Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 6384–6388, October 1985
Biochemistry

The "β-like-globin" gene domain in human erythroid cells

(β-globin gene cluster/DNA sequences/DNase I-hypersensitive sites/domain boundary/enhancers)

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Contributed by Irving M. London, May 24, 1985

ABSTRACT **We have mapped the distribution of the major and minor DNase I-hypersensitive sites in the human "β-like-globin" gene domain. The minor DNase I-hypersensitive sites map close to the 5′ end of each of the β-like-globin genes. Their presence is specifically associated with the transcription of the immediate downstream β-like-globin genes. The major DNase I-hypersensitive sites map in what appear to be the 5′ and 3′ boundary areas of the human β-like-globin gene domain, a region estimated to span at least 90 kilobases of DNA. These major sites are present in various erythroid cells, which express predominantly either the embryonic, the fetal, or the adult β-like-globin genes, and seem to be involved in defining the active β-like-globin gene domain in cells of erythroid lineage. The four major DNase I-hypersensitive sites in the 5′ boundary area, when correlated with sequencing data, are shown to be located in DNA regions containing enhancer core-like sequences and alternating purine and pyrimidine bases.**

The human "β-like-globin" genes (hemoglobin β-chain gene cluster) encode, respectively, one embryonic (ε), two fetal ($^{G}\gamma$ and $^{A}\gamma$), and two adult (δ and β) globin chains. These genes have been shown to reside within ≈50 kilobases (kb) of chromosomal DNA in the transcriptional order 5′ ε-$^{G}\gamma$-$^{A}\gamma$-δ-β 3′ (ref. 1; see Fig. 1). These structurally related genes are normally expressed exclusively in cells of erythroid lineage. Furthermore, their expression undergoes a developmental stage-related switching mechanism: the embryonic ε-globin gene is expressed in the early embryo; the fetal γ-globin genes are expressed during most of fetal life; and the adult δ- and β-globin genes, in adulthood (2).

In an attempt to locate the regulatory elements important in controlling the differential expression of the human β-like-globin genes during erythroid differentiation and development, we have mapped the DNase I-hypersensitive sites in the flanking DNA of the β-like-globin gene complex in several human cells: a human leukemia cell line (K562) in which the embryonic ε-globin gene is predominantly expressed (3, 4); a human erythroleukemia cell line (HEL), which expresses predominantly the fetal γ-globin genes (5); normal nucleated bone marrow cells of adult humans, in which the β-globin gene is predominantly expressed; and a human promyelocytic leukemia cell line (HL60), which expresses none of the β-like-globin genes. In agreement with others (6), we have found DNase I-hypersensitive sites close to the 5′ end of the transcribed globin genes, which we named minor hypersensitive sites because of the relatively high DNase I concentration required for their detection. In addition, we have found major DNase I-hypersensitive sites in what appear to be the 5′ and 3′ boundary areas of the β-like-globin gene domain, far upstream and far downstream of the expressed globin genes. These major hypersensitive sites are present in all three erythroid cell types, regardless of whether the predominantly expressed globin gene is the embryonic ε-, fetal γ-, or adult β-globin gene, but they are absent in HL60 cells, which do not express the β-like-globin genes. Their presence may thus serve to define and mark the active β-like-globin gene domain in erythroid cells during differentiation and development. Sequencing data of the DNase I-hypersensitive sites in the 5′ boundary area show that they each contain 2 or 3 enhancer core-like sequences and 10–26 consecutive or nonconsecutive pairs of alternating purine and pyrimidine bases.

EXPERIMENTAL PROCEDURES

Cells were grown as described (7). Human bone marrow cells were collected from cancer patients with normal marrow who were to undergo chemotherapy and bone marrow reinfusion. Isolated by dextran column chromatography, ≈25% of the nucleated cells were erythroid.

DNase I-digestion, gel electrophoresis, RNA isolation, blotting, and hybridization were carried out as described (7).

RESULTS

Globin Gene Transcription in K562,HEL, Adult Human Marrow, and HL60 Cells. Nuclear and cytoplasmic RNAs were isolated from cells, and individual globin gene transcription was detected by "dot-blot" hybridization with ε-, γ-, δ-, or β-globin specific cDNA probes (7). The results are not shown but may be summarized briefly. In K562 cells, the embryonic ε-globin gene is transcribed, and the fetal γ-globin genes are also transcribed but at a lower level; transcription of the adult δ- and β-globin genes is not detected. The transcriptional pattern of the β-like-globin genes in K562 cells thus bears resemblance to the embryonic pattern. In HEL cells, the fetal γ-globin genes are transcribed at a higher level than the ε-globin gene; the transcriptional pattern thus resembles that of the β-like-globin genes in the fetus. In adult nucleated human marrow cells, the β-globin gene is predominantly transcribed. In HL60 cells, none of the β-like-globin genes is detectably transcribed.

DNase I-Hypersensitivity Mapping. The locations of the plasmid probes and the restriction fragments chosen to map the β-like-globin area are presented in Fig. 1. We are able to map an area covering ≈100 kb in the human β-like-globin gene cluster, from a *Pvu* II restriction site 25 kb upstream of the ε-globin gene to a *Bam*HI site 25 kb downstream of the β-globin gene (Fig. 1). If not otherwise stated, the data shown are for hemin-treated K562 and HEL cells.

DNase I-Hypersensitive Sites Upstream of the ε-Globin Gene in K562 Cells. In the 13.8-kb *Kpn* I fragment in K562 cells (Fig. 2*a*), there are five degradation bands (8.1, 4.6, 3.9, 2.5, and 1.8 kb). The 8.1-kb band is very dark and well-defined and is clearly discernible at the lowest DNase I concentration

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Abbreviation: kb, kilobase(s).

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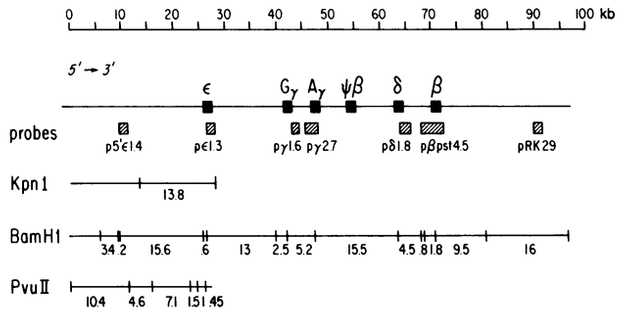

FIG. 1. Restriction map of the human β-like-globin gene cluster. Hatched boxes denote the locations, with respect to the globin genes, of the hybridization probes. ψβ, a pseudo β-globin gene. Restriction sites were determined from sequencing data (8, 27).

(lane 1 in Fig. 2*a*). It is therefore generated by cleavage at the most sensitive site in the region. This site is marked by a thick vertical arrow in Fig. 2*b* at about 6 kb upstream of the ε-globin gene. The 4.6-kb degradation band is discernible in lane 2 of Fig. 2*a* and is generated by cleavage at a less sensitive site, marked by a less thick vertical arrow in Fig. 2*b*. The other three degradation bands (3.9, 2.5, and 1.8 kb) are rather diffuse (Fig. 2*a*) and become discernible at the highest DNase I concentration (Fig. 2*a*, lane 3); they are generated by cleavage at the three least sensitive sites (thin vertical arrows in Fig. 2*b*). The data shown are for K562 cells without hemin treatment. In K562 cells treated with hemin, these hypersensitive sites are also present; however, there is one additional site at around −3.7 kb (7).

The DNase I-hypersensitive sites 5′ of the ε-globin gene are not found in HL60 cells (7), which do not transcribe the ε-globin gene; the appearance of these sites in K562 cells seems, therefore, to be associated with ε-globin gene transcription.

DNase I-Hypersensitive Sites in the γ-, δ-, β-Globin Gene Region of K562 and HL60 Cells. For mapping the DNase I-hypersensitive sites around the γ-globin genes, *Bam*HI enzyme was chosen. There are two DNase I-hypersensitive sites generating respective degradation bands at 4.1 and 1.1 kb (Fig. 3*a*, lanes 2 and 3). The hypersensitive site generating the 4.1-kb degradation band has been placed at the 5′ end of the Aγ-globin gene (Fig. 3*b*). The 1.1-kb degradation band is probably generated by a hypersensitive site at the 5′ end of the Gγ-globin gene (Fig. 3*b*). The placement of hypersensitive sites 5′ of the transcribed γ-globin genes in K562 cells is in agreement with findings of others (9, 10). These hypersensitive sites are less sensitive to DNase I than the two, most sensitive sites upstream of the ε-globin gene (Fig. 2) and are marked with thin vertical arrows in Fig. 3*b*. In HL60 cells, in which the γ-globin genes are not transcribed, no DNase I-hypersensitive site is detected in the $^{G}\gamma$- and $^{A}\gamma$-globin gene region (Fig. 3*a*). The bands below the 5.2- and 2.5-kb bands in lane 3 of the HL60 blot are nonspecific background contamination.

In both K562 and HL60 cells, where no δ- or β-globin gene transcripts are detected, the four major *Bam*HI fragments in the δ- and β-globin gene region exhibit no DNase I-sensitive degradation band (not shown). A hypersensitive site immediately 5′ of the δ-globin gene in K562 cells, correlating with a very small amount of δ-globin gene transcripts as detected by nuclease S1 mapping (11), has been reported (10). In our hands, however, this site is not well-defined and is much less sensitive to DNase I digestion than the hypersensitive sites 5′ of the ε- or of the γ-globin gene.

Hypersensitive Sites Far Downstream of the β-Globin Gene in K562, HL60, HEL, and Nucleated Adult Human Marrow Cells. In K562 cells, where no β-globin gene transcript is detected, we observe no DNase I-hypersensitive site in the 9.5-kb *Bam*HI fragment spanning about 8.5 kb of DNA immediately downstream of the β-globin gene (Fig. 3*b*). In the 16-kb *Bam*HI fragment downstream of this 9.5-kb fragment, however, we detect a DNase I-hypersensitive site that

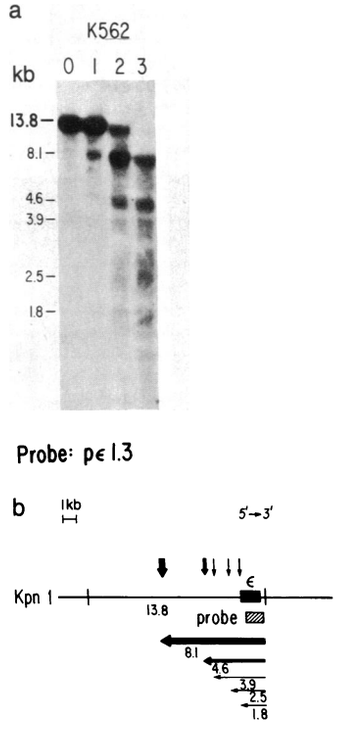

FIG. 2. (*a*) Southern blot of K562 DNA, from cells not treated with hemin, cleaved with *Kpn* I. DNase I at 0, 10, 15, or 20 μg/ml (lanes 0, 1, 2, and 3, respectively). (*b*) Distribution of DNase I-hypersensitive sites upstream of the ε-globin gene. Horizontal arrows denote the degradation fragments. Vertical arrows mark the location of the DNase I-hypersensitive sites.

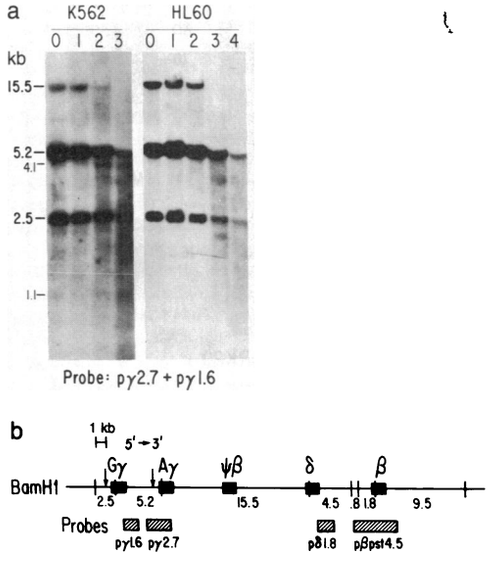

FIG. 3. (*a*) Southern blots of DNA from K562 or HL60 cells, cleaved with *Bam*HI. DNase I concentrations were 0, 10, 15, or 20 μg/ml for K562 nuclear DNA (lanes 0–3, respectively) and 0, 2.5, 5, 10, or 15 μg/ml for HL60 nuclear DNA (lanes 0–4, respectively). (*b*) Distribution of DNase I-hypersensitive sites in the γ-,δ-,β-globin gene region.

generates an 11-kb degradation band (Fig. 4*a*). Since the hybridization probe (pRK29) does not hybridize to the 5′ or the 3′ terminal sequences of the 16-kb fragment, we can place the hypersensitive site on either the 3′ side or the 5′ side of the pRK29 probe (these alternative sites are marked by solid and broken vertical arrows, respectively, in Fig. 4*b*). We have placed the hypersensitive site (denoted HS VI in Fig. 4*b*) on the 3′ side of the pRK29 probe, because we also observe a 4.5-kb degradation band produced from a 9-kb *Pst* I fragment (12), which does not contain the 7-kb of DNA spanning the alternative hypersensitive site (not shown); HS VI is situated about 20 kb downstream of the β-globin gene (Fig. 4*b*). In HL60 cells, we detect no DNase I-sensitive degradation bands (Fig. 4*a*) that are derived from the 16-kb parental *Bam*HI fragment. HS VI is therefore not present in HL60 cells, which express none of the β-like-globin genes.

The presence of HS VI in K562 cells does not seem to be associated with nearby non-globin genes that are being actively transcribed. The DNA sequence immediately upstream of HS VI, subcloned in pRK29, does not hybridize to K562 RNAs in RNA dot-blotting experiments (unpublished data) and therefore does not seem to serve as a template for RNA transcription. Further upstream of HS VI is a cluster of repetitive DNA sequences (13), belonging to the *Kpn* I middle-repetitive sequence family (14), which is unlikely to contain structural genes. Downstream of HS VI there are also clusters of repetitive sequences, including, among others, members of the *Kpn* I and *Alu*-repetitive sequence (15) families (R. Kaufman, personal communication), which are also unlikely to contain structural genes. HS VI, located about 20 kb downstream of the β-globin gene, thus seems to be associated with ε- and γ-globin gene transcription in K562 cells. To determine whether HS VI is associated with β-like-globin gene transcription in other cells of erythroid lineage, we have mapped the area downstream of the β-globin gene in a human erythroleukemia cell line (HEL), which expresses predominantly the γ-globin genes (ref. 5 and unpublished data). In HEL cells we find the same 11-kb degradation fragment (Fig. 4*a*); HS VI is therefore also present in HEL cells. Furthermore, in nucleated adult human marrow cells containing erythroid cell precursors, which express predominantly the β-globin gene (unpublished), we also detect the 11-kb degradation band (Fig. 4*a*). This band is relatively faint because the amount of erythroid DNA which can give rise to the 11-kb degradation band is only one-quarter of the total DNA sample. The 11-kb degradation band generated by cleavage at HS VI is clearly discernible in lanes 2 of the K562, HEL, and marrow blots in Fig. 4*a*. HS VI is therefore more sensitive to DNase I than those sites immediately 5′ of the ε- and γ-globin genes but less sensitive than the most sensitive site 6-kb upstream of the ε-globin gene.

In summary (see Fig. 6), HS VI is present in cells that express predominantly the embryonic ε-globin gene (in K562), the fetal γ-globin genes (in HEL), or the adult β-globin gene (in adult marrow). HS VI is, however, not present in HL60 cells, which express none of the β-like-globin genes.

DNase I-Hypersensitive Sites Far Upstream of the ε-Globin Gene. In a 15.6-kb *Bam*HI fragment far upstream of the ε-globin gene (Fig. 5*c*), there are two hypersensitive sites producing degradation bands at 9.2 and 4.7 kb, respectively (Fig. 5*a*, lane 1). The hypersensitive site generating the 9.2-kb

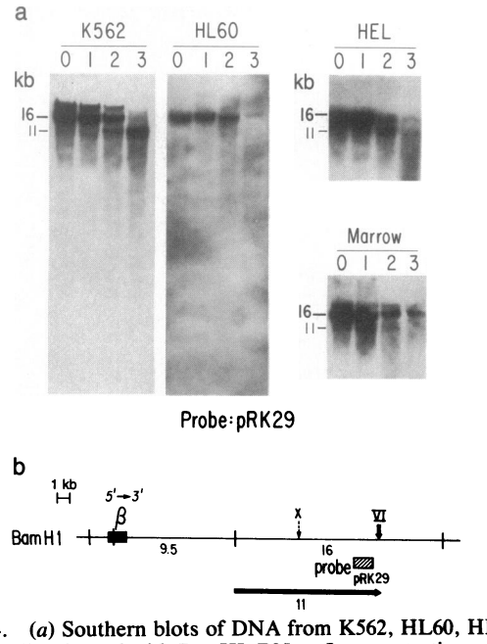

FIG. 4. (*a*) Southern blots of DNA from K562, HL60, HEL, or marrow cells, cleaved with *Bam*HI. DNase I concentrations were 0, 10, 15, or 20 μg/ml for lanes 0–3, respectively. (*b*) Location of the DNase I-hypersensitive site far downstream of the β-globin gene. Vertical arrow VI marks one possible location of the DNase I-hypersensitive site; broken vertical arrow X marks the alternative location of the DNase I-hypersensitive site.

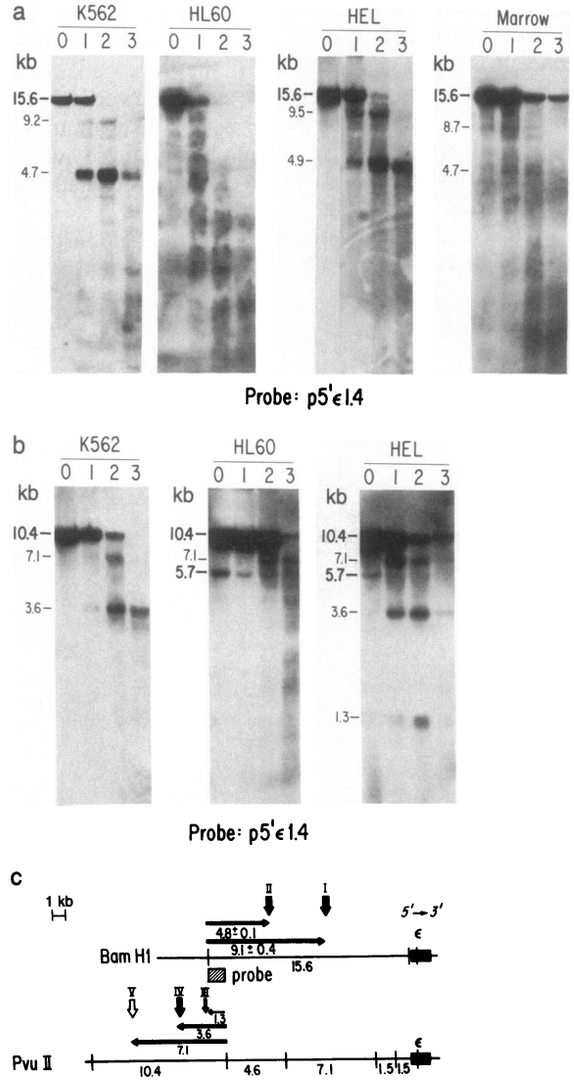

FIG. 5. (*a* and *b*) Southern blots of DNA cleaved with *Bam*HI (*a*) or *Pvu* II (*b*). DNase I concentrations were 0, 10, 15, or 20 μg/ml for the samples in lanes 0–3, respectively. (*c*) Distribution of DNase I-hypersensitive sites (HS I–V) in the area far upstream of the ε-globin gene.

degradation band is located 6.4 kb from the *Bam*HI site bordering the 3′ end of the 15.6-kb *Bam*HI fragment and is also about 6.4-kb from the ε-globin gene, since the 3′ *Bam*HI site abuts the ε-globin gene (Fig. 5*c*). This hypersensitive site is marked by vertical arrow I in Fig. 5*c*. This site, HS I, 6.4-kb upstream of the ε-globin gene, and the most sensitive site 6.1-kb upstream of the ε-gene (Fig. 2) are probably one and the same site. The hypersensitive site whose cleavage generates the 4.7-kb degradation band is marked by vertical arrow II in Fig. 5*c* and is located about 11-kb upstream of the ε-globin gene. In the HL60 blot (Fig. 5*a*), we detect no degradation bands. In the HEL blot, however, we detect degradation bands at 9.5 and 4.9 kb; in the marrow blot, we detect degradation bands at 8.7 and 4.7 kb (Fig. 5*a*). The sizes of the 9.5-kb band in HEL cells, of the 8.7-kb band in marrow cells, and of the 9.2-kb band in K562 cells are within the error of measurement (2–8%) of the technique; therefore, we consider these bands to be generated from cleavage at the same HS I present in each cell line. We think that the 4.7-kb degradation bands in K562 and marrow cells and the 4.9-kb band in HEL cells are generated by cleavage at the same HS II present in each cell line.

In a 10.4-kb *Pvu* II restriction fragment further upstream of the ε-globin gene, we detect major degradation bands at 7.1 and 3.6 kb in K562 (Fig. 5*b*, lanes 1 and 2). The hypersensitive site (HS IV) generating the 3.6-kb degradation band is marked by vertical arrow IV in Fig. 5*c*. It is located about 17.5-kb upstream of the ε-globin gene. The hypersensitive site (HS V) generating the 7.1-kb degradation band is marked by open vertical arrow V and is located about 21.5 kb upstream of the ε-globin gene. In HL60 cells, which express no β-like-globin genes, the 7.1-kb degradation band generated by HS V is present but the 3.6-kb degradation band generated by HS IV is not. The 5.7-kb band present in all four lanes in the HL60 blots (present also in the HEL blot in Fig. 5*b*) is a cross-hybridization band with the p5′ε1.4 probe, observed because both the HL60 and HEL blots were hybridized and rinsed under less stringent conditions than the K562 blot in Fig. 5*b*. In HEL cells, the 7.1-kb degradation band generated by HS V and the 3.6 kb degradation band generated by cleavage at HS IV are both present (Fig. 5*b*, lanes 1 and 2). In addition, there is a 1.3-kb degradation band (Fig. 5*b*) generated by cleavage at HS III (Fig. 5*c*), which is, however, not detected in either K562 or HL60 cells and may be a site peculiar to the HEL cells.

In summary (Fig. 6), HS I and HS II are present in K562, HEL, and nucleated marrow cells expressing at least one β-like-globin gene but are absent in HL60 cells which express none of the β-like-globin genes. Likewise, HS IV is present in both K562 and HEL cells but is absent in HL60 cells. The presence of HS I, HS II, and HS IV thus appears to be associated with β-like-globin gene transcription. The most upstream site, HS V at −21.5 kb, is present not only in K562 and HEL cells but also in HL60 cells. The relationship, if any, of HS V to β-like-globin gene transcription is uncertain.

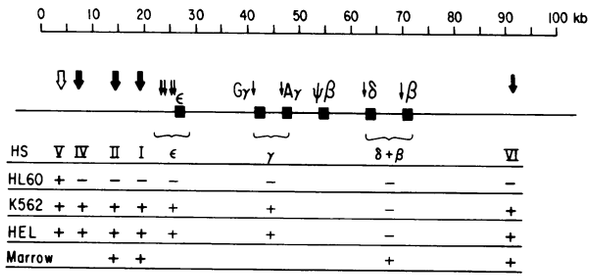

FIG. 6. The distribution of major and minor DNase I-hypersensitive sites in the human β-like-globin gene domain. HS V, located probably outside of the β-like-globin gene domain, is marked with an unfilled vertical arrow. Data on HS ε, HS γ, and HS δ+β in HEL and on HS δ+β in marrow cells are from our unpublished observations. + and − represent the presence or absence of various hypersensitive sites in each cell type.

The presence of HS I, HS II, and HS IV in K562, HEL, and nucleated marrow cells does not seem to be associated with other nearby structural genes, because the area of DNA spanned by HS I, II, and IV, with the exception of only two short gaps of nonrepetitive DNA, is comprised of repetitive DNA sequences (unpublished data, and R. Kaufman, personal communication) and therefore does not seem to contain structural genes. The nonrepetitive sequence gaps, however, could code for structural genes. DNA corresponding to the p5′ε1.4 probe, which was subcloned from one of the nonrepetitive sequence gaps, is indeed transcribed in K562 cells, as determined by the RNA dot-blotting technique; the transcripts, found mostly in the nucleus (unpublished data), are unlikely to code for a protein product in K562 cells.

The DNA upstream of the ε-globin gene up to an area 2 kb 5′ of HS IV has been sequenced (27). Correlating the sequence data with the locations of the above hypersensitive sites, we found that HS I, at −6 kb, is in an area that contains, within 800 bases, three enhancer core-like sequences (16), a stretch of 28 consecutive thymidylate residues, and a stretch of 21 consecutive pairs of alternating purine or pyrimidine bases [$(CA)_{15}(TA)_6$]. HS II, at −11 kb, is in an area that contains, within 900 bases, two enhancer core-like sequences and a stretch of 26 consecutive pairs of alternating purine and pyrimidine bases [$(TA)_{10}(CA)_2(TA)_2(CG)(TA)_{11}$]. HS III, at −14.5 kb, is in an area that contains, within 400 bases, three enhancer core-like sequences and a fourth enhancer core-like sequence, which is followed by 10 nonconsecutive pairs of purine and pyrimidine bases embedded in short stretches of purine or pyrimidine bases [GGGAAAGGTGGGGGAGG-$(CA)_2$G(CA)(TA)(GC)(AT)A(GC)A(GC)(AT)TTT-T(CA)TT]. HS IV, at −17.5 kb, is in an area which contains, within 600 bases, two enhancer core-like sequences and 12 nonconsecutive pairs of purine and pyrimidine bases embedded in short stretches of purine or pyrimidine bases [(CA)-(TA)(CA)CTCT$(CA)_5$AA(CA)(TA)A(AC)(TA)AA]. We do not know the sequence features of HS V because it is outside of the area whose sequence has been determined.

DISCUSSION

The distribution of the major and minor DNase I-hypersensitive sites in the entire human β-like-globin gene cluster is presented in Fig. 6. The minor hypersensitive sites include the 4 sites within 4 kb upstream of the ε-globin gene (denoted HS ε in Fig. 6) and the sites immediately 5′ of the Gγ- and Aγ- (HS γ in Fig. 6) and of the δ- and β-globin genes (HS δ+β in Fig. 6). The sites immediately 5′ of the globin genes are situated close to the promoter region of the individual globin genes and appear to be associated with the transcriptional activity of the adjoining globin gene. For example, in K562 and HEL cells, these minor hypersensitive sites are present 5′ of the actively transcribed ε- and γ-globin genes but are absent 5′ of the inactive β-globin gene; conversely, in adult human marrow cells containing erythrocyte precursors, the minor hypersensitive sites are present 5′ of the active β-globin gene (ref. 10 and unpublished data). The major DNase I-hypersensitive sites HS I, HS II, and HS IV, situated upstream of the ε-globin gene, and HS VI, situated downstream of the β-globin gene also seem to be associated with β-like-globin gene expression, since they are present in K562, HEL, and adult nucleated marrow cells, which express the β-like-globin genes, and are absent in HL60 cells, which do not express the β-like-globin genes. However, they differ from the minor DNase I-hypersensitive sites in at least four respects. First, they are much more sensitive to DNase I digestion and seem therefore to be located in a much more

open and accessible chromatin structure. Second, they are located much farther from the globin genes than the minor hypersensitive sites and seem to bracket the β-like-globin genes and the minor hypersensitive sites. Third, the appearance of HS I, II, and IV and of HS VI, which is separated from HS I, II, and IV by 70–85 kb, seems to be coordinately controlled. Both the far upstream sites (HS I, II, and IV) and the far downstream site (HS VI) either are present (in K562, HEL, and nucleated marrow cells) or are absent (in HL60). Fourth, the appearance of these major hypersensitive sites is not closely coupled to any specific globin gene transcription as is that of the minor hypersensitive sites; they are present in specific cell lines irrespective of whether the predominantly expressed globin gene is the embryonic ε-globin gene in the K562 cell, or the fetal γ-globin genes in the HEL cell, or the adult β-globin gene in the adult nucleated marrow cell (Fig. 6). As with the active ovalbumin multigene family in chicken oviduct (17) and the β-like-globin genes in chicken erythroblasts (18), it is possible that the human β-like-globin genes may also be organized in an active chromatin domain in K562, HEL, and adult nucleated marrow cells, with HS I, II, and IV and HS VI possibly marking the locations of the 5′ and 3′ boundaries of the active chromatin domain. The most upstream major hypersensitive site, HS V, which is present not only in cells expressing the β-like-globin genes but also in HL60 cells not expressing these genes, may then represent either the limit of the 5′ boundary of the human β-like-globin gene domain or part of the boundary area of a neighboring domain. If the limit of the 5′ boundary lies somewhere between HS IV and HS V and if the beginning of the 3′ boundary is in an area marked by HS VI, the human β-like-globin gene domain would then span at least 90 kb of DNA. We do not know how far downstream from HS VI the 3′ boundary extends because of the unavailability of a unique-sequence probe in this area (R. Kaufman, personal communication).

Active chromatin domains show overall sensitivity toward DNase I digestion when compared to unexpressed genes or DNA outside of the domain (17, 18). This overall nuclease sensitivity may represent a state of transcriptional preactivation of the structural genes contained within such active domains (19). Thus, in the active β-like-globin gene domain in K562 and HEL cells, the actively transcribed embryonic ε-globin (unpublished data) and fetal γ-globin genes as well as the nontranscribed β-globin gene display the same overall DNase I sensitivity (10). This suggests that the transcribed ε- and γ- as well as the nontranscribed β-globin genes are all in a transcriptionally preactivated state. For actual transcription to take place, the chromatin structure around the preactivated globin genes needs to be further modulated by additional factors. The presence of the minor hypersensitive site 5′ of the transcribed ε- and γ-globin genes and the absence of these minor DNase I-hypersensitive sites 5′ of the nontranscribed β-globin gene in K562 and HEL cells (Fig. 6) may reflect such a requirement of further modulation in chromatin structure. A possible function of major hypersensitive sites I, II, IV, and VI may be to organize and maintain the β-like-globin gene domain in an overall DNase I-sensitive, transcriptionally preactivated state, such that the chromatin structure 5′ of the embryonic ε-, fetal γ-, and adult β-globin genes could be further modulated by cellular signals affecting the transcription of each specific globin gene.

The nucleotide sequence of the DNA upstream of the ε-globin gene up to 2 kb 5′ of HS IV has been determined (unpublished data). Correlating the sequence data with the locations of the major DNase I-hypersensitive sites, we found common sequence features in HS I–IV. These major hypersensitive sites all contain two or three enhancer core-like sequences (16) and 10–26 consecutive or nonconsecutive pairs of alternating purine and pyrimidine bases, found also in many transcriptional enhancers (20). The immunoglobulin enhancer sequences display tissue-specific DNase I hypersensitivity (21, 22) and are recognized by lymphoid-specific cellular factors (23–25). The DNA sequences contained in the above major DNase I-hypersensitive sites, which share common sequence features with these enhancers (23–26), might also possess enhancer function and be recognized by erythroid-specific cellular factors. Whether HS I, II, and IV can serve as transcriptional enhancers for the β-like-globin genes remains to be investigated.

We thank Drs. Arthur Nienhuis and Bernard Forget for critical review of the manuscript, Dr. Paul Eder for the nucleated adult human marrow cells, Dr. Thalia Papayannopoulou for the HEL inoculum, and Dr. Russel Kaufman for the pRK29 probe. This work was supported by National Institutes of Health Grant AM16272 and by the National Foundation for Cancer Research. Q.L. was supported by National Institutes of Health Grants GM20069 and AM20120 to Dr. Oliver Smithies. W.S. was a recipient of a Physicians Research Training Fellowship from the American Cancer Society.

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