Developmental Switch in the Relative Expression of the \( \alpha \)- and \( \alpha_2 \)-Globin Genes in Humans and in Transgenic Mice

By M. Albitar, F.E. Cash, C. Peschle, and S.A. Liebhaber

Human \( \alpha \)-globin is encoded by two adjacent genes, \( \alpha_2 \) and \( \alpha_1 \). Despite their remarkable level of structural identity, the more 5' \( (\alpha_2) \) gene is the major \( \alpha \)-globin locus in the normal adult, expressed at 2.6-fold higher levels than the adjacent and more 3' \( (\alpha_1) \) globin gene. In light of the well-characterized pattern of gene activation in the human \( \alpha \)- and \( \beta \)-globin gene clusters during development, we considered the possibility that the relative expression of these two \( \alpha \)-globin loci might be developmentally controlled. Analysis of human embryonic and early fetal erythroid RNA samples confirmed this possibility; levels of mRNA encoded by the two \( \alpha \)-globin loci are equal in the embryo and subsequently shift to dominant expression of the \( \alpha_2 \)-globin locus at week 8 in utero. In transgenic mice carrying the entire human \( \alpha \)-globin cluster (except for the \( \theta \) gene) we show the same shift from equal expression of the \( \alpha_1 \)- and \( \alpha_2 \)-globin loci at the embryonic stage to predominance of the \( \alpha_2 \)-globin locus in the adult. These data demonstrate a switch in the expression of the two adjacent \( \alpha \)-globin genes during the embryonic-to-fetal switch in erythroid development and provide an experimental system for its further characterization.

© 1992 by The American Society of Hematology.

MATERIALS AND METHODS

Source and preparation of RNA samples. RNA was extracted from polysomes of reticulocytes in the peripheral blood of an individual with sickle cell anemia as previously described.12 A yolk sac (5- to 7-week gestation) and a series of liver samples from human embryos at 6, 7, and 8 weeks of gestation were isolated from curetage abortions as detailed elsewhere.13 The women had previously provided fully informed consent for research studies. The possibility of contamination of the embryonic RNA samples with mRNA from maternal blood was checked for and ruled out by assessing \( \beta \)-globin synthesis in an in vitro translation of each sample (data not shown). RNA was extracted from transgenic mouse embryos at 11 and 16 days of gestation by lysing the tissue in thiocyanate guanidine, which was followed by pelleting the RNA through a CsCl cushion.14 Peripheral blood RNA was isolated from adult mice after induction of reticulocytosis with phenylhydrazine for 3 days as previously described.15 The blood was collected from the left atrium of anesthetized mice after perfusion with phosphate-buffered saline through the left ventricle and RNA was isolated from polysomes (see above). All animal work was performed under protocols approved by the Committee for Laboratory Animal Research of the University of Pennsylvania.

Transgenic mice. The generation of the transgenic mice used in this study has been previously detailed.16 Briefly, a 23-kb EcoRI fragment containing 5'-3' \( \Psi_c \)-\( \Psi_a \)-\( \alpha_1 \)-\( \alpha_2 \)-3', a 13-kb HindIII DNA fragment containing \( \zeta \)-globin gene, and a 6.5-kb SacII DNA fragment containing the \( \beta \)-globin LCR were microinjected into the pronuclei of either F2 hybrid eggs from (C57BL/6XCBA) or F1 hybrid eggs from (SWR/JXCB6). Two transgenic lines (lines 1 and 6 described by Albitar et al16) were established. Hemizygous em-
bryos for each of the lines were generated by mating each of the founders to normal SWR or F1 (C57BL6XCBA) females. The day when the vaginal copulation plug was observed was considered day ½.

RNA analysis. Primer extension/HaeIII (PE/HIII) mapping of α1- and α2-globin mRNAs was performed as described by Liebhaber and Kan7 and shown in schematic form in Fig 1A. Briefly, the total RNA is reverse transcribed using AMV reverse transcriptase (Life Sciences, St Petersburg, FL) with a [32P] 5’ end-labeled oligonucleotide primer perfectly complimentary to both the human α1- and α2-globin mRNAs and mismatching the mouse α-globin mRNA by four of the nine nonpoly (T) bases. This primer is human specific and does not cross-react with the mouse α-mRNA.16 The cDNA product is then digested with HaeIII and the products resolved on an 8 mol/L urea/6% acrylamide gel and autoradiographed. The HaeIII digestion generates a [32P] 3’ fragment specific for α1- and α2-globin mRNAs reflecting their structural divergence within the 3’ nontranslated region.7 The analysis of each sample was repeated two or three times and the average value was used. The amount of α1- and α2-globin mRNA represented in each band was quantified either by analysis of autoradiograms on a Molecular Dynamics 300A computing densitometer or by direct counting on a Molecular Dynamics PhosphorImager (Molecular Dynamics, Sunnyvale, CA). All scans were performed on autoradiographic signals that were in the linear range of detection.

RESULTS

Developmental switch in the α2:α1 mRNA ratio in humans. To determine the relative expression of the two α-globin loci in embryonic erythroid tissue, RNA from yolk sac and from human embryos livers at 6, 7, and 8 weeks gestation was isolated and analyzed for the α2:α1 mRNA ratio by RT/HIII (Fig 1). The RNA in the yolk sac contained mostly ζ-globin mRNA, although α-globin mRNA was detectable, the 6- and 7-week samples contained similar levels of ζ- and α-globin mRNAs, and the 8-week sample contained a predominance of α-globin mRNA, although ζ-globin mRNA was still clearly present.17 [32P] 3’ end-labeled cDNAs reverse transcribed from human α1- and α2-globin mRNAs can be distinguished by digestion with HaeIII (see Materials and Methods). A representative study of the α2:α1 mRNA ratio is shown in Fig 1A. The average results from three separate analyses of these samples are shown in the graph in Fig 1B. These studies show that the ratio of mRNAs from the two α-globin loci are equivalent in the primitive erythroid tissue within the yolk sac and in the total erythroid tissue of the 6- and 7-week embryos. At 8 weeks of gestation the ratio shifts to an excess of α2-globin mRNA and the adult sample shows the expected 2.6:1 ratio.

Developmental switch in the expression of the human α2- and α1-globin genes in the transgenic mouse. In previously published studies we generated two transgenic mouse lines that contain and express segments of the human α-globin gene cluster.16 In mice containing the embryonic ζ-globin gene together with both fetal/adult α-globin genes juxtaposed to the β-LCR, developmental switching of the human
transgenes in the transgenic environment is maintained in an appropriate manner. We studied the relative expression of the two human fetal/adult \( \alpha \)-globin genes, \( \alpha_2 \) and \( \alpha_1 \), in these two lines (1 and 6). We have previously shown that the switch from \( \xi \)- to \( \alpha \)-globin expression in these two lines occurs between 11 and 12 days of development. We analyzed mRNA from whole embryo samples at days 11 (embryonic stage) and 16 (fetal/adult stage), and reticulocyte mRNA isolated from transgenic adult mice in line 6 by PE/HIII mapping. The results of a representative analysis of an 11-day embryo, a 16-day embryo, and an adult mouse from line 6 are shown in Fig 2A and the average values of 2 to 4 repeats of the assay are graphed versus developmental period in Fig 2B. The results show that a shift occurs in the \( \alpha_2: \alpha_1 \) ratio in parallel with the embryonic to fetal/adult (\( \xi \rightarrow \alpha \)) switch in globin gene expression. The ratio at day 11 is approximately 1.0, it shifts to 1.4 at day 16, and is 2.8 in the line 6 adult mouse. A similar switch, although not as marked (0.7 ratio in the 11-day embryo to 1.4 in the adult) was found in line 1 (data not shown). The reason for the less marked switch in line 1 has not been determined.

**DISCUSSION**

In the present study we extend the description of hemoglobin gene switching during human development. We test whether the relative levels of expression of the two adjacent fetal/adult \( \alpha \)-globin genes are affected by developmental events occurring in the cluster during the switch from embryonic \( \xi \)-globin to fetal/adult \( \alpha \)-globin gene expression. The results of our experiments show that this appears to be the case. The expression of the two \( \alpha \)-globin loci is equal in the embryonic erythroid tissue before the completion of the switch from predominantly \( \xi \)- to predominantly \( \alpha \)-globin gene expression. After completion of this switch the \( \alpha_2 \)-globin locus becomes dominant to \( \alpha_1 \). To extend these results and establish an experimental system in which the mechanism of this switch and the dominant expression of the \( \alpha \)-globin gene could be manipulated and studied, we asked if this switch could be reproduced in the experimental system of the transgenic mouse. We have previously shown that the human embryonic \( \xi \)- and adult \( \alpha \)-globin transgenes are appropriately controlled during the development of the mouse. In the present case we now extend these observations by showing that the switch between the two adjacent \( \alpha \)-globin loci can also be recapitulated in this transgenic mouse system.

Despite the remarkable similarity in structure of the two \( \alpha \)-globin genes (see above), the \( \alpha_2 \)-globin locus is dominant in the adult, encoding 2.6-fold as much mRNA and protein as the \( \alpha_1 \)-globin locus. The basis for this difference is unknown. Because the same 2.6 ratio of \( \alpha_2: \alpha_1 \) is detected in transcriptionally active bone marrow and in transcriptionally silent reticulocytes, it has been suggested that the dominant nature of the \( \alpha_2 \)-globin gene is transcriptional in origin. This possibility is supported by direct measurements of globin transcription that show a parallel between cytoplasmic ratio of \( \alpha_2: \alpha_1 \) globin mRNAs and their relative transcriptional rates. One potential mechanism is that transcription of the more 5' \( \alpha_2 \)-globin gene interferes with the transcription of the more 3' \( \alpha_1 \)-globin gene.
more 3' α1 locus. Although this is supported by the observed twofold increase in the expression of the α1-globin gene in individuals lacking the α2 locus, a formal test of this mechanism is lacking for the genes in the context of their native relationship in the intact cluster.

The mechanism of the switch in relative expression of the two adjacent and highly similar α-globin loci is unclear. One might ask whether the switch from equal expression of the two α-globin loci to predominance of the more 5' α2 locus reflects a 3' to 5' wave of activation within the cluster. Such a switch would be opposite in direction to the 5' to 3' (embryonic to fetal/adult) switching observed in most (although not all) mammals. Alternatively, the expression of the two α-globin genes may be equally 'leaky' in the embryo and, during the subsequent general α-globin gene activation, the more 5' (α2) locus is more strongly expressed. This latter possibility would be consistent with the general 5' to 3' activation along the cluster. Alternatively, the chromatin environment of both α-globin genes may be equally activated and the lower level of transcription of the α1-locus may reflect exaggerated transcriptional interference from the upstream α2 on the downstream α1 gene as the transcriptional activity of both genes increases. The shift in the relative expression of the two α-globin genes may also reflect differential effects of specific transacting factors that may favor the transcription of the α2-globin gene as has been speculated on the basis of cell fusion studies. The previously demonstrated ability to use the transgenic mouse model to approach the developmental control of the α-globin cluster in general and specifically to analyze the interaction of the two fetal/adult α-globin loci (the present study) may allow the testing of these various possibilities.

REFERENCES

Developmental switch in the relative expression of the alpha 1- and alpha 2-globin genes in humans and in transgenic mice

M Albitar, FE Cash, C Peschle and SA Liebhaber