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The modulation of Hb F synthesis in adult erythroid progenitor (burst-forming unit) cultures reflects changes in γ-globin gene transcription and chromatin structure

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ABSTRACT **Fetal hemoglobin production can be reactivated *in vivo* in adult persons with various hemoglobinopathies and other hemopoietic disorders, and in cultures of adult erythroid progenitors. We show that the activation of Hb F in adult cells is transcriptional in nature and is accompanied by the appearance of DNase I-hypersensitive sites and undermethylation of *Hpa* II sites 5′ to the γ-globin genes. Production of Hb F in culture is strongly modulated by the environment, and the repression of Hb F synthesis by specific culture conditions has been reported. By nuclear runoff, chromatin, and methylation analyses, we show that this inhibition of Hb F production *in vitro* is at the level of transcription with the concomitant loss of characteristic γ hypersensitive sites and methylation of γ *Hpa* II sites. These data indicate, first, that the organization of globin chromatin of adult cells that produce fetal hemoglobin resembles that of fetal erythroid cells and, second, that this organization switches from a fetal to an adult pattern in response to changes in the environment of the erythroid cells.**

During the perinatal period of human ontogeny there is a switch from fetal to adult globin synthesis (reviewed in refs. 1 and 2). This switch is transcriptional in nature, and it is associated with characteristic changes in the chromatin structure of the γ-globin genes (3). The switch from γ to β formation is not complete, since low levels of fetal hemoglobin (Hb) are synthesized by every normal adult. Furthermore, Hb F is typically increased in some patients with β-chain hemoglobinopathies as well as in patients with acquired disorders of hematopoiesis. In addition, a reactivation of Hb F synthesis occurs when adult erythroid cells are grown in clonal cultures (reviewed in ref. 4). An interesting feature of the production of Hb F in culture is its strong modulation by the *in vitro* environment. Thus, when adult erythroid progenitors are grown in medium containing fetal sheep serum or plasma, Hb F synthesis is strongly inhibited (5). Similarly, there is a decrease in γ- and an increase in β-chain synthesis when neonatal erythroid progenitors are cultured in fetal sheep serum, but there are no effects on globin expression when fetal erythroid progenitors are cultured under similar conditions (5). Continuation of fetal hemoglobin production in the adult is characteristic of the mutants of hereditary persistence of fetal hemoglobin and δβ-thalassemia (1, 2). δβ-Thalassemias are caused by deletion of genes of the β-globin cluster, while the phenotype of hereditary persistence of fetal hemoglobin is produced by deletion as well as nondeletion mutants (2). It has been shown that culture in fetal sheep serum abolishes or strikingly inhibits Hb F synthesis in colonies produced by erythroid progenitors from deletion or nondeletion mutants (6).

Previous analysis of Hb F modulation in culture has relied on two methods: globin biosynthesis or labeling the culture cells with fluorescent anti-globin chain antibodies (6, 7). We wished to learn whether the modulation of Hb F expression in the adult erythroid cells is controlled at the post-transcriptional or at the transcriptional level. Cells were cultured under conditions that allow high γ-globin production or conditions that inhibit Hb F production, and the structure of γ-globin locus chromatin, the degree of γ-globin gene methylation, and the transcriptional activity of γ-globin genes were assessed. We found that production of Hb F in adult cells is associated with increased sensitivity of the γ-globin locus to DNase I and decreased methylation—i.e., findings characteristic of the fetal erythroid cells (3, 8, 9). We further observed that the environmentally induced inhibition of γ-globin synthesis by fetal sheep serum is associated with increased γ-globin gene methylation, a loss of γ-globin gene DNase I hypersensitive sites, and a decrease in γ-globin gene transcription. These results suggest that the organization of the γ-globin chromatin is dynamic, switching from a "fetal" to an "adult" pattern in response to the change in the cell's environment. This change in chromatin apparently takes place in cells already committed to the adult lineage and does not reflect the selection of a subpopulation of fetal hemopoietic cells.

MATERIALS AND METHODS

Cultures. Cultures were established in methylcellulose as supporting medium as described (5). In brief, mononuclear cells were isolated from heparinized peripheral blood after layering on a cushion of Ficoll/sodium Metrizoate (Lymphoprep-Nyegaard, Oslo, Norway). The adherent cells present in the light-density mononuclear cell fraction were removed by adherence to plastic surfaces after a half-hour incubation at 37°C. Remaining nonadherent cells were collected and inoculated at densities from 10^5 to 3×10^5 cells per ml in 35-mm plates containing 1% methylcellulose (Dow Methocel), and the following culture components: 1% bovine serum albumin (Sigma), 30% fetal calf serum (Flow Laboratories), or fetal sheep serum, alpha medium (Flow Laboratories), and step III sheep plasma erythropoietin (2 international units/ml; Connaught, Willowdale, ON, Canada). The plates were incubated at 37°C in a fully humidified incubator with 5% CO_2/95% air. Individual mature erythroid bursts were selectively removed from the plates with an ultrathin capillary pipette. Colonies to be used for molecular analyses were suspended in medium containing either fetal calf serum or fetal sheep serum. Colonies to be used for globin biosynthesis were placed on the bottom of microfuge tubes and processed as described (5, 7).

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Abbreviations: bp, base pair(s); kb, kilobase(s); IVS, intervening sequence(s); BFU-e, adult erythroid progenitor(s) [burst-forming unit(s)].

Molecular Studies. Isolation of nuclei, DNase I digestion, blot hybridization conditions, and *in vitro* nuclear run-off transcription assays were performed as described (3, 10). The globin-specific run-off transcription products were analyzed by hybridization of ^{32}P-labeled nuclear RNA to recombinant DNA molecules containing the intervening sequences (IVS) of ε-, γ-, or β-globin human genes dot-blotted to nitrocellulose filters as described (10). The $^{G}\gamma$ IVS probe consisted of a 457-base-pair (bp) fragment extending from the *Bam*HI site in the second exon to a *Pvu* II site in the second intron of this gene. The IVS β-globin probe extended from the *Bam*HI site at the end of the second exon to the *Eco*RI site at the start of the third exon and includes 923 bp of the β-globin gene. The IVS δ-globin probe contains 960 bp extending from the *Bam*HI site in the second exon to the *Eco*RI site in the third exon of the δ-globin gene. The IVS ε-globin probe contains 1300 bp extending from the *Bam*HI site in the second exon to the *Eco*RI site 3′ to the third exon of the ε-globin gene.

RESULTS

Experimental Approach. The experimental approach consisted of culturing the immature erythroid progenitors present in the circulation and known as burst-forming units (BFU-e). The donor of the cells was a patient with homozygous β^{+}-thalassemia, who produces about equal amounts of γ- and β-globin chains in his blood ($\gamma/\gamma+\beta$ ratio, $\approx$0.5). The particular genetic lesion in this patient is not known. The blood from a patient with homozygous β^{+}-thalassemia, rather than a normal individual, was used for two reasons: the plating efficiency of thalassemic BFU-e is higher than BFU-e derived from normal blood; and individuals with thalassemia have higher endogenous levels of Hb F *in vivo*. The level of γ-globin synthesis in cultures of BFU-e from this patient depends on the culture medium used. In fetal calf serum cultures, γ/γ+B ratios range from 0.5 to 0.7. In fetal sheep serum cultures, $\gamma/\gamma+\beta$ ratios range from 0.03 to 0.4 depending on the fetal sheep serum preparation used. For all the experiments reported below, erythroblasts from fetal sheep serum-grown BFU-e cultures showing $\gamma/\gamma+\beta$ ratios in the range of 0.03 to 0.08 were used. Approximately 10^{8} erythroblasts were collected from several culture experiments and were used to prepare DNA for methylation analysis or nuclei for the analysis of hypersensitive sites and run-off transcription.

Methylation of $^{A}\gamma$- and $^{G}\gamma$-Globin Genes. DNA was isolated from erythroid cells grown either in fetal calf serum or fetal sheep serum, and methylation at specific sites within the β-globin locus was analyzed by digestion with methylation-sensitive enzymes as described (8). In Fig. 1, methylation of sites within or near the $^{G}\gamma$- or $^{A}\gamma$-globin genes is shown. Digestion of DNA from fetal calf serum- or fetal sheep serum-treated cultures with *Eco*RI and subsequent blot hybridization to a $^{G}\gamma$ intervening sequence (IVS probe) results in the generation of 6.7- and 2.7-kilobase (kb) fragments, which correspond to the $^{G}\gamma$- and $^{A}\gamma$-globin genes, respectively. *Eco*RI/*Msp* I double digestion of these same samples results in the generation of 5.0- and 1.4-kb fragments, which correspond to *Msp* I cleavage at sites M1, M2, and M4, as indicated in Fig. 1 (*Lower*). *Eco*RI/*Hpa* II digestion of DNA from cells grown in fetal calf serum results in the generation of the same fragments, indicating the relative hypomethylation of these sites in the fetal calf serum-treated cells. In contrast, the 1.4-kb band is barely visible upon similar analysis of cells grown in the presence of fetal sheep serum, indicating that sites M2 and M4 are methylated at cpG dinucleotides under these conditions.

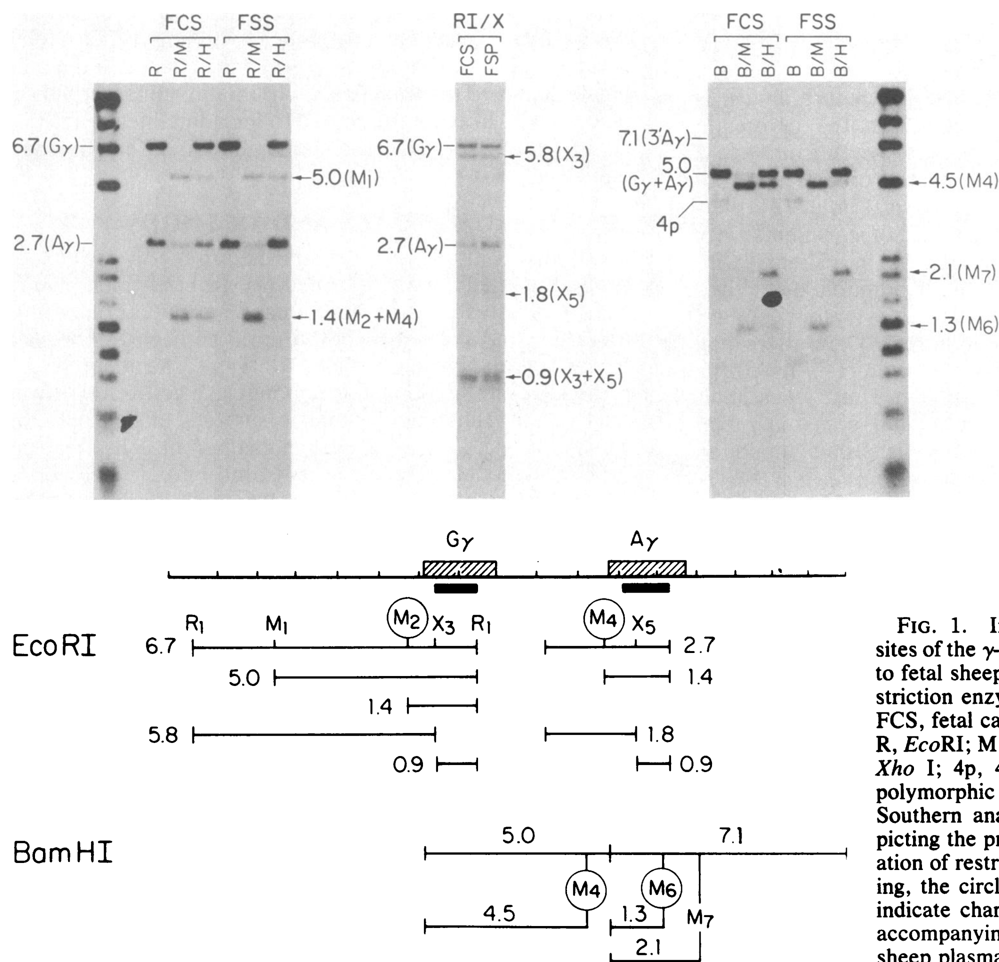

FIG. 1. Increased methylation of *Hpa* II sites of the γ-globin genes of BFU-e in response to fetal sheep serum. Methylation-sensitive restriction enzyme sites are defined as in ref. 8. FCS, fetal calf serum; FSS, fetal sheep serum; R, *Eco*RI; M, *Msp* I; H, *Hpa* II; B, *Bam*HI; X, *Xho* I; 4p, 4.0-kb fragment resulting from a polymorphic *Bam*HI site 3′ to the $^{A}\gamma$. (*Upper*) Southern analysis; (*Lower*) line drawings depicting the probes, restriction sites, and generation of restriction fragments. In the line drawing, the circles around sites M2, M4, and M6 indicate changes in methylation of these sites accompanying treatment of BFU-e with fetal sheep plasma.

Similarly, the status of methylation at *Xho* I sites internal to the $^G\gamma$-globin and $^A\gamma$-globin genes was analyzed in DNA from cells grown in either fetal calf serum or fetal sheep serum. *Eco*RI/*Xho* I digestion of DNA from either sample results in the appearance of similar bands. These include the 6.7 and 2.7 parental R1 bands corresponding to the $^G\gamma$- and $^A\gamma$-globin genes, respectively. In addition, a 5.8-kb band representative of cutting of the parental 6.7-kb band at *Xho* I site X_3 (indicated in Fig. 1 *Lower*), and a 1.8-kb band representative of cutting of the parent 2.7-kb $^A\gamma$ fragment at *Xho* site X_5 are observed in both samples. Finally, a 0.6-kb fragment, which represents cutting at sites X_3 and/or X_5, is observed in both samples. These results reveal that no change in *Xho* I methylation within the γ-globin genes occurs upon exposure of cells to fetal sheep serum.

Methylation at sites M6 and M7 (internal and 3′ to $^A\gamma$, respectively) were analyzed by digestion of these same samples with *Bam*HI and *Hpa* II. Digestion of either DNA isolated from fetal calf serum or fetal sheep serum erythroid cells with *Bam*HI reveals the characteristic 7.1-kb parental fragment containing the majority of the $^A\gamma$-globin gene and 3′ flanking sequences, the 5.0-kb parental band containing most of the $^G\gamma$ sequences and sequences in between $^G\gamma$ and $^A\gamma$, as well as a 4.0-kb fragment, resulting from a *Bam*HI polymorphism 3′ to the $^A\gamma$-globin gene. Thus, the relative high intensity of the 5.0-kb parental fragment compared to the 7.1- and 4.0-kb fragments is due to the fact that the 5.0-kb band fragment is contained on both chromosomes, while the 7.1- and 4.0-kb fragments are contained on only one chromosome. Digestion of the samples with *Bam*HI plus *Msp* I reveals the presence of a 4.5-kb fragment representative of cutting at site M4 and a 1.3-kb fragment representative of cutting at *Msp* I site M6. *Bam*HI/*Hpa* II analysis of these samples again reveals the increase in methylation of site M4 5′ to $^A\gamma$, as indicated by the relative decrease in molarity of the 4.5-kb fragment in the fetal sheep serum samples compared to the fetal calf serum samples. In addition, an increase in methylation of site M6, internal to the $^A\gamma$-globin gene, is seen in the fetal sheep serum sample, as indicated by the lack of the 1.3-kb fragment. Finally, no significant change in methylation of the *Hpa* II site 3′ to $^A\gamma$ (M7) is seen in the fetal sheep serum compared to fetal calf serum sample, since both show an equal molarity of the 2.1-kb fragment.

In summary, analysis of the methylated state of defined sites in or around $^G\gamma$- and $^A\gamma$-globin genes reveals an increase in methylation at *Hpa* II sites 5′ to the $^G\gamma$- and $^A\gamma$-globin genes upon exposure of BFU-e to fetal sheep plasma. Specifically, sites M2, M4, and M6 show an increase in methylation under these conditions.

Methylation of δ- and β-Globin Genes. The analysis of the status of methylation in the region 5′ to the δ-globin gene is presented in Fig. 2. As shown (Fig. 2 *Left*), digestion of either DNA sample with *Hin*dIII results in the generation of a 15.5-kb band when hybridized to an IVS probe from δ. *Msp* I/*Hin*dIII digestion generates a 6.1-kb fragment, representative of cutting of the *Hin*dIII parental fragment at site M12. Digestion of either of the fetal calf serum- or fetal sheep serum-grown erythroid cell DNA with *Hin*dIII and *Hpa* II reveals an identical pattern of fragments: a 6.1-kb fragment, indicating cleavage at site M12; a 7.5-kb fragment, indicating *Hpa* II cleavage at a polymorphic *Hpa* II site (mp); and faint but reproducible bands at 14 and 15.5 kb representative of cleavage at sites M10 and M9, respectively. Similar cutting at site M12 in both the fetal calf serum and fetal sheep serum DNA samples is confirmed by a *Bgl* II/*Hpa* II digest, shown in Fig. 2 (*Center*). The ratio of the parental 8.4 *Bgl* II fragment to the 7.0 *Bgl* II/*Hpa* II fragment is identical in the DNA from erythroid cells grown in the presence of fetal calf serum or fetal sheep serum. Fig. 2 (*Right*) shows an analysis of the methylation of the *Sal* I site immediately 5′ to the δ-globin gene (site S13). It is evident in this panel that site S13 is completely cleaved in both the fetal calf serum and fetal sheep serum samples. Thus, we conclude that in contrast to the $^G\gamma$- and $^A\gamma$-globin genes, no change in methylation in the region 5′ to the δ-globin gene is observed. A similar analysis of methylation sites 3′ to the adult β-globin gene has also revealed no differences in DNA from erythroblasts grown in either fetal calf serum or fetal sheep serum (data not shown).

Culture of Cells in Fetal Sheep Serum Results in a Marked Decrease in the $^G\gamma$- and $^A\gamma$-Globin Gene 5′ Hypersensitive Site. The experiments presented above indicate that changes in methylation 5′ to the $^G\gamma$- and $^A\gamma$-globin genes, but not the δ- or β-globin genes, are apparent in DNA samples isolated from erythroid cells grown in fetal sheep serum compared to fetal calf serum. The location of the methylated sites 5′ to $^A\gamma$ and $^G\gamma$ is in a region that is hypersensitive to DNase I, S1 nuclease, and other nucleases in cells that are actively

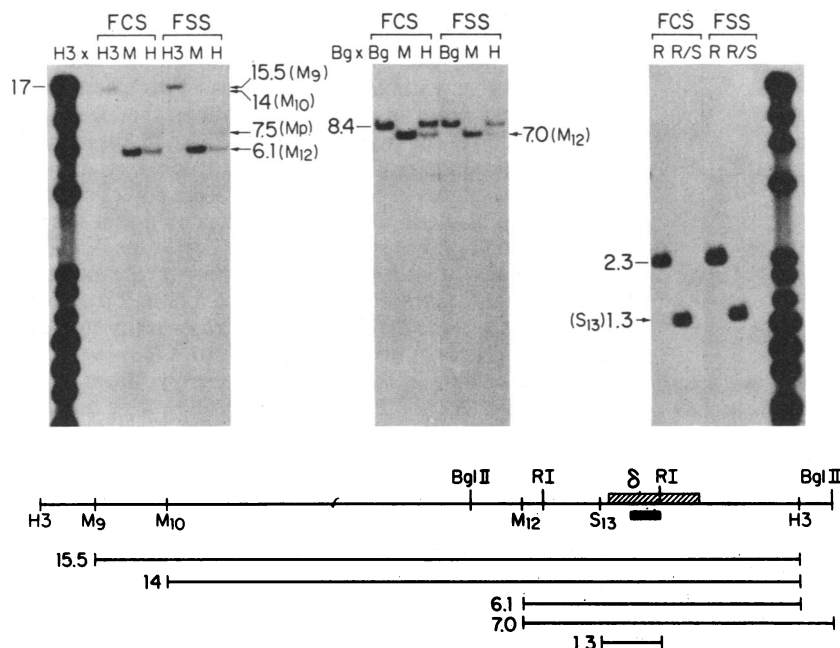

FIG. 2. No change in methylation in the region 3′ to the adult δ-globin gene is observed upon treatment of adult BFU-e with fetal sheep serum. Abbreviations are as in legend to Fig. 1; in addition, H3, *Hin*dIII; Bg, *Bgl* II; S, *Sal* I. The 7.5-kb fragment generated in the double *Hin*dIII and *Hpa* II digest and designated Mp refers to a polymorphic *Hpa* II site.

transcribing the γ-globin genes. Thus, we analyzed the chromatin structure of the $^{G}\gamma$- and $^{A}\gamma$-globin genes in nucleated erythroid cells grown in either fetal calf serum or fetal sheep serum. The assay consists of digestion of isolated nuclei with increasing concentrations of DNase I, and the blot hybridization of the samples to the IVS γ- or δ-globin probe. As shown in Fig. 3, while the hypersensitive site characteristic of the γ-globin genes is apparent in the fetal calf serum-derived samples, no such site is evident in those erythroid cells grown with fetal sheep serum. In contrast, a δ-globin hypersensitive site is present in a representative sample of the DNase I-treated nuclear DNA from erythroid cells treated with either fetal calf serum or fetal sheep serum. Thus, we conclude that the growth of erythroid cells in fetal sheep serum results in a change in the chromatin structure around the γ-globin genes—namely, the diminution of a hypersensitive site 5′ to these genes.

Growth of Cells in Fetal Sheep Serum Is Associated with a Decrease in Transcription of the γ-Globin Genes. To determine whether the decrease in the hypersensitive site and increase in methylation 5′ to the γ genes in fetal sheep serum-treated adult erythroid cells correlate with the lack of overt transcription of these genes, we investigated the steady state distribution of elongating endogenous polymerase molecules along the γ-globin genes by nuclear run-off transcription. Under the conditions used for this assay, RNA polymerase molecules continue to elongate nascent RNA transcripts, which then incorporate [^{32}P]UMP. No new initiation occurs under these conditions. As shown in Fig. 4, there is a decrease in γ-globin gene transcription by a factor of 8–10 in those nuclei from erythroid cells grown in the presence of fetal sheep serum compared to fetal calf serum. However, no change in the rate of β-globin gene transcription is observed in cells grown in fetal calf serum or fetal sheep serum. Thus, growth of adult erythroid cells in the presence of fetal calf serum results in an increase in methylation at the 5′ ends of $^{G}\gamma$- and $^{A}\gamma$-globin genes, the loss of the γ-globin hypersensitive site, and a decrease in transcription by a factor of 8–10 in the γ-globin genes.

DISCUSSION

Previous work with fetal or adult erythroid cells has revealed that changes in the sensitivity of chromatin to digestion by DNase I and in the degree of methylation of globin genes accompany the switch from γ- to β-chain formation (3, 8, 9). Fetal cells produce mainly γ chains and minor amounts of β chains; in these cells the γ-globin genes are hypomethylated (9) and globin chromatin is in the active conformation, as revealed by the presence of the 5′-placed γ-globin gene hypersensitive sites (3). Adult cells produce almost exclusively β chain; in the adult erythroid cells, the γ-globin genes are hypermethylated (9) and the γ-globin gene chromatin is in the inactive conformation as shown by the disappearance of the 5′-placed hypersensitive sites (3). The switch from γ to β formation is thus associated with specific changes in methylation and DNase I sensitivity of the γ-globin genes. It is possible that these changes are irreversible, so that once they occur at the switch period, all the subsequent progeny of the "switched" adult cells bear the "adult" pattern of chromatin conformation and methylation. This would predict that switched adult cells would be incapable of re-expressing the fetal program. Thus γ-globin gene expression in adults would derive only from a subpopulation of unswitched cells. Alternatively, the pattern of molecular organization of chromatin, which is associated with γ-globin gene expression, may demonstrate plasticity and reappear when γ-globin genes are turned on in the adult cells. Our results provide insights into these alternative models.

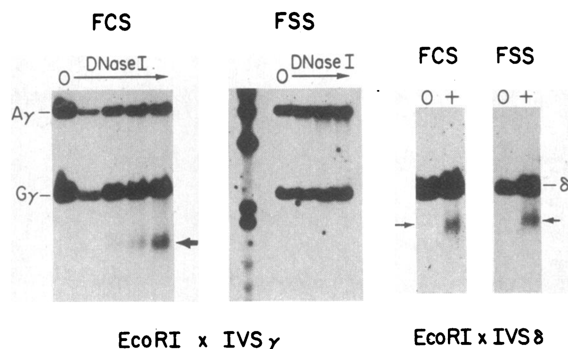

FIG. 3. Decrease in the $^{G}\gamma$ and $^{A}\gamma$ 5′ hypersensitive site upon treatment of adult BFU-e with fetal sheep serum (FSS). Nuclei were isolated from cultures of BFU-e treated with either fetal calf serum (FCS) or FSS. The nuclei were treated with increasing concentrations of DNase I, and isolated DNA was redigested with *Eco*RI, Southern blotted, and hybridized to either the IVS-γ or IVS-δ probe. No hypersensitive site is detectable 5′ to the $^{A}\gamma$- or $^{G}\gamma$-globin gene in even high exposures of the DNase-treated nuclear DNA from cells grown in the presence of FSS. In contrast, the typical 5′ γ-globin hypersensitive site is detectable in the cells grown in fetal calf serum. The 5′ δ-globin hypersensitive site is detected in a representative sample of both series.

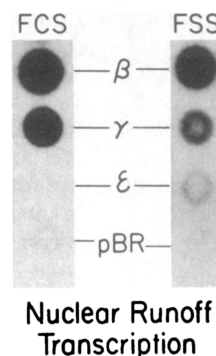

FIG. 4. Analysis of globin gene transcription in adult BFU-e grown in the presence of either fetal calf serum or fetal sheep serum. In the cultures grown in fetal calf serum and fetal sheep serum, significant β-globin gene transcription is detectable by nuclear run-off assay. In contrast, there is marked reduction in γ-globin gene transcription in the cells grown in the presence of fetal sheep serum. Slight ε-globin transcription detected in the fetal sheep serum-treated cultures is not significantly above the pBR background. FCS, fetal calf serum; FSS, fetal sheep serum.

It is known that Hb F production in the adult can be induced by a variety of conditions. For example, acute anemia and other *in vivo* manipulations that result in fast regeneration of the erythroid marrow also result in induction of Hb F production (10). Induction of Hb F is also characteristic of cultures of BFU-e derived from adult bone marrow or peripheral blood (4). It has been shown that the F cells (i.e., erythrocytes producing Hb F) and the A cells (i.e., erythrocytes lacking fetal hemoglobin) of the adult derive from the same pluripotent stem cells (11, 12). It is also known that a single adult BFU-e can produce progeny that express Hb F and progeny that express only Hb A (13, 14). Thus, the fetal hemoglobin in the adult is not derived from a subpopulation of fetal cells that continue proliferating during adult hematopoiesis but represents genuine reactivation of the γ-globin gene in the erythroid cells of the adult developmental stage (11–14). Whether the activation of Hb F in the adult cells, either *in vivo* or in culture, is associated with the reappearance of the characteristic fetal methylation patterns and chromatin structure has not been examined before. Our analyses of cells grown in fetal calf serum provide a model system to study this question. Under this culture condition, levels of Hb F that were close to those present in our patient's

blood were produced by the cultured erythroblasts. The analysis of the methylation status of the γ-globin genes showed that the *Hpa* II sites located 5′ to the $^G\gamma$- and $^A\gamma$-globin genes (sites M2 and M4) as well as a site within the $^A\gamma$-globin gene (site M6) are hypomethylated when the γ-globin genes are turned on in adult cells. The analyses of DNase I-digested erythroid nuclei showed that a major hypersensitive site located in the promoter region of the $^G\gamma$- and $^A\gamma$-globin genes of fetal cells reappears when, in the adult cells, the γ-globin genes are turned on. Hence, the first conclusion of our study is that the reactivation of Hb F in the adult cells is associated with reappearance of fetal-like patterns of γ-globin gene methylation and of chromatin organization.

Previous work using globin biosynthesis or immunochemical probes have demonstrated the striking modulation on Hb F synthesis achieved by changing the environment in which the adult cells grow (5–7). Environmental modulation of γ-globin gene expression is best demonstrated by culturing cells in fetal calf serum or fetal sheep serum (8–10). However, the previous studies were not informative as to whether the modulation of Hb F synthesis in culture is controlled at the transcriptional or post-transcriptional levels. Our run-off transcription experiments answer the question, since they show that the inhibition of Hb F by fetal sheep serum is controlled at the level of transcription. In addition, we observed that culture in fetal sheep serum results in reappearance of the adult type of organization of γ-globin chromatin. Thus, *Hpa* II sites 5′ to $^G\gamma$ and $^A\gamma$ and the internal $^A\gamma$ *Hpa* II site, which are hypomethylated in the fetal calf serum-grown cells, become hypermethylated in the fetal sheep serum-grown cells. Furthermore, the 5′ $^G\gamma$- and $^A\gamma$-globin gene DNase I hypersensitive sites, which are turned on in the fetal calf serum-grown cells, are turned off in the fetal sheep serum-grown cells. These data demonstrate that the whole organization of the γ-globin chromatin switches from a fetal to an adult pattern in response to the change of the environment and reinforce the idea of the plasticity of γ-globin chromatin structure. It is interesting to note that the methylation of cytosine residues 5′ to the $^A\gamma$-globin gene between nucleotides -760 to $+100$ results in the transcriptional inactivation of this gene when transfected into mouse L cells (15). Site M4 maps to a position within this region (approximately at -50), and site M2 is in an identical location 5′ to the $^G\gamma$-globin gene. In addition, the 5′ γ-globin hypersensitive site maps in the region containing the M2/M4 *Hpa* II site (3).

The mechanism whereby the culture environment influences Hb F expression is unknown. Certain experiments suggest that the effects of the environment are exerted in earlier cells—i.e., in erythroid progenitors and perhaps in proerythroblasts (9). It is possible that the factors present in fetal sheep serum or in fetal calf serum act directly on these cells and influence methylation, chromatin structure, and expression of the γ-globin genes. For example, such factors might bind to DNA sequences within the 5′ hypersensitive sites, altering the chromatin structure of the region. Alternatively, these factors might stimulate or might inhibit γ-globin expression by influencing the process of differentiation/maturation of erythroid cells. In this latter model the earlier, less mature, adult cells would normally have an organization of chromatin and a γ-globin gene methylation pattern resembling that of fetal cells. During subsequent differentiative divisions, this pattern may switch to the pattern characteristic of the adult cells. *In vivo* (as in the β-thalassemia syndromes) or *in vitro* (as in culture in fetal calf serum) stress erythropoiesis may promote premature terminal differentiation of adult cells; under these conditions, the fetal-like chromatin pattern is retained and γ-globin gene expression occurs. Culture in fetal sheep serum may promote normal differentiation of adult cells; under these conditions, the adult pattern of chromatin appears and the γ-globin genes are not expressed.

How can one explain, with the above hypothesis, why fetal sheep serum can strikingly inhibit γ-chain formation in cultures of BFU-e bearing mutant hereditary persistence of fetal or $\delta\beta$-thalassemia genes (6)? The continuation of Hb F production in these mutants has been explained by assuming that the structural lesions inhibit the interaction between the mutant genes and the trans-acting elements, which normally turn off γ-globin gene expression in the adult cells (16). It is possible that fetal sheep serum, through its action on maturation, stimulates the production of these trans-acting elements. To explain the inhibition of Hb F in the mutants, one could postulate that these trans-acting elements achieve high levels when erythroid cells are cultured in fetal sheep serum. The high concentration of the trans-acting factor might then permit interaction with the structurally altered mutant γ-globin genes resulting in inhibition of γ-globin gene expression.

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