uptake by acting directly as or on the carrier component of this transport system rather than its associated ferrireductase activity and, moreover, that the action of the ferrireductase endogenous to HeLa cells is not rate-limiting for Tf-independent uptake.

Cu Depletion Inhibits Tf- and Non-Tf-bound Fe Uptake Mediated by SFT—Numerous studies have established a connection between copper and iron metabolism (2, 15, 33). For example, yeast cells defective in Cu transport display diminished Fe uptake activity (34). In studies of cultured human cells, Tf-mediated Fe uptake was also found to be reduced by experimentally induced Cu depletion (24). To investigate whether Cu plays a role in SFT function, fetal bovine serum was depleted by dialysis against the chelator TRIEN. After 4 days in culture with Cu-depleted serum, HeLa cell SOD activity was decreased by 60–70%, consistent with previous results reported by Percival (28). Cells grown under this condition have markedly reduced levels of Tf-dependent and -independent Fe uptake activity (Fig. 5). Notably, although HeLa(SFT) cells have 51 ± 17% greater Tf-mediated uptake activity (panel A) and 38 ± 11% greater non-Tf Fe transport (panel B), Cu depletion reduces both activities to the same level as non-transfected control HeLa cells. Thus, SFT apparently cannot functionally stimulate Fe uptake in Cu-depleted cells. The decrease in Fe assimilation by both pathways is marked as follows: Tf-mediated uptake is diminished by 70–80%, whereas Tf-independent Fe transport drops by ~50%. The fact that the effects of Cu depletion on HeLa cell Tf-mediated uptake are more pronounced may indicate subtle differences between Tf-dependent and -independent Fe transport mechanisms. Studies in yeast have implicated the existence of multiple iron uptake systems in high eukaryotes (2, 9–12). It is possible that certain Fe transport mechanism(s) in mammalian cells, other than SFT-mediated uptake, maintain normal function despite the Cu deficiency. For example, the human equivalent of the rodent divergent cation transporter DCT1/nRAMP2 may participate in HeLa cell Tf-independent Fe uptake, and its activity may not be affected in the Cu-depleted state.
Cu Depletion Reduces HeLa Cell Surface Ferrireductase Activity—Because ferrireduction has been implicated in the membrane translocation of Fe released from Tf within endosomes (3, 4) as well as the import of non-Tf-bound Fe at the cell surface (5–8), one explanation for the effects of Cu depletion is that ferrireductase activity may be impaired, thus inhibiting Fe assimilation from both pathways. To examine the influence of Cu depletion on ferrireduction, cell-mediated reduction of ferri- to ferrocyanide was monitored as described for Fig. 4. Ferrireductase activity was measured as described above. The ferrireductase activity observed for depleted cells as discussed above (Fig. 6), a prediction would be that the transport of Fe$^{3+}$ remains unaffected by these conditions. Related to this hypothesis is the idea that SFT function, which is impaired by Cu depletion (Fig. 5) and which does not modulate ferrireductase activity (Fig. 4), may stimulate Fe uptake by acting as or on the putative Fe$^{2+}$ carrier mechanism.

To test whether SFT mediates Fe$^{2+}$ uptake, transport assays were performed with $^{55}$FeCl$_3$ prepared in buffer containing freshly dissolved ascorbate (1:20 molar ratio) to effectively reduce the radioactive iron. The time course of uptake presented in Fig. 7 (panel A) demonstrates that HeLa(SFT) cells (circles) have enhanced transport activity relative to nontransfected cells (squares). HeLa(SFT) cells display levels of uptake 50% greater than control cells, similar to the results described earlier (Fig. 1). Although these data do not necessarily discriminate that Fe$^{2+}$ is the actual cationic form translocated across the bilayer, our combined results do demonstrate that SFT functionally stimulates Fe assimilation regardless of whether cells are presented with ferric or ferrous forms. This activity is distinct from that characterized for the rat intestinal iron transporter DCT1, which appears to recognize and mediate uptake of divalent cations alone (20).

Since our results indicate that cell surface ferrireductase activity is the potential Fe$^{3+}$ transport factor affected by Cu depletion, Fe$^{2+}$ uptake assays were also performed with Cu-depleted HeLa(SFT) and control cells. Panel B (Fig. 7) further demonstrates the stimulation of Fe uptake by SFT (compare open bars). Importantly, nearly identical levels of transport are maintained for both HeLa(SFT) and control cells that have been Cu-depleted (compare open bars with solid bars). Thus, the ability of HeLa cells to take up iron presented in the ferrous form is insensitive to Cu depletion; moreover, stimulation of
this transport activity by SFT is also unaffected. These results indicate that a HeLa cell ferrireductase activity that is impaired by depletion of cellular Cu levels must function upstream of SFT to enable its apparent stimulation of Fe\(^{2+}\) uptake and that this activity, which presumably mediates the reduction of Fe\(^{3+}\) to Fe\(^{2+}\), is not required for the apparent stimulation of Fe\(^{2+}\) uptake by SFT.

**DISCUSSION**

The stimulation of non-Tf-bound Fe transport by SFT has all of the features previously described for Tf-independent transport by K562 (26) and HeLa (30) cells. Uptake is time- and temperature-dependent, inhibited by Cd\(^{2+}\), blocked by Fe\(^{3+}\) and Fe\(^{2+}\) chelators, and appears specific for the cation rather than its anionic ligand. Moreover, SFT function in Tf-independent transport also appears to depend on cell surface ferrireductase activity, as previously characterized for uptake of ferric iron (5–8). The observations that HeLa(SFT) cells display a V\(_{\text{max}}\) two times greater than non-transfected HeLa cells and that levels of SFT expression are approximately doubled in the stably transfected cell line directly support the hypothesis that SFT itself is a membrane carrier for iron. Indeed, SFT is an integral membrane protein localized to the plasma membrane and endosomal domains (23); its expression stimulates both Tf-dependent and -independent Fe assimilation (see Fig. 5), and it contains an REXXE domain putatively involved in Fe binding (10). Mutation of these critical glutamic acid residues results in the loss of function of SFT (23), further implicating a direct role for SFT in the translocation of Fe across membrane bilayers. Despite the strength of this combined evidence, however, it is important to keep in mind the consideration that the enhanced non-Tf-bound Fe uptake by HeLa(SFT) cells could be a secondary consequence of SFT expression. Recent controversies surrounding the precise role for cystic fibrosis transmembrane conductance regulator in ATP transport reflect similar concerns (35). Until rigorous evidence is obtained through reconstitution studies, the precise role of SFT in the transmembrane transport of Fe may remain elusive (see below).

The complexities associated with membrane transport of Fe have been revealed by genetic studies in yeast. Multiple carrier mechanisms have been defined (9–12), similar to recent reports investigating iron uptake in mammalian systems (20, 22, 23). FET4, for example, appears to play a direct role in the uptake of Fe\(^{2+}\) (12); this activity resembles the function of the rodent DCT1/nRAMP2 transporter in intestinal iron uptake (20, 22). It is likely that the latter transporter may play a role in endosomal transport of Fe as well since it has been discovered that the Belgrade rat, which has genetic defects in the release of Tf-bound Fe from endosomes (36), harbors the exact same mutation in DCT1/nRAMP2 as the mk mouse (37). One notable difference is the ability of DCT1/nRAMP2 to mediate uptake of other divalent cations (20). The idea that a common carrier exists to translocate iron and other metals across the plasma membrane is consistent with observations made for uptake by reticulocytes (38), HeLa cells (30), fibroblasts (30, 39), and hepatocytes (8, 40) but contrasts with our studies of K562 cells that suggest this erythroleukemia cell line has distinct transport properties (26). The specificity for Fe by FET4 not only resembles the characteristics of a second yeast iron transport mechanism involving FET3 and FTR1 but also parallels the properties we have defined for SFT, which was identified by screening a K562 cell cDNA library (23). Both FET4 and FET3/FTR1 depend on the function of FRE1, a yeast ferrireductase, to transport Fe\(^{3+}\) (13, 14). Similarly, our investigation demonstrates a strong dependence on HeLa cell surface ferrireductase activity for SFT's stimulation of non-Tf-bound Fe\(^{3+}\) uptake. This correlation is revealed by the finding that Cu depletion impairs ferrireductase function and that expression of SFT is unable to compensate for the loss in Fe\(^{3+}\) uptake under these conditions (Fig. 5).

Certain facets of the yeast FTR1/FET3 iron uptake system are comparable to elements associated with SFT function. FTR1 and SFT are both integral membrane proteins containing six transmembrane-spanning domains and are of similar size (~40 kDa). Both of these putative carriers have REXXE motifs that Stearman et al. (10) speculate may be involved in iron binding. In contrast, FET4 lacks the appearance of similar motifs within its primary sequence and has a greater mass of 63 kDa (11). The activities of both FTR1 and SFT in the transport of ferric iron also appear to depend on Cu. However, our investigation reveals clear mechanistic distinctions between yeast and man in the relationship of Cu in cellular iron uptake.

The importance of Cu in yeast Fe transport is believed to be associated with its activity as a cofactor for FET3. FET3 was identified early in the search for membrane carriers of iron (9);
in a concurrent search for iron transport proteins, Dancis et al. (34) uncovered the necessity for a copper transport protein, CTR1. The connection between FET3 and CTR1 was revealed by the findings that FET3 was a multicopper oxidase (9) and that although Δctr1Δfet3 yeast strains were unable to take up Fe^{2+}, activity could be restored to Δctr1 yeast grown in high copper media (34). Later studies also defined the requirement for an intracellular Cu transporter, CCC2 (41). Finally, the role of FTR1 in FET3-mediated iron uptake was defined by Klausner and colleagues (10) when they characterized yeast transport mutants that phenotypically resembled fet3 mutants but had defects that could not be compensated for by addition of excess Cu to the growth medium. FTR1 appears to be required for FET3 expression and loading with copper (10); elimination of the FTR1 gene results in a block in FET3 biosynthesis and expression at the cell surface, whereas FET3 deletion mutants reciprocally cannot express FTR1 at the plasma membrane. The idea that FTR1 is an iron transporter is supported by the finding that mutants in its REXEX domain cannot take up iron despite the fact that FET3 oxidase activity is unimpaired (10).

The multifunctional nature of Fe^{2+} uptake by yeast, requiring FRE1, FET4, or FTR1/FET3, as well as the Cu-transporting proteins CTR1 and CCC2, emphasizes the concerns raised earlier regarding the exact role of SFT in mammalian non-Tf-bound Fe transport. Individually, each of these yeast genes appears to modulate iron assimilation and could serve as stimulators of Fe transport. To this end, a complete description of yeast Fe import also remains elusive since iron is required for growth, and fet3fet4 deletion strains, predicted to be defective in both of the pathways described above, are still capable of survival (2, 12).

Similar to the mechanism of Fe uptake in yeast, Cu depletion of HeLa cells also results in defective Tf- and non-Tf-bound iron assimilation (Fig. 5), yet our studies demonstrate that at least for the latter activity, impaired cell surface ferrireductase function can account for this loss (Fig. 6). Although it is possible that a ferroxidase activity analogous to FET3 could be compromised by Cu deficiency, under our assay conditions both basal and SFT-stimulated Fe^{2+} uptake remains unperturbed. Because oxidation of Fe^{2+} by FET3 is apparently required for FTR1 function in yeast (10), we conclude that if a similar activity is involved in SFT-mediated iron uptake, it is unlikely to be dependent on cellular Cu status.

This investigation yields two related findings regarding the function of SFTs: 1) SFT stimulates the import of iron present as either Fe^{2+} or Fe^{3+}, and 2) it is unlikely that SFT simply acts as a ferrireductase to stimulate Fe uptake. Because our results clearly demonstrate that Cu depletion inhibits ferric iron uptake and that ferrireduction is impaired under these conditions, we conclude that SFT-mediated Fe transport is downstream of the cell surface ferrireductase activity, but whether its activity stimulates the membrane translocation of ferrous iron alone is uncertain. As discussed above, FRE1/2 ferrireductase activity is required for yeast iron uptake when presented in the ferric form (13, 14), yet the activity of the FET3 oxidase is also necessary for the role of FTR1s in transport; these observations prompted Stearman et al. (10) to suggest that FTR1 may bind both Fe^{2+} and Fe^{3+} during its mechanism of transmembrane transport. Importantly, the yeast reductase FRE1 has been identified to be a b-type cytochrome similar to the NADPH oxidase gp91^{phox}. Shatwell et al. (42) have reported biophysical characteristics of FRE1 indicating that it is a heme protein; this is consistent with early investigations of Lesuisse and Labbe (43) in which they describe the lack of ferrireductase activity in heme-deficient yeast strains. In this context, analogies in the role of copper for ferric iron uptake between yeast and man possibly may be drawn since Cu deficiency is known to impair heme biosynthesis (see Ref. 33 and references therein). Therefore, one may speculate that the influence of Cu depletion on HeLa cell Fe uptake may result from defective heme synthesis leading to the loss of a cytochrome b-like cell surface ferrireductase. Further experimentation is required to fully elucidate the role of Cu in mammalian iron assimilation and its exact function in SFT-mediated transport.

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Influence of Copper Depletion on Iron Uptake Mediated by SFT, a Stimulator of Fe Transport

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Additions and Corrections

Vol. 273 (1998) 6909–6915
Influence of copper depletion on iron uptake mediated by SFT, a stimulator of Fe transport.
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Vol. 273 (1998) 21380–21385
Structural and functional analysis of SFT, a stimulator of Fe transport.
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Expression of SFT (stimulator of Fe transport) is enhanced by iron chelation in HeLa cells and by hemochromatosis in liver.
Jianming Yu, Zhong Kang Yu, and Marianne Wessling-Resnick

These articles characterize a cDNA clone called SFT (stimulator of Fe transport) originally described by Gutierrez, J. A., Yu, J., Rivera, S., and Wessling-Resnick, M. (1997) J. Cell Biol. 139, 895–905 and deposited with ATCC (no. 97458). It was reported to us and we have confirmed that there are serious errors in the published sequence.

The corrected sequence data are now available from GenBank™ under accession number AF020761. These changes indicate that the open reading frame initially predicted for SFT must be incorrect. Instead, several short open reading frames can be mapped. Until a true open reading frame is determined, the specificity of the anti-SFT peptide antisera (Fig. 3, Yu and Wessling-Resnick (1998) J. Biol. Chem. 273, 6909–6915; Fig. 2, Yu et al. (1998) J. Biol. Chem. 273, 34675–34678) and the topological assignments based on their use (Yu and Wessling-Resnick (1998) J. Biol. Chem. 273, 34675–34678) are called into question.

We have reconfirmed the functional activity associated with the SFT cDNA, i.e. stimulation of Fe transport in transfected cells. Resequencing of the pGL2-SFT construct used for these studies also confirms the changes noted above except that there is an A → T conversion at position 725 and a nucleotide deletion at position 883. However, until an open reading frame can be identified, the molecular basis and significance of these functional data also remain uncertain.

Finally, a recent BLAST search of the nonredundant GenBank™/EBI/DDBJ/PDB sequences indicates a 46-base stretch of identity between nucleotides 530–573 of human SFT (accession number AF020761) and nucleotides 414–457 of human ubiquitin-conjugating enzyme E2D1 (UBE2D1, accession number X78140). A search of human EST sequences identifies at least two GenBank™ entries, accession numbers AA188127 and AA146924, that overlap with both UBE2D1 and SFT. Our laboratory is currently investigating the relationship between these species. Reported results of Northern blot analysis and ribonuclease protection experiments (Yu et al. (1998) J. Biol. Chem. 273, 34675–34678) must be re-evaluated.

We sincerely regret any inconvenience or additional work that this misleading data may have caused.

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