Transferrin Receptor Number, Synthesis, and Endocytosis during Erythropoietin-induced Maturation of Friend Virus-infected Erythroid Cells*

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Erythropoietin (EP) responsive Friend virus-infected erythroid cells had 200,000 steady-state binding sites for transferrin at 37 °C when isolated from the spleens of Friend virus-infected mice. Upon culture of these cells with EP, the synthesis of transferrin receptors increased 4- to 7-fold and the number of transferrin-binding sites per cell doubled after 24 h. However, the rate of uptake of $^{59}$Fe from transferrin remained constant at approximately 35,000 atoms of $^{59}$Fe per minute per cell during this period in culture. The amount of $^{125}$I-transferrin internalized during the steady-state binding did not change during this culture period while the transferrin bound to the surface increased 3-fold. At all stages of erythroid maturation, the maximum rate of endocytosis was determined to be 18,000 molecules of transferrin per minute per cell, and the interval that $^{125}$I-transferrin remains in the interior of the cell was calculated to be 6.9 min. After 48 h of culture with EP, the number of steady-state transferrin-binding sites was reduced in part due to the sequestration of surface receptors within the cell. The uptake of iron from transferrin was limited by the level of endocytosis of transferrin during the initial phase of culture and the number of transferrin receptors at the cell surface during the latter stages of erythroid maturation of these cells.

Transferrin delivers iron to cells by binding to a specific receptor (reviewed in 1, 2). Internalization of the transferrin by receptor-mediated endocytosis is thought to be a required step in the release of the iron from transferrin. In the view of many workers (3–19), the endocytotic vesicle is acidified to allow the release of iron from transferrin, and the apotransferrin, still attached to the receptor, is returned to the cell surface and released into the medium. The receptor then can bind another molecule of transferrin and complete another cycle. Some work indicates that transferrin binding is required before the receptor is endocytosed (14, 16) while a recent report suggests that the receptor recycles continually in the absence of ligand (19).

Transferrin receptors are found on all rapidly dividing cells (20). However, the regulation of transferrin receptors has been studied in more detail in developing erythroid cells since most of the iron in the body is used for the production of hemoglobin. Reticulocytes (6, 8, 21, 22), heterologous mixtures of erythroid cells from bone marrow or fetal liver (9, 10, 23), and continuous murine erythroleukemic cell lines (24–26) have been studied. These investigations provide substantial information on the change in the number of transferrin receptors, which increase during the earlier phase of erythroid development and decline as the cell becomes more similar to an erythrocyte. A unifying limitation of these investigations has been the lack of a pure, developmentally synchronized population of erythroid cells which respond to erythropoietin (EP), the normal regulator of erythropoiesis, with which to study the transferrin receptor.

A procedure has been developed in this laboratory to procure a large number of developmentally synchronized, relatively pure, immature erythroid cells from the spleens of mice infected with the anemia strain of Friend virus (FVA) (27, 28). In contrast with the erythroleukemia cell lines established from mice infected with Friend virus, these erythroid cells are fully responsive to EP. Upon culture with EP, greater than 85% of the cells synthesize hemoglobin and red cell specific membrane proteins, such as spectrin, band 3, and band 4.1. By 24 h in culture, nuclear condensation is evident, and enucleation is frequent after 48 h in culture (27). We have studied the number of transferrin-binding sites, the synthesis of transferrin receptors, the accumulation of iron, and the receptor-mediated endocytosis of transferrin during EP-induced erythroid maturation of these FVA-infected erythroid cells.

We find that the number of transferrin receptors and the synthesis of transferrin receptors increase to the levels expected from previous studies in fetal liver cells cultured with EP and murine erythroleukemia cell lines induced by chemicals. However, we find that the rate of iron accumulation does not correspond with the changes in transferrin-binding capacity. This is accounted for by the control of the cellular capacity to internalize transferrin through receptor-mediated endocytosis.

**EXPERIMENTAL PROCEDURES**

Materials—Mouse transferrin was purchased from Cappel/Worthington Biochemicals. Human transferrin and Pansorbin cells were from Calbiochem-Behring. Na$^{131}$I (100 mCi/ml) and $[^{35}]$S)methionine were purchased from Amersham, and $[^{59}]$FeCl$_3$ was purchased from ICN. A monoclonal antibody (RT1-208) against the mouse transferrin receptor (29) was a generous gift from Ian Trowbridge (Salk Institute, San Diego). Human EP was obtained from the National Institutes of Health (Cat 1, 1140 units/mg of protein). Murine erythroleukemia

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1 The abbreviations used are: EP, erythropoietin; FVA, anemia strain of Friend virus; BSA, bovine serum albumin; IMDM, Iscove's modified Dulbecco's medium; SDS, sodium dodecyl sulfate, MEL, murine erythroleukemia.
(MEL) cells, clone 746, were obtained from W. LeStourgeon (Vanderbilt University, Nashville).

**Cells—** EP responsive progenitor cells were obtained from mice infected with FVA by a method we have described previously (27, 28). Briefly, mice were infected with 10^6 spleen focus-forming units of FVA. After 2 weeks, the spleen had enlarged 10-fold with erythroid cells. The spleen was minced with scissors, and in some cases, the spleen cells were purified by velocity sedimentation at unit gravity over a continuous gradient of BSA. Rapidly sedimenting cells in fractions pooled for these experiments were determined to be greater than 95% erythroid cells by electron microscopy. Lymphocytes were the major contaminating cell type. Contamination by red cells did not stain positive for the presence of heme/hemoglobin using benzidine. Cells were cultured in IMDM (Gibco) containing 0.8% methylcellulose (Fisher, laboratory grade), 30% fetal bovine serum, 1% deionized BSA, 100 units/ml penicillin, 100 μg/ml of streptomycin, and 0.4 mM α-thioglycerol. EP was added to these cultures at a concentration of 0.2 units/ml. MEL cells were cultured in 20% fetal bovine serum in IMDM containing penicillin and streptomycin.

**Induction of Transferrin—** Mouse transferrin was subjected to gel filtration to remove aggregated material (G-200 Sephadex). The transferrin was then incubated in 0.25 M Tris-Cl, pH 8.0, 10 μM NaHCO3 containing 1% Triton X-100, 0.1% BSA, and 200 μg of α-thioglycerol. EP was added to these cultures at a concentration of 0.2 units/ml. MEL cells were cultured in 20% fetal bovine serum in IMDM containing penicillin and streptomycin.

**Binding Assays—** The binding of 125I-transferrin to intact erythroid cells at different stages of maturation was carried out in three different ways. In the first method, cells from the velocity sedimentation gradient, or from cultures, were washed in IMDM containing 0.8% methylcellulose (Fisher, laboratory grade), 30% fetal bovine serum, 1% deionized BSA, 100 units/ml penicillin, 100 μg/ml of streptomycin, and 0.4 mM α-thioglycerol. EP was added to these cultures at a concentration of 0.2 units/ml. MEL cells were cultured in 20% fetal bovine serum in IMDM containing penicillin and streptomycin.

**Assay of Transferrin Receptors in Disrupted Cells—** A method to determine soluble transferrin receptors based on the difference of solubility of transferrin and transferrin bound receptors in polyethylene glycol was modified from a previous description (31). Cells from the entire culture was centrifuged through dibutyl phthalate oil as described above. [59Fe]Heme was determined by extracting the cell pellet with cyclohexanone as described in previous reports (27, 28, 29). Of the radioactivity determined to be [59Fe]heme by this procedure, greater than 95% of the heme cochromatographed with hemoglobin on ion exchange chromatography.

**RESULTS**

**Binding of Transferrin to FVA-infected Erythroid Cells at Different Stages of Maturation—** The number of transferrin steady-state binding sites determined on FVA-infected erythroid cells increased after 6 h in culture with EP as seen in Fig. 1A. Binding of 125I-transferrin to these cells at 37°C was measured essentially in the same way as the binding of 125I-transferrin. [59Fe]-Transferrin or [59Fe]transferrin was incubated with cells in IMDM containing 2% BSA at 37°C. The cells were separated from the binding medium by centrifuging them through dibutyl phthalate oil. The centrifuge tubes were frozen at -80°C, and the tips were cut off and counted in a gamma counter. Nonspecific binding was 5-10% of the total.

**Binding Assays—** The binding of 125I-transferrin to intact erythroid cells was determined by incubation at 4°C and washing the cell pellet at 4°C. In the experiments shown, this residual surface binding was subtracted from the acid-resistant radioactivity.
saturable with 200 μg of transferrin/ml within 15 min (Fig. 6A). In the presence of EP, the level of transferrin-binding sites increased from 200,000 sites/cell to a maximum level of 400,000/cell during the period from 6 to 24 h in culture. During the period from 24 to 48 h in cultures with EP, the number of binding sites fell to 100,000 sites/cell. In the absence of EP, the number of transferrin-binding sites per cell declined after 4 h in culture to eventually reach a level of approximately 100,000 binding sites/cell. These cells did not proliferate but remained viable as determined by trypan blue dye exclusion. In Fig. 1B, the data for incorporation of 55Fe into heme/hemoglobin in parallel cultures is shown. 55Fe incorporation into heme increased greater than 10-fold during the interval from 12 to 24 h in culture and then slowly declined over the next 40 h. Thus, the increase in the number of binding sites for transferrin precedes the initiation of 55Fe incorporation into heme by approximately 6 h.

Cells taken directly from the animal and cells cultured in the presence of EP for 24, 48, and 72 h were incubated with varying concentrations of 125I-transferrin at 4 °C to determine the number of surface binding sites. The specific binding of transferrin was determined after 3 h, and the data was plotted by the method of Scatchard as shown in Fig. 2. The number of transferrin-binding sites on the cell surface were determined to be 78,000 on control cells, 186,000 on cells cultured 24 h, 90,000 on cells cultured 48 h, and 66,000 on cells cultured 72 h. A constant slope of the Scatchard plots for transferrin binding indicated that the association constant of 3–5 nM did not change as the erythroid cells matured in response to EP.

Total cellular transferrin receptors were determined by measuring the binding of 125I-transferrin to solubilized cell extracts, surface receptors were determined by binding at 4 °C, and steady-state binding of transferrin was determined at 37 °C as described under “Experimental Procedures.”

<table>
<thead>
<tr>
<th>Binding condition</th>
<th>Time of culture with</th>
<th>Molecules of 125I-transferrin bound/cell × 10⁻⁶</th>
</tr>
</thead>
</table>
| Cell surface      | Solubilized cell     | 266 ± 18  
| receptors         | extract, 60 min, 37 °C| 416 ± 20 198 ± 12 |
| Steady-state      | Intact cells, 3 h, 4 °C| 60 ± 11 188 ± 21 78 ± 8 |
| binding to        | Intact cells, 30 min, 37 °C| 223 ± 18 397 ± 24 111 ± 20 |
| receptors         |                      |                                             |

*Data expressed as the mean from three separate preparations of cells ± S.D.
ferrin to cells did not change in a constant way as these erythroid cells matured in culture. Surface receptors increased more than the total cellular receptors or the steady-state binding of 125I-transferrin as the cell progressed through the first 24 h of culture (3-fold compared to 1.6-fold and 1.8-fold, respectively). This phenomenon is the result of a shift in the distribution of receptors from an internal pool to the cell surface. Moreover, during the late erythroid maturation occurring between 24 and 48 h in cultures with EP, the steady-state binding of transferrin fell more sharply than either surface receptors or total receptors (3.6-fold compared to 2.4-fold and 2.1-fold, respectively). A sequestration of transferrin receptors within the cell from the normal flow of transferrin receptors from the cell surface through the cell and back to the surface may explain this observation.

Synthesis of Transferrin Receptors in Maturing, FVA-infected Erythroid Cells—Synthesis of transferrin receptors was studied under conditions that allowed it to be compared with the appearance and disappearance of transferrin-binding sites during erythroid maturation. Control cells and cells cultured with EP for 10, 22, 34, 46, and 70 h were labeled with [35S]methionine for 2 h as described under “Experimental Procedures.” Total protein synthesis per cell was determined (Fig. 4), and an aliquot containing 5 x 10^6 cpm of radioactive protein from each cell extract was immunoprecipitated with a monoclonal antibody directed against the mouse transferrin receptor. The immunoprecipitate was analyzed by SDS-polyacrylamide gel electrophoresis and fluorography. As shown in Fig. 3, two bands of molecular mass, 92 and 98 kDa, were immunoprecipitated by the monoclonal antibody against the transferrin receptor. The lower band of 35S-labeled material was decreased when the cells were labeled for longer periods or the labeled cells were chased with nonradioactive methionine for a short period (data not shown). Therefore, we assumed that the upper band represented the mature subunit of the transferrin receptor, and the lower band was an incompletely processed subunit of the transferrin receptor.

The synthesis of the transferrin receptor normalized to total protein synthesis increased between 2 and 12 h of culture in the presence of EP. The bands on the gel corresponding to the transferrin receptor were excised and counted by scintillation spectroscopy. These results are shown in Fig. 4 along with the total protein synthesis per cell and the synthesis of the transferrin receptor per cell calculated from these values.

The synthesis of transferrin receptors per cell increased more than 7-fold by 24 h in culture with EP and then declined sharply. This latter effect was due to the dramatic fall in total protein synthesis after 24 h of culture with EP.

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Fig. 3. Synthesis of transferrin receptors during erythroid maturation of FVA-infected cells. Cells from the animal and culture were labeled with [35S]methionine for 2 h in the presence of EP. Labeled protein extract (5 x 10^6 cpm) was immunoprecipitated with a monoclonal antibody against the mouse transferrin receptor, and the precipitate was analyzed by SDS-gel electrophoresis and fluorography as described under “Experimental Procedures.” The autoradiogram shown here was obtained by exposing the gel to film for 3 days at -80 °C. Molecular weight markers indicated were visualized by staining the gel with Coomassie Blue stain. Lane A is the same extract as lane D immunoprecipitated with a nonimmune control serum.

![Fig. 3. Synthesis of transferrin receptors during erythroid maturation of FVA-infected cells.](image)

Fig. 4. Transferrin receptor synthesis and total protein synthesis in FVA-infected erythroid cells during culture in the presence of EP. Bands corresponding to the transferrin receptor shown in the autoradiograph in Fig. 4 were excised and counted by scintillation spectroscopy (○). Total protein synthesis was determined as described under “Experimental Procedures” (△, data are the mean of triplicate determinations ± S.D.). The synthesis of the transferrin receptor per cell was calculated from the above data (○).
more closely examine the rate of $^{59}$Fe uptake from transferrin in maturing erythroid cells, the binding and release of $^{59}$Fe from $^{125}$I/$^{59}$Fe-labeled transferrin was studied. Fig. 6A illustrates the binding of transferrin to cells at 37°C. The binding of $^{125}$I-transferrin came to a steady state within 10-15 min, and the number of binding sites per cell corresponded very closely with that determined in Table I and Fig. 1A. As shown in Fig. 6B, the $^{59}$Fe uptake per cell did not correspond to the level of $^{125}$I-transferrin bound to the cell. In this experiment, control cells accumulated 35,800 atoms of $^{59}$Fe per minute per cell. Cells cultured 24 h with EP accumulated 33,300 atoms of $^{59}$Fe per minute per cell, and cells cultured for 48 h with EP accumulated 14,500 atoms/min/cell. In addition, cells cultured 24 h in the absence of EP accumulated 7,500 atoms/min/cell. Data from three other experiments are presented in Table II. From this table it can be seen that the number of iron atoms delivered per transferrin bound per min was reduced by half as the cell matured from control to cells cultured 24 h in the presence of EP. However, this rate increased to near control levels after cells were cultured 48 h with EP. The value of 0.07 atoms of $^{59}$Fe per minute per transferrin receptor occupied was determined in cells cultured for 24 h without EP. Apparent mean cycle time for transferrin in control cells was 11.6 min, in cells cultured 24 h with EP, 24.4 min, and in cells cultured 48 h with EP, 14.0 min.

The level of transferrin binding and accumulation of $^{59}$Fe from $^{125}$I/$^{59}$Fe-labeled transferrin in control FVA-infected, EP responsive cells were compared to those of a continuous erythroleukemia cell line derived from mice infected with the Friend virus, MEL cells (clone 745). As shown by the data in Table III, the erythroid cells purified from the spleens of mice infected with FVA had a greater number of steady-state transferrin-binding sites than the MEL cells. Moreover, the FVA-infected erythroid cells accumulated 4.5-fold more $^{59}$Fe per minute per cell than the MEL cells. MEL cells accumulated 0.06 atoms of $^{59}$Fe per minute per transferrin receptor occupied which is similar to the value determined for FVA-infected erythroid cells cultured in the presence or absence of EP for 24 h but only half of the value determined for freshly isolated FVA-infected erythroid cells and cells cultured for 48 h in the presence of EP.

**Distribution of Transferrin between the Cell Surface and Interior**—A high salt, acid wash was used to remove surface-bound transferrin from cells to which $^{125}$I-transferrin had been bound at steady-state levels. As shown in Fig. 7, the ratio of transferrin bound on the surface to transferrin in the cell interior (not accessible to the high salt, acid wash) was stable after the binding reached a steady state. However, this ratio was different in each stage of erythroid maturation. In Fig. 7A, it can be seen that 60% of total transferrin bound was in the interior of the control cells. After 24 and 48 h of culture with EP, the cell interior contained 50% of total transferrin bound at a steady-state level. Control cells were determined to bind 12,000 molecules of transferrin per cell interior at steady state compared with 11,000 and 6,000 molecules for cells cultured 24 and 48 h, respectively, with EP. The number of molecules bound to the surface during the steady state was comparable.
were resuspended in the same media at 6 × 10⁶ cells/ml, and 200 pg of

Table 11, a rate of the release of 125I-transferrin from cells to which the ligand

was centrifuged through dibutyl pthalate oil and radioactivity was deter-

mined. After another wash, the cells '251/5Ve-transferrin was added for 1 h at 37 °C. The cells were then

had been cultured with EP for up to

minutes per transferrin in the cell interior. As can be seen in

similar experiments in which ⁵⁹Fe accumulation and ¹²⁵I-

transferrin internalization at steady-state binding were com-

pared were used to calculate the rate of ⁵⁹Fe delivered per

minute per transferrin in the cell interior. As can be seen in

Table II, a rate of 0.28 atoms of ⁵⁹Fe per minute per internal

transferrin was determined for control cells and cells which

had been cultured with EP for up to 48 h. The apparent time

for a transferrin molecule to travel through the cell interior to release iron was calculated to be 6.9 min based on the

assumption that diferric transferrin delivered two atoms of iron per transferrin in the cell interior as the cell-bound

transferrin at a steady state.

Release of ¹²⁵I-Transferrin from FVA-infected Erythroid Cells—In order to investigate the possibility that the ¹²⁵I-

transferrin, bound to cells of different stages of erythroid maturation, was endocytosed and released in the same way,

the release of ¹²⁵I-transferrin from cells to which the ligand

...iron accumulation and transferrin binding in FVA-infected erythroid
cells...
and 48 h released the bound 125I-transferrin to a lesser extent than control cells in medium containing no added transferrin. Control cells released virtually all the bound 125I-transferrin during the first 15 min of incubation. Cells cultured with EP released approximately 50% of initially bound 125I-transferrin in a similar way as control cells during the first 5 min, but the remaining radioactivity was very slowly released in the absence of added transferrin. The addition of 100 μg of unlabeled transferrin per milliliter increased the fraction of 125I-transferrin released from the cells cultured with EP as shown in Fig. 8F. Under this condition, all the cells of varying erythroid maturation released virtually all the bound 125I-transferrin in a similar manner within 15 min.

Movement of Transferrin from the Cell Surface through the Cell into the Medium—The high salt, pH 2.5, wash method was used to quantitate the disappearance of 125I-transferrin from the cell surface and transitory appearance in the cell interior. In addition, the release of 125I-transferrin into the medium was determined. Fig. 9 shows the data obtained from two separate experiments in which the transferrin on the surface of the cell (Fig. 9, A and B), in the cell interior (Fig. 9, B and E), and released into the medium (Fig. 9, C and F) were monitored in the presence of saturating levels of transferrin (Fig. 9, D-F) and the absence of added transferrin (Fig. 9, A-C). Control cells and cells cultured with EP for 24 and 48 h were incubated with 125I-transferrin at 4°C for 2 h. After washing away unbound transferrin, the cells were resuspended in medium containing 100 μg of transferrin/ml or no transferrin and placed in a 37°C water bath at zero time. In the absence of added transferrin, the rate at which transferrin was cleared from the surface and released into the medium was slower than in the presence of added transferrin; the initial rate of disappearance from the surface of the cell was approximately 7,000 molecules/min/cell in the absence of transferrin and approximately 18,000 molecules/min/cell in the presence of added transferrin. This rate of disappearance from the cell surface was almost identical in control cells and cells which had been cultured in the presence of EP even though the amount of transferrin on the surface of the cells cultured with EP for 24 h was more than 2-fold greater than control levels.

Examination of the amount of 125I-transferrin in the cell interior showed that the endocytosis of 125I-transferrin proceeded immediately after the cells were warmed to 37°C. 125I-Transferrin was accumulated rapidly in the interior of the cell for the first 5 min in every experiment. In cells warmed in the presence of added transferrin, the internal 125I-transferrin rapidly declined after 5 min. This same result was seen in control cells warmed in the absence of added transferrin. However, in cells cultured with EP for 24 and 48 h, the internal 125I-transferrin reached a plateau after being warmed for 5 min in medium lacking added transferrin (shown in Fig. 9B). A corresponding lower release of transferrin into the medium from these cells in the absence of added transferrin in the medium was also observed (Fig. 9C).

The surface-bound 125I-transferrin was virtually completely processed by control cells and cells cultured for 48 h with EP by 10–15 min after warming the cells. Cells cultured with EP for 24 h required longer than 15 min for this event. These times are similar to the mean cycles time calculated for the processing of transferrin bound at a steady-state from the data in Table II.

The above experiment was repeated using 59Fe-transferrin to verify that the transferrin released into the medium had passed through the cell and delivered 59Fe to the cell. 59Fe-Transferrin initially bound to the surface of the cell was cleared from the surface in the same manner as 125I-transferrin (Fig. 10, A and D). Added transferrin in the medium facilitated the clearing of 59Fe-transferrin from the surface and the accumulation of 59Fe within the cell (Fig. 10, D and F). Seventy to 90% of 59Fe which was lost from the surface was accumulated within the interior of the cell. Therefore, the effect of transferrin added in the medium was directly on the process of endocytosis of 125I-transferrin and did not simply displace 125I-transferrin bound to surface receptors.

**DISCUSSION**

It is now well documented in most systems that transferrin delivers iron to the cell through a process that involves endocytosis of the diferric transferrin-receptor complex, acidification of the endosome which results in the release of iron, and the subsequent return of the transferrin-receptor complex to the surface of the cell (3-19). This report confirms this mechanism in the FVA-infected erythroid cell. The precise correlation between the rate of uptake of 59Fe and the internalization of transferrin in this study is of interest since the number of binding sites for transferrin on cells of increasing erythroid maturation are changing independently of the proportion of bound transferrin internalized and the rate of iron uptake. These results indicate that the rate of endocytosis of the transferrin-receptor complex is a control point in the accumulation of iron from transferrin. A previous study suggests that the number of transferrin receptors is the primary limiting and regulatory factor in the accumulation of iron in erythroid cells (23). However, the experiments reported here demonstrate a cell system where the accumulation of iron can be limited by the process of endocytosis of the transferrin-receptor complex.

Freshly prepared FVA-infected erythroid cells accumulated 59Fe from transferrin at a rate 2-fold that expected when
Erythropoietin Effect on Transferrin Receptors

Fig. 10. Endocytosis of $^{59}$Fe-transferrin and accumulation of $^{59}$Fe in FVA-infected erythroid cells. Surface transferrin receptors were found with $^{59}$Fe-transferrin to saturation at 4°C on control cells (○) and cells cultured for 24 h (□) and 48 h (△) in the presence of EP. After unbound ligand was washed away, the cells were resuspended in medium containing no added transferrin (A-C) and medium containing 100 μg of unlabeled transferrin (D-F). The cells were incubated for the indicated interval at 37°C and pelleted at 4°C. The medium was collected and counted (C and F). The cells were then washed with a high salt, pH 2.5, wash. Acid sensitive counts (A and D) are considered surface-bound transferrin, while acid stable counts (B and E) are considered in the interior of the cell.

FIG. 10. Endocytosis of $^{59}$Fe-transferrin and accumulation of $^{59}$Fe in FVA-infected erythroid cells. Surface transferrin receptors were found with $^{59}$Fe-transferrin to saturation at 4°C on control cells (○) and cells cultured for 24 h (□) and 48 h (△) in the presence of EP. After unbound ligand was washed away, the cells were resuspended in medium containing no added transferrin (A-C) and medium containing 100 μg of unlabeled transferrin (D-F). The cells were incubated for the indicated interval at 37°C and pelleted at 4°C. The medium was collected and counted (C and F). The cells were then washed with a high salt, pH 2.5, wash. Acid sensitive counts (A and D) are considered surface-bound transferrin, while acid stable counts (B and E) are considered in the interior of the cell.

The mechanism by which the number of steady-state binding sites for transferrin was reduced more in proportion than total receptors or surface receptors during the period of culture from 24 to 48 h is not known. A study by Hunt and coworkers (25) reports a similar finding in K-562 cells treated with hemin. However, other similar studies in K-562 cells fail to detect this phenomenon (31, 34).

In agreement with previous studies, the number of surface transferrin receptors in the FVA-infected cells are but a minority of the total cellular receptors (3, 12, 15, 25). As these cells undergo erythroid maturation, the number of surface...
receptors increases in relation to the total receptors. However, this is a developmental event that was not seen before 12 h and is distinct from the immediate event that occurs in fibroblasts stimulated with growth factors where an increase in the ratio of surface transferrin receptors to total transferrin receptors occurs within minutes (35).

We have confirmed the observation of Klausner et al. (12) in the K-562 cell line that the presence of externally added ligand is necessary for all transferrin bound to the cell to be endocytosed and released from the cell. Our data suggest that the site of this effect is the return of apo-transferrin to the cell surface since [125I]-transferrin accumulates in the cell interior only when transferrin is omitted from the medium (Fig. 9B). This interpretation is consistent with the conclusion of Klausner and coworkers (12). However, we observed this effect only in cells cultured in the presence of EP which had elevated levels of transferrin receptors (similar to the K-562 cell line).

It is well-documented that the number of transferrin receptors on a wide variety of different cell types increases as a result of stimulation of cell growth (20). New synthesis of transferrin receptors in the FVA-infected erythroid cells above that required for iron accumulation may be the result of mitogenic stimulation of these cells by EP (EP is required for growth of these cells as well as erythroid maturation). The synthesis of transferrin receptors is regulated upward by agents which chelate iron in the medium (25, 31, 34, 36, 37) and downward by the addition of heme and iron salts to the medium (31, 34, 37). This effect may be due to the regulation of synthesis through the intracellular pools of either heme or iron. The induction of transferrin receptor synthesis in FVA-infected erythroid cells stimulated with EP may reflect early changes in the pools of iron or heme prior to hemoglobin synthesis. Changes in transferrin receptor synthesis due to iron chelation can occur as early as 6 h (24) which is similar to the time after treatment with EP in which an increase in the number of transferrin receptors in FVA-infected erythroid cells is seen.

The increase in transferrin receptors in the FVA-infected erythroid cell precedes the incorporation of 59Fe into heme/hemoglobin (Fig. 1) and cell division (27) but coincides with the induction of β-major globin gene transcription (38). This coincidence may suggest a linkage of these two events through as yet unknown metabolic events. Until now, only an increase in the uptake of 42Ca2+ has been documented to occur earlier than the increase in transferrin receptors in the FVA-infected cell after stimulation with EP (30).

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REFERENCES