Molecular mechanisms regulating the synthesis of transferrin receptors and ferritin in human erythroleukemic cell lines

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The effects of either iron salts or iron chelators on the biosynthesis of transferrin receptors in human erythroleukemic lines was investigated. Addition of these compounds induced a rapid and marked decrease (iron salts) or increase (iron chelators) in the level of transferrin receptors synthesis. Both phenomena were inhibited by actinomycin D. In contrast, the increase in the synthesis of ferritin induced in these cells by addition of iron salts was not inhibited by actinomycin D. These results suggest that iron salts modulate the synthesis of transferrin receptors and ferritin via different molecular mechanisms, of transcriptional and translation type, respectively.

Iron control mechanism *Iron* *Iron chelator* *Transferrin receptor* *Ferritin* *Erythroleukemic cell*

1. INTRODUCTION

The amount of iron entering a cell may be partially regulated by modulation of the number of transferrin (Trf) receptors. This number is apparently controlled by a feedback mechanism, modulated by the intracellular level of both iron and heme. In this regard, the Trf binding capacity of human leukemic lines [1–4] as well as of Hela cells [5] grown in the presence of heme is markedly reduced. Similarly, Hela cells [6], human fibroblasts [7] and human leukemic lines [1,4] grown in the presence of iron salts (ferric ammonium citrate or ferric ammonium sulfate) showed a concentration- and time-dependent decrease of Trf binding capacity. The decline reflects a reduction in the rate of synthesis of receptors, which is not associated with a significant modification of their half-life [4]. In contrast, cells grown in the presence of iron chelators (e.g., picolinic acid, α,α_1-dipyridyl [4,8] or desferrioxamine) showed a marked increase in Trf binding capacity, via an increase in the rate of receptor synthesis [9,10].

These studies have been carried out in an attempt to elucidate the molecular mechanisms underlying the modulation of Trf receptors by iron.

2. MATERIALS AND METHODS

2.1. *Reagents*

Picolinic acid, ferric ammonium citrate, purified human transferrin and actinomycin D were obtained from Sigma (St Louis, USA), purified rabbit immunoglobulin anti-human spleen ferritin from Bio Yeda (Israel), [^{35}S]methionine (900 Ci/mmol) from New England Nuclear (Boston, USA), and activated Sepharose 4B from Pharmacia (Uppsala, Sweden).

2.2. *Cells*

K562 [3] and HEL [4] erythroleukemic lines were grown in RPMI 1640 medium (Boehringer,

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Mannheim) containing 10% (v/v) fetal calf serum (FCS) (Boehringer).

2.3. *Trf receptors biosynthesis*

10^7 cells, grown under different culture conditions, were incubated for 30 min at 37°C in RPMI-1640 methionine-free medium (Bio-Pro, FRG) containing 5% (v/v) dialysed FCS and 100 μCi [^{35}S]methionine. Trf receptors were purified by affinity chromatography on Sepharose-transferrin and analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as in [4].

2.4. *Ferritin concentration*

The intracellular concentration of ferritin was evaluated by a radioimmunoassay (Hoechst-Behring Institute, Frankfurt).

2.5. *Ferritin biosynthesis*

Ferritin biosynthesis was evaluated on cells labelled with [^{35}S]methionine as above. Metabolically labelled cells lysed by freeze-thawing (× 3) were centrifuged at 15000 × *g* for 30 min. Supernatants were immunoprecipitated with rabbit anti-human spleen ferritin (Bio-Yada) and protein A-Sepharose (Pharmacia). The protein was reduced by heating in sample buffer with 2-mercaptoethanol (2-ME) and SDS for 5 min at 100°C. The precipitates were analyzed by SDS-PAGE. The autoradiographs were scanned in an LKB spectrophotometer.

3. RESULTS

3.1. *Effect of iron chelators on Trf receptor synthesis*

Cells incubated for different times with iron salts (ferric ammonium citrate) and iron chelators (picolinic acid) were pulsed with [^{35}S]methionine for 30 min. Trf receptors present in cell extracts were purified by affinity chromatography on Sepharose-Trf and analyzed by SDS-PAGE (fig.1).

Cells incubated for 3 h in the presence of 50 μg/ml of ferric ammonium citrate exhibited a 2-fold decrease of the level of Trf receptor synthesis (not shown). In contrast, cells incubated with picolinic acid showed an increase of transferrin receptor synthesis as early as 1 h after addition

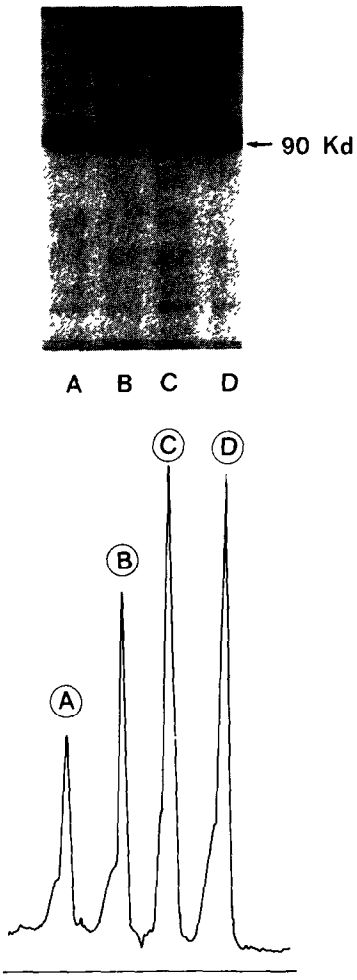

Fig.1. Induction of transferrin receptor biosynthesis in picolinic acid-treated cells. Lanes: (A) control; (B,C,D) cells grown for 2, 4 and 6 h, respectively, in the presence of the chelator. Top: autoradiography of SDS-PAGE of Trf receptors. Bottom: densitometric scanning of the gel shown above.

of the chelator (fig.1). The rate of synthesis reaches a peak level (i.e., 3-fold over control values) at 6 h after treatment with this agent (fig.1). The effect of picolinic acid could be completely abolished by saturating the chelator with iron.

In an attempt to elucidate the mechanism(s) underlying these phenomena, it seemed of interest to investigate the effect of actinomycin D addition. This compound completely inhibited the effect of both ferric ammonium citrate and picolinic acid on Trf-receptor biosynthesis: at a concentration of

0.2 μg/ml it suppressed incorporation of [^{3}H]uridine and [^{3}H]leucine into CH_3COOH-precipitable material by 99% and 5%, respectively (figs 2,3).

In contrast, actinomycin D did not inhibit ferritin induction in both K562 and HEL cells. Thus, K562 cells treated with actinomycin D or ferric ammonium citrate show an increase of ferritin content, over control cultures, as evaluated by a radioimmunoassay. A combination of these two agents resulted in an additive increase of ferritin accumulation (fig.4). Further experiments were carried out by ferritin immunoprecipitation on HEL cells labelled with [^{35}S]methionine: these showed that actinomycin D did not inhibit the stimulatory effect of iron salts on ferritin synthesis, but enhanced it (fig.5).

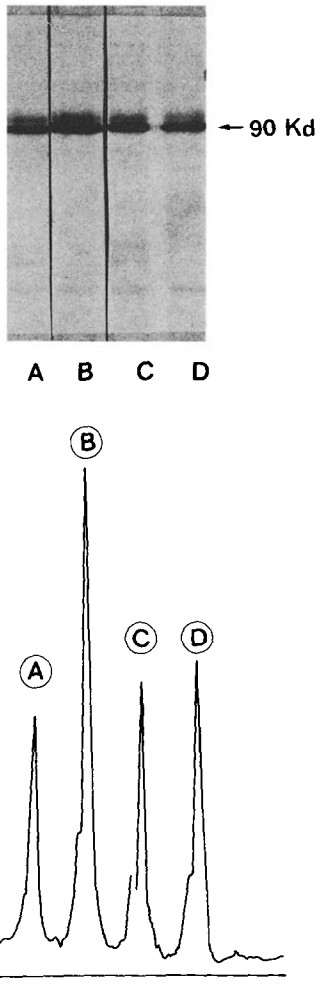

Fig.2. Effect of actinomycin D on the induction of transferrin receptor biosynthesis in picolinic acid-treated cells. Lanes: (A) control; (B) cells grown for 4 h in the presence of picolinic acid; (C) cells grown in the presence of actinomycin D (0.5 μg/ml); (D) cells grown for 4 h in the presence of both picolinic acid and actinomycin D. Top: autoradiography of SDS-PAGE of Trf receptors. Bottom: densitometric scanning of the gel shown above.

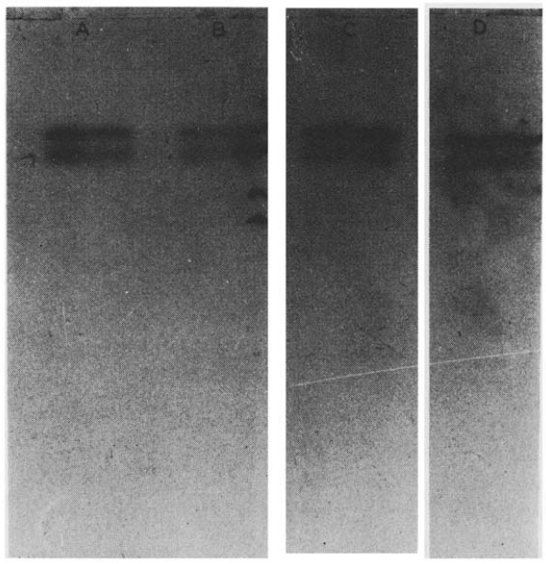

Fig.3. Effects of actinomycin D on the inhibition of transferrin receptor biosynthesis induced by iron. Lanes: (A) control; (B) cells grown for 4 h in the presence of ferric ammonium citrate (100 μg/ml); (C) cells grown for 4 h in the presence of actinomycin D (0.5 μg/ml); (D) cells grown for 4 h in the presence of both ferric ammonium citrate (100 μg/ml) and actinomycin D (0.5 μg/ml).

4. DISCUSSION

Previous studies showed that the enhancement of Trf receptor biosynthesis induced by iron chelators can be accounted for by an increase in the level of mRNA coding for transferrin receptor [10]. However, since these studies were performed in a cell-free translation system, a specific inhibition of Trf receptor mRNA degradation or activation of pre-existing message could not be ruled out.

The present study provides evidence that iron modulates the rate of synthesis of Trf receptors in both K562 and HEL cells through a transcriptional mechanism. This conclusion is supported by the

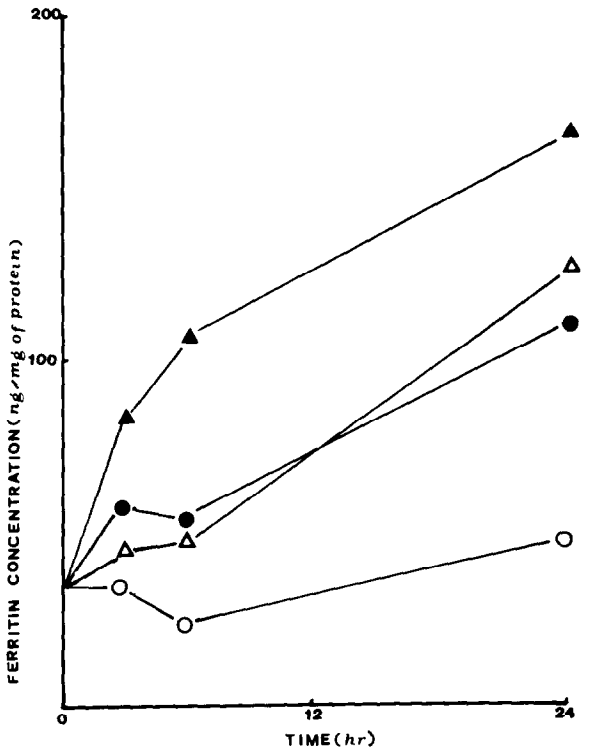

Fig.4. Effect of actinomycin D and iron on the accumulation of ferritin into K562 cells. K562 cells were grown in medium containing, respectively, either no additive (○), 0.2 μg/ml actinomycin D (△), 50 μg/ml ferric ammonium citrate (●) and both ferric ammonium citrate and actinomycin D (▲). Cells were removed at various tissues and ferritin concentration was determined.

observation that actinomycin D completely inhibited the inhibition and enhancement of Trf receptor biosynthesis induced by iron salts and iron chelators, respectively.

In contrast, the stimulation of the synthesis of ferritin by iron both in K562 and HEL cells seems to be dependent upon a translational mechanism, since actinomycin D has little or no effect on this phenomenon. Our observations confirm previous studies showing that: (i) actinomycin D has no effect on the induction of ferritin by iron [13,14]; (ii) ferritin mRNA is abundant in both control and iron-induced cells [14,15]; and (iii) in unstimulated cells it is present mainly in the mRNA fraction [14]. Thus, a currently accepted model [13] suggests that ferritin mRNA, present in the cytoplasm of uninduced cells in an inactive form, becomes

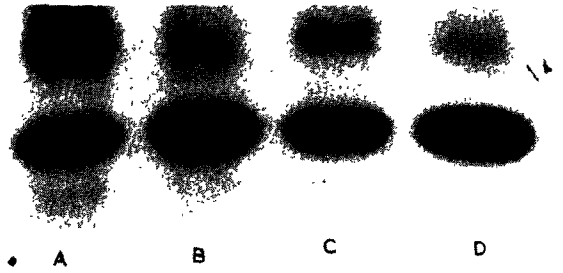

Fig.5. Effect of actinomycin D and iron on the biosynthesis of ferritin into HEL cells. The figure shows the autoradiography of polyacrylamide gel electrophoresis of ferritin immunoprecipitated using an antiserum to human spleen ferritin from HEL cells labeled with [^{35}S]methionine for 60 min. Lanes: (A) control; (B) cells grown for 6 h in the presence of 100 μg/ml ferric ammonium citrate; (C) cells grown for 6 h in the presence of actinomycin D (0.25 μg/ml); (D) cells grown for 6 h in the presence of both ferric ammonium citrate and actinomycin D.

translatable upon entry of iron into the cytoplasm, via an unknown mechanism, thus leading to an accumulation of ferritin [16].

In conclusion, our results suggest that in erythroleukemia lines the intracellular iron levels inversely modulate in the synthesis of both transferrin receptors and ferritin, via a coordinate molecular mechanism(s) action at the transcriptional and translation level, respectively.

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