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Genetics

Human β-globin gene expression in transgenic mice is enhanced by a distant DNase I hypersensitive site

(Southern blot/ribonuclease protection assay/tissue-specific expression)

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ABSTRACT **Several lines of evidence suggest that erythroid-specific DNase I hypersensitive sites (HS) located far upstream of the human β-globin gene are important in regulating β-globin gene expression. We used the polymerase chain reaction technique to amplify and clone an 882-base-pair DNA fragment spanning one of these HS, designated HSII, which is located 54 kilobases upstream of the β-globin gene. The cloned HSII fragment was linked to a human β-globin gene in either the genomic ($\overrightarrow{HSII}$-β) or antigenomic ($\overleftarrow{HSII}$-β) orientation. These two constructs and a β-globin gene alone (β) were injected into fertilized mouse eggs, and expression was analyzed in liver and brain from day-16 transgenic fetuses. Five of 7 β-transgenic fetuses expressed human β-globin mRNA, but the level of expression per gene copy was low, ranging from 0.93 to 22.4% of mouse α-globin mRNA (average 9.9%). In contrast, 11 of 12 $\overrightarrow{HSII}$-β transgenic fetuses expressed β-globin mRNA at levels per gene copy ranging from 31.3 to 336.6% of mouse α-globin mRNA (average 139.5%). Only three fetuses containing intact copies of the $\overleftarrow{HSII}$-β construct were produced. Two of three expressed human β-globin mRNA at levels per gene copy of 179.2 and 387.1%. Expression of human β-globin mRNA was tissue-specific in all three types of transgenic fetuses. These studies demonstrate that a small DNA fragment containing a single erythroid-specific HS can stimulate high-level human β-globin gene expression in transgenic mice.**

The human β-globin gene cluster consists of five functional genes arranged in the order 5′-ε-^{G}γ-^{A}γ-δ-β-3′ on the short arm of chromosome 11 (1–3). The expression of each of these genes is limited to erythroid tissues and to a particular developmental stage: the ε-globin gene is expressed in the embryonic yolk sac, the ^{G}γ- and ^{A}γ-globin genes are expressed in the fetal liver, and the δ- and β-globin genes are expressed in the bone marrow in adulthood (4, 5). Gene transfer experiments using γ- or β-globin genes with immediate flanking sequence have resulted in tissue-specific and developmental stage-specific expression (6–12). However, the level of expression is usually quite low, suggesting that essential cis-acting regulatory elements are located outside the structural globin genes and their immediate flanking sequence.

The study of a small subset of patients with β-thalassemia suggests that such regulatory elements are located some distance from the β-globin gene. In thalassemic individuals from three families, deletions have been defined that do not involve the β-globin gene but affect its expression. A Dutch family with a β-thalassemia syndrome was found to have a deletion that begins 2.5 kilobases (kb) upstream of the β-globin gene and extends upstream for 99 kb (13, 14). The inactive β-globin gene on this chromosome was shown to be structurally normal and to be active *in vitro* (15). We have described an English family with a β-thalassemia syndrome in which an intact β-globin gene is inactive on a chromosome bearing a large deletion that stops 25 kb upstream. The deletion begins in the ^{G}γ-globin gene and continues upstream for >75 kb (16). The β-globin gene on this chromosome was also shown to be normal by *in vitro* expression studies and sequence analysis (17). Recently, a case of β-thalassemia was described in which an intact sickle β-globin gene is inactive on a chromosome bearing a deletion that begins 9.5 kb upstream from the ε-globin gene and extends upstream for 30 kb (18). The common feature of these three mutations is that an intact β-globin gene is inactive in the presence of a deletion that removes sequences upstream from the ε-globin gene. Thus, it appears that sequences essential to β-globin gene expression are located at the upstream end of the β-globin gene cluster.

DNase I analysis of the β-globin gene cluster provided another line of evidence for the presence of distant regulatory elements and gave an indication of their location. Initial studies revealed DNase I hypersensitive sites (HS) in close proximity to each of the genes of the cluster when chromatin from erythroid tissue at the appropriate developmental stage was examined (19). Later studies showed that five sites of extreme hypersensitivity to DNase I were present at either end of the cluster in chromatin from erythroid tissue at all developmental stages. Four of these "major" HS are located 6–18 kb upstream from the ε-globin gene; one is 20 kb downstream from the β-globin gene (20, 21). These distant DNA sequences are postulated to facilitate the serial expression of the genes in the β-globin gene cluster throughout development. The importance of the major HS in regulating β-globin gene expression has been demonstrated in transgenic mice. DNA fragments containing the upstream and downstream HS were linked to a human β-globin gene and used in the production of transgenic mice. In contrast to previous transgenic studies in which human β-globin gene expression was often quite low, in these experiments each copy of the human β-globin gene was expressed at a level equivalent to that of the endogenous mouse β-globin gene. Examination of DNA from these transgenic mice revealed that the upstream HS were present, but the downstream site was absent (22). These results suggest that the important regulatory elements are located at the upstream end of the cluster. Ryan *et al.* (23) have shown that only the upstream sites are necessary to activate β-globin gene expression in transgenic mice.

Recently Tuan and coworkers (24) examined 20 kb of DNA immediately upstream from the ε-globin gene for enhancer activity. A 732-base-pair (bp) *Hin*dIII–*Bgl* II fragment located 10.2–11.0 kb upstream from the ε-globin gene showed

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Abbreviation: HS, DNase I hypersensitive site(s).

enhancer activity that was erythroid-specific and developmental-stage nonspecific; this fragment contains the second of four upstream HS sites, designated HSII. To determine whether this DNA fragment alone is sufficient to direct high-level β-globin gene expression, we used the polymerase chain reaction technique to amplify and clone an 882-bp fragment containing HSII. We then linked this fragment in either orientation, upstream of a human β-globin gene and used these constructs to produce transgenic mice. Our results indicate that this small fragment alone can direct high-level β-globin gene expression in transgenic mice.

MATERIALS AND METHODS

Cloning HSII. To amplify a DNA fragment spanning HSII, two 30-base oligonucleotides that flank this region were synthesized. The sequences of the upstream oligonucleotide (5′–GTTTAGGATCCAGCAGGTGCTTCAAAACCA-3′) and downstream oligonucleotide (5′-AGATGGATCCTTAAGATGAGAGCTTCCCAG-3′) begin 11,157 and 10,275 bp upstream of the ε-globin gene, respectively (25). The underlined nucleotide in each oligonucleotide was altered to create an artificial *Bam*HI site used for subcloning. Amplification of an 882-bp HSII fragment (Fig. 1*A*) was carried out in a programmable heat block (DNA thermal cycler, Perkin–Elmer/Cetus Instruments) using *Thermus Aquaticus* (*Taq*) polymerase (Cetus) as described by Saiki *et al.* (26). The amplified DNA was digested with *Bam*HI and subcloned into the *Bam*HI site in pUC18. The orientation and nucleotide sequence of the HSII fragments were determined (27).

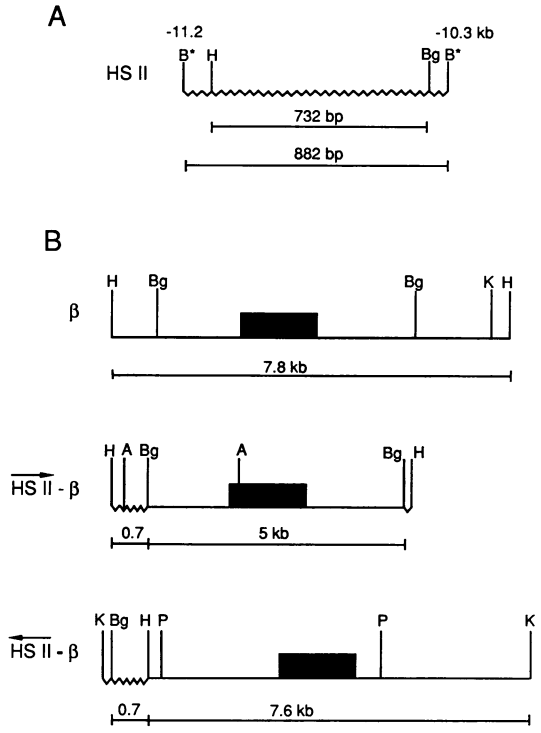

FIG. 1. HSII fragment and injected β-globin and HSII β-globin constructs. (*A*) The HSII fragment is indicated by a jagged line. The distance in kb of this fragment from the ε-globin gene is shown above. The size of the amplified fragment and that of the *Hin*dIII–*Bgl* II enhancer fragment of Tuan *et al.* (24) is indicated below. Artificial *Bam*HI sites are marked by B*. (*B*) (*Top*) β represents the β-globin gene in a 7.8-kb *Hin*dIII fragment. (*Middle*) $\overrightarrow{\text{HSII}}$-β shows the 5-kb *Bgl* II β-globin gene fragment linked to the *Bgl* II site in the HSII fragment in the genomic orientation. A 45-bp 3′ piece of the HSII fragment along with a *Hin*dIII site from the pUC18 polylinker is shown downstream from the β-globin gene fragment. (*Bottom*) $\overleftarrow{\text{HSII}}$-β was constructed by introducing the *Hin*dIII β-globin fragment into the *Hin*dIII sites in the pUC18–HSII vector in the antigenomic orientation. The injected construct (shown) was obtained by *Kpn* I digestion at the upstream site in the pUC18 polylinker and the downstream site in the β-globin gene fragment. H, *Hin*dIII; Bg, *Bgl* II; B, *Bam*HI; K, *Kpn* I; A, *Ava* II; P, *Pst* I.

β-Globin and HSII β-Globin Constructs. A normal human β-globin gene, in a 7.8-kb *Hin*dIII fragment (Fig. 1*B*, *Top*) was cloned in bacteriophage Charon 40 and subcloned in pUC18. This gene was linked to the HSII fragment in both the genomic orientation ($\overrightarrow{\text{HSII}}$-β) and in the opposite orientation ($\overleftarrow{\text{HSII}}$-β). To construct $\overrightarrow{\text{HSII}}$-β (Fig. 1*B*, *Middle*), a 5-kb *Bgl* II β-globin fragment was subcloned into the *Bgl* II site in HSII. A 45-bp *Bgl* II–*Bam*HI portion of the HSII fragment is positioned downstream from the β-globin gene. The downstream *Hin*dIII site is from the pUC18 polylinker. $\overleftarrow{\text{HSII}}$-β (Fig. 1*B*, *Bottom*) was made by subcloning the 7.8-kb *Hin*dIII β-globin fragment into *Hin*dIII sites in a pUC18–HSII vector. The upstream *Kpn* I site is from the pUC18 polylinker. The proper orientation of each construct was confirmed by restriction mapping.

Production of Transgenic Mice. Each DNA fragment was separated from plasmid sequence by digestion with *Hin*dIII (β and $\overrightarrow{\text{HSII}}$-β) or *Kpn* I ($\overleftarrow{\text{HSII}}$-β) followed by isolation from 0.8% low-gelling-temperature agarose (FMC). The fragments were then purified by phenol extraction, ethanol precipitation, cesium chloride gradient centrifugation, and extensive dialysis against injection buffer. The DNA, at a concentration of 1–3 μg/ml, was then injected into the pronuclei of F2 hybrid eggs from C57 × SJL parents (The Jackson Laboratory). Microinjected eggs were placed in culture for several hours or overnight, and surviving eggs were implanted into pseudopregnant recipients by oviductal transfer. Fetuses were removed at day 16 of gestation for analysis (28).

DNA Analysis. DNA was prepared from fetal mouse hindquarters and assayed for the presence of injected sequences by Southern analysis. Ten micrograms of DNA was digested with *Bgl* II, *Ava* II, or *Pst* I, electrophoresed on 0.8% agarose gels, and transferred to nitrocelluose. The filters were hybridized with a human β-globin and HSII probes, which were radiolabeled by random priming. The human β-globin probe was the 1.9-kb *Bam*HI fragment containing the 5′ portion and flanking sequence of the gene. The HSII probe was the entire 882-bp fragment. After hybridization, the filters were washed and exposed to x-ray film at −70°C for 12–48 hr. The autoradiographs were scanned in a Joyce–Loebl densitometer (Haake-Buchler, Saddle Brook, NJ) to determine transgene copy number by comparison to a human DNA standard.

RNA Analysis. Total RNA was extracted from homogenized fetal mouse livers and brains in guanidine thiocyanate and pelleted through a cesium chloride gradient as described by Chirgwin *et al.* (29). Transcription of the human β-globin and mouse α-globin genes was determined using the RNA probe ribonuclease protection assay (30). A 768-bp 3′ human β-globin antisense RNA probe was synthesized from a pBSM13+ vector (Stratagene) containing the *Eco*RI–*Pst* I fragment of the β-globin gene. The mouse α-globin antisense RNA probe was produced from a pSp6 vector containing a 309-bp 3′ fragment of the mouse α-globin gene. Five to ten micrograms of fetal mouse RNA was hybridized in solution overnight with 1–2 × 10^5 cpm of each probe. After hybridization, the samples were digested with ribonuclease A (50 μg/ml) and ribonuclease T1 (1 μg/ml) for 30 min at 22°C. The samples were purified and electrophoresed on 4% polyacrylamide/7 M urea gels. Autoradiography was performed, followed by densitometric scanning.

RESULTS

Production of Human β-Globin, $\overrightarrow{\text{HSII}}$-β-Globin, and $\overleftarrow{\text{HSII}}$-β-Globin Transgenic Mice. The 882-bp fragment spanning HSII obtained by amplification of human genomic DNA is

illustrated in Fig. 1*A*. The sequence of this fragment was identical to that of the published genomic sequence (25). Fig. 1*B* shows three constructs, β, $\overrightarrow{HSII}$-β, and $\overleftarrow{HSII}$-β, used to produce transgenic mice. Eight fetuses containing the human β-globin gene alone, 12 fetuses containing the $\overrightarrow{HSII}$-β construct, and 11 fetuses containing the $\overleftarrow{HSII}$-β construct were obtained.

Fig. 2*A* represents Southern analysis of DNA from the eight β-globin transgenic fetuses. Samples were digested with *Bgl* II and hybridized with the β-globin probe. The expected 5-kb β-globin band was present in seven of the eight transgenic lanes. Mouse A2 contained only a portion of the β-globin gene, which resulted in bands of abnormal size on this and other autoradiographs. (The aberrant band is not visible in Fig. 2*A*.) Densitometry was performed to determine integrated gene-copy number by comparison with the human standard. The copy number varied from <1 to 65 copies per cell (Table 1).

In Fig. 2*B*, DNA from the 12 $\overrightarrow{HSII}$-β transgenic animals was digested with *Ava* II and hybridized with the β-globin probe. The $\overrightarrow{HSII}$-β fragment contains a 2.3-kb *Ava* II fragment that extends from the 5′ portion of the HSII fragment to the middle of the β-globin gene (Fig. 1*B*, *Middle*). This band was present in all 12 transgenic lanes. Further analysis of DNA samples from these animals, including probing with the HSII fragment, revealed that each contained at least one intact copy of the injected construct and that all but one (B10) contained head-to-tail tandem arrays of the transgene (data not shown). Mouse B10 contained two copies of the injected construct that had integrated separately into the genome. The copy number of these animals varied from 0.5 to 22 copies per cell (Table 1). Animals containing one or fewer copies per cell must be mosaics with several transgenes linked in tandem in a fraction of their cells.

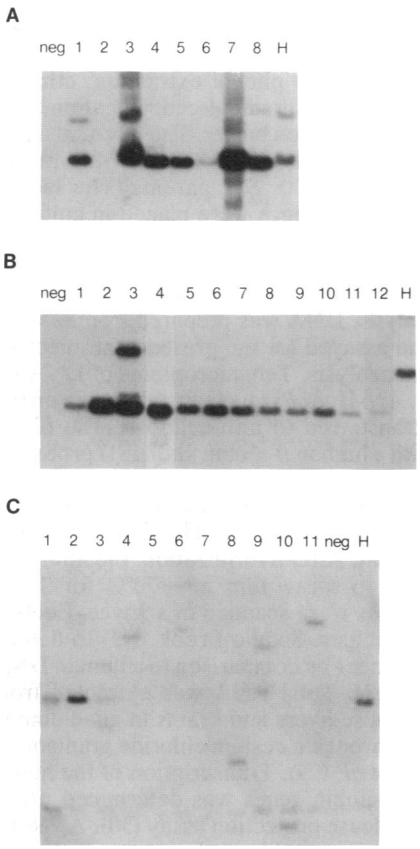

FIG. 2. Southern analysis of β, $\overrightarrow{HSII}$-β, and $\overleftarrow{HSII}$-β transgenic mice. Numbered lanes represent DNA samples from transgenic animals. Control lanes representing nontransgenic (neg) and human (H) samples are indicated. The 1.9-kb *Bam*HI human β-globin gene probe was used to produce the autoradiograph shown. (*A*) β transgenic mice. Digestion with *Bgl* II results in 5-kb β-globin band in the human sample and in transgenic mouse samples that contains an intact copy of the injected construct. The human lane contains a larger band that results from hybridization of the β-globin gene probe with an 8-kb δ-globin gene fragment. (*B*) $\overrightarrow{HSII}$-β transgenic mice. *Ava* II digestion results in a 2.3-kb band in transgenic samples and a major 3-kb band in human DNA. Additional bands in the transgenic lanes represent rearranged copies of the β-globin gene. (*C*) $\overleftarrow{HSII}$-β transgenic mice. *Pst* I digestion results in a 4.4-kb band in transgenic samples that contains an intact copy of the human β-globin gene. A faint crosshybridizing band is present in all mouse lanes near the bottom of the autoradiograph due to homology between mouse and human β-globin sequences.

Table 1. Human β-globin expression in transgenic mice

Mouse	Percent endogenous mouse α-globin mRNA*	Human β-globin gene copies per cell	Percent of expression per gene copy†
β			
A1	29.6	8	14.8
A2	0	<1	0
A3	0	50	0
A4	5.8	25	0.93
A5	42.1	12	14.0
A6	0	1	0
A7	364.1	65	22.4
A8	124.0	28	17.7
$\overrightarrow{HSII}$ β			
B1	70.2	1	280.8
B2	208.0	19	43.8
B3	201.6	22	36.6
B4	109.4	14	31.3
B5	205.4	4	205.4
B6	185.2	7	105.8
B7	118.6	3	158.1
B8	54.4	2	108.7
B9	42.8	1	171.3
B10	0	2	0
B11	24.4	0.5	195.3
B12	42.1	0.5	336.6
$\overleftarrow{HSII}$ β			
C1	0	1	0
C2	134.4	3	179.2
C3	48.4	0.5	387.1
C4	0	0.5	0

*$\left(\frac{\text{human β-globin mRNA}}{\text{mouse α-globin mRNA}}\right) \times 100.$

†$\left(\frac{\text{human β-globin mRNA/human β-globin gene}}{\text{mouse α-globin mRNA/mouse α-globin gene}}\right) \times 100.$

Fig. 2*C* shows analysis of 11 $\overleftarrow{HSII}$-β transgenic animals. Digestion with *Pst* I followed by hybridization to the β-globin probe resulted in a 4.4-kb β-globin band in lanes from animals carrying an intact copy of the injected construct. As shown in Fig. 2*C* (lanes 1–3), only 3 of the 11 animals (C1, C2, and C3) contained intact copies of the human β-globin gene. The β-globin gene in the remaining transgenic animals had undergone significant rearrangements, resulting in a variety of different bands. Analysis with additional enzymes and the HSII probe showed that mice C2 and C3 contained intact copies of the injected construct in tandem, whereas mouse CI contained only a single intact construct (data not shown). The copy number of these animals ranged from 0.5 to 3 copies per cell (Table 1).

Expression of Human β-Globin mRNA in β-Globin, $\overrightarrow{HSII}$-β, and $\overleftarrow{HSII}$-β Transgenic Mice. The liver is the major site of erythropoiesis in the mouse from day 13–17 of gestation, at

which time the predominant hemoglobin has switched from the embryonic to the adult form. Thus, we analyzed total liver RNA from day-16 transgenic animals for the presence of human β- and mouse α-globin mRNA with the RNA probe ribonuclease protection assay. Analysis of the eight transgenic animals containing the β-globin gene alone is shown in Fig. 3*A*. The positions of the probe fragments protected by human reticulocyte β-globin mRNA and mouse α-globin mRNA are indicated. Five of eight transgenic animals in this group expressed human β-globin mRNA. One of the animals that did not express β-globin mRNA (A2) contained a truncated β-globin gene. The relative amounts of human β- and mouse α-globin mRNA were determined by densitometry. This group of animals expressed human β-globin mRNA at levels ranging from 5.8 to 364.1% of mouse α-globin mRNA levels (Table 1). To directly compare the level of expression per gene copy, the levels of mouse α- and human β-globin mRNA were divided by their respective gene-copy numbers. (There are four mouse α-globin genes per diploid genome.) The level of human β-globin expression per gene copy ranged from 0.93 to 22.4% of the mouse α-globin gene expression (Table 1). Among the seven animals with an intact copy of the human β-globin gene, the average level of human β-globin expression per gene copy was 9.9%.

Analysis of the 12 $\overrightarrow{\text{HSII}}$-β transgenic animals is shown in Fig. 3*B*. Eleven of 12 animals expressed human β-globin mRNA at levels ranging from 24.4 to 208% of mouse α-globin expression (Table 1). The level of human β-globin gene expression per gene copy was significantly higher than in the animals containing the β-globin gene alone, ranging from 31.3 to 336.6% of mouse α-globin expression (Table 1). Among these 12 animals, the average level of human β-globin mRNA expression per gene copy was 139.5% of mouse α-globin mRNA expression.

Four $\overleftarrow{\text{HSII}}$-β animals were analyzed for expression, including the three containing intact copies of the injected

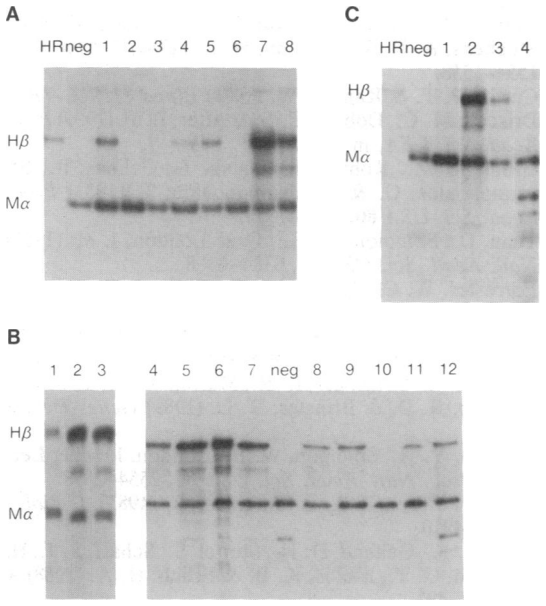

FIG. 3. Riboprobe ribonuclease protection analysis of fetal liver RNA from β, $\overrightarrow{\text{HSII}}$-β, and $\overleftarrow{\text{HSII}}$-β transgenic mice. Numbered lanes represent samples from the transgenic mice shown in Fig. 2. Human reticulocyte (HR) and nontransgenic mouse (neg) RNA samples are indicated. The positions of the human β- and mouse α-globin antisense RNA probe fragments that are protected by human reticulocyte mRNA and mouse α-globin mRNA are indicated by Hβ and Mα, respectively. (*A*) β-transgenic mice; (*B*) $\overrightarrow{\text{HSII}}$-β transgenic mice; and (*C*) $\overleftarrow{\text{HSII}}$-β transgenic mice.

construct (C1, C2, and C3), and one containing only a truncated β-globin gene (C4). As shown in Fig. 3*C*, two of three animals containing intact $\overleftarrow{\text{HSII}}$-β constructs expressed human β-globin mRNA. No expression was detected in the animal containing only a portion of the human β-globin gene (C4). Animals C2 and C3 expressed human β-globin mRNA at levels of 48.8% and 134.4% of mouse α-globin mRNA expression (Table 1). The level of human β-globin mRNA expression per gene copy was 179.2 and 387.1% of the mouse α-globin mRNA expression.

Tissue Specificity of β-Globin Gene Expression. Fetal liver and brain RNA from β, $\overrightarrow{\text{HSII}}$-β, and $\overleftarrow{\text{HSII}}$-β transgenic mice were assayed for mouse α- and human β-globin mRNA to assess the tissue specificity of expression. Analysis of one animal produced with each of the three constructs is shown in Fig. 4. Human β-globin gene expression was seen only in fetal liver and not in fetal brain in all mice examined. Interestingly, a strong mouse α-globin signal was detected in the fetal brain RNA from these animals and all others analyzed. The reason for this is not clear, but it cannot be due simply to peripheral blood contamination of the brain sample because the human β-globin signal would also be visible in that case.

DISCUSSION

Several lines of evidence suggest that distant cis DNA sequences play an important role in the regulation of the human β-globin gene cluster. (*i*) The observation in thalassemic individuals from three unrelated families of deletions of the upstream portion of the β-globin gene cluster that inactivate an intact β-globin gene in cis (13–18). (*ii*) The discovery of HS at either end of the β-globin gene cluster that are present exclusively in erythroid tissue and at all developmental stages (20, 21). (*iii*) Transgenic mice studies in which the human β-globin gene was linked to the upstream and downstream HS resulted in expression at levels equivalent to that of the mouse β-globin gene (22). Recently, it was shown that the upstream HS alone are sufficient to activate the human β-globin gene in transgenic mice (23). Tuan and coworkers (24) examined 20 kb of DNA spanning these upstream HS and found an erythroid-specific, developmental stage nonspecific enhancer in a 732-bp fragment that contained HSII.

To determine the effect of HSII alone on β-globin gene expression, we have linked HSII to the human β-globin gene in both orientations and used these constructs in the production of transgenic mice. Our results indicate that HSII linked to the β-globin gene in the genomic orientation can activate

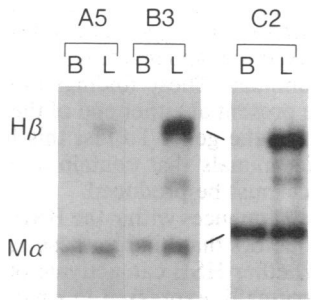

FIG. 4. Tissue specificity of β-globin gene expression. Riboprobe ribonuclease protection analysis of fetal brain (B lanes) and liver (L lanes) RNA was performed to assess tissue specificity of expression. A representative animal from the β (A5), $\overrightarrow{\text{HSII}}$-β (B3), and $\overleftarrow{\text{HSII}}$-β (C2) groups is shown. The positions of the human β-globin and mouse α-globin antisense RNA probe fragments that are protected by human reticulocyte mRNA and mouse α-globin mRNA are indicated by Hβ and Mα, respectively.

high-level human β-globin gene expression in the erythroid tissue of transgenic mice. Eleven of the 12 animals containing the HSII-β construct expressed human β-globin mRNA at levels per gene copy of 31.3 to 336.6% of mouse α-globin mRNA. Thus, on average, this group of mice expressed human β-globin mRNA per gene copy at a level somewhat higher (139.5%) than mouse α-globin mRNA. This level of expression per gene copy was substantially higher than that obtained in the β-transgenic mice (9.9%). The human β-globin expression seen in all animals was tissue-specific, present in fetal liver, and absent in fetal brain. The high percentage of HSII-β animals that expressed the human β-globin gene suggests that this regulatory element can activate the β-globin gene regardless of the site of integration in the mouse genome. On the other hand, the level of expression seen in these animals did not correlate directly with gene-copy number. In fact, the lowest levels of expression per gene copy in the HSII-β animals was seen in the animals with the highest gene-copy number. This finding suggests that large numbers of transcriptionally active genes may compete for the locally available transcription factors, resulting in a lower level of expression per gene copy.

Eleven transgenic animals were obtained with the antigenomic HSII-β construct. Only three of the HSII-β transgenic mice contained intact copies of the injected construct. Two of these three mice expressed human β-globin mRNA at levels per gene copy of 179.2 and 387.1% of the mouse α-globin mRNA. While we cannot draw firm conclusions due to the limited number of animals produced, these findings suggest that HSII may be able to activate the human β-globin gene in an orientation-independent fashion *in vivo*.

Eight of eleven HSII-β transgenic mice contained only rearranged, partially deleted constructs. In contrast, only one of the eight β-globin gene transgenics and none of the HSII-β transgenics failed to contain an intact copy of the injected construct. Thus, the HSII fragment linked to the β-globin gene in the antigenomic orientation appears to predispose the construct to significant rearrangements. This effect cannot be due solely to the β-globin gene fragment used in the HSII-β construct because a slightly larger fragment was used in the β construct. Sequence analysis of the HSII fragment and the human β-globin gene fragment failed to reveal shared direct or inverted repeats that might explain this recombination. Further analysis of the rearrangements present in these animals may shed light on their genesis.

In each group of HSII-β animals, one animal contained an intact copy of the injected construct but did not express human β-globin mRNA. Extensive analyses, both by Southern mapping and gene amplification, demonstrated that at least one intact copy of the injected construct was present in each of these animals. However, both nonexpressing animals (B10 and C1) lacked the tandem array of transgenes found in the rest of the animals. These findings raise the possibility that HS must be present at either end of the β-globin gene to allow activation of the gene. To test this hypothesis, additional transgenic animals that contain a single copy of an HSII-β construct must be produced.

The regulatory sequences within the HSII fragment and the nuclear protein factors that bind to these sequences need to be identified. Whether HSII can activate other globin genes and genes not normally expressed in erythroid tissues also awaits an answer. However, the introduction of an HSII-β construct into a retroviral vector might succeed in transferring a human β-globin gene capable of high expression into murine hematopoietic tissue. Such a result would be an important step on the road to gene therapy for the hemoglobin disorders.

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