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Inducible transcription of five globin genes in K562 human leukemia cells

(human globin mRNA/*in vitro* translation/S1 nuclease mapping/gene expression/δ-globin mRNA)

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ABSTRACT **We studied the abundance and structure of globin mRNAs present in K562 cells both before and after induction of hemoglobin synthesis by hemin. *In vitro* translation of poly(A)$^+$ RNA from K562 cells generated protein products corresponding to α, ^{A}γ-, ^{G}γ-, ε-, and ζ-globin mRNAs. Individual globin mRNAs increased 1.5- to 3-fold after induction. Similar results were obtained by measuring steady-state mRNA concentrations of induced and uninduced cells by using S1 nuclease mapping. Globin gene transcripts were correctly initiated and processed. In addition, S1 nuclease analysis revealed the presence of δ-globin mRNA in both control and induced cells. A small percentage of δ-globin transcripts appeared to be initiated upstream from the normal initiation site. β-Globin mRNA was not detected in any studies. The results (*i*) suggest that hemin induction of K562 cells is mediated at a transcriptional level and (*ii*) reveal the dissociation of δ- and β-globin gene expression in K562 cells compared with normal erythroid cells.**

The globin genes, a well characterized family of genes, are differentially expressed during development (1). Expression of the ε- and ζ-globin genes during the early embryonic period is followed by expression of the α- and γ-globin genes later in fetal life. Late in gestation, β-globin gene expression increases and γ-globin gene expression declines. In adults the α- and β-globin genes are expressed at high levels, but the γ-globin genes are repressed. The δ-globin gene is also expressed at a low level in the adult. The mechanisms that determine the types and quantities of globin polypeptides that particular cells will synthesize are not yet understood. However, because the amounts of globin polypeptides correlate with the amounts of the corresponding globin mRNAs, regulation probably occurs at the level of gene transcription.

The K562 cell line was isolated from an adult patient with chronic myelogenous leukemia (2) and can be induced by hemin to accumulate embryonic and fetal hemoglobins but not adult hemoglobin (3–5). We have reported (6) that hemin induction is reversible and occurs in the absence of terminal erythroid differentiation. Globin mRNAs accumulate in K562 cells during induction by hemin (4, 7, 8). However, estimates of the magnitude of increase for specific globin mRNAs have been complicated by the lack of specificity of globin cDNA probes for the highly homologous globin mRNAs.

To distinguish among globin messages, we used *in vitro* translation and S1 nuclease mapping to study the abundance and structure of individual globin mRNAs in K562 cells. α-, γ-, ε- and ζ-globin mRNAs increased upon hemin induction in proportion to the observed increases in the respective globin polypeptides. Furthermore, S1 nuclease mapping revealed the presence of small amounts of δ-globin mRNA, a species that was previously undetected in K562 cells. β-Globin mRNA was not detected. K562 cells may be useful for the study of factors controlling globin gene expression because the β-globin gene in K562 cells may be repressed, as it is in early development.

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EXPERIMENTAL PROCEDURES

Cell Culture. K562 cells were grown in suspension culture in RPMI 1640 medium containing 10% fetal calf serum (Biofluids, Rockville, MD) and 10 mM Hepes with penicillin and streptomycin. Cells were maintained at densities greater than 5×10^4 cells per ml and were subcultured when they reached 8×10^5 cells per ml. Induction was performed by addition of 20 μM hemin (GIBCO) to the culture medium (6).

Preparation of RNA. Cells (about 5×10^8 cells per preparation) were washed twice with phosphate-buffered saline, suspended in 15 ml of 8 M guanidine·HCl/0.01 M sodium acetate/1 mM dithiothreitol, and the RNA was isolated (9). The final RNA pellet was dissolved in distilled water at a concentration of 5 mg/ml and was stored at −20°C.

Isolation and *in Vitro* Translation of Poly(A)$^+$ RNA. Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose (P-L Biochemicals) chromatography (10) by binding in 0.01 M Tris chloride, pH 7.5/0.5 M NaCl/1 mM EDTA at a RNA concentration of 1 mg/ml and eluting with 0.01 M Tris chloride (pH 7.5). Translation reactions were carried out with a wheat germ translation system (Bethesda Research Laboratory) for 60 min with 0.1–0.5 μg of poly(A)$^+$ RNA per reaction in the presence of [^{3}H]leucine (New England Nuclear; 50 Ci/mmol; 1 Ci = 37 GBq).

Gel Electrophoresis of Translation Products. Electrophoresis of cell lysates was carried out in polyacrylamide gels containing acetic acid/urea/Triton X-100 (5). Gels were then treated with EN^{3}HANCE (New England Nuclear) and exposed to Kodak X-Omat film for 4–14 days at −70°C. Densitometry was performed with a model 2202 Ultrascan (LKB).

Hybridization and S1 Nuclease Analysis. S1 nuclease analysis was modified (11) from the method of Berk and Sharp (12). Varying amounts of total cellular K562 RNA (1–20 μg) were hybridized with 20,000–160,000 cpm of double-stranded ^{32}P-labeled probes. Samples were hybridized for 18 hr at 65°C in 10-μl reaction volumes containing 50% formamide. The samples then were treated with 250 units of S1 nuclease (Miles) at 42°C for 30 min in 150 μl (final volume) of 30 mM sodium acetate, pH 4.5/50 mM NaCl/10 mM zinc sulfate/1 μg of denatured calf thymus DNA per ml. Digestion products were precipitated with ethanol and analyzed in 8% polyacrylamide sequence analysis gels under denaturing conditions (11, 13).

Preparation of Probes. All reagents for probe synthesis were obtained from Bethesda Research Laboratories. Appropriate

Abbreviations: kb, kilobase pair(s); nt, nucleotide(s).

fragments of the human α-, β-, γ-, δ- and ε-globin genes were cloned into various restriction sites in M13mp7 (refs. 11 and 13; also described in the text and figures). Clones containing single-stranded template that was equivalent to the coding strand of the gene were selected. ^{32}P-Labeled double-stranded probes were synthesized on these recombinant globin-M13 molecules by using the Klenow fragment of DNA polymerase I to direct incorporation of [α-^{32}P]dATP (Amersham; >800 Ci/mmol) and unlabeled nucleotides (14). The probes were then digested with appropriate restriction enzymes, isolated on 1% nondenaturing agarose gels, and recovered by electroelution.

RESULTS

We isolated total RNA from K562 cells before or after 3 days of growth in the presence of 20 μM hemin. The abundance of mRNAs coding for individual globin chains was assessed by *in vitro* translation. Poly(A)$^+$ RNA from K562 cells directed incorporation of [^{3}H]leucine into major products that comigrated with the α-, ^{G}γ-, ^{A}γ-, ε-, and ζ-globin chains in acrylamide gels containing acetic acid/urea/Triton X-100 (Fig. 1). δ-Globin chains are not resolved under these conditions. No band migrated at the position of the β-globin standard. Densitometer scans of the fluorogram revealed a 3.5-fold increase of ε-globin mRNA translation products after induction; other species increased by smaller amounts (ζ > γ > α) as summarized in Table 1. These increases are similar to the increases observed in the respective globin polypeptide chains after hemin induction under a variety of conditions (4, 5, 16, 17).

Using S1 nuclease mapping, we examined the globin RNA transcripts in detail. Fig. 2*A* shows an S1 nuclease analysis of RNA from K562 cells harvested 3 days after induction with hemin. A uniformly ^{32}P-labeled β-globin gene probe that extends from the 5′ untranslated region of the gene to the *Eco*RI site in exon 3 (see Fig. 2*A Lower*) was used. Correctly initiated and processed β-globin mRNA protected probe fragments of 145 nucleotides (nt) (exon 1) and 225 nt (exon 2) from digestion by S1 nuclease. Bands corresponding to these fragments were clearly visible in adult bone marrow RNA (Fig. 2*A*, lane 7) and in RNA obtained from COS cells transfected with an expression vector containing a normal human β-globin gene (Fig. 2*A*, lane 5; ref. 13). No corresponding bands were visible in lanes containing various amounts of RNA from induced (lanes 1 and 2) or uninduced (lanes 3 and 4) K562 cells. However, a band of 167 nt was clearly visible in these lanes. This band also was seen in the analysis of bone marrow RNA (Fig. 2*A*, lane 7) obtained from an individual with β$^+$-thalassemia, who also had an elevated level (5%) of hemoglobin A_2 ($\alpha_2\delta_2$) in the peripheral blood. A faint band at 167 nt also was seen in an analysis of RNA obtained from COS cells (Fig. 2*A*, lane 6) transfected with a vector containing a normal human δ-globin gene (13).

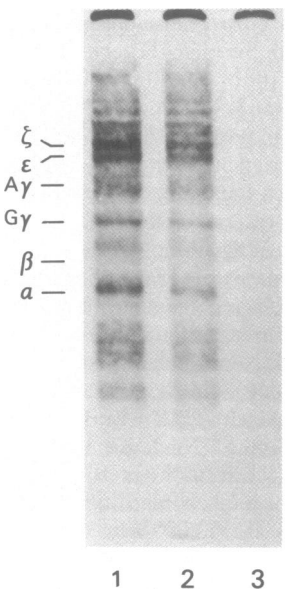

FIG. 1. *In vitro* translation products of mRNA from K562 cells. Poly(A)$^+$ RNA was used to direct protein synthesis in a wheat germ translation system in the presence of [^{3}H]leucine. The translation products were separated on an acetic acid/urea/Triton X-100 polyacrylamide slab gel (5) and detected by fluorography. Lanes: 1, translation products of poly(A)$^+$ RNA (0.375 μg) from cells treated with hemin for 4 days; 2, translation products of poly(A)$^+$ RNA (0.375 μg) from untreated cells; 3, no added RNA. Lanes 1 and 2 were loaded with 20,000 cpm of trichloroacetic acid-precipitable material. Standards (not shown) included purified HbA, HbF, and K562 globins obtained by binding to and elution from a Sepharose-haptoglobin conjugate (15).

Table 1. Increase in individual globin mRNAs after induction with hemin for 3 days

Globin chain	Determination by *in vitro* translation	Determination by S1 nuclease assay
α	1.1	1.1–1.5
β	ND	ND
γ	1.7	1.5
δ	ND	1.5
ε	3.5	3
ζ	2.6	2

ND, not detected.

The 167-nt fragment results from partial protection of the β-globin gene probe by δ-globin RNA (11, 13). The presence of δ-globin RNA in K562 cells both before and after induction was confirmed with a δ-globin gene probe containing all of exon 1 and extending to the *Bam*HI site in exon 2 (Fig. 2*B*, *Lower*). Authentic δ-globin mRNA protected probe fragments of 145 nt and 207 nt (see Fig. 2*B*, lane 6). These two bands also were present in all of the RNA samples obtained from K562 cells (Fig. 2*B*, lanes 1–4). Bone marrow RNA (Fig. 2*B*, lane 7) protected probe fragments of 145, 167, and 207 nt. The nucleotide sequences of the β- and δ-globin genes (18, 19) are almost identical (only an occasional 1-nt change) except for a 6-nt region of dishomology in exon 2 (Fig. 2*C*). This dishomology allowed S1 nuclease to nick the imperfect hybrid formed between a β-globin gene probe and a δ-globin RNA transcript (Fig. 2*A*), generating a 167-nt fragment. Hybridization between a δ-globin gene probe and β-globin RNA produced the same fragment (Fig. 2*B*, lane 7).

Hybridization of K562 RNA with the δ-globin gene probe (Fig. 2*B*) also yielded a 157-nt fragment, 12 nt longer than correctly initiated and processed exon 1. Because the δ-globin gene probe includes 12 nt of sequence flanking the 5′ end of exon 1, this band may represent a RNA species initiated at a site (or sites) upstream from the normal transcription initiation site.

The number of copies per cell of δ-globin mRNA was determined for the bone marrow RNA sample (data not shown) and for the transfected monkey COS cell RNA (13). By comparison to these standards, we estimated that induced K562 cells contain 70–100 copies of δ-globin mRNA. We looked for the presence of δ-globin chains in cell lysates obtained from induced cells grown in the presence of [^{3}H]leucine. Globins were separated by carboxymethylcellulose chromatography in the presence of urea (20), and pooled fractions were analyzed in acrylamide gels (ref. 5; data not shown). δ-Globin was not detected. The relative insensitivity of these techniques did not permit us to rule out translation of the δ-globin transcripts.

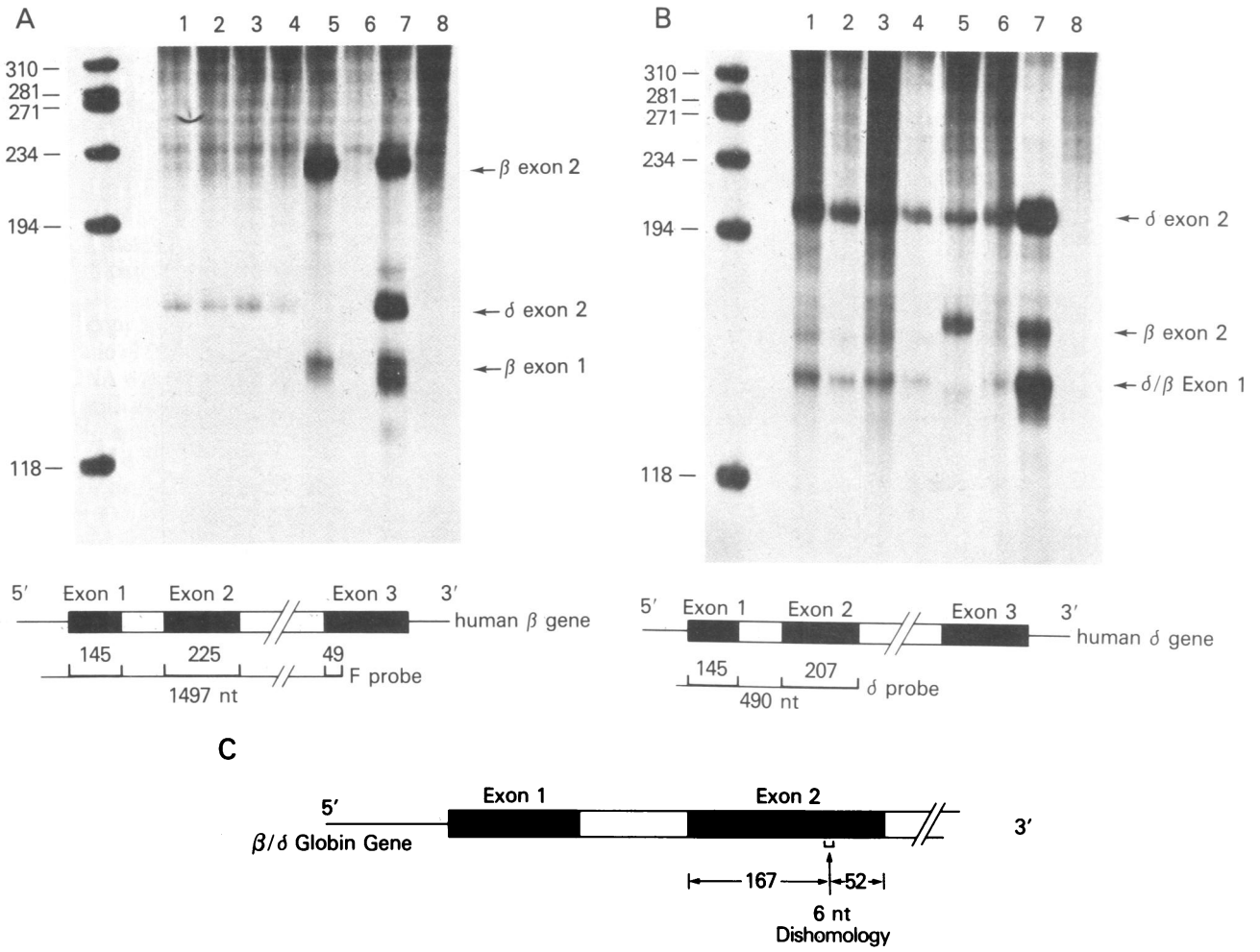

FIG. 2. (*A*) S1 nuclease analysis of K562 RNA (*Upper*) with a β-globin gene probe (*Lower*) (F probe in ref. 11); 160,000 cpm (about 5 ng) of probe was used per hybridization reaction. The positions of correctly initiated and processed mRNA molecules corresponding to β-globin exon 1 and exon 2 are indicated. Lanes: 1 and 2, 10 μg and 5 μg, respectively, of RNA from K562 cells induced for 3 days with hemin; 3 and 4, 10 μg and 5 μg, respectively, of RNA from untreated cells; 5, RNA (20 μg) obtained from COS cells transfected with a vector containing a normal human β-globin gene (13); 6, RNA (20 μg) from COS cells transfected with a vector containing a normal human δ-globin gene (13); 7, adult β^+-thalassemic bone marrow RNA (5 μg); 8, no added RNA. (*B*) S1 nuclease analysis of K562 RNA (*Upper*) with a δ-globin gene probe (*Lower*) (13); 61,000 cpm of probe was used per hybridization reaction. The positions of correctly initiated and processed δ-globin exon 1 and exon 2 are indicated. RNA samples were the same as in *A*. (*C*) Comparison of the β- and δ-globin genes. A 6-nt region of dishomology in exon 2 is indicated. Size markers are shown in nt.

Fig. 3 shows an S1 nuclease analysis of K562 RNA with a β-globin gene probe that includes most of exon 3 (Fig. 3 *Lower*). Correctly processed exon 3 was seen in the lane containing authentic β-globin mRNA (Fig. 3, lane 5); a faint band of the same length was seen in the lanes containing K562 cell RNA (Fig. 3, lanes 1–4). δ-Globin mRNA derived from transfected COS cells also gave rise to a faint band of this length (Fig. 3, lane 6).

ε-Globin mRNA from K562 cells was mapped with the 5′ 651-nt ε-globin gene *Bam*HI fragment (Fig. 4). ε-Globin mRNA protected probe fragments of 146 nt and 204 nt. These bands were visible in K562 RNA samples both before (lanes 5–8) and after (lanes 1–4) hemin induction. Adult bone marrow RNA (lane 9) yielded no bands; the same sample yielded bands characteristic of correctly initiated and processed γ- and β-globin mRNAs when analyzed with probes specific for these species.

We also studied α-, γ- and ζ-globin RNA transcripts obtained from K562 cells (ref. 21; data not shown); correctly initiated and processed transcripts were found for each globin gene. The steady-state concentrations of individual globin mRNAs before or after hemin induction were measured by densitometry. We noted a slight increase (1.1–1.5 fold) in α-globin transcripts after induction; however, γ- and ζ-globin transcripts increased about 2-fold. The increases for individual globin transcripts measured by both S1 nuclease mapping and *in vitro* translation are compared in Table 1.

DISCUSSION

We measured the abundance of individual globin mRNAs in K562 cells as a function of hemin induction. *In vitro* translation studies and S1 nuclease mapping indicated that hemin induction of K562 cells causes a 1.1- to 3-fold increase in the steady-state concentrations of each of five globin genes. The results suggest that the effect of hemin is mediated primarily at a transcriptional level. Concurrent studies of newly synthesized globin mRNAs in K562 cells suggest that hemin increases the rate of transcription of globin genes, rather than affecting RNA processing or stabilization (22).

In S1 nuclease studies, we used two β-globin-specific probes capable of visualizing all three exons of the β-globin gene. The lower limit of detection in this assay may be as little as one copy of a particular RNA molecule per cell. We did not observe β-globin mRNA in K562 cells either before or after hemin induction. However, these studies revealed the presence of low

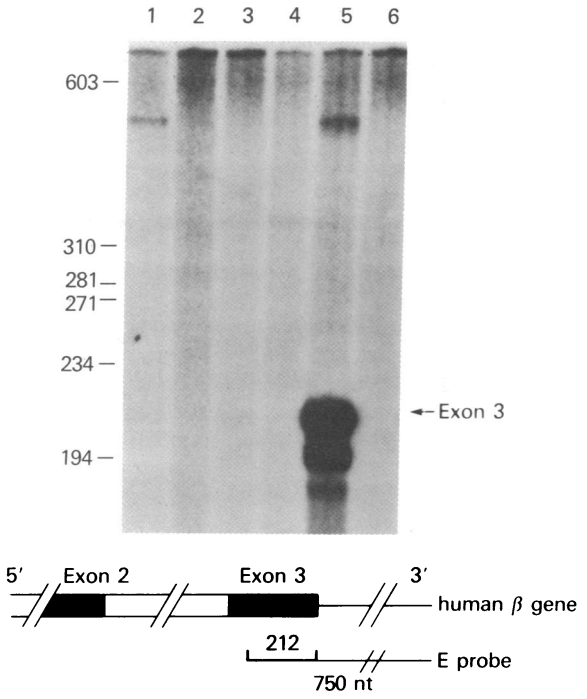

FIG. 3. S1 nuclease analysis of K562 RNA (*Upper*) with a 3′ β-globin gene probe (*Lower*); 96,000 cpm of probe was used per hybridization. The position of correctly terminated mRNA from β-globin exon 3 is indicated. Lanes: 1 and 2, 20 μg and 2 μg, respectively, of RNA from K562 cells induced with hemin for 3 days; 3 and 4, 20 μg and 2 μg, respectively, of RNA from untreated cells; 5 and 6, β-globin RNA (20 μg) and δ-globin RNA (20 μg), respectively, obtained as explained in the legend to Fig. 2. Size markers are shown in nt.

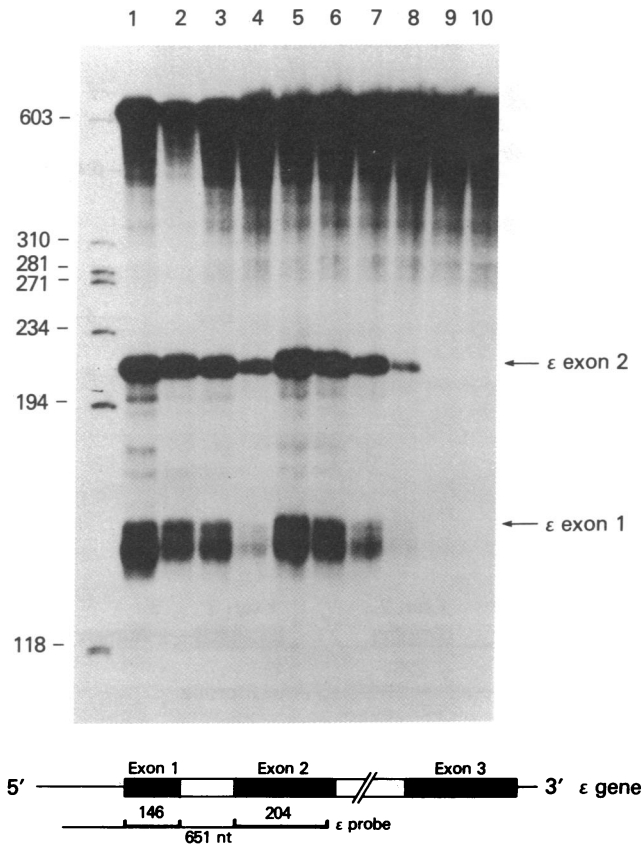

FIG. 4. S1 nuclease analysis of K562 RNA (*Upper*) with an ε-globin gene probe (*Lower*). The 0.67-kb *Bam*HI 5′ ε-globin gene fragment was cloned into the M13Mp7 *Bam*HI site. After synthesis of the complementary strand, the ^{32}P-labeled double-stranded probe was cut out with *Eco*RI and isolated on an agarose gel; 61,000 cpm of probe was used per hybridization reaction. The positions of correctly initiated and processed mRNA from ε-globin exon 1 and exon 2 are indicated. Lanes: 1–4, decreasing amounts (20 μg, 10 μg, 5 μg, and 1 μg) of RNA from K562 cells induced with hemin for 3 days; 5–8, decreasing amounts (20 μg, 10 μg, 5 μg, and 1 μg) of RNA from untreated cells; 9, bone marrow RNA (10 μg) as in Fig. 2, lane 7; 10, no added RNA. Size markers are shown in nt.

levels of δ-globin gene transcription in K562 cells. Active gene transcription has been associated with DNase-hypersensitive sites 5′ to globin genes (23). The presence of a DNase-hypersensitive site 5′ to the K562 δ-globin gene but none 5′ to the K562 β-globin gene has been observed (24).

Our data suggest that a small percentage of δ-globin gene transcripts may be at least 12 nt longer than normal at the 5′ end. Similar elongated transcripts have been observed for the ε- and β-globin genes. About 5% of ε-globin gene transcripts are initiated at a site 215 nt upstream from the normal CAP site in embryonic hematopoietic cells and in K562 cells (25); about 10% of human β-globin gene transcripts are initiated at a site 176 nt upstream from the normal transcription initiation site in bone marrow cells and reticulocytes (26). These elongated transcripts seem to be spliced normally, but their functional significance is unknown.

In normal adult red cells, the δ-globin gene is expressed at a level 1/40th that of the β-globin gene (27). Hypofunction of the δ-globin gene may be due to differences in the promoter region (13). However, in K562 cells, it is the β-globin gene which is hypofunctional relative to the δ-globin gene. Similarly, in individuals with β-thalassemia, little or no β-globin is synthesized, but normal or increased δ-globin chain synthesis occurs (11, 27, 28). The lack of production of β-globin in β-thalassemia is usually caused by mutations affecting transcription or processing of β-globin mRNA (briefly summarized in ref. 11). Mapping of the K562 β-globin locus (17, 29) has demonstrated no major deletions, insertions, or rearrangements. Nevertheless, lack of expression of the K562 β-globin gene may reflect a subtle mutational event. Alternatively, developmentally specific controls may be responsible for repression of the β-globin gene in these cells. Detailed analysis of the cloned K562 β-globin gene will be necessary to distinguish between these possibilities.

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