Expression of Human Globin Genes in Transgenic Mice Carrying the β-Globin Gene Cluster with a Mutation Causing $^{G}\gamma\beta^{+}$ Hereditary Persistence of Fetal Hemoglobin[a]

MINORU TANAKA,[b,c] JUDITH A. NOLAN,[b]
AJAY K. BHARGAVA,[b] KIRSTEN ROOD,[d]
FRANCIS S. COLLINS,[d,e] SHERMAN M. WEISSMAN,[b]
BERNARD G. FORGET,[b,f] AND
JOHN W. CHAMBERLAIN[b,g]

[b]*Departments of Human Genetics and Internal Medicine*
Yale University School of Medicine
New Haven, Connecticut 06510
and
[d]*Departments of Internal Medicine and Human Genetics and*
The Howard Hughes Medical Institute
University of Michigan School of Medicine
Ann Arbor, Michigan 48109

INTRODUCTION

The human β-like globin genes are located on the short arm of chromosome 11 as a cluster of five functional genes in the order 5′-ε-$^{G}\gamma$-$^{A}\gamma$-δ-β-3′.[1] Each globin gene is expressed at a sequential stage of development. The ε-globin gene is expressed in embryonic (yolk sac–derived) erythroid cells, the $^{G}\gamma$- and $^{A}\gamma$-globin genes are expressed in fetal erythroid cells, and the δ- and β-globin genes are expressed primarily in adult erythroid cells.[2] In normal adults, the level of fetal hemoglobin is less than 1% of the total hemoglobin.[2] In a heterozygous non-deletion form of hereditary persistence of fetal hemoglobin (HPFH) called $^{G}\gamma\beta^{+}$ HPFH, the level of fetal hemoglobin ranges from 15% to 25% of the total hemoglobin and is composed predominantly of $^{G}\gamma$ chains.[3] A single base substitution (C → G) was identified 202

[a]This work was supported in part by grants from the National Institutes of Health (S. M. W. and B. G. F.), the National Foundation March of Dimes (F. S. C.), the Cooley's Anemia Foundation (M. T.), and the Damon Runyon–Walter Winchell Cancer Fund (J. W. C.).

[c]Present address: Department of Biochemistry, Mie University School of Medicine, Tsu, Mie 514, Japan.

[e]Associate Investigator of the Howard Hughes Medical Institute.

[f]Address correspondence to Bernard G. Forget, M.D., Professor of Medicine and Human Genetics, Hematology Section, Department of Internal Medicine, School of Medicine, Yale University, WWW4, P.O. Box 3333, New Haven, CT 06510-8056.

[g]Present address: Research Institute, The Hospital for Sick Children, 555 University Avenue, Toronto, Canada M5G 1X8.

base-pairs 5′ to the cap site (position −202) of the $^{G}\gamma$-globin gene in an affected individual,[4] and the presence of this mutation was shown to be closely associated with the $^{G}\gamma$ HPFH phenotype in different individuals.[5]

The generation of transgenic mice has been a useful tool for the study of the developmental regulation of human globin genes. When cloned human β-globin and γ-globin genes containing a limited amount of 5′- and 3′-flanking DNA are introduced into mouse oocytes and transgenic lines are established, these individual genes are regulated during murine development in a manner similar to that of their murine homologs. The human β-globin gene is expressed at the fetal liver stage as well as the adult stage of erythropoiesis in a manner analogous to that of the adult murine β^{major}-globin gene,[6-9] while the γ-globin gene is expressed only at the embryonic (yolk sac) stage of erythropoiesis in a manner analogous to that of the embryonic murine β^{h1}-globin gene.[7,10,11] In this paper, we describe expression studies of human globin genes in transgenic mice which carry the 40-kilobase (kb) *Kpn* I fragment of the human β-like globin gene cluster from an individual with the $-202\ ^{G}\gamma\beta^{+}$ HPFH mutation. The pattern of expression of the human β-, $^{G}\gamma$- and $^{A}\gamma$-globin genes differed from that of their corresponding murine homologs. The $^{G}\gamma$-globin gene with the −202 HPFH mutation was expressed at all developmental stages. The normal β-globin gene was expressed in adult erythroid cells but was virtually inactive in fetal erythroid cells, whereas the normal $^{A}\gamma$-globin gene was expressed beyond the embryonic (yolk sac) stage into the fetal stage of development and then became inactive in adult erythroid cells.

MATERIALS AND METHODS

Production of Transgenic Mice

The 40-kb *Kpn* I fragment containing the $-202\ ^{G}\gamma$ HPFH mutation was purified from a cosmid clone[4] by agarose gel electrophoresis or sucrose gradient centrifugation, followed by centrifugation on a CsCl gradient and dialysis.[12,13] The DNA was injected into the pronuclei of (C57BL/6J × SJL/J)F2 or (C57BL/6J × DBA/2J)F2 fertilized eggs as described previously.[12,13] Transgenic mice were identified by Southern blot analysis of tail DNA. To generate transgenic embryos and fetuses, heterozygous transgenic males were mated with normal C57BL/6J females. The day that the mating plug was observed was designated day 0; pregnant females were sacrificed on the indicated gestation day. Transgenic embryos in each litter were identified by dot-blot analysis of DNA prepared from the unused carcass.

Preparation of RNA

Adult mice were made anemic by three injections (at 12-h intervals) of a solution of 0.4% phenylhydrazine[11] and sacrificed 5 days after the first injection. Blood was collected by retro-orbital bleeding into phosphate-buffered saline containing 10 U/ml of heparin (PBS-heparin), and cells were recovered by centrifugation. Transgenic embryos were bled from the umbilical cord into PBS-heparin. Blood cells or whole tissues were washed in PBS-heparin, and total RNA was isolated by homogenization in 4 *M* guanidinium isothiocyanate followed by centrifugation through CsCl.[14]

RNase Protection Assay

The labeled antisense RNA probes used to measure $^{G}\gamma$ and $^{A}\gamma$ RNA were synthesized from the *Eco*R I–*Sau*3A fragments of the third exon of the $^{G}\gamma$ and $^{A}\gamma$ genes, where four consecutive base differences occur between the $^{G}\gamma$ and $^{A}\gamma$ sequences starting at a point three bases 3′ to the termination codon.[15] The labeled antisense RNA probe used to measure human β-globin mRNA was synthesized from an *Eco*R I–*Pst* I fragment spanning the poly(A) addition site. Each probe (1×10^5 cpm per assay) was hybridized with 10 μg of total cellular RNA at 50°C for 18 h in 30 μl of a solution containing 40 m*M* PIPES, pH 6.5, 0.4 *M* NaCl, 1 m*M* EDTA, and 80% formamide. The RNA samples were digested with RNase A (40 μg/ml) and RNase T1 (2 μg/ml) for 30 min at 37°C and then digested with proteinase K (50 μg/ml) for 15 min at 37°C. The protected fragments were analyzed by electrophoresis in a 7.5% polyacrylamide–7 *M* urea gel.

Northern Blot Analysis

Northern blot analysis was performed using the following 19-mer oligonucleotide probes specific for the RNA to be analyzed: 5′-ACAGCAAGAAAGCGAGCTT-3′ (human β), 5′-CATCATGGGCAGTGAGCTC-3′ (human $^{G}\gamma$), 5′-AGAGCAG-GAAAGGGGGTTT-3′ (mouse β^{major}), and 5′-GTGTACTGGAATGGAGTTT-3′ (mouse β^{h1}). These correspond to sequences that start at the second nucleotide of the termination codon of these four globin mRNAs. The oligonucleotides were labeled with [γ-^{32}P]ATP by polynucleotide kinase. Total RNA from mouse tissues was fractionated by formaldehyde gel electrophoresis[16] and transferred to GeneScreen Plus membranes (New England Nuclear). Filters were hybridized with the appropriate oligonucleotide probe (2×10^5 cpm/ml) at 42°C in a solution containing 5× SSC, 0.5% SDS, 5× Denhardt's solution, 50 m*M* sodium phosphate, pH 7.0, and 200 μg/ml of salmon sperm DNA. Following hybridization, the filters were washed twice for 5 min at room temperature in a solution containing 2× SSC and 0.5% SDS, followed by a 10-min wash in the same solution at 45°C.

RESULTS

Structural Analysis of the Human β-like Globin Gene Cluster in Transgenic Mice

In order to determine the pattern of integration of the 40-kb fragment, tail-skin DNA from individual transgenic mice was analyzed by Southern blotting. The −202 mutation of the $^{G}\gamma$-globin gene abolishes a normal *Apa* I site in the 40-kb *Kpn* I fragment.[5] Therefore, digestion with *Apa* I allows direct confirmation of the presence of the mutation in the insert. As shown in FIGURE 1, fragments of 21.7 kb and 18.2 kb were detected in *Apa* I digests of DNA from four transgenic mouse lines following hybridization with a $^{G}\gamma$ cDNA probe. Fragments of the expected normal sizes were detected in the *Eco*R I digests. These results demonstrate that all the transgenic mice have intact 40-kb inserts that are integrated in tandem arrays. The number of copies of the insert per cell in transgenic lines 3, 4, 6, and 10 was estimated to be approximately 60, 20, 10 and 5, respectively.

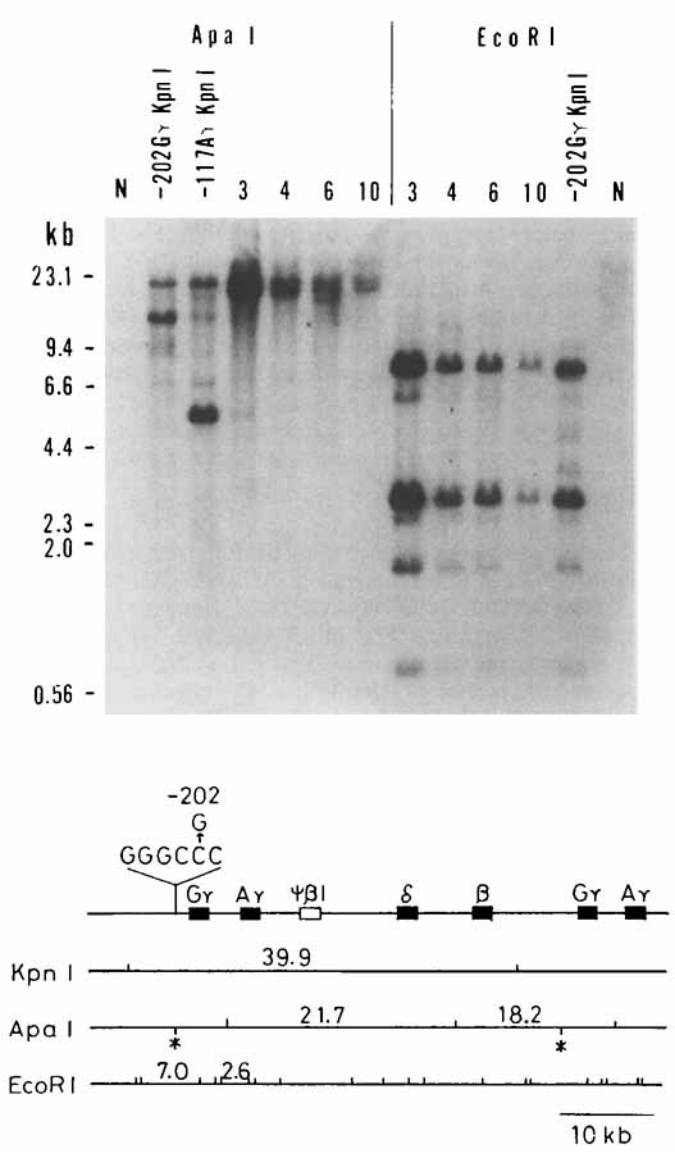

FIGURE 1. Structural analysis of the human β-globin gene cluster in transgenic mice. (**Upper panel**) 10 μg of tail DNA was digested with *Apa* I (**left**) or *Eco*R I (**right**), electrophoresed in a 0.5% agarose gel, transferred to a nylon membrane, and hybridized to a ^{G}γ-globin cDNA probe. The number of each transgenic line is indicated above the respective lanes. N, tail DNA from non-transgenic control mice. 10 ng each of the microinjected *Kpn* I fragment (−202Gγ Kpn I) and the 40-kb *Kpn* I fragment from a Greek-type ^{A}γ HPFH gene containing wild-type ^{G}γ-, δ- and β-globin genes and the ^{A}γ-globin gene with a G → A mutation at position −117 (−117Aγ Kpn I) were analyzed as positive controls. The locations of DNA molecular weight standards (*Hin*d III fragments of λ DNA) are shown to the *left* of the blot. (**Lower panel**) The diagram shows the 40-kb *Kpn* I fragment aligned in a head-to-tail tandem array and the resulting map of restriction sites. The *Apa* I site is abolished by the −202 C → G mutation, as indicated by the *asterisks*.

Expression of Human Globin Genes in Adult Mice

Expression of human globin mRNA in adult tissues of two transgenic mouse lines (lines 3 and 6) was examined by the RNase protection assay (FIG. 2). Adult mice were made anemic by phenylhydrazine treatment. Hemin-induced K562 cells[17] and cytosine arabinoside–induced KMOE cells[18] were used as positive controls for γ-globin mRNA and β-globin mRNA, respectively. The specificity of the $^{G}\gamma$ and $^{A}\gamma$ probes was determined by the protection patterns observed with $^{G}\gamma$ and $^{A}\gamma$ control RNAs, which were synthesized *in vitro* using T7 RNA polymerase and the *Eco*R I–*Sau*3A fragments cloned in the plasmid vector pGEM. The $^{A}\gamma$ probe was derived from the cosmid clone containing the -202 $^{G}\gamma\beta^{+}$ HPFH 40-kb *Kpn* I fragment, which has a deletion of 6 base-pairs 5′ to the poly(A) addition signal, AATAAA, of the $^{A}\gamma$-globin gene. Therefore, a fragment of 134 bases (instead of the expected 161 bases, as obtained with synthetic $^{A}\gamma$ RNA) is protected when this $^{A}\gamma$ probe is hybridized to $^{A}\gamma$-globin mRNA from K562 cells (FIG. 2B). The expected fragment of 161 bases was protected when the $^{G}\gamma$ probe was hybridized to various RNAs (FIG. 2A). The $^{G}\gamma$ probe yielded a strong positive hybridization signal with blood and spleen (but not kidney or liver) RNA from adult anemic transgenic mice, whereas no hybridization signal was obtained from any of the mouse tissues with the $^{A}\gamma$ probe.

FIGURE 3 shows the results of the RNase protection assays using the human β-globin probe. The specific 212-bp fragment protected by the human β-globin probe was detected in adult blood and spleen RNA but not in liver and kidney RNA from either line 3 or line 6 transgenic mice. The protected fragment was not detected in spleen RNA from a non-transgenic anemic mouse which was producing large amounts of endogenous mouse β^{major}-globin mRNA. These results show that the human $^{G}\gamma$- and β-globin genes are expressed specifically in erythroid tissues of anemic adult transgenic mice. The level of human β-globin mRNA in the line 3 transgenic mice was approximately 1.0% of that of endogenous mouse β^{major}-globin mRNA, and the level of $^{G}\gamma$-globin mRNA was approximately 20% of that of human β-globin mRNA. These levels were estimated by Northern blot analysis of the RNA using the specific 19-mer oligonucleotide probes listed in MATERIALS AND METHODS (data not shown).

Expression of Human Globin Genes during Mouse Development

We also analyzed the pattern of expression of the individual human globin genes during development in the transgenic mice. RNA samples were isolated from erythroid tissues of mouse embryos at day 11 and day 14 of gestation, and these were analyzed for the presence of the human globin mRNAs. FIGURE 4 shows the expression patterns of $^{G}\gamma$- and $^{A}\gamma$-globin genes during development of line 3 transgenic mice. The $^{G}\gamma$-globin mRNA was detected in all of the RNA samples tested, including day 11 yolk sac and blood, day 14 liver and blood, and adult blood. The $^{A}\gamma$-globin mRNA was detected, but at significantly lower levels, in embryonic (day 11) and fetal (day 14) erythroid tissues. In fetal (day 14) liver RNA, the level of $^{A}\gamma$-globin mRNA was 20-fold lower than that of $^{G}\gamma$-globin mRNA. In contrast to the case of $^{G}\gamma$-globin mRNA, $^{A}\gamma$-globin mRNA was virtually absent in adult blood cell RNA.

FIGURE 5A shows RNase protection assays obtained with RNA samples from line 3 transgenic mice using the human β-globin probe. Human β-globin mRNA was not detected in RNA from day 11 blood and yolk sac or from day 14 fetal blood. Barely detectable levels of human β-globin mRNA were observed in RNA from day

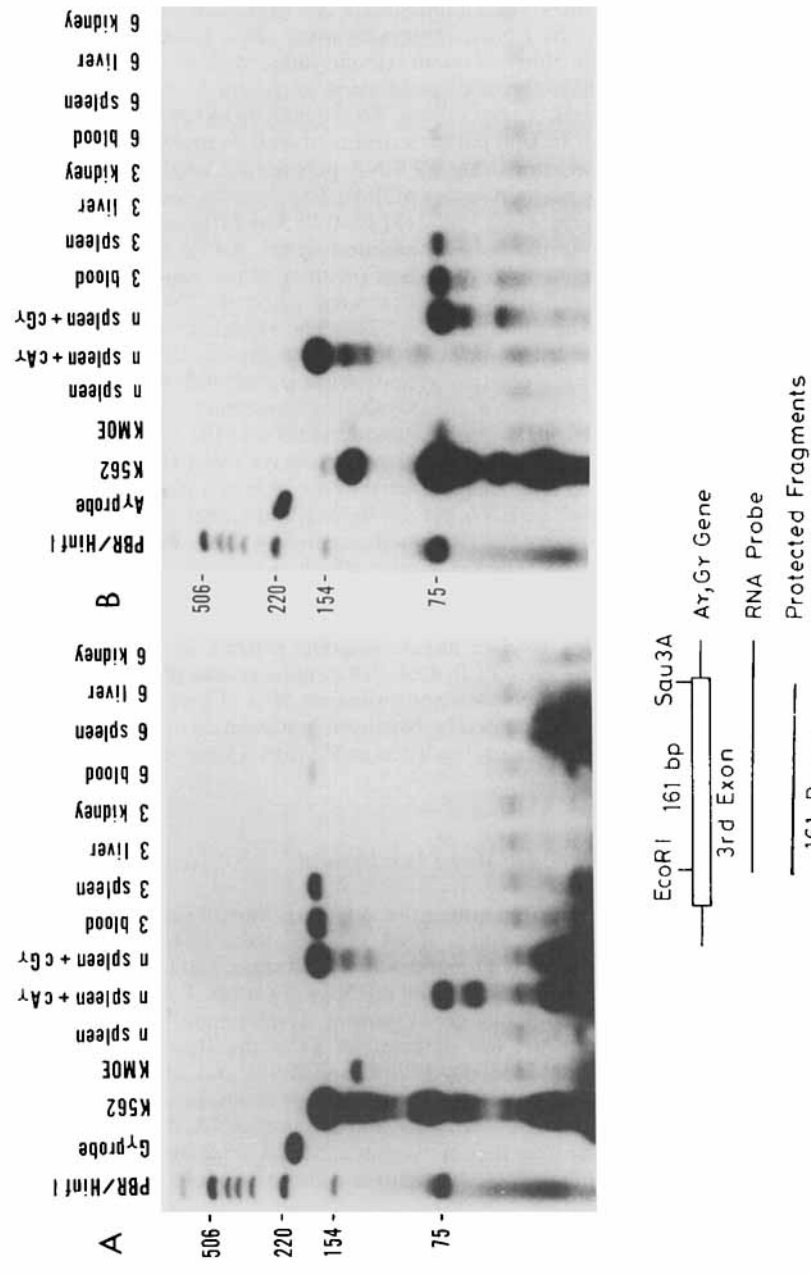

FIGURE 2. Analysis of human $^{G}\gamma$- and $^{A}\gamma$-globin gene expression in adult transgenic mice. Total RNA was isolated from tissues of adult anemic mice. 10 μg of total RNA was hybridized to the $^{G}\gamma$-globin (**panel A**) or $^{A}\gamma$-globin (**panel B**) antisense RNA probes illustrated below the autoradiographs. The protected RNAs were analyzed by 7 *M* urea–7.5% polyacrylamide gel electrophoresis. The specificity of the $^{G}\gamma$ and $^{A}\gamma$ probes was tested with 20 pg of synthetic control $^{G}\gamma$ (cGγ) or control $^{A}\gamma$ (cAγ) RNA mixed with 10 μg of spleen RNA from non-transgenic mice (n spleen). The autoradiographs were exposed for 3 days. The numbers (3, 6) designate the transgenic line from which the indicated tissue RNA was isolated.

14 fetal liver. FIGURE 5B shows the expression patterns of endogenous adult mouse β^{major}- and embryonic β^{h1}-globin mRNAs in the same RNA samples, analyzed by Northern blot using specific 19-mer oligonucleotide probes. The mouse β^{major}-globin mRNA was present at approximately equivalent levels in RNA from fetal liver, fetal

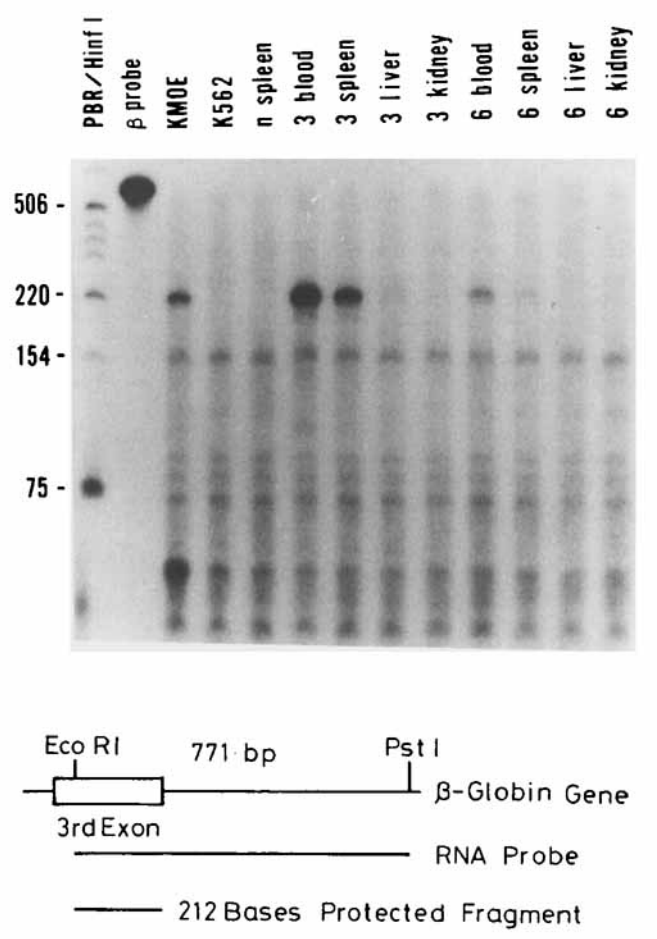

FIGURE 3. Analysis of the expression of the human β-globin gene in adult transgenic mice. The same RNA samples as in FIGURE 2 were analyzed. 10 μg of total RNA was hybridized to the β-globin antisense RNA probe illustrated below the autoradiograph. The protected RNAs were analyzed by 7 *M* urea–7.5% polyacrylamide gel electrophoresis. The autoradiograph was exposed for 2 days.

blood and adult blood, whereas β^{h1}-globin mRNA was detected only in RNA from day 11 blood and yolk sac. It is noteworthy that the same fetal liver RNA sample that contained high levels of mouse β^{major} mRNA yielded barely detectable levels of human β-globin mRNA. This same RNA sample essentially lacked mouse embryonic

β^{hl} mRNA yet contained significant levels of mRNA from the non-mutated $^{A}\gamma$-globin gene.

DISCUSSION

We have analyzed the expression of human globin genes in transgenic mice carrying the 40-kb *Kpn* I fragment from the human β-like globin gene locus of an individual with $^{G}\gamma\beta^{+}$ HPFH. In addition to the normal β-globin human gene, the $^{G}\gamma$-globin gene which carries the −202 point mutation was expressed in erythroid tissues of anemic adult transgenic mice. The expression level of the $^{G}\gamma$-globin gene was approximately 20% of that of the β-globin gene, which is very similar to the level

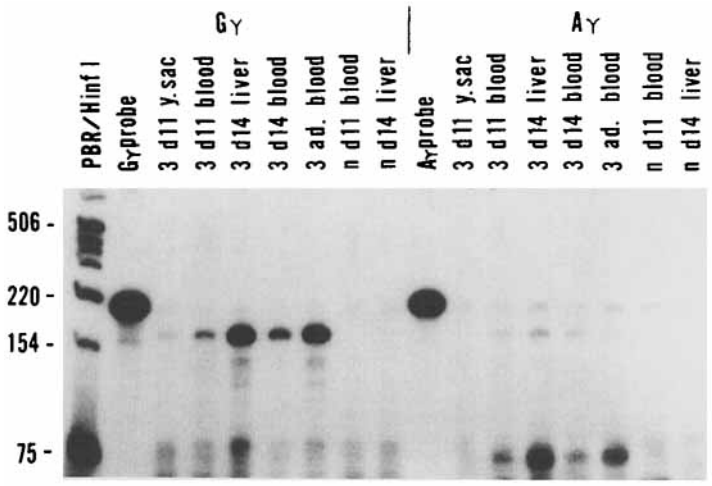

FIGURE 4. Analysis of $^{G}\gamma$- and $^{A}\gamma$-globin gene expression during development in transgenic mice. Total RNA was isolated from day 11 (d11) yolk sac (y. sac) and blood, day 14 (d14) liver and blood, and adult (ad.) blood of line 3 transgenic mice (3) or non-transgenic mice (n). RNase protection assays were performed with the antisense $^{G}\gamma$- or $^{A}\gamma$-globin RNA probes as described in the legend to FIGURE 2. The autoradiograph was exposed for 7 days.

of Hb F in adult red cells of individuals with $^{G}\gamma\beta^{+}$ HPFH. These results suggest that the −202 mutation might contribute to the unexpected expression of the $^{G}\gamma$-globin gene in adult transgenic mice. However, it should be noted that enhancer elements in the third exon and 3′ flanking region of the human β-globin gene[19–23] are now located approximately 10 kb upstream of the $^{G}\gamma$-globin gene in the head-to-tail tandem repeat of the 40-kb insert in the transgenic mice (FIG. 6). The β-globin enhancers are capable of activating $^{A}\gamma$- and $^{G}\gamma$-globin promoters in fetal liver or adult erythroid tissues of transgenic mice when located 0.2 to 2.5 kb from these promoters.[11,20–22] In addition to the −202 mutation, the β-globin enhancers may also contribute to the deregulated expression of the human $^{G}\gamma$-globin gene in adult erythroid cells of the transgenic mice.

It has been previously reported that in transgenic mice, individual cloned human

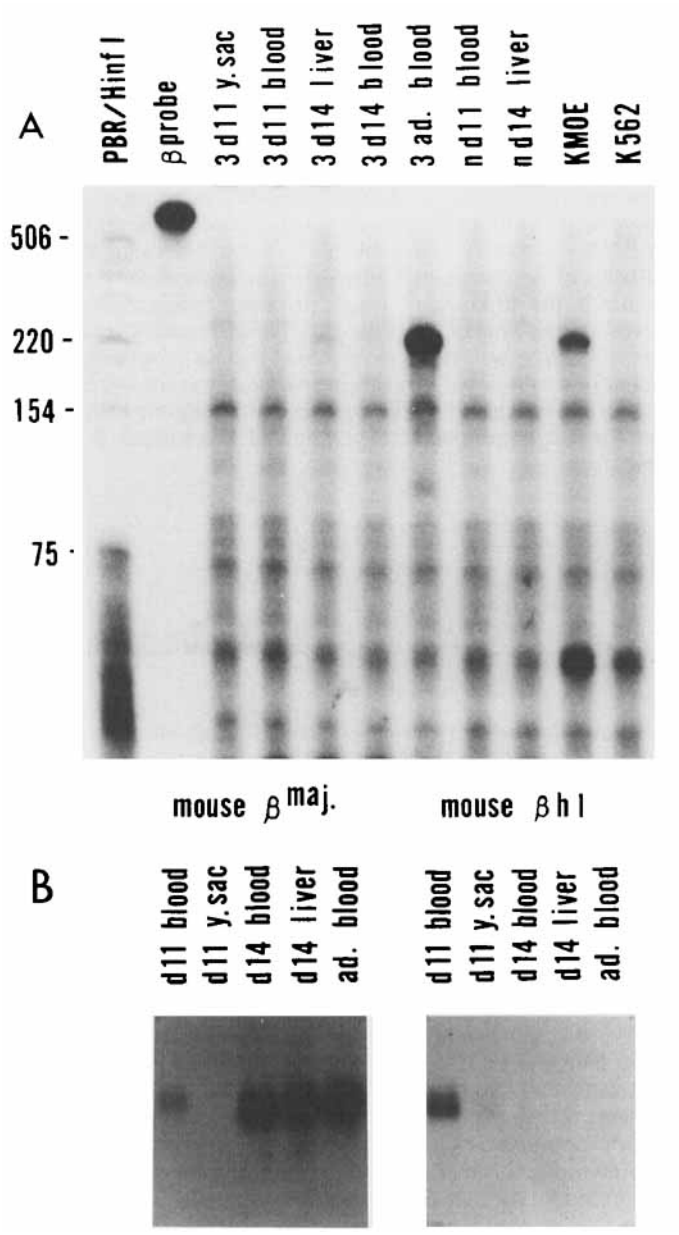

FIGURE 5. Analysis of human β-globin and endogenous mouse β^{major}- and β^{hl}-globin gene expression during development in transgenic mice. The same RNA samples as in FIGURE 4 were analyzed. (**Panel A**) RNase protection assays were performed as described in FIGURE 3 using the human β-globin antisense RNA probe. (**Panel B**) Total RNA (10 μg) was electrophoresed in formaldehyde gels, transferred to nylon membranes, and hybridized to gene-specific 19-mer oligonucleotide probes (see MATERIALS AND METHODS). The autoradiographs in (**A**) and (**B**) were exposed for 3 days and 6 h, respectively. β^{maj}, β^{major}.

β- and γ-globin genes are expressed during development in a manner similar to that of their murine homologs, β^{major} and β^{h1}, respectively.[6-11] However, our results, using the 40-kb *Kpn* I fragment from the human β-globin gene locus, demonstrate a pattern of expression of the normal human $^{A}\gamma$- and β-globin genes that is different from that of their murine homologs. Although the endogenous mouse adult β^{major}-globin gene was fully active in both fetal liver and adult erythroid cells, the human β-globin gene was virtually inactive in fetal liver. Furthermore, whereas endogenous mouse embryonic β^{h1}-globin mRNA was virtually absent in fetal liver RNA, human $^{A}\gamma$-globin mRNA was readily detectable in the same RNA sample. It is noteworthy that the patterns of developmental stage–specific expression of the human β- and $^{A}\gamma$-globin genes in the 40-kb *Kpn* I fragment of our transgenic mice are similar to those observed during human development. The differences in the developmental pattern of expression between individual cloned human genes and those in the 40-kb *Kpn* I fragment of the β-globin gene cluster suggest that the overall organization of the globin gene cluster, including the presence of intergenic DNA, is an important determinant for the stage-specific expression of the human β-like globin genes during development.

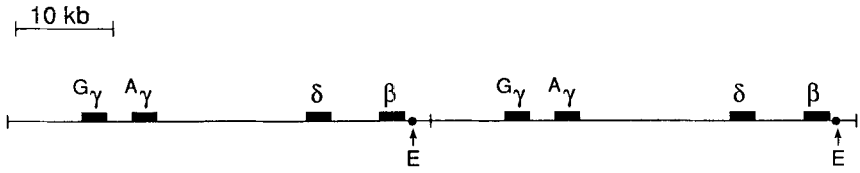

FIGURE 6. Diagram showing the relationship between the β-globin gene enhancer (E) and the $^{G}\gamma$-globin gene promoter in the tandemly repeated 40-kb transgene.

SUMMARY

We have introduced into the mouse germ line the 40-kilobase (kb) *Kpn* I fragment containing the β-globin gene cluster from an individual with a non-deletion form of hereditary persistence of fetal hemoglobin (HPFH) believed to be due to a point mutation at position −202 of the $^{G}\gamma$-globin gene. The $^{G}\gamma$-globin gene, as well as the β-globin gene, was expressed in adult erythroid tissues of the resulting transgenic mice. The level of expression of the $^{G}\gamma$-globin gene was about 20% of that of the β-globin gene. Others have previously shown that cloned individual normal human β- and γ-globin genes containing a limited amount of 5′- and 3′-flanking DNA are expressed in a manner similar to that of their corresponding murine homologs during development in transgenic mice. In contrast, we have observed that the pattern of expression of the normal (non-mutated) $^{A}\gamma$- and β-globin genes in the 40-kb insert was different from that of their corresponding murine homologs. The β-globin gene remained inactive at the fetal stage, whereas the normal $^{A}\gamma$-globin gene was expressed beyond the embryonic (yolk sac) stage into the fetal stage of development and then became inactive in adult erythroid cells. The pattern of expression of the human globin transgenes during mouse development resembles that observed during human development. These results suggest that the gross organization of the human β-like globin gene cluster is important for stage-specific expression of each human globin gene during development.

ACKNOWLEDGMENTS

The authors thank Dr. Clyde Hutchison for the mouse adult β-globin probe, Hemaxi Vasavada and Linda Klein for skilled technical assistance, and Barbara Gramenos and Leif Madsen for expert preparation of the manuscript.

REFERENCES

1. FRITSCH, E. F., R. M. LAWN & T. MANIATIS. 1980. Molecular cloning and characterization of the human beta-like globin gene cluster. Cell **19:** 959–972.
2. STAMATOYANNOPOULOS, G. & A. W. NIENHUIS. 1987. Hemoglobin Switching. *In* Molecular Basis of Blood Diseases. G. Stamatoyannopoulos, A. W. Nienhuis, P. Leder & P. W. Majerus, Eds.: 66–105. W. B. Saunders. Philadelphia.
3. HUISMAN, T. H. J., A. MILLER & W. A. SCHROEDER. 1975. A $^{G}\gamma$ type of the hereditary persistence of fetal hemoglobin with β chain production in cis. Am. J. Hum. Genet. **27:** 765–777.
4. COLLINS, F. S., C. J. STOECKERT, JR., G. R. SERJEANT, B. G. FORGET & S. M. WEISSMAN. 1984. $^{G}\gamma\beta^{+}$ hereditary persistence of fetal hemoglobin: Cosmid cloning and identification of a specific mutation 5′ to the $^{G}\gamma$ gene. Proc. Natl. Acad. Sci. USA **81:** 4894–4898.
5. COLLINS, F. S., C. D. BOEHM, P. G. WABER, C. J. STOECKERT, JR., S. M. WEISSMAN & B. G. FORGET. 1984. Concordance of a point mutation 5′ to the $^{G}\gamma$-globin gene with $^{G}\gamma\beta^{+}$ hereditary persistence of fetal hemoglobin in the black population. Blood **64:** 1292–1296.
6. CHADA, K., J. MAGRAM, K. RAPHAEL, G. RADICE, E. LACY & F. COSTANTINI. 1985. Specific expression of a foreign β-globin gene in erythroid cells of transgenic mice. Nature **314:** 377–380.
7. COSTANTINI, F., G. RADICE, J. MAGRAM, G. STAMATOYANNOPOULOS, T. PAPAYANNOPOULOU & K. CHADA. 1985. Developmental regulation of human globin genes in transgenic mice. Cold Spring Harbor Symp. Quant. Biol.: **50:** 361–370.
8. MAGRAM, J., K. CHADA & F. COSTANTINI. 1985. Developmental regulation of a cloned adult β-globin gene in transgenic mice. Nature **315:** 338–340.
9. TOWNES, T. M., J. B. LINGREL, H. Y. CHEN, R. L. BRINSTER & R. D. PALMITER. 1985. Erythroid-specific expression of human β-globin genes in transgenic mice. EMBO J. **4:** 1715–1723.
10. CHADA, K., J. MAGRAM & F. COSTANTINI. 1986. An embryonic pattern of expression of a human fetal globin gene in transgenic mice. Nature **319:** 685–689.
11. KOLLIAS, G., N. WRIGHTON, J. HURST & F. GROSVELD. 1986. Regulated expression of human $^{A}\gamma$-, β-, and hybrid β-globin genes in transgenic mice: Manipulation of the developmental expression patterns. Cell **46:** 89–94.
12. HOGAN, B. L., M. F. COSTANTINI & E. LACY. 1986. Manipulating the Mouse Embryo: a Laboratory Manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, N.Y.
13. CHAMBERLAIN, J. W., J. A. NOLAN, S. GROMKOWSKI, K. KELLEY, J. EISENSTADT, K. HERRUP, C. A. JANEWAY & S. M. WEISSMAN. 1988. Cell surface expression and alloantigenic function of a human class I MHC heavy chain gene (HLA-B7) in transgenic mice. J. Immunol. **140:** 1285–1292.
14. CHIRGWIN, J. M., A. E. PRZYBYLA, R. J. MACDONALD & W. J. RUTTER. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18:** 5294–5299.
15. RIXON, M. W. & R. E. GELINAS. 1988. A fetal globin gene mutation in $^{A}\gamma$ nondeletion hereditary persistence of fetal hemoglobin increases promoter strength in a nonerythroid cell. Mol. Cell. Biol. **8:** 713–721.
16. MANIATIS, T., E. F. FRITSCH & J. SAMBROOK. 1980. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, N.Y.
17. RUTHERFORD, T. R., J. B. CLEGG & D. J. WEATHERALL. 1979. K562 human leukaemic cells synthesize embryonic haemoglobin in response to haemin. Nature **280:** 164–165.

18. KAKU, M., K. YAGAWA, K. NAKAMURA & H. OKANO. 1984. Synthesis of adult-type hemoglobin in human erythremia cell line. Blood **64:** 314–317.
19. KOLLIAS, G., J. HURST, E. DEBOER & F. GROSVELD. 1987. The human β-globin gene contains a downstream developmental specific enhancer. Nucleic Acids Res. **15:** 5739–5747.
20. TRUDEL, M., J. MAGRAM, L. BRUCKNER & F. COSTANTINI. 1987. Upstream $^{G}\gamma$-globin and downstream β-globin sequences required for stage-specific expression in transgenic mice. Mol. Cell. Biol. **7:** 4024–4029.
21. BEHRINGER, R. R., R. E. HAMMER, R. L. BRINSTER, R. D. PALMITER & T. M. TOWNES. 1987. Two 3′ sequences direct adult erythroid-specific expression of human β-globin genes in transgenic mice. Proc. Natl. Acad. Sci. USA **84:** 7056–7060.
22. TRUDEL, M. & F. COSTANTINI. 1987. A 3′ enhancer contributes to the stage specific expression of the human β-globin gene. Genes & Dev. **1:** 954–961.
23. ANTONIOU, M., E. DEBOER, G. HABETS & F. GROSVELD. 1988. The human β-globin gene contains multiple regulatory regions: Identification of one promoter and two downstream enhancers. EMBO J. **7:** 377–384.