Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 2718–2722, May 1984
Biochemistry

Mapping of DNase I-hypersensitive sites in the upstream DNA of human embryonic ε-globin gene in K562 leukemia cells

(gene expression/enhancer cores/HL60 cells/c-*myc* gene/Southern blots)

DOROTHY TUAN* AND IRVING M. LONDON*†

*Harvard–Massachusetts Institute of Technology Division of Health Sciences and Technology; and †Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Contributed by Irving M. London, January 17, 1984

ABSTRACT **We have mapped the DNase I-hypersensitive sites around the ε-globin and c-*myc* genes in two human leukemia cell lines K562 and HL60. In K562 cells in which the ε-globin gene is transcribed, six DNase I-hypersensitive sites are found in 6 kilobases (kb) of upstream flanking DNA; in HL60 cells in which the c-*myc* gene is expressed, two DNase I-hypersensitive sites are observed in 2 kb of upstream DNA. Neither the inactive ε-globin gene in HL60 cells nor the inactive c-*myc* gene in K562 cells displays such upstream DNase I-hypersensitive sites. Our results are consistent with previous studies that have shown DNase I-hypersensitive sites within 1 kb of the 5′ end of other expressed genes. In addition, we have found sites displaying even more DNase I sensitivity further upstream of expressed ε-globin and c-*myc* genes. Among the six DNase I-hypersensitive sites of the expressed ε-globin gene in K562 cells, the most sensitive site is located about 6 kb upstream of the ε-globin gene. When correlated with the DNA sequence upstream of the ε-globin gene, this site was found to correspond to a region that contains a stretch of 28 consecutive Ts, three enhancer core-like sequences, and a stretch of consecutive $(C\text{-}A)_{15}(T\text{-}A)_6$ alternating purine and pyrimidine bases. These findings suggest the possibility that an enhancer element for ε-globin gene expression resides within this DNase I-hypersensitive site.**

The flanking DNA of structural genes contains regulatory elements for gene expression. The canonical "TATA" and "CCAAT" boxes in the promoter region required for specific and efficient transcription are located within the 100 bases immediately upstream of the 5′ end of most structural genes. Further upstream in the flanking DNA, there are sequences that recognize regulatory molecules such as inducers and steroid hormone–receptor complexes for genes whose expression is regulated by them (1–3). Flanking DNA also contains enhancer sequences that can stimulate gene transcription in a manner independent of position and orientation (4). In the simian virus 40 minichromosome, as well as the polyoma chromatin, the enhancer sequence, located in the flanking region between late and early viral genes, displays preferential sensitivity to DNase I digestion (5–7). In a mouse myeloma cell line expressing the immunoglobulin light chain constant C_κ gene, the enhancer region upstream of the C_κ gene (8, 9) also exhibits preferential DNase I sensitivity (9); but in mouse brain or liver nuclei, which do not express the C_κ gene, no DNase I hypersensitivity is found in the C_κ upstream flanking chromatin (9). DNase I hypersensitivity has been found also in the flanking chromatin around the promoter region of expressed or developmentally committed eukaryotic genes (for review, see ref. 10).

The embryonic ε-globin gene in the human leukemia cell line K562 is constitutively expressed, and this expression can be enhanced by treatment with hemin (11–13). In an attempt to locate the sequence elements in the flanking DNA that might regulate ε gene expression, we have probed, by DNase I hypersensitivity mapping, the flanking chromatin structure of the transcriptionally active ε-globin gene in hemin-treated K562 cells and transcriptionally inactive ε-globin gene in HL60 cells of a human promyelocytic leukemia (14). The results demonstrate several discrete regions of DNase I hypersensitivity in the upstream DNA as far as 6 kilobases (kb) from the actively transcribed ε gene; no DNase I hypersensitivity is observed in the upstream DNA of the inactive ε gene. In HL60 cells in which the c-*myc* gene is transcribed, there is a DNase I-hypersensitive site about 1.5 kb 5′ to the first exon.

MATERIALS AND METHODS

Cell Cultures. K562 cells were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum in a humidified incubator with a 5% CO_2 and air mix. K562 cell inocula were a kind gift of Ann Dean and Alan Schechter. For hemin treatment, 100-fold concentrated stock of hemin was added to a final concentration of 20 μM to cells in logarithmic phase. Four days later, cells were harvested and stored frozen at −80°C in growth medium plus 10% glycerol. Unless otherwise stated, the nuclei used in this study were derived from frozen cell stocks. The viability of the thawed cells as measured by trypan blue exclusion was >75%. HL60 cell inocula were a kind gift of Howard Steinberg. The HL60 cells were grown in the same medium as the K562 cells.

DNase I Digestion. Frozen K562 or HL60 cells after quick thawing at 37°C were pelleted and then resuspended in RSB buffer (10 mM Tris·HCl, pH 7.4/10 mM NaCl/3 mM $MgCl_2$). After 10 min on ice, the swollen cells were mechanically broken in a tight-fitting Dounce homogenizer. The intact nuclei were purified from cytoplasmic components by centrifugation through a 0.25 M sucrose/RSB buffer cushion. Pellets of the purified nuclei were resuspended to a density of ≈1.5 × 10^8 per ml in Dulbecco's phosphate-buffered saline with 5 mM $MgCl_2$/1 mM phenylmethylsulfonyl fluoride. DNase I was then added to aliquots of the nuclear suspension to desired concentrations. The sets of nuclear suspension were then incubated at 22°C for 10 min when the reaction was stopped and the DNA was purified as described (15).

Gel Electrophoresis and Southern Blotting. The DNA after DNase I digestion and subsequent restriction enzyme cleavage was run (10–20 μg of DNA per lane) on a 1.2% agarose gel in 40 mM Tris·HCl, pH 7.8/20 mM sodium acetate/2 mM EDTA buffer and transferred to a nitrocellulose filter following the procedure of Southern (16).

Nick-Translation and Hybridization. The 5′ pε0.6 and 3′ pε1.3 plasmid probes (17) were a kind gift of Bernard G. Forget. Adrian Hayday kindly supplied the human immunoglobulin heavy constant C_μ region and the c-*myc* probe DNA.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: kb, kilobase(s).

The probes were labeled by nick-translation reaction (18). The blots were hybridized with the probes as described (19, 20).

Nuclear and Cytoplasmic RNA Isolation. Nuclei and cytoplasm were separated from each other as described above. The outer nuclear membrane was removed from the nuclei by spinning in RSB buffer containing 0.26% Tween 40 and 0.13% deoxycholate (21). The nuclear and cytoplasmic fractions were made into 4 M guanidinium isothiocyanate and the RNA was purified through a CsCl gradient (22).

Dot-Blot Hybridization. The RNAs or DNAs in 10–15× concentrated NaCl/Cit (NaCl/Cit = 0.15 M NaCl/0.015 M sodium citrate) were dot-blotted and hybridized to the ^{32}P-labeled probes according to the procedure of Thomas (23).

RESULTS

The Upstream Flanking Sequence of the ε-Globin Gene in K562 Cells Contains DNase I-Hypersensitive Sites. In mapping the DNase I-hypersensitive sites around the ε-globin gene, DNA purified from K562 nuclei mildly treated with DNase I is cleaved again with a suitable restriction enzyme. The appropriate restriction enzymes for secondary nuclease digestion were selected from the known restriction maps of the ε-globin gene (refs. 20, 24; Q. Li and O. Smithies, personal communication) such that the resultant restriction fragment spans at least 10 kb of either the upstream or downstream flanking sequence with ε-globin gene situated at the 3′ or 5′ terminus of the fragments. The terminal location of the ε-globin gene, to which the probe hybridizes, eliminates ambiguities in subsequent placement of the DNase I-hypersensitive sites in the restriction fragment. By using the cloned ε-globin gene (as opposed to cloned flanking DNA fragments) as the hybridization probe, we are also assured of the detection of only the flanking sites (or regions) that display more DNase I sensitivity than the ε-globin gene.

For the restriction enzyme cleavage of DNase I-treated nuclear DNA, we have chosen restriction enzymes *Bam*HI and *Kpn* I. From the simplified restriction map of the ε-globin gene and its flanking regions in Fig. 1 *Lower*, it can be seen that there are three *Bam*HI sites around the ε-globin gene—one very close to the 5′ end of the gene, one inside the gene, and the third site far downstream to the 3′ end of the gene. Thus, *Bam*HI cleavage of the DNA from control nuclei without DNase I treatment generates two fragments on the Southern blot, when the DNA is hybridized to the nick-translated ε-globin gene probe, one small 0.6-kb fragment covering the 5′ end of the ε gene and one large 13-kb fragment spanning about 12 kb of the downstream flanking sequence (Fig. 1 *Upper* and *Lower*). On the other hand, *Kpn* I cleavage gives rise to one large 13.8-kb fragment with the ε-globin gene situated close to the 3′ end of the fragment, spanning about 12 kb of the upstream flanking sequence.

Double-digestion of the ε gene with *Bam*HI and *Eco*RI produces two small fragments, one 0.6 kb (by *Bam*HI alone) covering the 5′ end of the ε gene and one 1.3-kb fragment covering the 3′ moiety of the ε gene (Fig. 1 *Lower*). In Fig. 1 *Upper*, the left panel is a blot of *Bam*HI and *Eco*RI double-digested DNA isolated from K562 nuclei treated with 0, 10, 15, and 20 μg of DNase I per ml and hybridized to a mixture of nick-translated 5′ ε 0.6-kb and 3′ ε 1.3-kb probes (17). In this DNase I concentration range, it can be seen that both the 5′ 0.6-kb and 3′ 1.3-kb fragments of the ε gene are not appreciably degraded. The middle panel in Fig. 1 *Upper* is the same set of DNase-treated DNA cleaved instead by *Bam*HI enzyme. Again, the 5′ 0.6-kb fragment is not appreciably degraded by DNase I, yet the 13-kb fragment spanning the downstream sequence is DNase I sensitive. Part of the preferential degradation of the 13-kb fragment may be due to random hits by DNase I, since the 13-kb fragment is much longer than the 0.6-kb fragment, and may thus present a larger target for nonspecific attack by DNase I. However, the similarly large 13.8-kb *Kpn* I fragment spanning the upstream flanking DNA (right panel in Fig. 1 *Upper*) exhibits even more sensitivity toward DNase I with a faster rate of disappearance of the 13.8-kb fragment than of the 13-kb *Bam*HI fragment. Moreover, in contrast to the degradative trailing observed for the 13-kb *Bam*HI fragment, six degradation bands at 8.1 ± 0.4, 5.7 ± 0.4, 4.6 ± 0.2, 3.9 ± 0.1, 2.5 ± 0.25, and 1.8 ± 0.12 kb are apparent. This finding suggests that in the 5′ flanking sequence of the K562 ε-globin gene, the DNA in the chromatin is organized in such a way that there are at least five discrete sites (or regions) that become more accessible to nuclease attack than the ε-globin gene itself; the DNase I sensitive site that generates the 1.8-kb degradation fragment maps just at the 5′ end or within the ε-globin gene.

The degradation band pattern generated by the upstream DNase I-hypersensitive sites is seen reproducibly; the sizes of the degradation bands cited above are averaged from four separate experiments. The locations of the hypersensitive sites around the ε-globin gene sequence are numbered from 1 to 6 and marked by downward arrows in Fig. 1 *Lower*. The

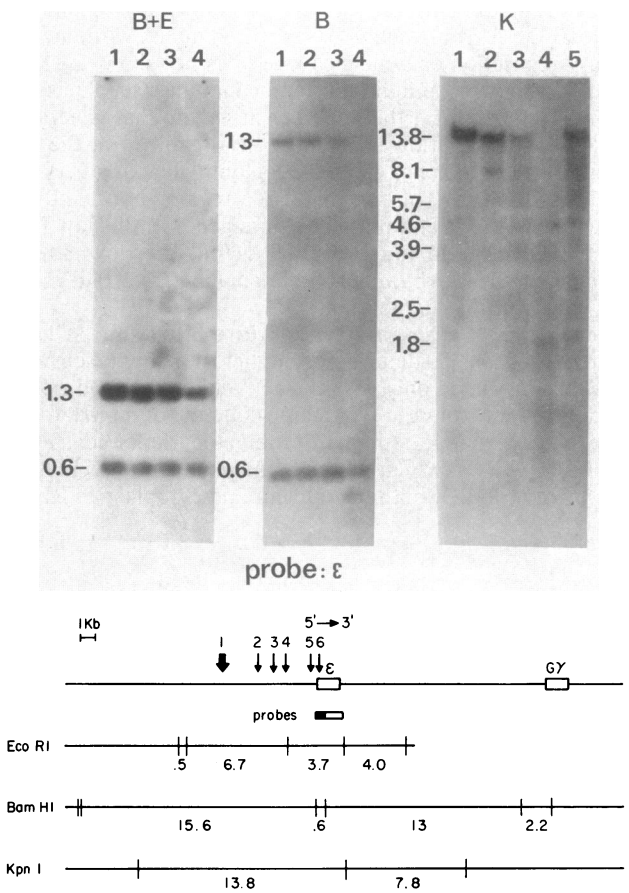

FIG. 1. (*Upper*) Southern blots of K562 DNA purified from nuclei treated with 0, 10, 15, and 20 μg of DNase I per ml (lanes 1–4) and subsequently cleaved singly or doubly with the following restriction enzymes: B, *Bam*HI; E, *Eco*RI; K, *Kpn* I; lane 5 in the *Kpn* I blot contains twice the amount of the same DNA sample as in lane 3. Sizes are shown in kb. (*Lower*) A simplified restriction map around the human ε-globin gene (refs. 20, 24; P. Powers, Q. Li, and O. Smithies, personal communication). Open boxes on the top line, the embryonic ε and fetal $^{G}\gamma$ globin structural genes. Filled and open rectangles below the ε gene, the 5′ and 3′ end ε hybridization probes. Vertical arrows mark the positions of the DNase I-hypersensitive sites. The heavy arrow at site no. 1 marks the most sensitive site.

5.7-, 3.9-, and 2.5-kb degradation bands are poorly defined in the blots and appear to be derived from a relatively diffuse region of DNase I hypersensitivity.

The degradation band pattern of the ε upstream sequence in Fig. 1 *Upper* was obtained from DNase I treatment of nuclei isolated from viable K562 cell stocks that had been stored frozen. Nuclei obtained from fresh K562 cells not previously frozen also produced the same degradation band pattern upstream of the ε gene but did so at a much lower DNase I concentration range of 2–10 μg/ml (data not shown).

The DNase I-hypersensitive site generating the 8.1-kb degradation band seems the most DNase I-sensitive since the 8.1-kb band is clearly discernible in lane 2 at digestion at 10 μg of DNase I per ml in the *Kpn* I blot (Fig. 1 *Upper*) when no other degradation bands are apparent. To determine whether the hypersensitive site generating the 8.1-kb fragment is indeed the most sensitive, we used a lower DNase I concentration range of 1–4 μg/ml to digest the K562 nuclei from viable frozen stock. Fig. 2 *Right* contains a blot of the DNA purified from nuclei treated with 0–4 μg of DNase I per ml and subsequently cleaved with *Kpn* I enzyme. The 8.1-kb fragment is discernible even at a DNase I concentration of 1 μg/ml when none of the other degradation bands can as yet be seen. Moreover, in agarose gel electrophoresis, the total DNA from such DNase-treated nuclei without secondary restriction enzyme cleavage does not show any visible degradation by ethidium bromide staining (see lanes 1–5 in Fig. 4). Therefore, the hypersensitive site that generated the 8.1-kb degradation band must be organized in the upstream flanking chromatin of the ε-globin gene in a very accessible structure. The site maps to a region about 6 kb upstream from the 5′ end of the ε-globin gene. In this low DNase I concentration range of 1–4 μg/ml, the downstream sequence in the 13-kb *Bam*HI fragment (Fig. 2 *Left*) does not show any sign of degradation.

Correlation of DNase I-Hypersensitive Sites with ε-Globin Gene Expression. To determine whether these hypersensitive sites are associated only with the actively transcribed ε gene and are absent from the upstream sequence of an inactive ε gene, we examined the human promyelocytic leukemia cell line HL60 (14), in which the ε-globin gene is not expressed. As shown in Fig. 3, both the nuclear and cytoplasmic RNAs isolated from HL60 cells do not contain detectable amounts of globin ε gene transcripts. Columns 1 of Fig. 3 present, from the top down, dot-blots of 5 μg each of K562 nuclear, K562 cytoplasmic, HL60 nuclear, and HL60 cytoplasmic RNAs. As a quantitative standard, columns 2 contain from top down normal DNA dotted in the amounts of 10, 7.5, 5, and 2.5 μg. Fig. 3 *Left* presents the dot-blots of RNAs hybridized to the ε 1.3-kb probe containing the 3′ end of the ε gene (Fig. 1), which does not cross-hybridize with the other β-like globin genes. ε gene transcripts are present in both the K562 nuclear and cytoplasmic RNAs (top two dots) but are undetectable in the HL60 RNAs (bottom two dots). The same samples hybridized to a C_μ probe containing the 1.4- and 1.5-kb *Eco*RI fragments (25) of the human immunoglobulin heavy chain μ constant gene (C_μ) are shown in Fig. 3 *Center*. The C_μ gene is not detectably transcribed in either the nuclear or cytoplasmic RNAs of both K562 and HL60 cells. Fig. 3 *Right* contains the same samples hybridized to the c-*myc* probe. In K562 cells, the c-*myc* gene transcripts are not detectable, but in HL60 cells, in agreement with a previous report (26), the *myc* gene is transcribed and the transcripts accumulate to a high level. The C_μ and c-*myc* gene probes have been included in this study to ensure that the DNase I digestion condition selected for HL60 nuclei is indeed comparable to that used for K562 nuclei.

HL60 nuclei seem to be more susceptible to DNase I digestion than K562 nuclei. The average size of HL60 DNA from nuclei treated with 10 μg of DNase I per ml (Fig. 4, lane 14) is around 5 kb, whereas the DNA from K562 nuclei treated similarly shows only slight digestion with the average size above 25 kb (Fig. 4, lane 10). To achieve a DNA size range for HL60 that is comparable to K562 DNA from nuclei digested with 10, 15, and 20 μg of DNase I per ml (Fig. 4, lanes 8, 9, and 10, respectively), the HL60 nuclei were digested with 2.5, 5, and 10 μg of DNase I (Fig. 4, lanes 12, 13, and 14, respectively). Even in this lower DNase I concentration range, the HL60 DNAs seem to be slightly smaller in average size than the K562 counterparts. To ensure that the HL60 DNAs are not overdigested, Southern analysis was carried out on both the HL60 and K562 DNAs from DNase-treated nuclei. The DNAs were cleaved with *Eco*RI restriction enzyme and the blots were hybridized to the C_μ probe. *Eco*RI enzyme cleaves the human C_μ region into two small fragments of 1.4 and 1.5 kb, respectively (25). The intensities of the 1.4- and 1.5-kb bands stayed quite constant at the different DNase I concentrations for both HL60 and K562 DNAs and did not show marked DNase I sensitivity (Fig. 5 *Upper Center*). (The origin of the dark band above the 1.5-kb band in lane 3 of the HL60 blot is not known.) These findings are consistent with the observation in RNA dot-blot experiments (Fig. 3) that the C_μ gene in both K562 and HL60 cells is not actively transcribed.

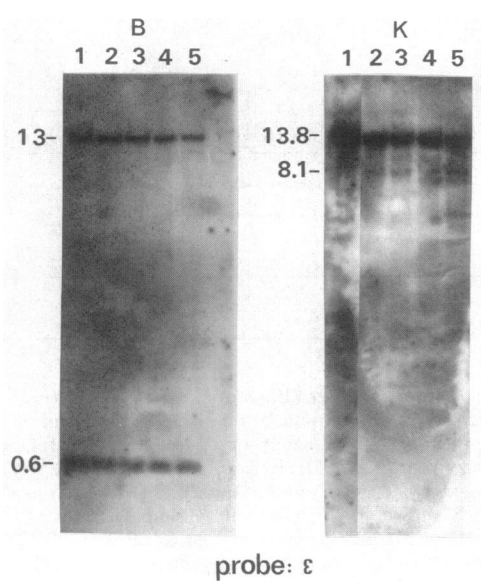

FIG. 2. Southern blots of K562 DNA purified from nuclei treated with 0, 1, 2, 3, and 4 μg of DNase I per ml (lanes 1–5) and subsequently cleaved with *Bam*HI (*Left*) and *Kpn* I (*Right*) enzymes. Sizes are shown in kb.

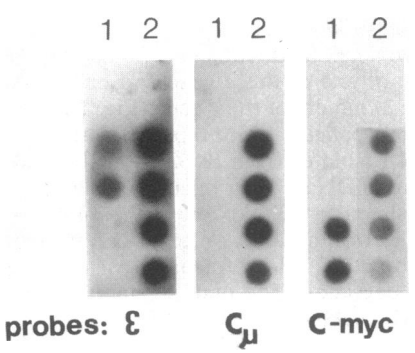

FIG. 3. Dot-blot hybridization. Columns 1 contain from top down 5 μg each of K562 nuclear and cytoplasmic and HL60 nuclear and cytoplasmic RNAs; columns 2 from top down contain 10, 7.5, 5, and 2.5 μg of a normal DNA standard.

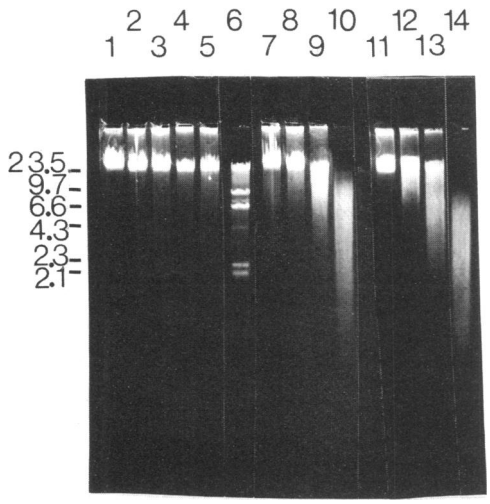

FIG. 4. Agarose gel electrophoresis of ethidium bromide-stained DNA from DNase-treated K562 and HL60 nuclei. Lanes 1–5, K562 DNA from nuclei treated with 0–4 μg of DNase I per ml. Lane 6, size marker: λ DNA cut with *Hin*dIII enzyme. Lanes 7–10, K562 DNA from nuclei treated with 0–20 μg of DNase I per ml. Lanes 11–14, HL60 DNA from nuclei treated with 0–10 μg of DNase I per ml. Sizes are shown in kb.

The HL60 DNase-treated DNA was cleaved with the *Bam*HI enzyme and the blot was hybridized to the ε probe. As with K562 DNA, two fragments of 0.6 and 13 kb, respectively, were generated (Fig. 5 *Upper Left*); no degradation bands were observed for the 13-kb fragment. The same set of HL60 DNase-treated DNAs, cleaved with *Kpn* I enzyme, and after the blot was hybridized to the ε 1.3-kb probe, generated one 13.8-kb fragment (Fig. 5 *Upper Left*). In contrast to the K562 *Kpn* I fragment, the HL60 *Kpn* I fragment presents no readily discernible degradation bands.

Further evidence that actively transcribed genes possess DNase I-hypersensitive sites in the 5′ flanking sequence was provided in experiments in which the HL60 and K562 DNAs from DNase-treated nuclei were digested with *Eco*RI enzyme and the blots were hybridized to the c-*myc* probe (Fig. 5 *Upper Right* and *Lower*). The c-*myc* gene in K562 is not detectably transcribed (Fig. 3), and the 12-kb *Eco*RI fragment containing the c-*myc* gene in K562 exhibits no discernible degradation bands (Fig. 5 *Upper Right*). On the other hand, the c-*myc* gene in HL60 is actively transcribed (Fig. 3) (26), and the blot shows at least 10 degradation bands (Fig. 5 *Upper Right*). The four most sensitive sites generating degradation bands of 7.6, 6.2, 5.3, and 3.3 kb, respectively, are marked by downward arrows in a simplified c-*myc* gene map (27, 28) in Fig. 5 *Lower*. Of the two DNase I-hypersensitive sites 5′ to the c-*myc* gene, the more sensitive site is located about 1.5 kb 5′ to the first c-*myc* exon.

DISCUSSION

The ε-globin gene is transcribed in K562 cells but not in HL60 cells. From restriction enzyme maps of this region as well as results of DNA dot-blot hybridization (our unpublished data), the ε-globin gene appears not to be amplified in K562 cells either before or after hemin treatment, and it is not deleted in HL60 cells. Therefore, the differential expression of the ε-globin gene in these two cell lines cannot be attributed to differences in gene dosage nor to gross DNA rearrangement around the ε gene. By DNase I hypersensitivity mapping, we have identified six DNase I-hypersensitive sites as far as 6 kb upstream of the K562 ε-globin gene; sites of such DNase I hypersensitivity have not been found around the ε gene in the HL60 cells. A correlation between far upstream DNase I-hypersensitive sites and gene transcription has also been observed in the case of the human *myc* cellular oncogene. The *myc* gene is not expressed in K562 cells and we do not find any upstream DNase I-hypersensitive sites; in HL60 cells, the *myc* gene is amplified (29, 30) and expressed (26), and we find two DNase I-hypersensitive sites in the 2-kb of flanking sequences 5′ to the *myc* gene. Such far upstream DNase I-hypersensitive sites have been reported to exist in the expressed chicken globin gene domain (31). It appears that the upstream DNA of an actively transcribed gene is organized in the chromatin in such a way that discrete regions become exposed and accessible to the exogenous nuclease. Similarly, these relatively open regions in the chromatin may also be accessible to regulatory

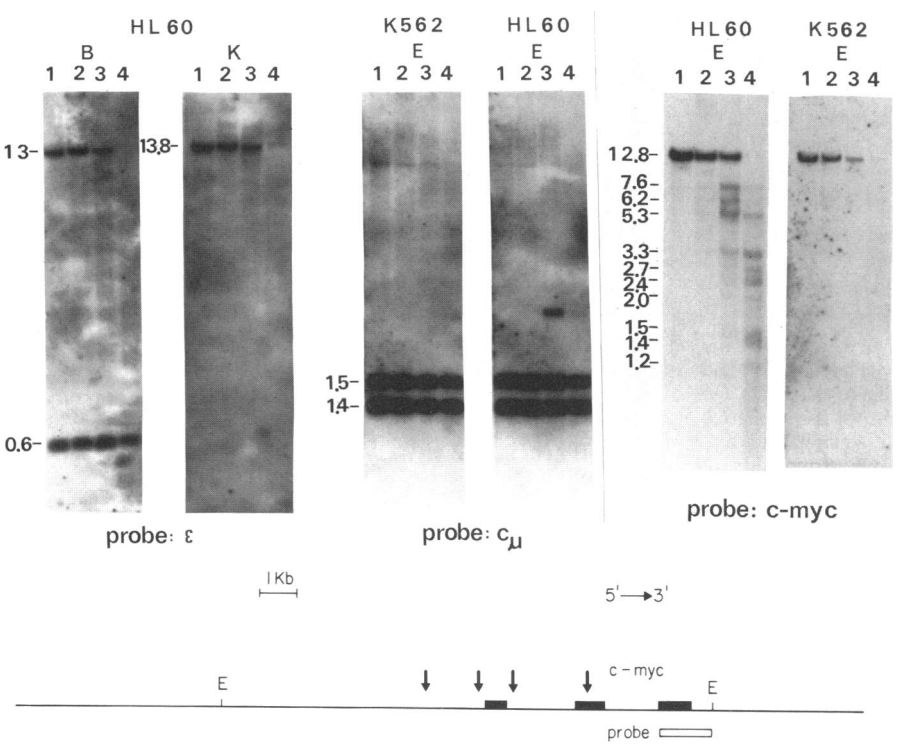

FIG. 5. (*Upper Left*) Southern blots of HL60 DNA from nuclei treated with 0, 2.5, 5, and 10 μg of DNase I per ml (lanes 1–4, respectively). (*Upper Center* and *Right*) Southern blots of the same DNase-treated HL60 DNAs as above but subsequently cleaved with *Eco*RI (E) and hybridized to the C_μ or c-*myc* probes. Sizes are shown in kb. (*Lower*) The *Eco*RI restriction fragment containing the human c-*myc* gene area (27, 28). Filled boxes, the exon regions of the c-*myc* gene. Open box, the position of the *myc* probe in the restriction fragment. Vertical arrows, positions of the four stronger DNase I-hypersensitive sites.

molecules such as inducers and hormone–receptor complexes (1–3) or to RNA polymerases in the transcriptional apparatus. Among the six DNase I-hypersensitive sites of the ε-globin gene, sites 2, 4, 5, and 6, (Fig. 1) correspond to regions containing minor transcriptional initiation sites of the ε-globin gene in K562 cells (32) and may thus represent the entry site for RNA polymerase. Hypersensitive site 6 (Fig. 1 *Right*) has been mapped to a region 200 bases upstream of or within the 5′ end of the ε-globin gene. DNase I-hypersensitive sites have also been mapped immediately 5′ to actively transcribed chicken (31, 33) and mouse (34–36) globin genes. Furthermore, the site immediately 5′ to the actively transcribed mouse globin gene has been shown to respond to inducers of erythroid differentiation and globin gene expression in the Friend erythroleukemia cell line by becoming 2- to 10-fold more sensitive to DNase I (34–36). The responses of the K562 hypersensitive site 6 to hemin treatment await further investigation.

The upstream DNA of the human ε-globin gene has been subjected to sequence analysis (ref. 37; Q. Li, P. Powers, and O. Smithies, personal communication). These sequence data enable us to correlate certain features with some of the DNase I-hypersensitive sites upstream of the ε-globin gene. Sites 6 and 3 do not present any remarkable sequence features. Site 5 contains within 500 bases a stretch of 20 T-rich bases in the middle and an enhancer core-like sequence (4), G-T-G-G-A-A-A-T-T, on the antisense strand at the end. Site 4 contains at the 5′ end two enhancer core-like sequences, G-T-G-G-A-T-A-T-T-A-G, on the sense strand, and G-T-G-G-T-A-T-A-G, on the antisense strand, and a stretch of 19 consecutive Ts followed by 60 A-rich bases in the middle of the 600-base fragment. Site 2 contains within 800 bases four enhancer core-like sequences: two at the 5′ end, G-T-G-G-T-T-T-A and G-T-G-G-T-A-A-A-G-A, both on the sense strand, and two at the 3′ end, G-A-G-G-G-A-A-G-G-T-G-G-A-A-A-T, on the antisense strand. Hypersensitive site 1, located 6 kb upstream of the ε-globin gene and the most sensitive to DNase I, has been mapped within an 800-base fragment that contains a stretch of 28 consecutive Ts toward the 5′ end, and three enhancer core-like sequences: at the 5′ end, G-T-G-G-T-T-T-G, on the sense strand, in the middle, G-T-G-G-A-T-A-T-A-G-A, on the antisense strand, and at the 3′ end, G-T-G-G-T-T-T-A-G-A, on the sense strand. In addition, there is a stretch of 21 consecutive pairs of alternating purine and pyrimidine bases: $(C\text{-}A)_{15}(T\text{-}A)_6$, on the sense strand (37) in the middle of the fragment. Oligomers of alternating purine and pyrimidine base pairs have been reported to possess the potential for forming Z-DNA (38). The simian virus 40 enhancer has been reported to contain both the enhancer core sequence G-T-G-G-A-A-A-G and four alternating purine and pyrimidine base pairs (4). The possibility that hypersensitive site 1 may be an enhancer for ε-globin gene expression is suggested by the present observations and remains to be explored.

We thank Drs. Bernard G. Forget and Adrian Hayday for the probes used in this study and Drs. Gary Quigley, Philip Auron, and William Solomon for helpful discussion. We wish to thank Dr. Qiliang Li, Ms. Pat Powers, and Dr. Oliver Smithies for their generosity in sending us their unpublished data on the ε-globin gene upstream DNA sequence. This work was supported by National Institutes of Health Grant AM-16272 and by grants of the National Foundation for Cancer Research and the National March of Dimes.

1. Compton, J. G., Schrader, W. & O'Malley, B. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 16–20.
2. Robins, D., Pack, I., Seebury, P. & Axel, R. (1982) *Cell* **29,** 623–631.
3. Guarente, L. & Mason, T. (1983) *Cell* **32,** 1279–1286.
4. Gluzman, Y. & Shenk, T., eds. (1983) *Enhancers and Eukaryotic Gene Expression* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
5. Varshavsky, A., Sundin, O. & Bohn, M. J. (1979) *Cell* **16,** 453–466.
6. Herbomel, P., Saragosti, S., Blangy, D. & Yaniv, M. (1981) *Cell* **25,** 651–658.
7. Saragosti, S., Cereghini, S. & Yaniv, M. (1982) *J. Mol. Biol.* **160,** 133–146.
8. Queen, G. & Baltimore, D. (1983) *Cell* **33,** 741–748.
9. Chung, S. Y., Folsom, V. & Wooley, J. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2427–2431.
10. Weisbrod, S. (1982) *Nature (London)* **297,** 289–295.
11. Benz, E., Murname, M. J., Tonkonow, B., Berman, B., Mazur, E., Cavallesco, C., Jenko, T., Snyder, E., Forget, B. G. & Hoffman, R. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3509–3513.
12. Rutherford, T., Clegg, J., Higgs, D., Jones, R., Thompson, J. & Weatherall, D. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 348–352.
13. Dean, A., Erard, F., Schneider, A. & Schechter, A. (1981) *Science* **212,** 459–461.
14. Gallagher, R., Collins, S., Trujillo, J., McCredie, K., Ahearn, M., Tasi, S., Metzgar, R., Aulakh, G., Ting, R., Ruscetti, F. & Gallo, R. (1979) *Blood* **54,** 713–733.
15. Blin, N. & Stafford, D. W. (1976) *Nucleic Acids Res.* **3,** 2302–2308.
16. Southern, E. (1975) *J. Mol. Biol.* **98,** 503–517.
17. Baralle, F., Shoulders, C. & Proudfoot, N. (1980) *Cell* **6,** 621–626.
18. Maniatis, T., Jeffrey, A. & Kleid, D. G. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 1184–1188.
19. Wahl, G. M., Stern, M. & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3683–3687.
20. Tuan, D., Biro, A., deRiel, K., Lazarus, H. & Forget, B. G. (1979) *Nucleic Acids Res.* **6,** 2519–2544.
21. Penman, S. (1966) *J. Mol. Biol.* **17,** 117–130.
22. Chirgwin, J. M., Przybyla, A., MacDonald, R. & Rutter, W. (1979) *Biochemistry* **18,** 5294–5301.
23. Thomas, P. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
24. Baralle, F., Shoulders, C., Goodbourn, S., Jeffrey, A. & Proudfoot, N. (1980) *Nucleic Acids Res.* **8,** 4393–4404.
25. Ravetch, J., Siebenlist, O., Korsemeyer, S., Waldmann, T. & Leder, P. (1981) *Cell* **27,** 583–591.
26. Westin, E. H., Wong-Staal, F., Gelmann, E. P., Dalla Favera, R., Papas, T. S., Lautenberger, J. A., Eva, A., Reddy, E. P., Tronick, S. R., Aaronson, S. A. & Gallo, R. C. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2490–2494.
27. Battey, J., Moulding, C., Taub, R., Murphy, W., Stewart, T., Potter, H., Lenoir, A. & Leder, P. (1983) *Cell* **34,** 779–787.
28. Saito, H., Hayday, A. C., Wiman, K., Hayward, W. S. & Tonegawa, S. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7476–7480.
29. Collins, S. & Groudine, M. (1982) *Nature (London)* **298,** 679–681.
30. Dalla Favera, R., Wong-Staal, F. & Gallo, R. (1982) *Nature (London)* **299,** 61–63.
31. Stalder, J., Larsen, A., Engel, J. V., Dolan, M., Groudine, M. & Weintraub, H. (1980) *Cell* **20,** 451–460.
32. Allan, M., Lanyon, G. & Paul, J. (1983) *Cell* **35,** 187–197.
33. McGhee, J., Wood, W., Dolan, M., Engel, J. & Felsenfeld, G. (1981) *Cell* **27,** 45–55.
34. Sheffery, M., Rifkind, R. & Marks, P. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 1180–1184.
35. Hofer, E., Hofer-Warbinek, R. & Darnell, J. (1982) *Cell* **29,** 887–893.
36. Balcarek, J. & McMorris, A. (1983) *J. Biol. Chem.* **258,** 10622–10628.
37. Shen, S. H. & Smithies, O. (1982) *Nucleic Acids Res.* **10,** 7809–7818.
38. Nordheim, A., Pardue, M., Lafer, E., Möller, A., Stoller, D. & Rich, A. (1981) *Nature (London)* **294,** 417–422.