Multiple ε-Promoter Elements Participate in the Developmental Control of ε-Globin Genes in Transgenic Mice*

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To delineate the regulation of the human ε-globin gene, we investigated ε-gene expression during the development of transgenic mice carrying constructs with ε-promoter truncations linked to a micro-focus control region (μLCR). Expression levels were compared with those of μLCRε mice carrying a 2-kilobase ε-promoter and βγAC controls. ε mRNA in the embryonic cells of μLCR (−179)ε mice were as high as in μLCRε mice suggesting that the proximal ε-promoter contains most elements required for ε-gene activation. ε mRNA in adult μLCR (−179)ε mice was significantly lower than in the embryonic cells indicating that elements involved in ε-gene silencing are contained in the proximal ε-promoter. Extension of the promoter sequence to −463 ε decreased ε-gene expression in the definitive erythroid cells, supporting previous evidence that the −179 to −463 region contains an ε-gene silencer. However, the ε-gene of the μLCR (−463)ε was not silenced in the definitive cells of fetal and adult erythropoiesis indicating that additional silencing elements are located upstream of position −463ε. These results provide in vivo evidence that multiple elements of the distal as well as the proximal promoter contribute to ε-gene silencing.

All animal species have different hemoglobins in the embryonic and definitive stages of development. These hemoglobins are synthesized under the control of globin genes whose expression is restricted to either the definitive or the primitive stages of erythropoiesis. In the mouse there are two embryonic genes, ε and β1, and two adult genes βmajor and βminor; expression of embryonic genes is totally restricted to the yolk sac stage of erythropoiesis, whereas adult gene expression starts only after the onset of definitive hematopoiesis in the liver of the 11-day-old mouse fetus. In humans, the first gene of the β globin locus to be expressed is the embryonic (ε) followed by the two fetal (γ) genes and the adult δ and β genes (1). ε-globin synthesis occurs predominately in primitive yolk sac origin erythroblasts, where it accounts for over 80% of β-like globins at 5 weeks of gestation, falling to 15% by week 7 (2–5). In humans, the ε-gene is totally and permanently silenced after the 7th week of gestation. The silencing of the ε-gene is controlled at the transcriptional level as shown by the total absence of a DNase I hypersensitive site in the ε-gene promoter of erythroid cells of 54-day-old human embryos (6).

The absolute nature of ε-globin gene silencing is remarkable in view of the relative proximity of the ε-gene to the locus control region (LCR), residing 6–22 kb upstream. The LCR, characterized physically by five DNase I hypersensitive sites (HS), influences chromatin structure over the entire β globin domain (7), acts as a powerful erythroid-specific enhancer (8, 9), and protects linked globin genes from the effects of surrounding chromatin (9, 10). Expression of the ε-gene in transgenic mice requires the presence of the LCR (11, 12); in mice bearing a 2.5-kb micro LCR cassette linked to the ε-globin gene, ε-globin expression is restricted to the primitive, yolk sac origin erythroblasts (11). Transgenic mice bearing a fragment containing the LCR hypersensitive sites 1 and 2 and contiguous ε 5′-flanking sequence fused to the coding region of the γ globin gene display an embryonic pattern of transgene regulation (13), suggesting that the cis elements necessary for proper developmental control of ε-globin gene are contained within its promoter and 5′-flanking sequence (12–14). Several negative regulatory elements have been identified upstream of the ε-globin gene (14–18). Transient expression assays have localized a silencer between 392 and 177 base pairs upstream of the ε-globin cap site (15, 16). Transgenic mice carrying a 2-kb ε-gene promoter from which the −177 to −392 silencer has been deleted express the ε-globin gene in the adult stage of development (14).

Transgenic mice provide an excellent model for delineation of the sequences necessary for ε-gene silencing, because of the absolute restriction of ε-globin gene expression in the yolk sac cells of the mouse. If such sequences exist, their deletion or mutation is expected to be associated with loss of ε-gene silencing resulting in continuation of ε-gene expression in the adult stage of development. In the experiments described in this report developmental studies were performed using transgenic mice containing an LCR cassette linked to an ε-globin gene containing only 179 or 463 bp of promoter sequence. We found that although a significant level of developmental control is retained in the proximal promoter, the bulk of ε-gene silencing resides with sequences located upstream of −179 ε. These sequences include but are not limited to the −177 to −392 ε-silencer. These results provide in vivo evidence that elements located both in the proximal as well as in the distal ε-gene promoter are involved in ε-gene silencing.

EXPERIMENTAL PROCEDURES

DNA Constructs (Fig. 1)—pμLCR(−463)ε was produced from a modified version of pμLCRε, which contains 2 kb of sequence upstream of the ε-globin gene transcription start site. The μLCR cassette of pμLCRε was modified to provide an additional 0.6 kb of sequence 5′ to HS4 of the original μLCR, producing 3.1-kb pμLCRε (thereafter, the term μLCR

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* The abbreviations used are: LCR, locus control region; kb, kilobase(s); HS, hypersensitive sites; bp, base pair(s).

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FIG. 1. $\epsilon$-gene constructs used for production of transgenic mice. A, schematic representation of the human $\beta$-globin locus. Numbers correspond to GenBank™ coordinates (Humberb). The thick vertical arrows indicate the DNase I hypersensitive sites of the LCR. Filled boxes show the five transcribed globin genes, whereas the open box marks the position of the pseudo $\beta$ gene. $B$, the left line shows the $\mu$LCR, a 3.1-kb truncated version of the LCR. The numbers above the line indicate the 5' and 3' ends of each HS fragment. The right line is a 3.7-kb EcoRI fragment encompassing the $\epsilon$-globin gene spanning from −2025 to +1745 relative to the cap site. $C$, $\epsilon$-globin gene promoter. Shown are the location of the conserved boxes and the binding motifs for various proteins. + and − correspond to the sites of positive and negative elements identified by transient transfection assays (43). The truncated positions of the two $\mu$LCR constructs used in this study are indicated by arrows (BamHI and BrfI).

used in the text represents this new LCR cassette except where otherwise indicated). $\mu$LCR was linearized using Smal, partially digested using BrfI, and the digestion products were blunted using Klenow enzyme. An 8.3-kb product was gel purified and religated to generate $\mu$LCR−463 e. To generate $\mu$LCR−179 e the construct $\mu$LCR e was linearized using Smal, partially digested using BamHI, and the digestion products were blunted using Klenow enzyme. A 7.9-kb product was gel purified and religated to produce $\mu$LCR−179 e.

All constructs were freed from vector sequences using restriction enzymes, gel purified, and resuspended in filtered Tris-EDTA before injection into fertilized ooytes.

Transgenic Mice—Transgenic mice carrying the $\mu$LCR−179 e and $\mu$LCR−463 e constructs were produced as described previously (19). Founder animals were identified by Southern blotting with an $\epsilon$-gene sequence probe. F1 progeny were obtained by breeding founder animals and were screened for correct integration and to exclude the presence of mosaicism in the founders. To study the developmental pattern of human $\epsilon$-gene expression, staged pregnancies were interrupted on days 9, 12, and 16 of development. Samples from blood and yolk sac were collected on day 9 embryos; blood, yolk sac and liver were collected on day 12 fetuses; and blood and yolk sac were collected on day 16 fetuses.

$\epsilon$ mRNA Quantitation—Total RNA was isolated from transgenic tissues by the method of Chomczynski and Sacchi (20). The $\epsilon$ mRNA level was measured by the quantitative RNase protection assay described previously (21). Briefly, riboprobe for $\epsilon$ mRNA was labeled by transcribing the linearized plasmid pT7e188 using T7 RNA polymerase (22). The $\epsilon$ probe protects a 188-bp fragment in exon 2 of $\epsilon$ mRNA. The mouse $\alpha$ and $\zeta$ riboprobes were used in RNase protection assays as internal globin mRNA controls. mRNA levels were determined in all transgenic siblings of each litter. RNA samples from different tissues were analyzed at least twice to reduce experimental error in mRNA quantitations. Human $\epsilon$ and mouse $\alpha$ and $\zeta$ signals were quantitated with a PhosphorImager. Levels of human mRNA per transgene copy were expressed as percentages of mouse $\alpha$-like mRNA levels per copy, taking into account that the mouse possesses four copies of the $\alpha$-globin gene and two copies of the $\zeta$-globin gene. In the adult stages of development when $\zeta$ mRNA is absent, murine $\alpha$ mRNA per copy was calculated by dividing the levels of murine $\alpha$ mRNA by four.

Copy Number Determination—Copy number determination was accomplished by the multiply redundant protocol described previously (21) to reduce experimental errors. Multiple DNA samples were obtained from each of at least three animals from each transgenic line. These samples were digested with restriction enzyme EcoRI and resolved by electrophoresis over 1% agarose gel. Southern blots were hybridized with a radiation-labeled $\epsilon$ probe by using a 0.6-kb BamHI fragment of the $\epsilon$-globin gene as template. The signals were quantitated on a PhosphorImager. Copy numbers were calculated by dividing the relative intensity of signals from a given transgenic line compared with the signals obtained from diploid human genomic DNA.

RESULTS

Constructs (Fig. 1)—The construct $\mu$LCR−179 e consists of a 1.9-kb $\epsilon$-gene fragment that contains the $\epsilon$-globin gene, 179 bp of sequences of the $\epsilon$-gene promoter and 280 bp of 3'-non-translated sequences. The −463 $\epsilon$ construct contains $\epsilon$ genomic sequences identical to those of the −179 $\epsilon$ construct, but the promoter is extended to a BrfI site at position −463. This construct therefore contains the −177 to −392 sequence previously shown in transient assays (15) and transgenic mouse studies (14) to behave like an $\epsilon$-gene silencer.

The −179 e or −463 e-fragments were linked to a 3.1-kb $\mu$LCR, which consists of 0.71 kb of HS1, 0.73 kb of HS2, 0.56 kb of HS3, and 1.1 kb of HS4. This 3.1 $\mu$LCR contains the core element of DNase I hypersensitive sites 1, 2, and 3, which are also present in the previously used 2.5 kb $\mu$LCR (23). The 3.1 $\mu$LCR also contains 600 additional bp of HS4, which includes the core element of HS4; this core of HS4 is missing from the 2.5 $\mu$LCR (23).

Control mice were of two kind. First, 3.1 $\mu$LCR e and 2.5 $\mu$LCR e mice in which the $\epsilon$-gene promoter is extending 2-kb upstream of the cap site, i.e. in the EcoRI site at position −2040. Second, three $\beta$YAC lines that were produced using a 248-kb $\beta$-locus YAC. These lines have been analyzed in detail for structural integrity using previously published protocols (24) and found to contain an intact $\beta$-globin locus, from 5' HS4 to 3' HS1.

Analysis of $\epsilon$-Globin Gene Expression in Cells of Embryonic and Definitive Murine Erythropoiesis—For developmental studies, timed pregnancies were interrupted at 9, 12, and 16 days, and yolk sac, blood, and fetal liver samples were collected for measurement of human $\epsilon$ and murine $\alpha$ and $\zeta$ mRNA by RNase protection (Fig. 2). More than one tissue was analyzed in each gestational day to increase the accuracy of $\epsilon$ mRNA measurements. Multiple members from each litter were used for measurements of globin mRNA. Data from each line and each day are presented in Tables I and II as means ± S.D.

The day 9 blood and yolk sac represent an early stage of embryonic erythropoiesis. We have previously observed that in
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**Panel A**

![Image of RNase protection assay](image)

**Panel B**

![Image of RNase protection assay](image)

**Panel C**

![Image of RNase protection assay](image)

FIG. 2. Representative RNase protection assay for transgenic mice carrying the human globin constructs. Protected fragment sizes are as follows: human e-globin (Hue), 188 nucleotides (nt); mouse g-globin (Mo), 151 nucleotides; mouse a-globin (Mox), 128 nucleotides. Ys = day 9 yolk sac; fb = day 12 fetal blood; fl = day 16 fetal liver; a/b = adult blood. Panel A, LCR(-179) (lines A–F); panel B, LCR(-463) (lines G–I); panel C, YAC (lines J–L), and LCR (lines M and N).

| Line | Construct | Copy number | Day 9 | Day 12 |
|------|-----------|-------------|-------|--------|
|     |           |             | Yolk sac | Blood | Yolk sac | Blood |
| A   | LCR(-179) | 4           | 15.3   | 14.0   | 21.5 ± 2.5 | 22.0 ± 2.8 |
| B   | LCR(-179) | 3           | 18.0 ± 2.4 | 14.8 ± 2.8 | 16.8 ± 3.2 | 19.4 ± 2.6 |
| C   | LCR(-179) | 6           | 14.6   | 16.2   | 11.8   | 9.8   |
| D   | LCR(-179) | 11          | 16.7 ± 1.9 | 17.5 ± 3.87 | 33.3 ± 8.77 | 20.7 ± 8.14 |
| E   | LCR(-179) | 3           | 23.3 ± 1.67 | 19.4 ± 1.36 | 21.0 ± 2.53 | 25.6 ± 1.01 |
| F   | LCR(-179) | 8           | 12.8 ± 1.4 | 11.8 ± 1.8 | 13.6 ± 1.0 |
| G   | LCR(-463) | 15          | 12.4 ± 2.3 | 9.9 ± 1.1 | 13.7 ± 1.1 | 13.2 ± 0.96 |
| H   | LCR(-463) | 6           | 11.2 ± 1.6 | 10.3 ± 1.1 | 16.6 ± 2.7 | 12.7 ± 2.2 |
| I   | LCR(-463) | 24          | 6.6 ± 1.5 | 7.7 ± 1.3 | 14.8 ± 4.9 | 6.0 ± 1.3 |
| J   | YAC       | 2           | 7.5 ± 1.2 | 6.0 ± 1.6 | 12.8 ± 1.3 |
| K   | YAC       | 1           | 9.7 ± 2.1 | 9.8 ± 2.4 | 12.1 ± 5.0 | 18.9 ± 4.1 |
| L   | YAC       | 2           | 7.8 ± 0.36 | 8.5 ± 0.4 | 15.7 ± 0.78 | 18.4 ± 1.6 |
| M   | LCR       | 3           | 18.2 ± 1.2 | 15.5 ± 1.4 | 15.8 ± 3.0 | 19.6 ± 0.15 |
| N   | LCR       | 10          | 21.3 ± 3.5 | 18.6 ± 0.60 | 25.7 ± 7.0 | 21.2 |

| Table 1 | Human e mRNA levels in the embryonic erythropoiesis of transgenic mice with truncated e-gene promoter and YAC or LCR controls |

μLCRe or βYAC transgenic mice the levels of human e mRNA peak at day 12 (22, 24–27). The day 12 yolk sac still contains large numbers of nucleated embryonic red cells, and the day 12 fetal blood is composed predominantly from nucleated erythrocytes of yolk sac origin. Therefore, blood and yolk sac samples from day 12 fetuses were used to assess e-globin expression in day 12 embryonic erythropoiesis.

e-gene expression in cells of definitive erythropoiesis was studied using day 12 fetal liver, day 16 fetal liver, day 16 fetal blood, and adult blood. The day 12 fetal liver is an organ of adult erythropoiesis, and it mostly consists of definitive erythroblasts that can be distinguished from the embryonic erythroblasts by their smaller size and small cytoplasmic nuclear ratio. There is no expression of murine embryonic εy and βh1 genes or human ε-transgenes in fetal liver erythropoiesis (11, 14, 28). Yolk sac origin erythroblasts that contaminate the fetal liver preparations (11) account for the small levels of e mRNA detected in fetal liver specimens from μLCRe or βYAC transgenic mice.

**ε-Gene Expression in the Embryonic Cells of μLCR(-179)ε Transgenic Mice and Controls**—The μLCR(-179)ε construct contains all the transcriptional motifs of the proximal ε-gene promoter, i.e., the TATA box at −30, the CAAT box at −84, the CACC box at −113, and a GATA-1 site in position −179. Although the proximal promoters of the β-like globin genes share a basic organization, several differences also exist between promoters. Differences between the e- and γ-gene promoters include the presence of a duplicated CAAT box in the γ-promoter and divergence of sequences surrounding the TATA, CAAT, and CACC boxes. Presumably these structural differences contribute to the difference in the developmental regulation of e- and γ genes in humans.

Using the μLCR(-179)ε recombinant we produced 8 trans-
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TABLE II
Human e mRNA levels in definitive erythropoiesis of transgenic mice with truncated e-gene promoter and βYAC or μLCRε controls

| Line | Construct | Day 12 liver | Day 16 | Adult Blood |
|------|-----------|--------------|--------|-------------|
| A    | μLCRε(−179) | 12.3 ± 3.0  | 10.8 ± 2.5  | 13.3 ± 0.25 | 5.5  |
| B    | μLCRε(−179) | 19.0 ± 3.8  | 5.2 ± 1.2   | 5.8 ± 0.8   | 8.6 ± 0.92 |
| C    | LCR(−179)   | 5.0 ± 0.8   | 5.7 ± 0.29  | 2.2         |
| D    | μLCRε(−179) | 15.3 ± 5.78 | 6.2 ± 1.86  | 5.31 ± 0.97 | 6.6 ± 3.4 |
| E    | μLCRε(−179) | 11.4 ± 1.78 | 5.21 ± 0.98 | 7.1 ± 1.18  | 5.35 ± 1.28 |
| F    | μLCRε(−179) | 15.8 ± 2.8  | 3.5 ± 0.1   | 2.6 ± 0.6   | 3.8 ± 0.41 |
| G    | μLCRε(−463) | 5.1 ± 0.54  | 3.3 ± 0.81  | 1.9 ± 0.3   | 1.6 ± 0.85 |
| H    | μLCRε(−463) | 6.4 ± 1.0   | 5.1 ± 0.52  | 4.2 ± 0.92  | 2.4 ± 0.9 |
| I    | μLCRε(−463) | 3.9 ± 0.29  | 4.1 ± 0.46  | 5.0 ± 0.71  | 1.1 ± 0.35 |
| J    | βYAC       | 2.3 ± 0.9   | 0           | 0           | 0         |
| K    | βYAC       | 2.0 ± 1.8   | 0           | 0           | 0         |
| L    | βYAC       | 3.7 ± 0.35  | 0           | 0           | 2.2       |
| M    | 3.1 μLCRε  | 1.3 ± 0.26  | 0.05 ± 0.07 | 0.18 ± 0.10 | 0.18 ± 0.07 |
| N    | 2.5 μLCRε  | 0.0 ± 0.0   | 0.67 ± 0.13 |            |           |

Fig. 3. e mRNA levels in cells of day 9 and day 12 embryonic (yolk sac) erythropoiesis. Constructs are indicated at the top of each column. At the bottom of each column the means ± S.D. of the measurements are shown. □, expression in yolk sac; ○, expression in blood.

The lower levels of e mRNA in the βYAC transgenic mice most likely represent the decreased chance of interaction between the e-gene and the LCR when a γ globin gene is also present in the construct. γ mRNA in embryonic cells of βYAC mice is considerably higher than e mRNA (24–27), suggesting that the murine embryonic trans acting environment favors the transcription of human γ gene.

e-Gene Expression in the Embryonic Erythropoiesis of −463 e-Transgenic Mice—The μLCR(−463) construct contains the sequence −177 to −392 previously shown to harbor an e-gene silencer (14, 15). This silencer contains sites that bind erythroid-specific as well as constitutive transcriptional factors (16–18, 28, 30), a GATA-1 binding site at −208, two GATA-1 binding sites that overlap with a YY1 site at −269; and a CCACC site at −379 that binds Sp-1 or a related factor.

Seven founder lines were produced but only three transmitted the transgene and were used for developmental studies (Fig. 2, panel B and Table I). Levels of e mRNA (corrected per copy of transgene) in the 9-day embryonic cells ranged from 7.7 to 12.4% (mean 9.7 ± 2.24%) and in the 12-day embryonic cells from 6.0 to 16.6% (mean 12.8 ± 3.6%). Therefore, e-gene expression in the embryonic tissues of the μLCR(−463) transgenic mice was considerably lower compared with the μLCR(−179) transgenic mice at both the day 9 and day 12 developmental stages (Fig. 3). The lower e-gene expression in the μLCR(−463) mice cannot be attributed to the increased distance of the LCR from the proximal promoter because, as shown in Fig. 3, e-gene expression in the μLCRε mice (which contain a promoter extending 2 kb from the cap site) is higher than in μLCR(−463) mice. The lower levels of e mRNA in the μLCR(−463) mice may reveal the presence in the −179 to −463 sequence of negative elements that can be detected only when other upstream sequences are deleted.

e mRNA Levels in Cells of Definitive Erythropoiesis of the μLCRε and βYAC Mice—As shown in Table II, the 12-day fetal liver samples of the βYAC mice contain from 2 to 3.7% e mRNA deriving from contaminating embryonic erythroblasts. There is no e mRNA in the 16-day blood and liver samples or in adult blood of the βYAC transgenic mice. In the 3.1 μLCRε and 2.5 μLCRε mice, traces of e mRNA are detected in the 16-day liver (0.11%) and adult blood (0.18–0.67%). Therefore, compared with the β-gene YACs, the two μLCRε constructs are “leaky” and allow synthesis of residual levels of e mRNA in the cells of definitive erythropoiesis.

e mRNA Levels in Definitive Erythroid Cells of −179 e Mice—Fig. 4 shows that e-gene expression in the 12-day fetal liver of the six μLCR(−179) lines is significantly higher than in the 2.5 μLCRε and 3.1 μLCRε controls. The difference is even more striking in the 16-day fetal liver in which the levels of e mRNA...
in μLCR(-179)e mice are 30–50-fold higher than in the 3.1 μLCRe control (Table II). The μLCR(-179)e construct, however, has not totally lost the ability to down-regulate e-gene expression, because, as shown in Fig. 5A, there is a significant decline in e-mRNA levels in the adult μLCR(-179)e-transgenic mice. This is in contrast to a μLCRAγ gene containing only the proximal γ-promoter (μLCR(-201)Aγ), which is not down-regulated in the adult and it directs similar levels of γ mRNA in the adult and in the embryonic cells of transgenic mice (19).

The levels of e-gene expression in the adult and in the embryonic cells of transgenic mice (19).

Expression of μLCR(-179)e and μLCR(-463)e Transgenes Is Independent of Position of Integration—The results in Tables I and II show consistency of expression of the e-gene in the mouse lines carrying the μLCR(-179)e or the μLCR(-463)e constructs. The mean e-gene expression in the μLCR(-179)e mice is 18.9 ± 5.5 in the day 12 embryonic blood and 5.3 ± 2.2 in adult blood. The coefficients of variation in per copy expression between the μLCR(-179) e lines are 0.29 for the fetal day-12 embryos and 0.41 for the adult stage, respectively. Small coefficients of variation (less than 0.5) indicate that the expression of a transgene is independent of the position of integration (21). Since e-gene expression is not influenced by the position of integration of the transgenes, the developmental profiles shown in Tables I and II must reflect an inherent property of the μLCR(-179)e construct. It is noteworthy that copy number-dependent expression was also previously observed in the μLCR(-201) Aγ transgenic mice containing only the proximal γ gene promoter (19). Copy number dependence of e expression is also characteristic of the construct μLCR(-463)e. Thus, the coefficient of variation is 0.38 in the day-12 embryonic blood and 0.39 in adult blood of the μLCR(-463)e-transgenic mice, indicating that the expression of this transgene is also independent of position of integration.

**DISCUSSION**

Several studies have shown that sequences in the e-gene promoter as well as in the LCR participate in the developmental regulation of the e-globin gene. Experiments in transgenic mice have shown that, in the absence of the LCR, the human e-genes remain silent in the embryonic cells of transgenic mice, indicating that the LCR is necessary for in vivo e-globin gene transcription (11, 12). The contribution of LCR sequences in the developmental control of e-gene has been clearly shown in studies of βYAC transgenic mice containing deletions of DNase I hypersensitive site 3; mice carrying a 2.5-kb deletion removing HS3 and the surrounding flanking sequences display decreased e-globin expression in the embryonic erythropoiesis (26), whereas mice carrying deletions of the core element of HS3 display total absence of e-expression in day-9 embryonic cells and severe reduction of e-expression in day-12 embryonic cells (31). Such data suggest that sequences of the core element of HS3 are necessary for activation of e-gene transcription. The contribution of elements of the e-gene promoter have been investigated in vitro, with transient expression assays and in vivo, in transgenic mice. Studies in transiently transfected cell lines have revealed sequences that either positively (17, 29, 30,
32–34) or negatively (15–17, 35) influence transcription from a linked reporter gene. Several of these sequences have been evolutionarily conserved (36). Studies in transgenic mice have shown that all the cis elements required for α-globin silencing are located in a 2.0-kb fragment that contains the α-globin promoter (11, 28).

In this study we wished to examine to what degree the proximal and distal ε-globin promoter are involved in the developmental regulation of the ε-globin gene in vivo. We used control constructs containing the whole β-locus or a 2-kb ε-promoter and constructs with an ε-promoter containing only the essential transcriptional motifs or these essential motifs as well as a previously identified upstream silencer. We found high levels of ε mRNA in the adult cells of transgenic mice carrying only the proximal ε-promoter indicating that structural elements located in the distal ε-gene promoter are critical for ε-gene silencing. The level of ε mRNA in the adult cells decreased when sequences containing the previously described silencer were added, providing further evidence for the in vivo function of this element. However, presence of this silencer did not totally suppress ε-gene expression in definitive cells suggesting that additional elements located in the distal ε-promoter are involved in ε-gene silencing. Although mice carrying only the proximal ε-promoter have high levels of ε-gene expression in the adult stage of development, the level of ε mRNA in the adult cells is strikingly lower than in embryonic cells, suggesting that sequences located in the proximal ε-promoter participate in ε-gene silencing. Overall, our results provide in vivo evidence that multiple elements located in the distal as well as in the proximal ε-gene promoter are involved in ε-gene silencing.

Liu et al. (37) have recently reported that a sequence located between position −179 to −304 of the ε-gene promoter contains a positive regulatory element, the deletion of which in the context of the β-locus YAC results in catastrophic reduction of ε-globin (as well as on γ-globin) gene expression. This finding contrasts to the results of the present study, which shows that the levels of ε mRNA in the embryonic cells of μLCR (−179) ε-transgenic mice are as high as in transgenic mice carrying an ε promoter extending 2-kb upstream of the ε-gene cap site. Such findings imply that most of the cis elements necessary to interact with the LCR are located in the proximal ε-gene promoter. The results of Liu et al. also contrast to the results of a previous study in which a −179 to −463 ε sequence was deleted from a 2-kb ε-promoter. Transgenic mice carrying this construct have normal ε mRNA levels in embryonic erythroblasts (14), although they should have lacked ε expression if a positive element with the characteristics described by Liu et al. (37) was located in the deleted sequence.

There are several explanations for these discrepant findings. First, it is known that YACs have a considerable tendency for rearrangements and YAC transgenic lines may carry several YAC integrants, each showing different structural rearrangements (24). The finding of Liu et al. (37) could be explained if such rearranged YACs were contained in the transgenic lines they studied, a possibility that has not been totally excluded with the structural analyses done. Second, as previous studies have shown, globin genes behave differently when they are individually linked to the LCR in short constructs or when they are present in constructs containing the whole β-locus. Thus, when the β genes are linked to a 2.5-kb μLCR (38) or a 20-kb LCR (39), they are expressed in embryonic as well as adult cells, whereas they are totally silenced in embryonic cells when they are located in γδ β-cosmid (38, 39) or in βYACs (27, 40). Similar behavior has been documented with γ-globin genes (19, 38, 39). Such results do not invalidate the use of short constructs but they point to the different insights obtained when short globin gene-LCR constructs or constructs containing the whole β-locus are used in transgenic mouse experiments. The short constructs are useful for delineating the functional role of specific sequences flanking the globin genes or elements contained in the HSs of the LCR. Such constructs have allowed the delineation of specific cis elements of the β−(41) or γ−(19) gene promoters or the HSs of the LCR (41–45). Since, in the intact β-locus, the competition between globin gene promoters to interact with the LCR becomes the dominant determinant of gene expression, “whole locus” constructs are most useful for addressing questions on the control of globin gene switching. It is likely that certain regulatory elements have different functions in the different stages of development, and these functions could be identified by the use of the two types of constructs. The discrepancies between our results and those of Liu et al. (37) perhaps reveal that the −179 to −392 sequence has a dual function. In the presence of the transcriptional environment of definitive erythropoiesis this element may act as an ε-gene silencer, and this function was depicted when the LCR ε-gene constructs were used in transgenic mice. In the presence of an embryonic transcriptional environment and an intact β-locus, this sequence may behave as an “anchor” that facilitates the interaction of the LCR with the ε- and γ genes of embryonic cells; this function was perhaps depicted when in the studies of Liu et al. (37) a β-locus YAC with a deleted −179 to −392 ε sequence was used.

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