Previous studies demonstrated that SFT (Stimulator of Fe Transport) facilitates both transferrin and non-transferrin-bound iron uptake in HeLa cells (Yu, J., and Wessling-Resnick, M. (1998) J. Biol. Chem. 273, 6909–6915). To further characterize the structure and function of SFT, we studied this human factor in rodent BHK cells. Kyte-Doolittle analysis suggests that SFT has six transmembrane-spanning segments. This transport protein also displays an REXXE motif resembling domains involved in iron binding by ferritin and in iron uptake mediated by the yeast transporter Ftr1. Using N- and C-terminal epitope tags, we have identified that modification of either protein terminus does not interfere with SFT function in nontransferrin-bound iron uptake. The N- and C-terminal domains are intracellularly disposed since antibodies against these epitopes fail to recognize expressed proteins unless BHK cells are solubilized with detergents. To define the topology of two large extramembranous loop domains, anti-peptide antibodies were employed; anti-loop 4 antibodies show no immunoreactivity unless cells are permeabilized but anti-loop 5 antibodies recognize and bind surface SFT. Thus, loop 4 must be intracellular while loop 5 is extracellular. These topological studies situate the putative iron-binding REXXE domain on the cytosolic face of the plasma membrane. However, 55Fe-binding studies reveal that the ability of SFT to bind and mediate transport of extracellular iron is defective in mutants with Glu → Ala conversions in this motif. Curiously, we also find that depletion of intracellular iron by desferrioxamine impairs SFT transport and iron-binding functions. These observations lead to the speculation that the REXXE motif may play an important role in regulating SFT activity through interaction with intracellular iron and demonstrate that iron transport mediated by SFT is itself an iron-dependent process.

Although the major route for the cellular delivery of iron is via receptor-mediated endocytosis of diferric transferrin (Tf), several lines of evidence indicate that mammalian cells also acquire iron through Tf-independent pathways. Recently, two iron transporters have been identified by functional expression cloning (1, 2). DCT1/Nramp2 is thought to be involved in intestinal Fe^{2+} transport (2, 3) and appears to mediate uptake of many other divalent cations (2). In contrast, SFT shows high specificity toward iron for Tf-independent uptake (1) and is able to stimulate translocation of both Fe^{3+} and Fe^{2+} across membrane bilayers (4). A functionally important REXXE motif has been found in SFT (1) that resembles domains in the yeast iron transporter Ftr1 (5) and ferritin light chains (6) that are implicated to interact with iron. However, the topological arrangement of SFT within the membrane has yet to be determined; hence, whether this putative iron-binding domain is involved in extra- or intracellular functions is unknown.

Kyte-Doolittle analysis suggests that SFT is an intracellular membrane protein with six bilayer-spanning domains (1). Using BHK cells stably expressing this transport protein with epitope tags, the topological disposition of the N and C termini of SFT, as well as the orientation of two large extramembranous loop domains, were defined in this investigation. The membrane topology of SFT predicts that the putative iron-binding REXXE domain resides within the cytoplasm. However, while SFT presents specific iron-binding sites on the BHK cell surface, mutants with Glu → Ala conversions in the REXXE domain lack extracellular iron-binding activity. The unexpected observation that depletion of cellular iron blocks SFT-mediated transport suggests that Glu/Glu may bind intracellular iron to regulate SFT function and further indicates that membrane transport of iron mediated by SFT is itself an iron-dependent process.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transfection—**BHK cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 50 units/ml penicillin G, and 50 μg/ml streptomycin. For transport assays, cells were grown to near confluence in 6-well (35-mm) plates. For Scatchard analysis and membrane topology studies, cells were cultured in 24-well (16-mm) plates. BHK cells were transfected using LipofectAMINE as the DNA carrier (Life Technologies, Inc.); generation of stable cells with pGL2 vector (Life Technologies, Inc.), pGL2-SFT (1), pGL2-SFT (Glu → Ala), or pEFPtag-SFT was the same as described previously (1).

**Indirect Immunofluorescence Microscopy—**Transiently transfected BHK cells grown on coverslips were fixed and incubated with PBS containing 1 mM MgCl2, 0.1 mM CaCl2, and distilled H2O, 0.1% Triton X-100, 1 mg/ml BSA, and either antibody against GFP (Molecular Probes, Eugene, OR) at 1:400 dilution or anti-HA antibody (Babco, Richmond, CA) at 1:600 dilution. After rinsing, cells were incubated with a 1:500 dilution of either anti-rabbit IgG-fluorescein isothiocyanate or anti-mouse IgG-fluorescein isothiocyanate (both from Jackson ImmunoResearch Laboratories, West Grove, PA). After copious washing, coverslips were mounted using Fluoromount-G (Southern Biotechnology Associates, Birmingham, AL) containing 2.5 mg/ml N-propyl gallate. Immunofluorescence microscopy was performed using an Axioskop epifluorescence microscope (Carl Zeiss, Thornwood, NY) at a nominal magnification of × 100.

**Construction of HA-tagged SFT and Mutant GFP-SFT**—PCR was performed using pBSK-SFT (1) as template with following two primers: 5’-CGTGGACGGATCCGACATCTGAAAGAT and 5’-GGCCGCTCTAGA-

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† Established Investigator of the American Heart Association. To whom correspondence should be addressed: Dept. of Nutrition, Harvard School of Public Health, Boston, MA 02115. Tel.: 617-432-3267; Fax: 617-432-2435.
‡ The abbreviations used are: Tf, transferrin; SFT, stimulator of Fe transport; GFP, green fluorescent protein; PCR, polymerase chain reaction; PBS, phosphate-buffered saline; NTA, nitrilotriacetic acid; ORF, open reading frame; GST, glutathione S-transferase; BSA, bovine serum albumin; RIA, radioimmunoassay; HA, hemagglutinin.
CAAGGGAGAC. The PCR product was digested with BamHI and XhoI and subcloned into pEPTag, a generous gift of Dr. A. Rao (7), to generate HA-tagged SFT containing two repeated epitopes of YPYDVPDYA at its N terminus. Point mutagenesis to introduce alanines in place of Glu63 and Glu64 of SFT was as follows. Two complementary primers (5'-GTGCGAGGACATCCATCATGCAGTTAAAAAT and 5'-ATTTTTTAACGCGATGATGTCGTCGAC) to introduce the alanine substitutions were employed in a PCR step with pGL2-SFT (1) as template along with universal SP6 primer and 5'-AATGGCGGCGGCTTAAATATATC to generate two products of 300 and 800 base pairs. The isolated fragments were then used as templates in a second PCR reaction with only the last two primers amplifying SFT ORF (1). After digestion with EcoRI and NotI, the 1.1 kilobase pair product was subcloned into pGL2 vector.

Iron Uptake Measurements—[^56FeCl3 was purchased from NEN Life Science Products (> 3 mCi/mg) and[^55Fe]nitritotriacetic acid (NTA) was prepared essentially as described by Teichmann and Stremmel (8). Uptake assays were carried out by incubating cells with specified concentrations of[^55Fe]NTA in serum-free medium for the indicated time periods. Cells were lifted using PBS containing 1 mM EDTA, and cell-associated radioactivity was measured by scintillation counting and normalized to protein content (10).

Modified Radioimmunoassay (RIA)—BHK(GFP-SFT) and BHK(HA-SFT) grown in 24-well plates were washed with PBS and incubated overnight at 4 °C with antibodies or preimmune sera at desired dilutions in PBS containing 0.5% BSA. Specifically, BHK(GFP-SFT) cells were incubated with anti-GFP antibodies (1:500) (Molecular Probes), and BHK(HA-SFT) cells were incubated with anti-HA antibodies (1:500) (Babco), anti-L4 (1:250), anti-L5 (1:250), or preimmune sera (1:250). Generation of antisera against the two extramembranous loops of SFT, L4 and L5, was described previously (4). After extensive washes with PBS, antibody binding was detected using 0.5 nM[^125I]-labeled Protein A (NEN Life Sciences Products, specific activity >80 mCi/mg of protein); incubation was on ice for 60 min. After three washes with PBS, cells were lifted off the plates in PBS containing 1 mM EDTA, and cell-associated radioactivity was determined in duplicate 200-μl samples by γ-counting. For some experiments, cells were permeabilized with 0.5% Triton X-100 to detect intracellular immunoreactivity.

Iron-binding Assay—Assay conditions to detect iron binding were as described previously (9) with the following modifications. BHK cells were incubated with[^55Fe]NTA in the presence or absence of 1000-fold excess unlabeled FeNTA for 1 h at 4 °C, washed once with PBS, then lifted with PBS containing 1 mM EDTA. Cell-associated radioactivity measured in the presence of unlabeled FeNTA was subtracted from equivalent samples incubated with[^55Fe]NTA alone to determine specific[^55Fe] binding. Binding affinity (Kd) and the number of iron-binding sites (Bmax) were determined by Scatchard analysis (11).

RESULTS
Expression of HA- and GFP-tagged SFT in BHK Cells—We have previously reported that a C-terminal chimera of SFT with GFP is localized to the plasma membrane and recycling endosomes (1). To identify potential alterations in the intracellular trafficking and targeting of N-terminally tagged SFT, immunofluorescence experiments were performed. Fig. 1 shows that HA-SFT predominantly localizes to the juxtanuclear recycling endosomal compartment with rather diffuse distribution at cell surface. A similar pattern was observed for BHK cells expressing GFP-SFT (panel b), indicating that modification of the N or C termi n of SFT does not interfere with its biosynthesis, membrane transport, and intracellular localization. While the epitope availability in RIA experiments described below suggests that SFT molecules are evenly distributed between cell surface and intracellular compartments, its distribution within plasma membrane does not appear to provide a sufficiently intense signal for prominent staining. Similarly, Tf receptor staining is rather conspicuous in punctate vesicular compartments wherein protein is concentrated but not on the cell surface where it is known to reside (1).

Comparison of HA-SFT and GFP-SFT Iron Uptake Activities—To characterize the function of human SFT as an exog enously expressed protein and to compare the transport activities of N- and C-terminal chimeras of SFT, BHK cells stably expressing the GFP-SFT fusion protein, BHK(GFP-SFT), or SFT with two N-terminal HA tags, BHK(HA-SFT), were established. Fig. 2 shows the time course of non-Tf-bound Fe uptake for BHK(HA-SFT), BHK(GFP-SFT), and non-transfected control BHK cells. Time- and temperature-dependent uptake of[^55Fe] is observed; at 4 °C, less than 5% of radioactivity is found associated with cells compared with[^55Fe] internalized at 37 °C. GFP-SFT and HA-SFT stimulate[^55Fe] uptake 1.7-fold and 2.1-fold, respectively. The observation that BHK(HA-SFT) cells display ~40% higher iron uptake activity is due to greater expression of HA-SFT (see below). To compare the kinetic determinants of non-Tf-bound Fe uptake by BHK(HA-SFT), BHK(GFP-SFT), and control BHK cells, initial rates of uptake were measured as a function of iron concentration. Double-reciprocal plots of these data are shown in Fig. 3. Control BHK cells have an apparent Kd for non-Tf-bound Fe transport of 14.8 ± 3.4 μM (n = 3); this value is ~2- to 3-fold greater than that determined for HeLa cells (4, 13). However, BHK cells expressing GFP-SFT or HA-SFT display lower Kd values of 5.2 ± 0.2 μM and 4.8 ± 0.3 μM, respectively (n = 3), consistent with the measured value of SFT-mediated uptake for HeLa cells (4). While control cells exhibit a Vmax for non-Tf-bound iron uptake of 7.5 ± 0.8 pmol/min/mg of protein, expression of SFT increases this kinetic parameter to 9.5 ± 0.8 and 13.6 ± 2.0 pmol/min/mg protein for BHK(GFP-SFT) and BHK(HA-SFT) cells, respectively. Again, these observations are consist-
ent with the idea that more iron transporters are expressed in the latter cell line.

Determination of the Membrane Topology of SFT—Kyte-Doolittle analysis predicts that SFT is an integral membrane protein with six transmembranous segments (1). A hypothetical model for SFT topology is presented in Fig. 4A. To confirm this topological arrangement, antibodies were raised against SFT-GST fusion peptides containing residues Glu149-Met224 and His246-Leu296, representing the integral membrane protein’s extramembranous loops L4 and L5, respectively. Previous work has shown that anti-L4 antibody recognizes SFT and SFT-GFP by Western blot analysis (4); similar results were obtained for anti-L5 antibody (not shown). As shown in Fig. 4B, preimmune antiserum showed little reactivity against BHK(HA-SFT) cells. Anti-HA and anti-L4 recognize and bind to these cells, but only upon permeabilization (solid bars). In contrast, anti-L5 binds to the surface of nonpermeabilized cells (open bars), and more immunoreactivity is observed upon addition of detergents (solid bars) due to solubilization of endosomal compartments and detection of intracellular SFT. Thus, the L5 domain appears to be extracellularly situated, while L4 and the N terminus of SFT are occluded from antibody in the absence of detergent, indicating their cytoplasmic orientation. The enhancement in L5 antibody binding upon the addition of detergent further supports the endosomal localization of SFT (Fig. 1) and suggests that the protein is roughly distributed in equal amounts between the cell surface and the intracellular compartments.

To examine the orientation of the C-terminal domain, experiments were performed with BHK(GFP-SFT) cells, and anti-GFP immunoreactivity was assessed. 125I-Labeled Protein A binding is detected only upon permeabilization (solid bars), and the total amount of SFT is lower in these cells. BHK(GFP-SFT) cells have 20% lower cell surface immunoreactivity than BHK(HA-SFT) as

**Fig. 2.** Time course of iron uptake. BHK(HA-SFT) (circles), BHK(GFP-SFT) (triangles), and control BHK cells (squares) were incubated at 37 °C (filled symbols) or 4 °C (open symbols) in the presence of 1 μM 55FeNTA. At the indicated times, 55Fe uptake was quenched by rapidly chilling the cells on ice, followed by three washes with ice-cold PBS. Nonspecific surface-bound 55Fe was displaced by a brief (20-min) incubation on ice with 1 mM FeNTA in 25 mM HEPES, 150 mM NaCl, pH 7.4. Cells were then lifted off plates with 600 μl of PBS containing 1 mM EDTA. Cell-associated radioactivity was determined in duplicate 200-μl aliquots. The mean value (±S.E.) of 55Fe taken up by BHK cells (fmol/μg of protein) is shown as a function of time. Results from an individual experiment are provided and reflect similar data obtained on three separate occasions.

**Fig. 3.** Kinetic analysis of iron uptake mediated by SFT. BHK(HA-SFT) (circles), BHK(GFP-SFT) (triangles), and control BHK cells (squares) were incubated with different concentrations of 55FeNTA for 10 min. Iron uptake measurements were carried out as detailed for Fig. 1. The difference between values obtained at 37 and 4 °C was taken as specific 55Fe transport. Shown is the double-reciprocal plot of data from a single experiment (±S.E.) representative of four independent experiments. Initial rate of uptake, V₀ (pmol of 55Fe/min/μg of protein), is shown as a function of [Fe] (μM).

**Fig. 4.** Determination of SFT membrane topology. Panel A shows a hypothetical model for SFT membrane structure. Panel B presents the results of modified RIA experiments. Briefly, BHK(GFP-SFT) and BHK(HA-SFT) cells were grown in 24-well plates. After washing with PBS three times, individual wells containing BHK(GFP-SFT) were incubated with anti-GFP, wells containing BHK(HA-SFT) were incubated overnight with anti-HA, anti-L4, anti-L5, or preimmune serum in PBS containing 0.5% BSA at 4 °C. Cells were then washed eight times with cold PBS, once with PBS containing 0.5% BSA, and then incubated with 0.5 μM 125I-labeled protein A for 60 min on ice to detect immunoreactivity. After three washes with PBS, cell-associated radioactivity was determined by γ-counting. To determine antibody binding to intracellular sites, cells were first permeabilized by incubation with 0.5% Triton X-100 (solid bars).
detected by anti-L5 (data not shown), supporting the conclusion that more transporters are expressed at plasma membrane in the BHK(HA-SFT) cells (see Figs. 2 and 3). Antipeptide antisera had negligible immunoreactivity in control BHK cells or cells stably expressing cytosolic GFP alone (see Fig. 5B), indicating that endogenous rodent SFT, if expressed, cannot be recognized by these antibodies. Thus, these data confirm the predicted topology of SFT (Fig. 4). Results from an independent experiment are provided here, and similar results were obtained on three separate occasions.

**Function of the REXXE Motif—**Crystal structure analysis has implicated a role for REXXE motifs in iron binding by ferritin (6). Moreover, the REXXE motif of the yeast Fre1 transport protein has been shown to play an important functional role (5). We have previously demonstrated that mutation of the two glutamic acid residues within the SFT domain eliminates its ability to mediate iron uptake in *Xenopus* oocytes (1). To study how this motif may affect SFT-mediated transport in mammalian cells, stable BHK cells were established expressing Glu → Ala point mutations in the GFP-SFT chimera. As shown in Fig. 5A, antibody-binding experiments demonstrate that nearly equal numbers of SFT molecules are expressed on the surface of BHK(GFP-SFT) and BHK(GFP-SFT^Glu → Ala^) cells as detected by anti-L5 binding. As a control, anti-L5 binding BHK(GL2) cells was measured and found to be negligible (<25 cpm/μg of protein). These results indicate that the biosynthesis, membrane trafficking, and cell surface expression of the mutant is unaffected. This idea is confirmed by the immunofluorescence data presented in Fig. 1 demonstrating that the cellular distribution of GFP-SFT^Glu → Ala^ is identical to wild-type (compare panel c to panels a and b). Fig. 5B compares the time course of non-Tf-iron uptake by BHK(GFP-SFT) and BHK(GFP-SFT^Glu → Ala^) and demonstrates that although GFP-SFT^Glu → Ala^ stimulates Fe uptake, Glu → Ala mutations within the REXXE domain impair this transport activity. The transport activity of BHK(GFP-SFT^Glu → Ala^) cells is nearly the same as that measured for the control. The failure of the mutant to stimulate iron transport is consistent with results observed when its expression was studied in *Xenopus* oocytes (1) and further identifies that Glu^83^ and/or Glu^86^ are critical residues for SFT transport function rather than its biosynthesis and trafficking.

**Characterization of Iron-binding Activity of SFT—**To examine the ability of SFT to bind iron and to determine whether the two glutamic acid residues are involved in this activity, the association of ^55^Fe with the membrane surface of BHK(GL2), BHK(GFP-SFT), and BHK(GFP-SFT^Glu → Ala^) cells was measured. As shown in Fig. 6, saturable ^55^Fe binding is observed for control BHK(GL2) cells. Similar activity is detected for BHK(GL2) cells and BHK(GFP-SFT^Glu → Ala^) cells show enhanced surface ^55^Fe binding. Scatchard analysis (Fig. 7) reveals a single class of binding sites (1.1 × 10^7^/cell) with a K_d of 5.9 ± 1.0 μM for BHK(GFP-SFT^Glu → Ala^) cells (n = 3). This value is quite comparable to the apparent K_m of transport associated with SFT function which is ~5 μM (Fig. 3). In contrast, BHK(GFP-SFT^Glu → Ala^) and BHK(GL2) cells have limited binding sites (~7 × 10^5^/cell), with a K_d of 12.3 ± 2.1 μM and 15.7 ± 3.1 μM, respectively (n = 3). The latter results suggest that Glu^83^ and Glu^86^ are critical not only for iron
transport, but also for the apparent iron-binding activity of SFT. Given that the topological data described above contradict the idea that the RE\_XX\_E domain would be extracellularly disposed and thus capable of binding 55Fe, a simple explanation for the failure of the mutant to bind iron is that protein folding might be perturbed. To examine whether Glu\_3\_Ala mutations impair SFT function by disrupting its membrane topology, experiments were performed to determine the orientations of L4, L5, and the C-terminal domain. Fig. 8 shows that anti-GFP and anti-L4 only bind to the BHK cells stably expressing GFP-SFT\_Glu\_3\_Ala upon solubilization, while anti-L5 binding is detected for both intact and permeabilized cells, consistent with wild type membrane topology.

Depletion of Intracellular Iron Inhibits SFT Function—To further explore the role of the intracellular RE\_XX\_E motif, the iron content of BHK cells was depleted by overnight incubation with 50 \textmu M desferrioxamine. 55Fe uptake assays were then performed to compare the activities of control and iron-depleted BHK(GFP-SFT), BHK(GFP-SFT\_Glu\_3\_Ala), and BHK(GL2) cells. As shown in Fig. 9A, 55Fe uptake was unaffected in control BHK(GL2) and BHK(GFP-SFT\_Glu\_3\_Ala) cells upon iron depletion, consistent with observations made for fibroblasts (14). Although expression of wild type SFT stimulates iron import nearly 2-fold over control, treatment with desferrioxamine (solid bars) blocks SFT-mediated transport activity, suggesting that the transport function of SFT is iron-dependent. Incubation of BHK(GFP-SFT) cells with 65 \textmu M FeNTA for 2 h after desferrioxamine treatment completely restored SFT activity (not shown). Moreover, Fig. 9B shows that iron depletion does not result in lower cell surface 125I-labeled protein A binding as detected by anti-L5, although surface 55Fe binding is reduced \approx 50%. Thus, effects of iron depletion are reversible and are not accounted for by an iron-dependent decrease of cell surface SFT.

DISCUSSION

When exogenously expressed in BHK cells, SFT stimulates non-Tf-bound iron uptake with properties essentially the same as described for HeLa cells (1). The previously reported \( K_m \) \( \approx 5 \textmu M \) is nearly identical to values determined for BHK(GFP-SFT) and BHK(HA-SFT) cells by our initial rate analysis. However, unlike HeLa cells which have an endogenous pool of SFT (1), BHK cells appear to have an uptake system with different properties (\( K_m \) \( \approx 15 \textmu M \)).
gesting the presence of two transport mechanisms in BH-K(GFP-SFT) or BHK(HA-SFT) cells are not observed in our study. Although one interpretation is that Lineweaver-Burk analysis may not be powerful enough to reveal the existence of multiple uptake mechanisms under our experimental conditions, it should be noted that the possibility that SFT modulates the activity of endogenous BHK cell transporters cannot be excluded. However, the findings that the biosynthesis, membrane trafficking, and transport characteristics of this protein are identical whether expressed in rodent or human cells lend support to the idea that SFT function does not require other protein cofactors. These observations are in contrast to the Saccharomyces cerevisiae transporter Ftr1, which requires Fet3 for proper function (12); moreover, the Schizosaccharomyces pombe homolog (Fip1) of Ftr1 cannot complement ftr1− cells unless the S. pombe homolog of Fet3 (Fin1) is co-expressed (12). Thus, we might tentatively conclude that SFT acts as a direct transport facilitator for iron, although rigorous reconstitution experiments will be necessary to confirm its activity.

Previous primary structure analysis predicted SFT to be a hydrophobic protein with six transmembranous domains (1). Taking advantage of the N and C terminally tagged SFT constructs and anti-peptide antibodies, we have confirmed the topology suggested by Kyte-Doolittle analysis (Fig. 4A). This model situates the putative iron-binding REXXE motif on an intracellular domain. However, Glu → Ala mutations in this domain block SFT-mediated iron binding and translocation at the cell surface. Immuno-fluorescence microscopy indicates that these mutations do not interfere with biosynthesis and targeting of SFT (Fig. 1), and loss of function for mutant SFT is not due to lower amounts of expressed protein at the cell surface (Fig. 5A). In addition, Glu → Ala mutations do not alter the topological arrangements of L4, L5, and the C terminus (Fig. 8), such that incorrect membrane folding is unlikely to account for failure of SFT mutant to bind and take up iron. An unexpected and interesting observation in our study is that SFT-mediated transport activity is dependent on intracellular iron status. Based on this finding and features of the Glu → Ala mutant, we speculate that the L2 domain of SFT acts as a “sensor” to monitor cellular iron status.

The dependence of SFT function on iron must occur at a post-translational level, because the activity of exogenously expressed protein appears to be exclusively affected. Moreover, the fact that this effect is fully reversed by brief incubation of cells with iron suggests that SFT may directly bind the cation. Thus, the REXXE motif domain may act to bind cytosolic “free” iron to maintain SFT in a conformation competent for binding and transport of extracellular iron. Since loss of the ability to sense intracellular levels of iron could result in excessive iron accumulation due to unchecked SFT activity, the failure of the Glu → Ala mutant to bind iron may provide a feedback signal that blocks iron uptake. Thus, we propose that the glutamic acids of the L2 region of SFT not only bind iron but serve as a sensory domain to regulate SFT activity. The envisioned sensor mechanism predicts that when the intracellular free iron level rises, SFT-mediated transport is diminished due to interaction at the REXXE site. It is known, for example, that increased cellular iron content decreases Tf receptor number to limit iron import under these conditions. However, unlike the effects observed for SFT function, iron chelation up-regulates Tf receptor expression. Although SFT can stimulate Tf-mediated iron uptake (1), we have yet to determine whether intracellular iron status influences SFT function in this pathway. Because iron depletion appears to enhance Tf receptor number, our results may suggest that SFT function in Tf-mediated uptake is differentially regulated from that of Nramp2, which has also been implicated recently to play a role in endosomal transport (16) and which appears to be up-regulated in response to diminished iron levels (2).

Post-translational effects of iron on non-Tf-mediated uptake have been reported previously (14, 15). In the studies of fibroblasts, HeLa, CHO, Hep-G2, and L-cells, increased levels of extracellular iron were found to increase iron assimilation (14, 15). Kaplan and co-workers hypothesized that this enhanced uptake was due to the recruitment of cryptic intracellular iron transporters to the cell surface, an idea supported by the enhanced V_{max} observed under these conditions. In our study, cell surface SFT numbers do not change upon iron depletion, but further experiments are required to study the influence of high levels of cytosolic iron on the function and distribution of SFT. Our investigation, however, raises critical questions regarding the regulation of SFT function by cellular iron status and provides the basis for future analysis of regulatory domains. A key to understanding SFT structure-function relationships will be the ultimate identification of the extracellular iron-binding and transport site that appears to be influenced by the intracellular REXXE domain.

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