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Chromatin Structure and Developmental Expression of the Human α-Globin Cluster

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The human α-like globins undergo a switch from the embryonic ζ-chain to the α-chain early in human development, at approximately the same time as the β-like globins switch from the embryonic ε- to the fetal γ-chains. We investigated the chromatin structure of the human α-globin gene cluster in fetal and adult erythroid cells. Our results indicate that DNase I-hypersensitive sites exist at the 5′ ends of the α1- and α2-globin genes as well as at several other sites in the cluster in all erythroid cells examined. In addition, early and late fetal liver erythroid cells and adult bone marrow cells contain hypersensitive sites at the 5′ end of the ζ gene, and in a purified population of 130-day-old fetal erythroid cells, the entire ζ-to α-globin region is sensitive to DNase I digestion. The presence of features of active chromatin in the ζ-globin region in fetal liver and adult bone marrow cells led us to investigate the transcription of ζ in these cells. By nuclear runoff transcription studies, we showed that initiated polymerases are present on the ζ-globin gene in these normal erythroid cells. Immunofluorescence with anti-ζ-globin antibodies also showed that late fetal liver cells contain ζ-globin. These findings demonstrate that expression of the embryonic ζ-globin continues at a low level in normal cells beyond the embryonic to fetal globin switch.

The human α-globins are encoded by a family comprising five genes in a 26-kilobase (kb) DNA region of chromosome 16 (17). The family contains one gene encoding the embryonic ζ-chain, a closely related pseudogene (ψζ), a pseudogene apparently derived from α-globin (ψα), and two genes encoding α-globin (α2 and α1). The ζ-globin is the earliest α-like globin chain detected during human development and is replaced by the α-chain starting at approximately 7 weeks of gestation (2, 29). The α-chain is synthesized throughout the remainder of gestation and postnatal life. An early report indicated that traces of ζ-globin (as hemoglobin Portland, $\zeta_2\gamma_2$) could be detected in the blood of normal newborn infants (11). Other laboratories have failed to detect ζ-globin in embryos of >10 weeks gestational age (15), and recent reports indicate that ζ-globin synthesis occurs only in the primitive erythroblasts derived from the yolk sac (24, 25). However, significant amounts of ζ-globin are found in infants with chromosomal abnormalities and the most severe form of α-thalassemia, hydrops fetalis (2, 11, 15, 17, 29), suggesting that definitive erythroblasts also synthesize some ζ-globin. More recently, small amounts of ζ-globin have been detected in patients with certain forms of α-thalassemia and hemoglobin H disease (4).

Analysis of the chromatin structure of the β-globin gene locus during development and in deletion forms of hereditary persistence of fetal hemoglobin as well as in β- or δβ-thalassemia have suggested that chromatin structure plays a significant role in the control of human globin gene expression and switching (5, 9). Thus, we examined the chromatin structure of the α-globin cluster in normal and transformed erythroid cells to define associations between various aspects of chromatin structure and gene activity in the α locus. Our approach was to assay the sensitivity of the α-globin locus in isolated nuclei to digestion by the enzyme pancreatic DNase I. The endonuclease DNase I, at low concentrations, can cause double-stranded cuts in relatively restricted regions of DNA in intact nuclei. These preferential cuts are referred to as hypersensitive (HS) sites and are manifested as distinct subbands on Southern blots after DNA purification and secondary digestion with restriction endonucleases (6, 9, 31). We identified several HS sites in the α-globin gene cluster of nonerythroid cells, fetal and adult erythroid cells, and the erythroleukemia cell line K562. The ζ- to α-globin region is more sensitive to DNase I digestion than is collagen in fetal and adult erythroid cells, and both ζ and α HS sites are present in these cells. In the ζ-producing K562 cell line, the ζ-globin is more sensitive to DNase I than the ψα and α2 globin genes, suggesting that the ψα and α2 genes are active in only a subpopulation of these cells, if at all. In addition, ζ-globin gene transcription is detectable by nuclear runoff transcription in fetal and adult erythroid cells, and by immunofluorescence, ζ-globin protein is found in late fetal liver cells. These results demonstrate the unexpected finding that embryonic ζ-globin expression continues, albeit at low levels, into adulthood.

MATERIALS AND METHODS

Fetal liver cells were prepared by finely mincing specimens (66 to 130 days of gestation) into phosphate-buffered saline (GIBCO Laboratories, Grand Island, N.Y.). Aggregates of hepatocytes were allowed to settle out for 1 h on ice. Cells remaining in suspension, which are generally 50 to 75% erythroid, were used to prepare nuclei as described previously (9, 10). Briefly, nuclei were obtained by lysis of cells in 0.1 to 0.5% Nonidet P-40 in RSB (10 mM NaCl, 10 mM Tris hydrochloride [pH 7.5], 3 to 5 mM $MgCl_2$) and low-speed centrifugation. Nuclei were prepared from fetal brain samples in a similar fashion, except that the cell lysates were centrifuged through a 25% sucrose–RSB layer to remove insoluble cytoskeletal debris. For the endogenous nuclear

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transcription assay (runoff transcription), normal erythroid cells were prepared by countercurrent elutriation from normal adult marrow (20). The cell lines K562 (18) and Manca (23) were maintained in tissue culture.

Preparation of nuclei, endogenous nuclear transcription, DNase I digestion, and Southern blot analysis have all been described in detail previously (9, 10). The probes used in these analyses were: αP1.5, a 1.5-kb *Pst*I fragment carrying the α1-globin gene (17); α2.3.7, a *Bgl*II-*Sac*I fragment containing intervening sequence 1 (IVS1) from ψζ (26); Z30, a 0.7-kb *Bam*HI-*Sac*I fragment from λHζG1 which maps 0.1 to 0.8 kb 3′ of ζ (26); β IVS, the *Bam*HI-*Eco*RI fragment containing IVS2 from β-globin (8); β-actin; and Hf 32, a cDNA clone of pro-α2(I) collagen (22). Isolated DNA fragments were labeled with [α-^{32}P]dCTP and [α-^{32}P]TTP (New England Nuclear Corp., Boston, Mass.) to an approximate specific activity of 1×10^8 to 5×10^8 dpm/μg by nick translation.

Smears of fetal liver erythroid cells were prepared by centrifugation of cells onto microscope slides (Cytospin; Shandon Southern Instruments, Inc., Sewickley, Pa.). The cells were fixed in methanol and stained with polyclonal rabbit anti-ζ-globin (4) and fluorescein-coupled goat anti-rabbit immunoglobulin G (Tago, Inc., Burlingame, Ca.) and rhodamine-coupled mouse monoclonal anti-γ-globin (28) antibodies. The rabbit antibody has been demonstrated to be specific for ζ-globin by radioimmunoassay (4). The anti-γ-globin monoclonal antibody (51.7 in reference 28) reacts only with human and monkey hemoglobin F.

RESULTS

Location of DNase I-HS sites around the α-globin genes. Digestion of fetal liver nuclei with DNase and *Eco*RI followed by hybridization with an α-globin-specific probe resulted in a 23-kb parent band and subbands at 8.9, 6.0, 4.6, 3.7, 3.5, 2.5, and 2.3 kb. None of these subbands was large enough to be the result of hybridization to a fragment extending from the *Eco*RI site between ψζ and ζ to an HS site in the vicinity of α1 or α2; thus, the subbands must arise from detection of a fragment with one end at the *Eco*RI site 3′ of α1 and the other at an HS site within the gene cluster. Therefore, in this case the sizes of the subbands provide the locations of the HS sites relative to the *Eco*RI site located 2.2 kb 3′ of the α1 mRNA cap site. The existence of subbands of 2.3 to 2.5 and 6.0 to 6.2 kb indicates that HS sites are present near the 5′ ends of α1 and α2, respectively, while the other subbands indicate the existence of several other sites between α2 and α1 and near the ψα gene as well. The locations of the HS sites in fetal liver cells were confirmed by digestion of these same DNA samples with other restriction enzymes and are summarized in Fig. 1D.

The mapping of HS sites in K562 cell nuclei (Fig. 1A) also revealed prominent sites at the 5′ ends of α2 and α1, at the 3′ end of ψα, and between α2 and α1. The sites at the 5′ ends of the α genes were mapped more precisely with a *Pvu*II digest (Fig. 1). A 1.0- to 1.1-kb subfragment was generated after DNase I digestion of K562 nuclei. By sequencing (21), a *Pvu*II site is located 932 base pairs 3′ of the cap site of both genes. Thus, DNase I cuts in a region 100 to 200 base pairs 5′ to the cap site of the α2 and α1 genes. The 3.3-kb subband in the digest is most probably due to cleavage at the HS site nearest the 3′ end of the α2.

One difference between K562 and the fetal liver samples was the existence in K562 of an HS site in the vicinity of ψζ. This site was evident as an ~16-kb subband after *Eco*RI digestion of K562 DNase-treated samples (Fig. 1A). This fragment size suggests that a DNase I site is located near the ψζ gene of K562, possibly in IVS1. This subband was not observed, even after prolonged exposure (<1 month; data not shown), in the fetal liver samples. The diffuse hybridization observed in the 10- to 20-kb region in the fetal liver samples presented was not reproducible and may be an artifact of the gel. The existence of this site in K562 nuclei and its virtual absence in fetal liver was confirmed by experiments with several other restriction endonucleases and various probes (data not shown).

Location of sites around the ζ-globin gene. As described in the Introduction, no synthesis of the embryonic ζ-globin protein is detected in normal tissues after week 10 of gestation. Therefore, we compared the HS sites of 95- and 130-day-old fetal liver and adult bone marrow erythroid cells with those of the ζ-globin-expressing K562 erythroleukemia line to define differences associated with ζ-globin gene expression. The Z30 probe is derived from sequences immediately distal to the poly(A) addition site of the ζ gene and does not hybridize to sequences near ψζ. *Hin*dIII digestion of human DNA yielded a 13-kb parent fragment after hybridization with Z30 (Fig. 1B). The *Hin*dIII site between ψζ and ζ is located 2.7 kb 3′ of the probe; therefore, subbands of less than 10 kb detected with this probe must be derived from the inter-ψζ-ζ *Hin*dIII site and HS sites on the 5′ side of ζ.

Several subfragments were observed in DNase I-treated nuclei from fetal erythroid cells (Fig. 1B). The sites appeared to be identical in 95- and 130-day-old fetal liver and adult bone marrow cells (data not shown). Two sites were observed within the ζ gene, near the 3′ end. Several more sites were present, including one within IVS1, another near the 5′ end of the gene, and others in the 9 kb of 5′-flanking sequences surveyed in these experiments.

In contrast to the plethora of HS sites 5′ to the ζ gene in the adult and late fetal erythroid cells, K562 cells appeared to contain few if any HS sites 5′ to ζ (Fig. 1B). Two strong HS sites were present near the 3′ end and a pair of weaker sites were near the 5′ end of the ζ gene. However, only rather diffuse hybridization was detected beyond the 5′ end of the gene, even though the same samples exhibited strong HS sites around the α-globin genes. The failure to detect HS sites 5′ to the ζ-globin genes may be a reflection of the nuclease sensitivity of a relatively large DNA region, as discussed below.

With the possible exception of those at the 3′ end of ζ, the HS sites in the α-globin cluster are specific for erythroid tissue. Faint, barely discernible subbands corresponding to HS sites between ψα and α2, and at the 3′ end of ζ, were detected in fetal brain nuclei (Fig. 1C and D). The weakness of the detected subbands indicates that a small minority of the brain cell population possesses these sites. In contrast, strong HS sites were observed when a β-actin probe was hybridized to the fetal brain DNA samples (data not shown).

DNase I-sensitive domains in the α locus. The overall level of sensitivity of the α-globin cluster to digestion with DNase I was determined. Isolated nuclei were digested with DNase I, and the DNA was purified as previously described (9, 10). The DNA was then digested with a combination of restriction endonucleases selected so that each of the hybridizing fragments contained primarily a single gene, distinct in size from the other fragments. By this protocol, the approximate level of sensitivity of each of the genes can be compared with the others as well as to an inactive control gene [in this case α2(I) collagen (22)]. In the experiments illustrated in Fig. 2, the DNAs were digested with *Bam*HI and *Hin*dIII and probed with αP1.5, which hybridizes to both α1 and α2, and

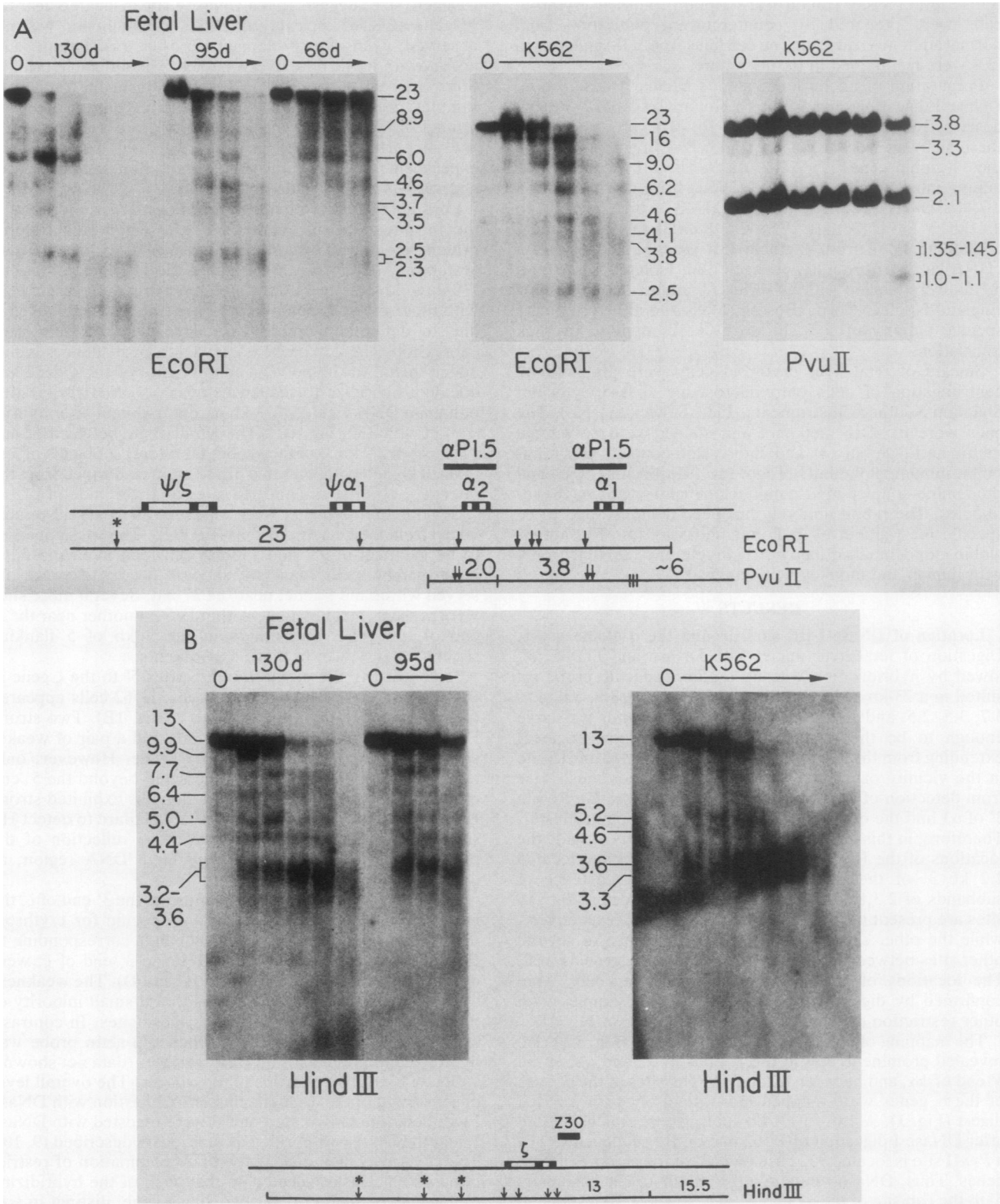

FIG. 1. DNase I-HS sites in the α-globin locus of erythroid cells. Isolated nuclei were digested with DNase I followed by restriction endonucleases as described in the text. Numbers on the sides of the figures indicate the sizes of the bands observed (kilobases). The arrows at the top of each blot indicate increasing DNase I concentration. The restriction endonuclease used is indicated below each blot. The sequences recognized by the hybridization probe are indicated by the broad lines. The restriction endonuclease map of the relevant portion of the α-globin cluster and the deduced positions of the DNase I-HS sites are presented below the blots. (A) HS sites around the α-globin genes in fetal liver and K562 cells. The approximate ages of the fetal liver samples (in days of gestation) are indicated above each blot. The

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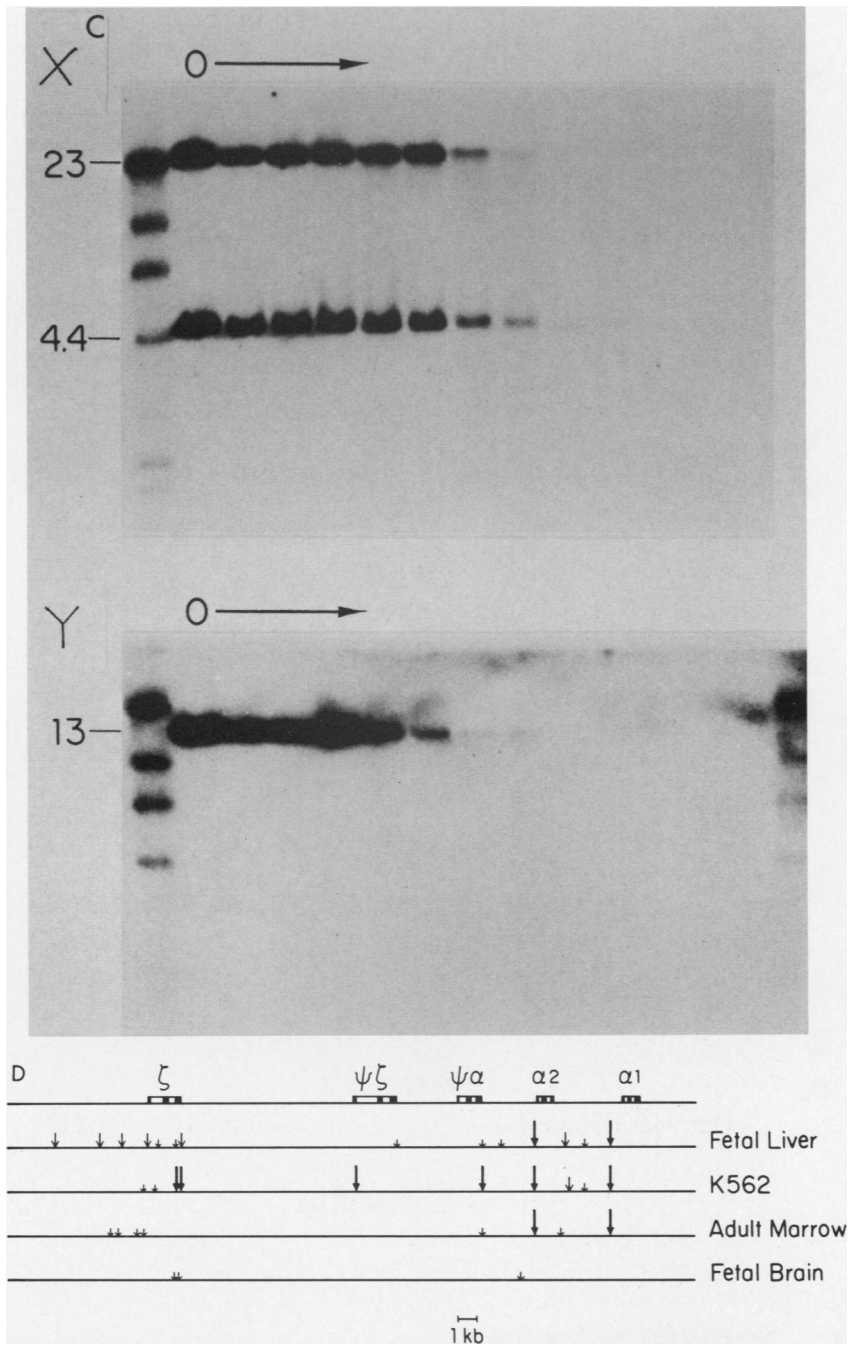

probe used was αP1.5, which hybridized to both α1 and α2. (B) ζ-Globin HS sites in fetal liver and K562 nuclei. The samples used in panel A were rehybridized to the ζ-specific probe Z30. HS sites present in fetal liver and absent in K562 nuclei are indicated with asterisks. (C) α- and ζ-Globin HS sites in fetal brain nuclei. Nuclei were digested with DNase I followed by either *Eco*RI (X) or *Hin*dIII (Y) and hybridized with αP1.5 first and then Z30 (X) or with Z30 alone (Y). In panel X, faint subbands are visible at 6.5 kb (α specific) and 2.7 and 2.5 kb (ζ specific) on the original autoradiograph. In panel Y, very faint hybridization at 3 kb is visible on the original. (D) Summary of HS sites in the α-globin cluster. The genes are illustrated schematically. The locations of the sites are derived from the previous panels and from other experiments not shown. The approximate intensities of the bands derived by cleavage at each site are indicated by the size of the arrow. Where several sites are closely spaced (e.g., at the 5′ ends of α1 and α2), only one is indicated.

α2.3.7, which hybridizes to both ψζ and ζ. In the K562 and fetal liver erythroid cells, the α-globin cluster was more sensitive to DNase I digestion than were the collagen gene fragments, whereas in the B-lymphoblastoid Manca cell line the globin and inactive collagen genes exhibited a similar level of sensitivity. Furthermore, in the ζ-producing K562 cell line, the fragment corresponding to the ζ gene was digested by lower levels of DNase I than were the ψα and α2 genes, even though the ζ gene was present on a smaller fragment. In the late (130-day-old) fetal liver, the ζ gene exhibited a level of sensitivity similar to that of ψα and α. These results suggest either that the differential sensitivity of

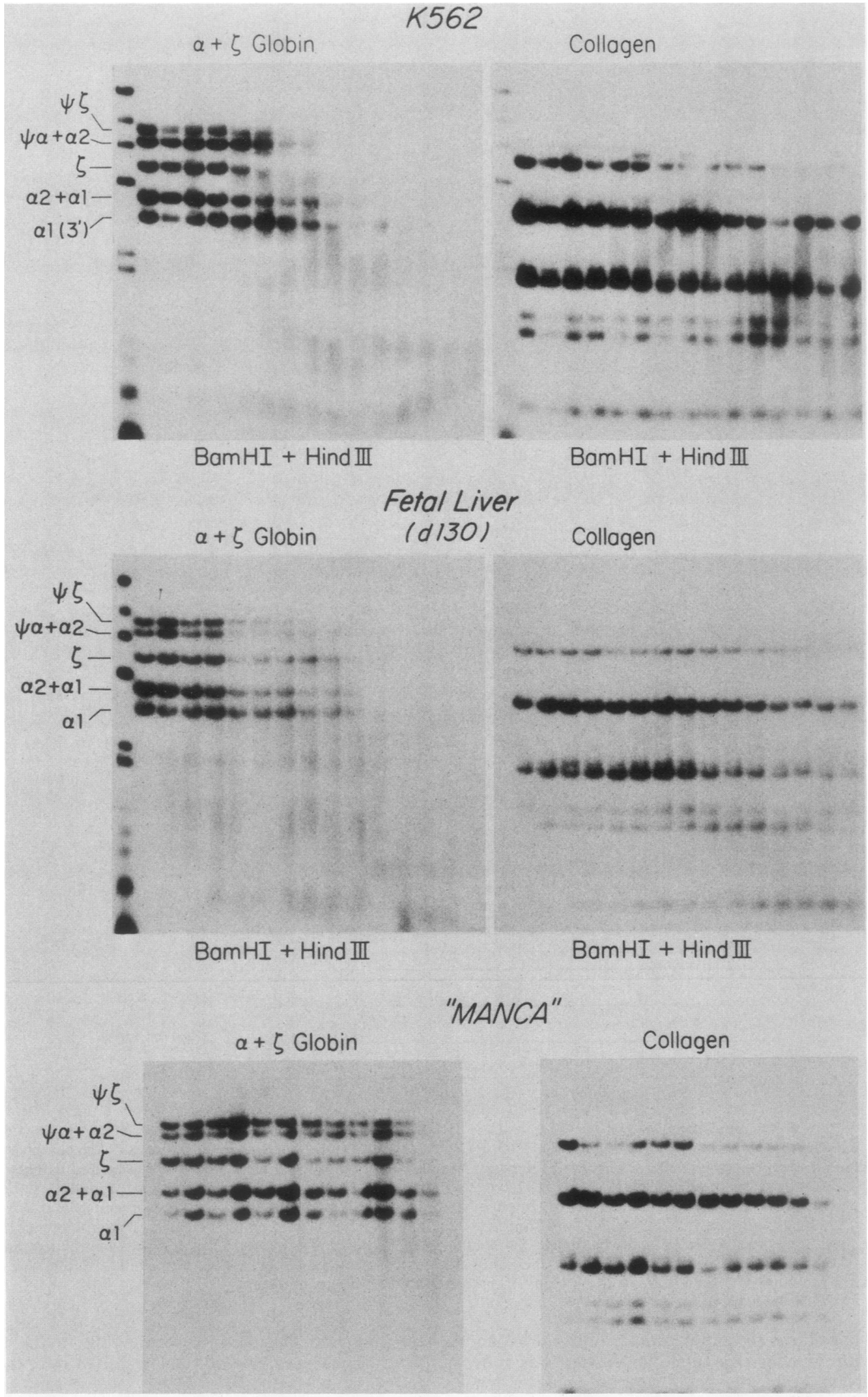

FIG. 2. Sensitivity of the α-globin cluster to DNase I. DNase I-treated samples were digested with *Bam*HI and *Hin*dIII. The blots were first hybridized with a mixture of αP1.5 (α-globin) and α2.3.7 (ψζ- and ζ-globin). After autoradiographic exposure, the probes were removed, and the blots were rehybridized to Hf 32, a cDNA clone encoding pro-α2(I) collagen. Cells used were K562, 130-day-old fetal liver erythroid cells, and Manca, a B-lymphoblastoid cell line. For the α and ζ probes, the genes contained in each fragment are indicated.

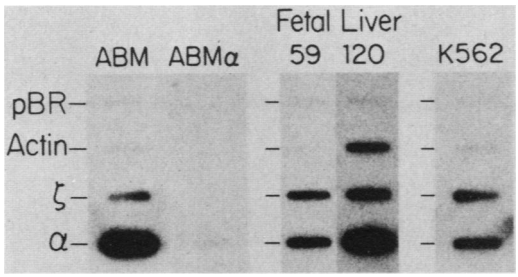

FIG. 3. ζ-Globin gene transcription in fetal liver and adult bone marrow cells. Plasmid DNA (5 μg) carrying the human actin gene sequence, the 1.5-kb *Pst*I fragment containing the α-globin gene, the *Bgl*II-*Sac*I fragment containing the large IVS from ψζ, or pBR322 alone was loaded onto nitrocellulose and hybridized to ^{32}P-labeled nuclear runoff products as described in Materials and Methods. Transcripts were from: adult bone marrow erythroid nuclei (ABM); adult bone marrow nuclei incubated with 2 μg of α-amanitin per ml (ABM α); fetal liver erythroid nuclei of 59 or 120 days of gestation (FL); or K562. The higher level of actin transcription in the older fetal liver sample reflects an increase in contamination of the cells with liver parenchyma. All hybridization, including that to actin, is sensitive to a concentration of α-amanitin (2 μg/ml) which specifically inhibits RNA polymerase II transcription.

the ζ-globin gene in K562 is associated with high levels of transcription or that the ψα and α2 genes are expressed only in a subpopulation of K562 cells (see Discussion).

Expression of ζ-globin gene in erythroid cells. The presence of HS sites 5′ of the ζ-globin gene in fetal and adult erythroid cells suggested the possibility that transcription of the gene is occurring in these cells. To test this, nuclei isolated from fetal liver and adult bone marrow erythroid cells and from K562 cells were incubated with [^{32}P]UTP. The resultant ^{32}P-labeled nascent RNA was then hybridized to an excess of DNA bound to nitrocellulose. In the experiments shown in Fig. 3, sequences specific to the ζ and α genes, as well as pBR322 and actin, were immobilized on nitrocellulose. The transcription of these regions was assayed by hybridization of nascent RNA from several cell types to these DNAs. ζ-Globin gene transcription was detected in adult bone marrow and both early and late (59- and 120-day-old) fetal liver cells (Fig. 3) as well as in K562 cells (3). In all cases, the ζ-globin transcription was greater than background or actin transcription and was sensitive to a concentration of α-amanitin (2 μg/ml) selectively inhibitory for RNA polymerase II transcription, as shown for the adult bone marrow cells. Thus, the signal observed from the ζ gene is representative of RNA polymerase II transcription. ζ-Globin transcription in the adult bone marrow cells, while significantly less than α- or β-globin gene transcription, was higher than any signal observed from the G_γ or A_γ genes (data not shown; see reference 9).

Recent reports indicate that little or no ζ-globin synthesis is detectable in definitive erythroblasts (24, 25). The results of the runoff assay could be explained by the presence, especially in early fetal livers, of a subpopulation of cells synthesizing large quantities of ζ-globin while the majority synthesize little or none. Immunofluorescent staining of fetal liver smears was therefore performed to assess the cellular distribution of ζ-globin synthesis. A large percentage of the nucleated erythroid cells in a 59-day-old fetal liver stained with both antibodies (Fig. 4), indicating that the cells contain a detectable amount of ζ- as well as fetal globins. The two embryonic progenitor cells present in the photographed field, distinguished by their large size and abundant cytoplasm, were stained by the anti-ζ-globin but not the anti-γ-globin antibody. These results indicate that primitive and at least some definitive fetal liver erythroid precursors contain ζ-globin and thus suggest that the transcripts observed in vitro are processed into mRNA in vivo. Therefore, these results indicate that ζ-globin expression is not lineage restricted and that the ζ- to α-globin switch cannot be accounted for by the displacement of primitive-lineage erythroblasts by definitive erythroblasts (24, 25).

DISCUSSION

HS sites. We showed that DNase I-HS sites are present at the 5′ and 3′ ends of the transcribed genes (ζ, α2, and α1) as well as at other positions in the α locus. The sites were erythroid specific; only a few weak sites were observed in brain nuclei. The locations and strength of the sites were similar in fetal and adult erythroid cells, suggesting that they are stable during development. Although some of the HS sites appeared to coincide with certain sequence features, e.g., the *Alu* family sequences at the ends of the X homology block (12), DNA sequence alone is not sufficient to determine the existence of an HS site. For example, a polypyrimidine-polypurine tract lying between α2 and α1 which gives rise to an S1 nuclease-sensitive site in supercoiled plasmid DNA (27) does not correspond to an HS site in erythroid nuclei. In addition, the 3′ HS sites observed in the ζ-globin gene map within the sequences duplicated in the ψζ gene (26), yet HS sites are not detected at corresponding positions in the pseudogene. Thus, these results support the notion that primary sequence per se, even in a topologically relaxed region of chromatin, is not sufficient for the formation of an HS structure. Evidence has been obtained for proteins capable of inducing HS structures near the chick β-globin gene (7, 14).

The absence of HS sites 5′ to the ζ gene in K562 cells, where the ζ gene is transcribed at relatively high levels, suggests that much of the 5′-flanking sequence is nuclease HS in these cells. Such a situation exists at the 5′ end of the chick β-globin gene where a region of approximately 200 bp is accessible to digestion by restriction endonucleases and can be excised as a protein-free DNA fragment from erythrocyte nuclei (7, 14, 16, 19). Preliminary evidence (not shown) suggests that the region 5′ to the ζ-globin gene is also accessible to restriction endonuclease digestion in K562 nuclei.

The significance of the HS site in the vicinity of the ψζ gene in K562 is unknown. S1 nuclease assays indicate that the ψζ gene does not give rise to stable RNA products, even in K562 cells (13; N. J. Proudfoot, personal communication); therefore, this site may not indicate transcriptional activity. Another possibility is that this site marks a transcriptional terminator. HS sites at putative termination or alternator sites have been described in human β- and avian α^D-globin genes (9, 30). Thus, although no steady-state ψζ mRNA can be detected in K562 or fetal liver, it is possible that transcription of the gene occurs but is prematurely terminated. It is also possible that this HS site is a consequence of an unusual chromatin structure encompassing the ζ-globin gene in K562 cells. Analyses of the chromatin structure of the ζ- to α-globin gene region in hybrids between K562 and MEL cells, where the ζ-globin gene is silenced (1), may provide clues to the significance of the differences between K562 and normal erythroid cells.

α-Globin chromatin domain. These studies demonstrated that the chromatin structure of the α-globin cluster is different in erythroid as compared with nonerythroid cells. In

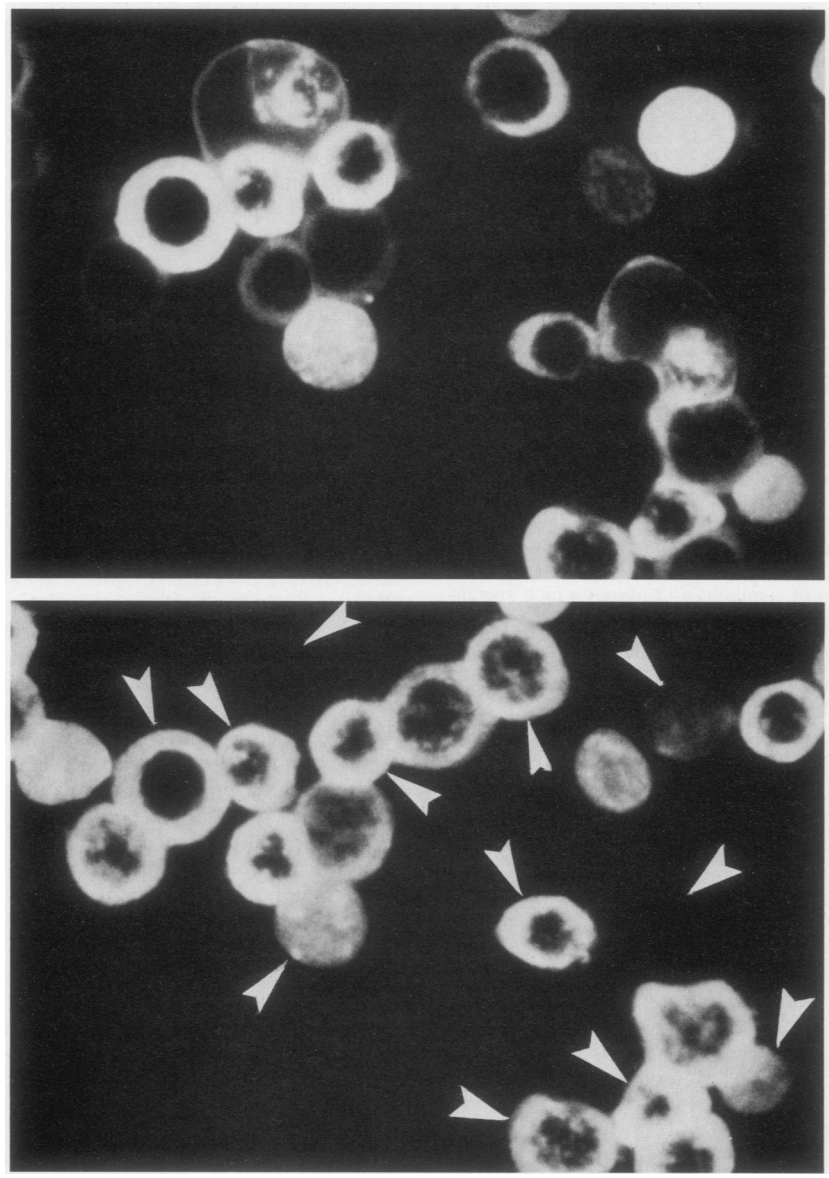

FIG. 4. Immunofluorescent analysis of fetal liver erythroid cells. Smears of cells prepared from a 59-day-old fetal liver were doubly stained with fluorescein-coupled goat anti-rabbit immunoglobulin G and rabbit anti-ζ-globin followed by rhodamine-coupled mouse monoclonal anti-human γ-globin antibody. Top, fluorescein, and bottom, rhodamine fluorescence of a single field. Arrowheads in the bottom photograph indicate ζ^+ erythroblasts. The two large cells which react with the anti-gζ-globin but not the anti-γ-globin antibody are yolk sac-derived embryonic erythroblasts.

the B-lymphoblastoid Manca cell line the unexpressed globin and collagen genes are comparable in their sensitivity to DNase I. In fetal liver erythroid cells producing little ζ-globin, the ζ- and α-globin genes exhibit similar levels of sensitivity to DNase I digestion and are more sensitive than the pro-α2(I) collagen gene. In K562 cells, the entire α locus is more sensitive to DNase I than is collagen, and the ζ gene is more sensitive than ψα or the 5′ portion of the α2 gene. While α transcripts and protein have been identified in K562 cells, it is not known whether the ψα or α2 genes are expressed in this cell line. It is possible that the increased sensitivity of ζ relative to ψα and α2 is a reflection of the lack of overt transcription of ψα and α2 in K562. Alternatively, ψα or α2 or both may be transcribed in only a subset of K562 cells, while ζ may be transcribed in most K562 cells. Either of these situations would result in an increased sensitivity of ζ compared with ψα and α2. Regardless of the explanation for this phenomenon, these results indicate that at least three levels of DNase I sensitivity are detectable: (i) a relatively DNase I-resistant state typified by the transcriptionally silent α2(I) collagen gene; (ii) an intermediate state of sensitivity exhibited by the ψα- and α2-globin genes in K562 cells; and (iii) a DNase I-sensitive state shown by the ζ-globin gene in K562.

Expression of ζ. The existence of HS sites 5′ to the ζ-globin gene in fetal and adult erythroid cells and the DNase sensitivity of this gene in fetal liver led us to examine the possibility that the ζ gene is transcribed in these cells. The results of the endogenous nuclear transcription assay indicate that the ζ-globin gene gives rise to a low but detectable

number of transcripts in late fetal and adult erythroid cells. Immunofluorescent staining of fetal liver smears indicated that these transcripts are processed into ζ-globin mRNA and translated into protein. Detectable levels of reactivity with the anti-ζ-globin antibody were observed in both embryonic and fetal erythroid precursors. Further support for the idea that ζ-globin expression continues beyond the embryonic-to-fetal globin switch comes from S1 nuclease protection assays performed on mRNA isolated from 84-day-old fetal liver and 16- to 20-week-old fetal blood samples (13; Proudfoot, personal communication). The results again indicate that bona fide ζ-globin mRNA is present at significant levels in fetal liver cells later than would be expected if ζ-globin expression was limited to embryonic precursors (24, 25).

The results of the runoff transcription, immunofluorescence, and S1 nuclease protection assays appear to conflict with the failure to detect ζ-globin synthesis in radioactively labeled fetal erythroid cells taken after week 7 of gestation (24, 25). The absence of labeled ζ-globin in later erythroid cells has been presented as evidence that this protein is synthesized only in primitive-lineage erythroblasts; however, the results presented here demonstrate that ζ-globin synthesis normally continues at low levels well beyond this point, possibly into adulthood. It is possible that the rate of ζ-globin protein synthesis is low compared with rate of synthesis of the other globin chains in fetal and adult erythroblasts. By the nuclear runoff assay, the ratio of ζ to α transcription decreases with increasing developmental age, indicating that some regulation is exerted at the transcriptional level. However, the rate of ζ transcription in adult marrow appears to be disproportionately high in view of the virtual absence of ζ^+ cells detected by immunofluorescence in this population (data not shown), suggesting that ζ-globin expression is also regulated at the posttranscriptional level. Alternatively, many adult bone marrow cells may synthesize ζ-globin protein at levels too low to be detected by immunofluorescence or biosynthetic labeling.

A recent study indicates that a subset of patients with α-thalassemia possess detectable levels of circulating ζ-globin into adulthood (4). A more detailed analysis of the relationship between the DNA sequence rearrangements in the α-globin cluster and the levels of embryonic ζ-globin expression in these patients may provide insights on the roles of chromatin structure and transcriptional and post-transcriptional events in the developmental regulation of the human α-globins.

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