Delivery of iron to K562 cells by diferric transferrin involves a cycle of binding to surface receptors, internalization into an acidic compartment, transfer of iron to ferritin, and release of apotransferrin from the cell. To evaluate potential feedback effects of iron on this system, we exposed cells to iron chelators and monitored the activity of the transferrin receptor. In the present study, we found that chelation of extracellular iron by the hydrophilic chelators desferrioxamine B, diethylenetriaminepentaacetic acid, or apolactoferrin enhanced the release of the cells of previously internalized 

\[ ^{125}\text{I} \]

transferrin. Presaturation of these compounds with iron blocked this effect. These chelators did not affect the uptake of iron from transferrin. In contrast, the hydrophobic chelator 2,2-bipyridine, which partitions into cell membranes, completely blocked iron uptake by chelating the iron during its transfer across the membrane. The 2,2-bipyridine did not, however, enhance the release of 

\[ ^{125}\text{I} \]

transferrin from the cells, indicating that extracellular iron chelation is the key to this effect. Desferrioxamine, unlike the other hydrophilic chelators, can enter the cell and chelate an intracellular pool of iron. This produced a parallel increase in surface and intracellular transferrin receptors, reaching 2-fold at 24 h and 3-fold at 48 h. This increase in receptor number required ongoing protein synthesis and could be blocked by cycloheximide. Diethylenetriaminepentaacetic acid or desferrioxamine presaturated with iron did not induce new transferrin receptors. The new receptors were functionally active and produced an increase in 

\[ ^{55}\text{Fe} \]

uptake from 

\[ ^{55}\text{Fe} \]-transferrin. We conclude that the transferrin receptor in the K562 cell is regulated in part by chelatable iron: chelation of extracellular iron enhances the release of apotransferrin from the cell, while chelation of an intracellular iron pool results in the biosynthesis of new receptors.

Iron is an essential element for cell growth and metabolism. It is a key component of heme which is the prosthetic group of hemoglobin, myoglobin, peroxidases, and most cytochromes. In addition, iron itself is a prosthetic group for many non-heme-containing proteins and enzymes such as xanthine oxidase. The major vehicle for iron delivery to the cells of the body is the serum glycoprotein transferrin which binds two 

\[ ^{55}\text{Fe}^{3+} \]

ions per molecule in association with a small anion (1). Iron is tightly complexed to transferrin and is released to cells after the protein binds to specific receptors (2, 3).

We have performed a series of studies of the transferrin receptor system using the K562 cell line (4). This human erythroleukemia cell line has been extensively examined as a model system for the regulation of hemoglobin synthesis (5, 6). We demonstrated that transferrin binds to specific high-affinity receptors on the cell surface and is internalized into an acidic, nonlysosomal compartment (7). Iron is removed from transferrin and delivered to ferritin, and apotransferrin is released from the cells. This transferrin cycle through the cell appears to be analogous to other systems of receptor-mediated endocytosis (3). With the low-density lipoprotein system, there is feedback regulation of its receptor by the cholesterol which low-density lipoprotein delivers to the cell (8). We decided to determine whether such an interaction also exists between iron and the transferrin receptor.

In the present study, we have investigated the relationship between extracellular and intracellular chelatable iron pools and the function and expression of the transferrin receptor. We have employed two hydrophilic iron chelators, desferrioxamine (Desferal®), and DTPA. The former appears to distribute into both intra- and extracellular spaces while the latter compound has access only to the extracellular space (9, 10). Additional experiments were performed with 2,2-bipyridine, a hydrophobic iron chelator which partitions into cell membranes and binds iron as it passes through this lipid environment (9, 11, 12). We found that chelation of extracellular iron stimulates the release from the cell of previously internalized transferrin, while chelation of an intracellular iron pool promotes the biosynthesis of new transferrin receptors. The 2,2-bipyridine effectively blocked all iron uptake from transferrin, but had no effect on either of these processes.

MATERIALS AND METHODS

Human transferrin and human lactoferrin were purchased from Calbiochem-Behring, 2,2-Bipyridine, cycloheximide, disodium ascorbate, disodium nitroacetate, and ferric chloride were purchased from Sigma. Desferrioxamine (Desferal®) was obtained from Ciba Geigy while diethylenetriaminepentaacetic acid, pentasodium salt (41% in water) (DTPA) was purchased from Eastman. Iodo-Beads were purchased from Amersham. 2,3,4,5,6-Pentadeuteriophenol (DTPA) was purchased from Ectron. 14C-Ferric ammonium sulfate was purchased from Fisher.

K562 cells were a generous gift of Dr. Brian Smith of Harvard Medical School. The cells were grown in RPMI 1640 medium supplemented with L-glutamine (M. A. Bioproducts) and 10% fetal bovine serum (Gibco). The cells were grown at 37 °C in a humidified incubator with a 5% CO₂ atmosphere and maintained in log-phase growth at about 5 × 10⁶ cells/ml. Cell viability as determined by exclusion of 0.1% trypan blue was greater than 95% for all experiments.

Both the transferrin and lactoferrin were saturated with iron as previously described (4) except that the 5-200 column chromatogra-

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Effect of Iron Chelators on Transferrin Receptors in K562 Cells

A critical study into the impact of iron chelation on transferrin receptor dynamics was conducted in K562 cells. The investigation explored the effect of various iron chelators on the transferrin receptor complex, particularly focusing on the role of iron saturation and receptor dynamics.

**Procedures:**
- **Preparation of Radiolabeled Proteins:** Single Iodo-Bead was added to 100 μl of 0.15 M NaCl, 0.02 M Tris-CI, pH 7.4, followed by 1 mM of Na2311 and a 10-min room temperature incubation. One mg of iron-saturated transferrin or lactoferrin was added to this mixture with an additional 15-min incubation.
- **Solubilization:** The solution containing the dioxin protein was passed over a PD-10 column (Pharmacia) and 0.5-ml fractions were collected. The peak fractions emerging immediately after the void volume were pooled. The specific activity of the different 51Fe-transferrin preparations ranged from 1.2 to 2.4 × 10^6 cpn/pmol.
- **Transferrin Uptake and Release by K562 Cells:** Differ 51Fe-transferrin cell surface receptors were performed by incubating cells at 10^5/ml in RPMI with 1% fetal bovine serum on ice with 0.1 μM dipheric 51Fe-transferrin for 20 min. Duplicate 200-μl aliquots of cells were layered on a 150-μl cushion of oil (nine parts dibutyl phthalate, one part mineral oil) in 0.4-ml microtubes and pelleted through the oil by a 15-s spin in a Beckman Microfuge B. The tube tips containing the cell pellets and the bound 51Fe-transferrin were cut off and counted in a Beckman Gamma 8000 counter. The unbound ligand remained in the upper aqueous phase.
- **Measurement of Cellular Iron Content:** One hundred ml of K562 cells were grown for 24 h in medium containing 20 μM desferrioxamine while a second 100 ml were grown in standard medium. At that time, the cells were counted and 20 ml were used to measure surface binding activity as outlined above. The remaining cells were transferred to nitric acid-washed 50-ml Corex tubes and were centrifuged to remove insoluble material, particularly nucleic acids. The absolute receptor number obtained was less than that given by the previous assay method. How-ever, the final step involved filter trapping of precipitated transferrin-receptor complex, removal of insoluble material which is nonspecifically trapped by the filter greatly reduced the background and variability of the assay. The assay is internally consistent, but cannot be directly compared with the surface receptor assay to determine the ratio of surface to total receptor number as was done earlier.

**RESULTS**

The results demonstrated that iron chelators profoundly affect transferrin receptor dynamics in K562 cells. Specific observations included:

1. **Effect of Chelation of Extracellular Iron on the Release of Cell-associated Transferrin:** The interaction of dipheric 55Fe-transferrin with K562 cells can be divided into three phases: 1) binding of ligand to cell surface transferrin receptors, 2) internalization into an intracellular compartment, and 3) release of apotransferrin from the cell. Desferrioxamine in concentrations ranging from 10 μM to 1 mM had no effect on the first two processes. Its influence on the third step, however, was profound.

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**Iron Determination:**
- Iron determinations were performed with a Perkin-Elmer 5000 atomic absorption spectrophotometer. Protein determinations were made with the Bio-Rad Assay Kit, using transferrin as the protein standard.

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This experiment makes two points: 1) only chelator present removed, and desferrioxamine was added to three of these at differing concentrations. The cells were rewarmed to 37°C, and at the indicated times, duplicate 200-μl aliquots were removed and spun through oil to determine the residual cell-associated counts. Δ, control; ○, 2 μM desferrioxamine; ■, 5 μM desferrioxamine; ▲, 10 μM desferrioxamine.

present in the incubation medium during the rewarming showed an enhanced release of the internalized transferrin. This experiment makes two points: 1) only chelator present in the extracellular environment affects transferrin release from the cells, and 2) desferrioxamine does not produce a long-term change in the cells with respect to the enhanced release of internalized transferrin.

The possibility remained, however, that the desferrioxamine produced a drug effect on the cells unrelated to its iron chelation properties. The failure of ferrioxamine, desferrioxamine previously saturated with iron, to enhance the release of internalized transferrin argued against this (Fig. 2B).

Another potential cause of desferrioxamine's effect on transferrin release was chelation of intracellular iron. To examine this question, DTPA, a second chelator which does not enter the cell, was used. Fig. 3A shows that 10 μM DTPA enhanced the release of internalized 125I-transferrin in a manner analogous to desferrioxamine. DTPA, like desferrioxamine, chelates iron on a 1:1 molar basis, and the concentration profile of its effect on desferrioxamine release paralleled that of desferrioxamine. And like ferrioxamine, iron-loaded DTPA showed an enhanced release of the internalized transferrin. DTPA, like desferrioxamine, enhances the release of internalized transferrin in a manner analogous to desferrioxamine and DTPA, confirming that it is the chelation of extracellular iron as opposed to divalent cations which stimulates transferrin release.

The apparent enhanced release of internalized transferrin by extracellular iron chelators could be explained if under normal circumstances a fraction of the apotransferrin released from the cell bound free iron in the medium and reattached to the transferrin receptor. When the extracellular iron was removed by a chelator, the newly released apotransferrin would find no iron to bind and would not reattach to the receptor. The result would be a net increase in transferrin release. It is possible, in fact, that a portion of the apotransferrin returning to the cell surface rebinds iron from the medium even before it is released from the receptor and never detaches at all.

When 59Fe-transferrin is added to cells, there would be competition for receptors between the 59Fe-transferrin and "recycled" transferrin carrying unlabeled iron acquired from the medium. Desferrioxamine does not remove iron from transferrin in the absence of a small facilitator molecule such as nitritotriacetate (18). Therefore, when added to cells incubated with 59Fe-transferrin, it would chelate unlabeled iron in the medium, prevent the competition for receptors by unlabeled transferrin, and thereby increase the rate of 59Fe uptake from 59Fe-transferrin. Fig. 4A shows that desferrioxamine does not enhance the rate of 59Fe uptake from 59Fe-transferrin. Experiments in which the 59Fe-transferrin concentration was reduced to 7.5 nM again showed no enhancement by desferrioxamine (data not shown). This suggests that rebinding of released apotransferrin does not occur to a significant extent in these cells.

The hydrophilic chelators used to this point bound iron in the extracellular aqueous environment. 2,2-Bipyridine is a very different iron chelator. This hydrophobic compound partitions into apolar environments such as cell membranes and has a high affinity for Fe2+ (11). It binds iron after its release from diferric transferrin during its vulnerable passage across the membrane into the cytosol. In contrast to the hydrophilic iron chelators, which did not affect cellular iron uptake from 59Fe-transferrin, 150 μM 2,2-bipyridine totally blocked iron uptake by K562 cells (Fig. 4B), a phenomenon previously observed with reticulocytes (11). Despite this dramatic effect on iron uptake, however, 2,2-bipyridine had no effect on the release of internalized transferrin from the cells (data not shown). It appears, then, that it is extracellular iron accessible to hydrophilic chelators but not to a hydrophobic chelator in the cell membrane which is important in the release of transferrin from the cell.
Effect of Iron Chelators on Transferrin Receptors in K562 Cells

Transferrin Receptors—A second series of experiments examined the effects on the transferrin receptor of growth of K562 cells in medium containing iron chelators. Fig. 5 shows that growth of cells for 24 h in medium supplemented with increasing amounts of desferrioxamine resulted in enhanced cell surface expression of the transferrin receptor. The threshold for this effect was between 1 and 5 μM desferrioxamine and peaked at 20 μM.

Using cells grown in 10 μM desferrioxamine, we next examined the time course of this effect. Since the cells have been shown to have a large intracellular pool of receptors (4), we examined both the number of cell surface transferrin receptors as well as solubilized receptors, which reflects the total cellular receptor content, using the soluble receptor assay. We could thereby identify any changes we observed were not due merely to a shift of receptors from the internal pool to the surface. Fig. 6 demonstrates that there was a dramatic change both in surface and total receptor number with cells grown in desferrioxamine. Incubation times of less than 9 h had no effect on the numbers of transferrin receptors, while by 24 h surface and total receptors had doubled in comparison to the controls. By 48 h, there was a 3-fold increase in transferrin receptors in the desferrioxamine-treated cells. Importantly, up to this time, the rate of cell division, gross cellular morphology, and cell viability were identical in the control and treated cells.

At this point, the treated cells were shifted to desferrioxamine-free medium and allowed to continue to grow. Over the next 48 h there was a decline in transferrin receptor number to near base-line.

Fig. 7A shows that this transferrin receptor induction was due to intracellular iron chelation by desferrioxamine. Cells grown in ferrioxamine or DTPA had no increase in transferrin binding to the cell surface. A similar lack of effect on total cellular transferrin receptors was also found (data not shown). When the time of exposure to DTPA was increased to even as much as 72 h, no enhancement of receptor expression occurred. These results with DTPA are particularly significant since this extracellular iron chelator stimulated the release of internalized transferrin, but failed to induce new receptors. This result would argue against a shift of "chelatable" iron out of the cell in response to extracellular iron chelation in the time frame used in this study.

In another series of experiments, cells were grown for 24 h in 2,2-bipyridine at concentrations of 50, 150, and 300 μM. Over this period of time, the cells remained viable and, again, showed no gross morphological alteration or impairment in the rate of cell division. Iron uptake by the cells was totally shut off at the two higher concentrations of chelator, and yet there was no effect on the number of cell-surface transferrin receptors (Fig. 7B) or total cellular receptors as determined by the soluble receptor assay (data not shown). During this period of time, the 2,2-bipyridine would likely distribute itself throughout the pool of intracellular membranes since there is a constant exchange of lipid between the intracellular and plasma membranes (19). The lack of effect of this chelator on receptor expression suggests that it is iron in the cytosolic fraction of the cell which is important in receptor induction.

Another possibility, however, was that the induction was due to a significant depletion of cellular iron stores by desferrioxamine. To examine this question, control cells and cells grown in 20 μM desferrioxamine for 24 h were assayed for...
cellular iron content as described under "Materials and Methods." The treated cells showed a 1.8-fold increase in transferrin receptor content over the controls. The iron determinations gave a value of 65 ± 2 ng of Fe/mg of protein for the controls and 77 ± 3 ng of Fe/mg of protein in the desferrioxamine-treated cells.

The increase in the number of transferrin receptors in K562 cells exposed to desferrioxamine could have resulted from an increase in the rate of receptor synthesis or a decrease in the rate of degradation. If a decreased degradation rate occurred, then in the presence of a protein synthesis inhibitor, the decay rate of transferrin receptors in desferrioxamine-treated cells should be less than that seen in control cells. Therefore, control cells and cells induced with desferrioxamine were compared to cells grown for 12 h in cycloheximide with and without desferrioxamine. The induction time was limited to 12 h so that an early receptor-induction effect by the desferrioxamine could be seen, while cycloheximide cell toxicity was minimized. The cell viability as assayed by trypsin blue exclusion as well as gross cellular morphology were identical in the cells with and without cycloheximide. The desferrioxamine-treated cells showed a small increase in transferrin receptors relative to the control (Fig. 8). Cycloheximide treatment produced a marked decrease in receptor number; however, the decline was unaffected by exposure to desferrioxamine. It appears that desferrioxamine does not change the rate of receptor degradation. Therefore, the increase in receptors seen in the chelator-treated cells may be due to an increased synthetic rate.

Function of Desferrioxamine-induced Transferrin Receptors—The function of the transferrin receptor is to deliver iron to cells. One important question, then, was whether the increased transferrin receptor content would produce an augmented rate of iron uptake. Therefore, cells were grown for 24 h in 20 μM desferrioxamine, producing a 2-fold increase in transferrin receptor number over the controls. The rate of 56Fe uptake from 56Fe-transferrin in the treated cells was increased in proportion to the increase in transferrin receptor number (Fig. 9). In contrast, cells grown for the same period of time in 20 μM DTPA showed no increase either in receptor number or 56Fe uptake rate. This indicated that it was the induction of new transferrin receptors and not simply growth with an iron chelator that produced the increased capacity for iron uptake by the desferrioxamine-treated cells.

**DISCUSSION**

Transferrin has long been recognized as the transport protein essential in the delivery of iron to cells. The elegant study of Jandl and Katz (2) demonstrated that the protein is released from the cell after depositing its metal ligand. Recently, increased attention has been directed to the regulation of the receptor. In the present study, the influence of iron chelators on the function and expression of the transferrin receptor in K562 cells was examined. The effects of the chelators could be divided into two categories. The first was an immediate effect in which the rate and magnitude of release of internalized transferrin was augmented by hydrophilic chelators. The second, which required hours, was an induction of new trans-

![Graph](image1.png)

**Fig. 3. Specificity of chelator effect on release of 125I-transferrin from cells.** A, effect of DTPA on release of 125I-transferrin from cells. Cells were loaded with 125I-transferrin at 37 °C, then chilled and washed as in Fig. 1. While at 4 °C, the cells were divided into three groups with desferrioxamine added to a final concentration of 10 μM in one and DTPA to the same concentration in another. All of the cells were warmed to 37 °C and duplicate 200-μl aliquots were spun through oil at the indicated times to determine residual cell-associated ligand. Δ, control; ▲, 10 μM desferrioxamine; ●, 10 μM DTPA. B, effect of diferric lactoferrin and apolactoferrin on release of 125I-transferrin from cells. After loading with 125I-transferrin at 37 °C, the cells were chilled to 4 °C and washed as in Fig. 1. They were divided into three groups with diferric lactoferrin added to one to a final concentration of 3 μM and apolactoferrin added to another of the same concentration. All of the cells were warmed to 37 °C, and duplicate 200-μl aliquots were spun through oil to determine residual cell-associated 125I-transferrin at the indicated times. Δ, control; ▲, 3 μM apolactoferrin; ●, 3 μM diferric lactoferrin.
Effect of Iron Chelators on Transferrin Receptors in K562 Cells

FIG. 4. Uptake of $^{59}$Fe-transferrin in the presence of hydrophilic or hydrophobic chelators. A, effect of desferrioxamine on $^{59}$Fe uptake from $^{59}$Fe-transferrin. Cells were suspended at $10^7$/ml in RPMI, 1% fetal bovine serum and warmed to 37°C for 15 min. They were divided into two groups with desferrioxamine added to one to a final concentration of 20 µM. Diferric $^{59}$Fe-transferrin was then added to both groups of cells to a concentration of 0.1 µM and duplicate 200-µl aliquots were spun through oil to determine cell-associated $^{59}$Fe at the times indicated. Specific activity of $^{59}$Fe-transferrin = 1100 cpm/pmol. O, control; •, 20 µM desferrioxamine. B, effect of 2,2-bipyridine on $^{59}$Fe uptake from $^{59}$Fe-transferrin. Cells were suspended at $10^7$/ml in RPMI, 1% fetal bovine serum and warmed to 37°C for 15 min. Diferric $^{59}$Fe-transferrin was added to a concentration 0.1 µM and duplicate 200-µl aliquots were spun through oil at 2, 4, 6, and 8 min. At 8 min, half the cells were quickly pipetted into a separate test tube and 2,2-bipyridine in ethanol added to a final concentration of 150 µM. At the subsequent indicated times, duplicate 200-µl aliquots from the control cells (O) and the 2,2-bipyridine-treated cells (●) were spun through oil to determine cell-associated $^{59}$Fe. Specific activity of $^{59}$Fe-transferrin = 1250 cpm/pmol.

FIG. 5. Effect of growth in desferrioxamine on cell-surface transferrin receptors. Cells were grown for 24 h in RPMI 1640, 10% fetal bovine serum supplemented with various concentrations of desferrioxamine. The cells were pelleted and resuspended at a concentration of $10^7$ cells/ml in RPMI, 1% fetal bovine serum without desferrioxamine, and binding of diferric $^{125}$I-transferrin to surface receptors was measured as outlined under "Materials and Methods." The specific activity of the diferric $^{125}$I-transferrin was 19,500 cpm/pmol. Error bars indicate the range of the duplicates.

Since apotransferrin released from the cell after iron delivery is structurally intact, it will quickly bind any available iron in the extracellular medium. Some fraction of this "reconstituted" transferrin could then quite possibly rebind to cell surface transferrin receptors. In an earlier study, this question was examined with anti-transferrin antibody that would attach to transferrin in the medium and prevent its binding to the cell surface (4). The antibody would not, however, displace transferrin already bound to the surface. The failure of this antibody to increase the magnitude of release of transferrin from the cells was interpreted as evidence against rebinding of apotransferrin. This did not, however, eliminate the possibility that apotransferrin acquired iron before its release from the receptor and, therefore, remained attached. In this study, it was shown that chelation of extracellular iron enhanced the release of apotransferrin from the cells. This effect could reflect an inhibition of rebinding of apotransferrin due to the removal of available iron. Although, as previously discussed, the data demonstrating that desferrioxamine does not enhance the rate of iron uptake from $^{59}$Fe-transferrin tend to argue against this scenario, it certainly does not eliminate it. It is conceivable that a new "steady state" is reached due to the chelation of the extracellular iron and that the $^{59}$Fe uptake experiment may not reflect this change since it may require a longer time to achieve a new steady state. Other approaches may be necessary to satisfactorily resolve this intriguing problem.

If, however, the effect of the chelators involves an actual increase in the release of apotransferrin from within the cell, this may reflect changes in the plasma membrane. The release of apotransferrin involves the fusion of intracellular vesicles with the plasma membrane and is, therefore, a modified form of exocytosis. Membrane fusion is a complex process influenced by specific proteins as well as by the lipid composition.
Effect of Iron Chelators on Transferrin Receptors in K562 Cells

and fluidity of the membranes (20, 21). Cations, such as calcium, have been demonstrated to have important cellular effects by direct interaction with the membrane (22). It is possible that iron associated with the external surface of the plasma membrane decreases its ability to fuse with exocytic vesicles. Removal of this iron by a hydrophilic chelator could, then, affect fusion processes possibly by a change in membrane fluidity.

The other major observation was that cells grown in deferoxamine have increased numbers of transferrin receptors. This appeared to be due to chelation of intracellular iron in a hydrophilic environment since DTPA, which is confined to the extracellular space, and 2,2-bipyridine, which is confined to hydrophobic areas in the cell, had no such effect. The response was not due to a depletion of cellular iron since iron content as measured by atomic absorption spectroscopy was not reduced during the period of study. There was a parallel increase in surface and total receptors, and the experiments with cycloheximide suggested that this increase was due to an enhanced receptor synthetic rate.

Iron has been shown to play a crucial role in the regulation of transferrin receptor expression in HeLa cells (23). When grown in the presence of ferric ammonium citrate or medium supplemented with human transferrin, the receptor number in these cells declined significantly. This event did not appear to be due directly to the increase in cellular iron content produced by these manipulations, and the existence of a transient iron-containing compound or metabolite which regulated transferrin receptor expression was proposed.
Fig. 9. $^{59}$Fe uptake from $^{55}$Fe-transferrin in desferrioxamine-treated cells. Cells were divided into three groups, and the medium of two of these was supplemented with 20 μM desferrioxamine or 20 μM DTPA. After 24 h of growth, they were pelleted and resuspended at 10^7 cells/ml in RPMI, 1% fetal bovine serum. The cells were warmed to 37°C for 10 min, at which time dipheric $^{59}$Fe-transferrin was added to a final concentration of 0.1 μM. At the times indicated, duplicate 200-μl aliquots of cells were spun through oil and the pellets counted for cell-associated $^{59}$Fe. Dipheric $^{59}$Fe-transferrin specific activity = 730 cpm/pmole. ○, control; ●, desferrioxamine-treated cells; ▲, DTPA-treated cells.

differentiate and produce hemoglobin (26, 27). As cellular iron stores are mobilized to produce heme, a relative iron depletion may occur, leading to augmented transferrin receptor production. K562 cells can be stimulated to produce hemoglobin by heme and, in some strains, by butyrate (6). In these cells, however, induction of hemoglobin production by either of these agents results in a decline in transferrin receptor expression (26, 29). Since hemin could potentially serve as an alternative iron source, its effect on the receptors could in part be due to iron loading. The fact that butyrate produces a similar decline in receptor number, however, suggests that other changes in cellular physiology accompanying hemoglobin induction may affect transferrin receptor expression in K562 cells.

Determination of the mechanisms by which these multiple parameters change transferrin receptor expression will ultimately depend on the development of appropriate molecular probes. Once cDNA for the transferrin receptor gene is available, definitive studies can be done to determine whether desferrioxamine influences receptor number at the level of transcription or translation. In the meantime, further examination of the effects of desferrioxamine on intracellular iron metabolism may shed some light on the intermediate steps before receptor induction.

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