Specific Globin mRNAs in Human Erythroleukemia (K562) Cells

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Specific globin mRNA accumulation was quantitated in several lines of K562 cells in the absence and the presence of hemin. Using specific cloned DNA probes, the amounts of $\xi$, $\alpha$, $\epsilon$ and $\gamma$ mRNAs were shown to be increased 2-3-fold in the presence of 20 $\mu$M hemin. No $\delta$ or $\beta$-globin mRNAs were detectable in any of the lines. In one line, Bos, there was a marked decrease in $\epsilon$-globin mRNA, which increased with hemin, although still to much lower levels than in the other lines. The decreased $\epsilon$-globin mRNA accumulation in Bos is shown to be due to decreased $\epsilon$-globin gene transcription.

The K562 cells were developed from the cells of a patient with chronic myelogenous leukemia. These cells grow continuously in culture, contain erythroid markers, and can be induced to differentiate along the erythroid pathway. In addition, they respond to a variety of inducers, including hemin, with increased globin and hemoglobin production. Previous studies have indicated that $\epsilon$ and $\xi$ globins (the embryonic $\beta$- and $\alpha$-like globins, respectively) are synthesized in these cells, in addition to $\gamma$ or fetal globin and $\alpha$-globin (an adult type of globin). No $\delta$ and $\beta$-globin proteins have been detected in these cells. Analysis of globin gene content in these cells by restriction mapping indicates that the $\delta$- and $\beta$-globin genes are not deleted despite their apparent lack of activity. Previous studies have indicated that $\epsilon$, $\gamma$, $\xi$, and $\alpha$ mRNAs are present in these cells and are increased in amount in the presence of hemin. However, in these latter studies, only $\alpha$, $\beta$, and $\gamma$ cDNA probes were used, and it was not possible to distinguish $\epsilon$ and $\gamma$ mRNAs, or $\xi$ and $\alpha$ mRNAs.

In the studies reported here, we have used purified cloned $\alpha$, $\beta$, $\xi$, $\epsilon$, and $\gamma$ probes to quantify the amounts of each of these mRNAs in five different K562 cell lines. We have shown that, in these lines, $\xi$, $\alpha$, $\epsilon$, and $\gamma$-globin mRNAs are synthesized in cells without hemin and are increased 2-3-fold in the presence of hemin. No $\delta$ or $\beta$ mRNAs are detectable in any of the lines. In one line, Bos, the baseline production of $\epsilon$ mRNA is markedly reduced, and although there is a detectable increase in $\epsilon$ mRNA in the presence of hemin, much less $\epsilon$ mRNA is accumulated than in the other lines. The decreased $\epsilon$ mRNA accumulation in Bos is shown to be due to decreased $\epsilon$-globin mRNA transcription.

MATERIALS AND METHODS

Cell Growth

Cells were cultured in RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 8.3% fetal calf serum. The cells were induced to increase hemoglobin synthesis by hemin. Bovine hemin was prepared by dissolving hemin in 1 N NaOH, neutralized with an equal volume of 1 M Tris, pH 7.0, and diluted from a 20-mM stock solution. Cells were assessed for their viability in a hemacytometer after staining with 0.4% trypan blue. The percent of hemoglobinized cells was determined by staining with 0.2% benzidine hydrochloride and 0.3% hydrogen peroxide.

Cell Lines Studied

Five different cell lines were used: K562 from G. Klein; K562 #2 derived from a clone of K562 Klein isolated in this laboratory; K562 from A. Dean; K562 Bos; and K562 #9 derived from Bos. Both K562 Bos and #9 were obtained from B. Alter.

RNA Isolation

RNA was isolated basically as described. Between 0.17-1.6 x 10^6 cells were lysed in 100 mM NaCl, 10 mM sodium citrate, 1 mM EDTA, 1% SDS, pH 5.1, and cesium chloride was immediately added to a final concentration of 1 g/ml and dissolved as described. The cesium chloride lysate was layered over 3 ml of a 1.35 g/ml solution of cesium chloride in 10 mM Tris, pH 7.4, 1 mM EDTA. The cesium chloride step gradient was centrifuged at 150,000 g for 16 hr. After centrifugation, the RNA pellet was dissolved and reprecipitated, and the concentration of RNA measured at 260 nm.

RNA Blotting

RNA blotting was done basically as described. RNA samples were prepared in a solution containing 50% formamide, 2.2 M formaldehyde, 20 mM morpholinopropanesulfonic acid (MOPS), 5 mM sodium acetate, 1 mM EDTA, pH 7.0. The sample was heated to 65°C for 10 min, placed on ice for 10 min, and prepared for loading by adding 1/5 volume 25% Ficoll, 0.05% Bromophenol blue, 10 mM Tris, pH 7.0, 1 mM EDTA, as described. In most experiments, 5-10 $\mu$g of total RNA were electrophoresed in 1.5% agarose and 2.2 M formaldehyde, 20 mM MOPS, 5 mM sodium acetate, 1 mM EDTA, pH 7.0, and 1 $\mu$g/ml of ethidium bromide for 4 hr. The RNA was transferred from gels to nitrocellulose paper (blotting) after equilibration in an equal volume of 20 x SSC (SSC = 0.15 M sodium chloride, 0.015 M sodium citrate) for 60 min. The transfer to 0.45 $\mu$m Schleicher and Schuell nitrocellulose filters was performed using 10 x SSC for 16 hr. The nitrocellulose filters were subsequently baked at 80°C for 5 hr under vacuum.

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Hybridization was done as described.5 The filters were prehybridized in 50% formamide, 10% dextrose sulfate, 1% each of bovine serum albumin, Ficoll, and polyvinylpolyethylene (PVP), 5 x SSC, and 0.1% SDS for 5 hr at 42°C. The filters were then hybridized with 2-5 x 10^6 counts of nick-translated probe/ml in 50% formamide, 10% dextrose sulfate, 2 x SSC, 100 g/ml sheared salmon sperm DNA, 0.1% SDS, and 1 mM EDTA for 16 hr at 42°C. After hybridization, the filters were soaked for 20 min in 2 x SSC, and then subsequently in lower concentrations of SSC to a final wash of 0.1 x SSC at 68°C. The rinsed filters were then radioautographed in cassettes using x-ray film and analyzed for band intensity. In addition, quantitation of counts in bands was performed by cutting regions of the nitrocellulose filters corresponding to bands and to other regions for background counts. These filter fragments were then added to aquasol, and counted in a Beckman liquid scintillation counter for sufficient time to accumulate at least 1,000 counts for a standard error of counting of 3% or less.

**Globin DNA Probes**

The plasmids used included: (1) pBR 14, containing the 5’ end of the ε-gene in a Bam HI 0.7 kilobase (kb) fragment in pBR 322;8 (2) JW 151, consisting of a γ cDNA cloned into pMB 9 (γ cDNA);11 (3) pBR 15, containing the pseudo γ-gene and flanking sequences in a 4.5-kb Bam HI Eco RI fragment in pBR 322;12 (4) pBR 15, containing the α-globin gene and flanking sequences in an Eco RI-Bgl II fragment in pBR 322;11 (5) β-globin gene, 4.4 kb in size (β 4.4);14 (6) β-gene, 2.3 kb in size (β 2.3). In most cases, globin DNA inserts were isolated from the plasmids, nick-translated as described previously,16 and hybridized to 10S RNA as described.

**Nuclear Transcription**

Nuclei from K562 cells were prepared primarily as described.16 Nuclei from 10 cells were incubated in 200 μl 30% glycerol, 2.5 mM diethiothreitol, 1 mM MgCl2, 70 mM KCl, 0.25 mM GTP and CTP, 0.5 mM adenosine triphosphate (ATP), and 250 μCi UTP (α32P 450 Ci/m mole) at 26°C for 10 min.16 Hybridizations with the RNA transcripts were performed as described for RNA blots. The amount of RNA synthesized was assayed by Dot blots with 0.1 μg plasmid/ dot.16

**Quantitation of Globin Synthesis**

K562 cells were cultured with 50 μM hemin for 4 days. The cells from 8 ml culture were centrifuged and resuspended in 1 ml of Dulbecco’s modification of Eagle’s medium lacking leucine, 10% fetal calf serum previously dialyzed against Kreb’s Ringer’s phosphate buffer at pH 7.4, and 100 μCi 1H-leucine (New England Nuclear Corporation, Boston, MA, >100 Ci/m mole). Labeling was carried out for 4 hr in a 37°C incubator, after which the cells were washed and lysed in 0.1 M sodium phosphate containing 0.1% NP40 and carrier nonradioactive fetal and adult hemoglobin. Carrier nonradioactive hemoglobins were added. The lysates were electrophoresed on polyacrylamide slab gels containing acetic acid, urea, and Trition X-100, and the gels were stained and fluorographed. The X-ray films were scanned in a Gilford spectrophotometer at 550 nm, and the areas under the peaks integrated by planimetry. Details of these methods have been described previously.4

**RESULTS**

**Accumulation of Specific Globin RNAs**

In 13 experiments with uninduced K562 #2 cells, and those after 4 days of treatment with 20 μM hemin, the RNA recovery was 10.5 ± 1.9 pg/cell and 10.9 ± 2.5 pg/cell, respectively. Similar recoveries of RNA/cell were present in K562 Bos, K562 #9, and K562 #2 cells. Between 1 and 20 μg of RNA were analyzed in individual experiments using different probes. It was shown that the amount of counts bound to filters was linear with the amounts of RNA added in this range of RNA. The baseline amounts of ε-, γ-, α-, and α-globin mRNAs were quantitated in K562 #2, Bos, and #9 in several experiments. RNA from these lines were also probed with δ- and β-globin probes, but were found to contain no detectable δ- or β-globin mRNA.

In several experiments, it could be shown that K562 #2, Bos, and K562 #9 produced significant amounts of γ-, α-, and α-globin mRNA in the absence of added hemin. The amounts of these mRNAs were comparable in several experiments (Tables 1 and 2, Figs. 1 and 2). However, the amount of ε-globin mRNA present in the Bos line was significantly lower than that in the other two cell lines (Tables 1 and 2, Fig. 1). On addition of 20 μM hemin, there was a consistent 2-3-fold increase in the amount of γ-, α-, and ε-globin mRNA present even with hemin; the fold increases were largest in the Bos line.

**Table 1. Ratio of Globin mRNA Content in Hemin-Treated and Control K562 Cells**

<table>
<thead>
<tr>
<th></th>
<th>Clone #2</th>
<th>Clone #9</th>
<th>Bos</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε</td>
<td>3.2 (1.1)</td>
<td>3.2 (0.6)</td>
<td>*</td>
</tr>
<tr>
<td>γ</td>
<td>2.1 (0.8)</td>
<td>2.7 (0.2)</td>
<td>1.8 (1.0)</td>
</tr>
<tr>
<td>α</td>
<td>2.0 (0.7)</td>
<td>1.0 (0.3)</td>
<td>2.8 (0.4)</td>
</tr>
<tr>
<td>δ</td>
<td>2.3 (0.9)</td>
<td>1.8 (0.4)</td>
<td>4.5 (0.3)</td>
</tr>
</tbody>
</table>

Six experiments were done with clone #2 cells; two experiments were done with clone #9 and Bos cells. All experimental points were performed in duplicate, and the results were averaged to obtain a given value. The means and standard deviations (in parentheses) are shown.

*The cpms for control and hemin-treated cells in Bos with the ε probe were too low to permit quantitation.

**Table 2. Globin mRNA Accumulation in K562 Cells**

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<table>
<thead>
<tr>
<th></th>
<th>ε</th>
<th>γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clone #2</td>
<td>413</td>
<td>1,186</td>
</tr>
<tr>
<td>Bos</td>
<td>5</td>
<td>28</td>
</tr>
<tr>
<td>Clone #9</td>
<td>144</td>
<td>520</td>
</tr>
<tr>
<td>α</td>
<td>2.3 (0.9)</td>
<td>1.8 (0.4)</td>
</tr>
<tr>
<td>δ</td>
<td>2.3 (0.9)</td>
<td>1.8 (0.4)</td>
</tr>
</tbody>
</table>
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Results of one of two experiments in which all three cell lines (#2, Bos, #9) were compared using the same probes on the same blots. All experimental points were performed in duplicate. The numbers in minus (−) and plus (+) columns are the mean cpms of labeled probes hybridizing to RNA blots in the absence (−) and presence (+) of hemin. The +/− is the ratio of hemin to control RNA of the duplicate values.
increase of \( \varepsilon \)-globin mRNA with hemin could not be quantitated because of the extremely low amount of \( \varepsilon \) mRNA present in uninduced cells (Tables 1 and 2, Fig. 1A).

K562 #9 differed from K562 #2 in the lower inducibility of \( \alpha \)-globin mRNA with hemin (0.05 < \( p \) > 0.1) (Table 1, Fig. 2B). No differences in \( \epsilon, \gamma, \) and \( \zeta \)-globin mRNA were detectable in this line as compared to the other lines. K562 Bos showed a greater induction of \( \zeta \)-globin mRNA than the other two cell lines (\( p < 0.025 \)) (Table 1, Fig. 2A).

No detectable \( \beta \)-globin mRNA was seen under the conditions used for Northern blotting (Fig. 3). Calculation of the amount of RNA used, and the specific activity of probe used, indicates that there are less than 60 \( \beta \)- or \( \delta \)-globin mRNA molecules/cell, since this amount would have been detected in these experiments.

**Globin Synthesis**

Globin synthesis was measured in each of the cell lines in the presence and absence of hemin. Figure 4 shows representative results for K562 #2 and Bos. All of the cell lines synthesized significant amounts of \( \gamma, \zeta, \) and \( \alpha \)-globin. All lines, except Bos, also synthesized significant amounts of \( \varepsilon \)-globin. The Bos cell line synthesized much less \( \varepsilon \)-globin than the other cell lines, although \( \varepsilon \)-globin synthesis was detectable. In the presence of 50 \( \mu M \) hemin, each of the cell lines increased the synthesis of \( \gamma, \zeta, \) and \( \alpha \)-globin 2–4-fold, and these increases were comparable to those seen in the content of globin mRNAs in these same cell lines. All of the cell lines, except the Bos cell line, showed a 2–3-fold increase in \( \varepsilon \)-globin synthesis as well. The Bos cell line showed some increase in \( \varepsilon \)-globin synthesis with hemin, but the fold increase could not be quantitated because of the low amount of \( \varepsilon \)-globin present. Thus, there was a good correlation in the Bos cell line between the decreased amount of \( \varepsilon \)-globin mRNA detectable by Northern blotting and the decreased amount of \( \varepsilon \)-globin present as compared to the other globin mRNAs and globins. In none of the cell lines was there any detectable \( \beta \)- or \( \delta \)-globin synthesis, reflecting the lack of detectable \( \delta \)- or \( \beta \)-globin mRNAs in these experiments.

**Globin mRNA Synthesis**

Globin mRNA synthesis was measured in K562 #2 and Bos by runoff nuclear transcription studies. The amount of \( ^{32}P \) hybridizing was linear to the amount of \( ^{32}P \) RNA added in the range of RNA used, indicating plasmid DNA excess on filters. Nuclear transcripts from hemin-treated K562 #2 cells hybridized to filters containing \( \gamma \) and \( \varepsilon \) plasmids with equal intensity (Fig. 5). Hybridization of transcripts from K562 Bos was only detected with the \( \gamma \) plasmid as probe; no hybridization of transcripts from K562 #9 was observed.
Fig. 3. Northern blot of K562 RNAs. Five micrograms of K562 RNA was used from clone #2, Bos, and clone #9. Minus (−) indicates control. Plus (+) indicates 20 μM hemin. Ly is 5 μg lymphoblast CP23, and r is 10 ng reticulocyte RNA. β 4.4 is the probe.

ization was detectable with the ε probe. These results imply that the low ε-globin mRNA levels observed in K562 Bos are due to reduced ε transcription. No RNA transcripts hybridizing to a β DNA plasmid were detected in either clone #2 or Bos lines in the presence of hemin (Fig. 5). The lack of hybridization of the ε probe to Bos RNA shows the specificity of the ε and γ probes. The lack of hybridization of the β probe to either Bos or clone #2 RNAs indicates the lack of cross-hybridization between the γ, ε, and β probes.

Nuclear runoff studies in clones #2 and Bos, both in the presence and the absence of hemin, were also performed. No reproducibly significant differences in hybridization were seen in nuclear transcripts of clones #2 and Bos in the absence or the presence of hemin, using the ε and γ probes, in five separate experiments (data not shown).

DISCUSSION

K562 cells provide a useful model for studying the regulation of human globin synthesis in a cell system that can be manipulated in culture. Previous studies have indicated that these cells synthesize ε-, γ-, ζ-, and α-globin and contain the mRNAs for these globins. Previously published studies also show that the levels of globin and globin mRNAs increase with various inducers, including hemin. In these latter studies, however, quantitation of specific globin mRNAs was not possible because of the lack of specific ε and ζ and ε and ζ probes, and cross-hybridization between γ and ε and the ζ and α sequences. By contrast, in the studies reported here, these difficulties were overcome, and the amount of α, ε, ζ, ε, and β mRNAs was quantified using cloned purified probes.

In these experiments, we have also compared the levels of these specific globin mRNAs in several different lines of K562 cells. We show that the amount of α, ε, ζ, ε, and γ mRNAs is detectable in these cells.
without hemin and is increased 2–3-fold with hemin. In addition, we have found that one K562 line, which we have called Bos, is markedly deficient in ε-globin mRNA accumulation and in the synthesis of ε-globin protein. This latter cell line provides an interesting model for defining the basis of decreased synthesis of one globin chain in an accessible cell line. These experiments also demonstrate other reproducible differences in hemin-induced specific globin mRNAs in different lines. There is an increased amount of ζ mRNA in Bos with hemin and less α-globin mRNA stimulation by hemin in K562 #9 than in the other cell lines with hemin. Another low ε-producing cell line has also been described. Different rates and extents of α- and β-globin mRNA accumulation have also been reported in mouse erythroleukemia cells (MELC) in the presence of different inducing compounds.

Increased globin mRNA accumulation could be due to changes in globin gene transcription, globin mRNA processing or transport, and/or globin mRNA stability. In MELC, the increased accumulation of specific globin mRNAs in the presence of dimethylsulfoxide and hexamethylene bisacetamide is associated with increased globin gene transcription, using runoff transcription analysis of isolated nuclei. By contrast, hemin induction of MELC is not associated with increased transcription by this assay. Increased stability of globin mRNAs has also been demonstrated in MELC. Our runoff transcription studies show that the decreased amount of ε-globin mRNA in Bos is due to decreased transcription of the ε-globin gene in these cells. Preliminary DNA restriction analysis indicates that there is no detectable deletion of ε-globin gene sequences in these cells. Nuclear transcription studies also show no significant differences in γ and ε RNA transcription in these cells in the presence or the absence of hemin. The results suggest that the increased γ and ε RNA accumulation in these cells with hemin is due to an increase in RNA processing, RNA transport, or RNA stability. Alternatively, it is possible that the runoff transcription experiments we performed are not sensitive enough to detect differences in RNA transcription that do exist. This may be due to either the relatively high level of globin RNA transcription in control cells and/or the relatively small (2–4-fold) increase in RNAs with hemin.

Since our studies were completed and submitted for publication, a paper has been published that provides evidence for transcriptional regulation of globin mRNA synthesis in one line of K562 cells. In this line, which grows attached to plates, there is a lower amount of baseline globin mRNA synthesis and accumulation in the absence of hemin and a greater (five-fold) increase in globin mRNA synthesis and accumulation with hemin than in our K562 lines. Whether this result is due to the different cell lines studied or to technical differences in the experimental protocols remains to be determined.

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