Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 12267–12271, October 1996
Biochemistry

Silencing of human fetal globin expression is impaired in the absence of the adult β-globin gene activator protein EKLF

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Contributed by Stuart H. Orkin, August 13, 1996

ABSTRACT **Globin genes are subject to tissue-specific and developmental stage-specific regulation. A switch from human fetal (γ)- to adult (β)-globin expression occurs within erythroid precursor cells of the adult lineage. Previously we and others showed by targeted gene disruption that the zinc finger gene, erythroid Krüppel-like factor (EKLF), is required for expression of the β-globin gene in mice, presumably through interaction with a high-affinity binding site in the proximal promoter. To examine the role of EKLF in the developmental regulation of the human γ-globin gene we interbred EKLF heterozygotes (+/−) with mice harboring a human β-globin yeast artificial chromosome transgene. We find that in the absence of EKLF, while human β-globin expression is dramatically reduced, γ-globin transcripts are elevated ≈5-fold. Impaired silencing of γ-globin expression identifies EKLF as the first transcription factor participating quantitatively in the γ-globin to β-globin switch. Our findings are compatible with a competitive model of switching in which EKLF mediates an adult stage-specific interaction between the β-globin gene promoter and the locus control region that excludes the γ-globin gene.**

Vertebrate globins are encoded by small α-like and β-like gene families. The expression of individual members is programmed such that different hemoglobin tetramers are assembled within red cells at different developmental stages. The human β-globin cluster spans >60 kb on chromosome 11 and contains an embryonic gene (ε), duplicated fetal genes (Aγ and Gγ), and one minor (δ) and one major (β) adult gene. The murine β-cluster, which is similarly organized, contains embryonic (ε^y, βh_0, and βh_1) and adult (β_{maj} and β_{min}) expressed genes, but no fetal stage-specific gene. Rather, the murine βh_0 and βh_1 globin genes, which most closely resemble the human γ-globin gene, are expressed only in yolk sac-derived embryonic red cells. An upstream, distal regulatory element, known as the locus control region (LCR), is required for position-independent, copy number-dependent, high level expression of linked globin genes in mice (1). The mechanisms by which individual globin genes within the loci are developmentally regulated remains incompletely understood, but has been postulated to involve interaction of stage-specific transcriptional regulators with critical stage-specific cis-elements.

The participation of cis-elements in the switching from human γ- to β-globin has been studied most extensively in the mouse by analyzing the expression profiles of transgenes containing various combinations of globin gene promoter and LCR regions. From these (reviewed in ref. 2) and complementary transfection experiments in chicken erythroid cells (3), two mechanisms of globin gene switching have been proposed. One model posits that individual globin genes are regulated autonomously; that is, their expression is regulated independently of the presence of linked globin genes. The other suggests that developmental regulation reflects the competition of globin genes for the influence of the LCR (reviewed in ref. 2). According to this model, which is based on the enhancer competition model for the ε- to β-globin switch in chickens (3), the LCR might loop to each gene in a mutually exclusive fashion.

Short promoter regions of individual human globin genes contain sufficient information to direct erythroid- and developmental stage-specific expression in transgenic mice (4, 5). In addition, γ-globin transgenes linked to LCR elements are substantially silenced in the mouse fetal liver (6). These observations are consistent with autonomous modes of regulation.

On the other hand, many transgenes containing the LCR linked to a single globin gene are inappropriately regulated (7, 8). Expression is seen at both embryonic and adult stages, a finding consistent with loss of stage-specific competition from a linked second gene. Furthermore, appropriate stage-specific expression is observed when the γ- and β-globin genes are linked in cis and are in their proper orientation with respect to the LCR (9). These experiments support competitive regulation, particularly with respect to silencing of the β-globin gene at the embryonic stage. The role, if any, played by the β-globin gene in competitive silencing of the γ-globin gene at the fetal liver stage of development remains uncertain.

The use of small transgenes that do not reflect the normal organization of the β-globin cluster complicates interpretation of many transgenic experiments. Large DNA constructs derived from yeast artificial chromosomes (YACs), however, more accurately mimic the behavior of complex multigene loci, such as the β-globin locus. Mice harboring a single copy of the entire human β-globin locus, as a YAC encompassing 150 kb (10) or 248 kb (11) of DNA, express each of the globin genes at levels comparable to the equivalent endogenous mouse genes and in a proper developmental profile. These mice provide the most appropriate murine model for studying the human γ- to β-globin gene switch.

Despite advances in mapping the cis-elements that participate in switching, the nuclear regulatory factors influencing the γ- to β-globin switch have remained elusive. Despite the attraction of models in which stage-specific activators or repressors control the developmental profile of globin gene expression, no stage-specific proteins have been identified. A region in the proximal promoter of the γ-globin gene (the stage-selector element) has been reported to bind a nuclear factor complex, the stage-selector protein (12). While the stage-selector protein complex is proposed to be a positive regulator of the γ-globin promoter, one of its subunits is a

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Abbreviations: EKLF, erythroid Krüppel-like factor; LCR, locus control region; YAC, yeast artificial chromosome; CFU-E, colony-forming unit-erythroid; RT-PCR, reverse transcriptase–PCR; E, embryonic day.

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ubiquitously expressed polypeptide, CP2 (13). Whether the apparent stage-specificity of stage-selector protein binding reflects a stage-specific component in the complex, or modification of subunits that are not themselves developmentally controlled, is currently unknown.

The expression of the adult β-globin gene is exquisitely dependent on a transcription factor known as erythroid Krüppel-like factor (EKLF), first identified as an erythroid-enriched cDNA by substractive hybridization (14). The deduced EKLF consensus DNA-binding site (CCNCNCCCN; ref. 15) matches that found in the β-globin promoter. Accordingly, EKLF transactivates reporter constructs containing the β-globin CACC-boxes, but fails to bind to mutated sequences seen in patients with β-thalassemia (15). An essential, nonredundant role for EKLF in murine β-globin expression was established by study of EKLF null ($EKLF^{-/-}$) embryos generated by gene targeting of embryonic stem cells (16, 17).

Here we address the role EKLF may play in the regulation of γ-globin expression, particularly its silencing in the adult erythroid lineage. We previously proposed a possible role for EKLF during the γ-globin to β-globin switch (16) on the basis of the selective β-globin deficiency we observed in $EKLF^{-/-}$ mice and elevated fetal hemoglobin in human adults heterozygous for deletions of the β-globin promoter or for mutations in the CACC box of the β-globin promoter (16–19). By studying the expression profile of a human globin YAC transgene in $EKLF^{-/-}$ embryos, we demonstrate that EKLF is essential not only for expression of the human β-globin gene, but also for proper silencing of the human γ-globin gene. These findings suggest that EKLF may participate in the interactions of the β-globin promoter with the LCR that contributes to γ-globin silencing by a competitive mechanism. As such, they establish EKLF as the first transcription factor affecting the γ-globin to β-globin switch.

MATERIALS AND METHODS

Generation of $EKLF^{-/-}$ Embryos Containing the Entire Human β-Globin Locus. $EKLF^{+/-}$ mice (16) were bred with mice harboring a 150-kb β-globin locus YAC (line A20.1) (10). $EKLF^{+/-}$ offspring were identified by Southern blot analysis as described (16), and the presence of the β-YAC transgene was determined by hybridization with a 1.9-kb *Kpn*I–*Pvu*II human HS-2 probe derived from pUC19-HSII (1.9)β (20). $EKLF^{+/-}$, β-YAC animals were interbred and staged litters were killed to examine embryonic [embryonic day (E) 10–E11] and definitive (E12–E15) hematopoiesis.

Hematopoietic Cell Culture and Immunofluorescence Assays. Single cell suspensions of fetal liver cells and yolk sac-derived defective progenitor cells were prepared as described (21). Colony-forming units-erythroid (CFU-E) were scored after 2 days growth in methylcellulose cultures supplemented with 2 units/ml erythropoietin. Liquid erythroid cultures were initiated with 1×10^5 fetal liver cells per ml and performed in DMEM/20% fetal bovine serum containing recombinant kit ligand (50 ng/ml) and erythropoietin (2 units/ml). After 7 days, the cellular composition was examined by May–Grunwald Giemsa staining of cytocentrifuge specimens. Immunofluorescence for γ-globin was performed as described (21). Fluorescein isothiocyanate-conjugated monoclonal antibodies from four independent hybridomas generated from rats immunized with purified human hemoglobin F, a kind gift from Dr. Thomas Campbell (Isolabs), were tested for specificity and low background on nontransgenic murine erythroid cells. One monoclonal antibody (9C3) was used for these studies.

RNase Protection and reverse transcriptase–PCR (RT-PCR) Analyses. RNA was prepared from primary tissues and *in vitro* cultured cells as described (22), and RNase protection assays were performed as described (23). Mouse α- and ζ-globin and human γ- and β-globin probes were previously reported (23). A human β-globin genomic PCR product including 5′ untranslated and first exon sequences was cloned into the *Bam*HI and *Eco*RI sites of pBSII-SK$^-$ (Stratagene). The human β-globin riboprobe, transcribed with T3 RNA polymerase from a *Eco*RI linearized template, protects the first 90 bp of human β-globin mRNA from RNase digestion.

cDNA was prepared from single CFU-E as described (21). Semiquantitative RT-PCR for globin transcripts was performed with primer pairs corresponding to a unique 5′ untranslated region of each globin gene transcript and a conserved region spanning exons 2 and 3. In this manner, amplification of any contaminating DNA was avoided, and specificity was confirmed by failure to amplify human globin PCR products from cDNA derived from nontransgenic erythroid cells. The mouse α-globin primer pairs were TCTGACAGACTCAGGAAGAAA and GTGGCTCAGGAGACGGGATCCACA. One of the human γ-globin primer pairs (AATAAGCTCCTAGTCCAGACGC) was specific for the 5′ untranslated region of the duplicated human Aγ and Gγ transcripts. The other ($^{A}/_{G}$TTNCC$^{C}/_{T}$A$^{A}/_{G}$GAGC$^{C}/_{T}$TGAAGTTCTC) was degenerate for sequences bridging the boundaries of exons 2–3 of all mouse and human β-like globins genes. Semiquantitative RT-PCR was performed in the presence of [^{32}P]dCTP as described (24). Each amplification cycle was performed at 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min. Since the quantity of human γ-globin transcripts was less than murine α-globin transcripts, PCR was performed in the presence of the human γ-globin primer pairs for four cycles before the addition of the α-globin primer pairs. The number of cycles required to amplify both PCR products within the linear range was determined empirically for each cDNA sample.

RESULTS

EKLF Is Required for Expression of the Human β-Globin Gene. Our prior evidence suggested that $EKLF^{-/-}$ embryos die from β-thalassemia, a hemolytic disorder due to decreased β-globin production and resulting unbalanced α-globin synthesis (16). We first tested by interbreeding of $EKLF^{+/-}$ and β-YAC transgenic mice whether the presence of the human β-YAC in an $EKLF^{-/-}$ background would rescue this embryonic lethality. No rescue was observed, as none of 38 β-YAC$^+$ newborn animals from $EKLF^{+/-}$ (β-YAC$^+$) crosses were of the $EKLF^{-/-}$ genotype. Analysis of timed litters demonstrated that β-YAC$^+$, $EKLF^{-/-}$ embryos succumb to anemia at E15 and are not appreciably different in appearance from β-YAC$^-$, $EKLF^{-/-}$ littermates. Despite the failure to rescue $EKLF^{-/-}$ embryos (see *Discussion*), human globin gene expression could be examined in E15 fetal livers.

Human β-globin transcripts were profoundly reduced in $EKLF^{-/-}$ embryos at E15 compared with $EKLF^{+/-}$ littermates (Fig. 1*a*). The deficit in human β-globin gene expression is even more pronounced than previously observed for the endogenous mouse β-globin gene (16, 17). Indeed, the human β-globin gene appears inactive in $EKLF^{-/-}$ embryos.

Elevated γ-Globin Gene Expression in $EKLF^{-/-}$ Fetal Liver Cells. We next examined the ratio of human γ-globin/mouse α-globin transcripts in fetal liver samples of varying genotypes. As shown in Fig. 1 *b* and *c*, γ-globin transcripts, which at E15 are present at only 1–2% the level of mouse α-RNA in $EKLF^{+/-}$ samples, are elevated ≈5-fold in the absence of EKLF. Thus, it would appear that EKLF, and presumably β-globin transcription, is required for proper γ-gene silencing.

Several potential problems complicate simple interpretation of these data. First, it remained possible that embryonic red cell contamination of $EKLF^{-/-}$ fetal liver preparations might lead to a spurious increase in γ-globin transcripts, since the γ-gene is actively expressed at the embryonic stage in the

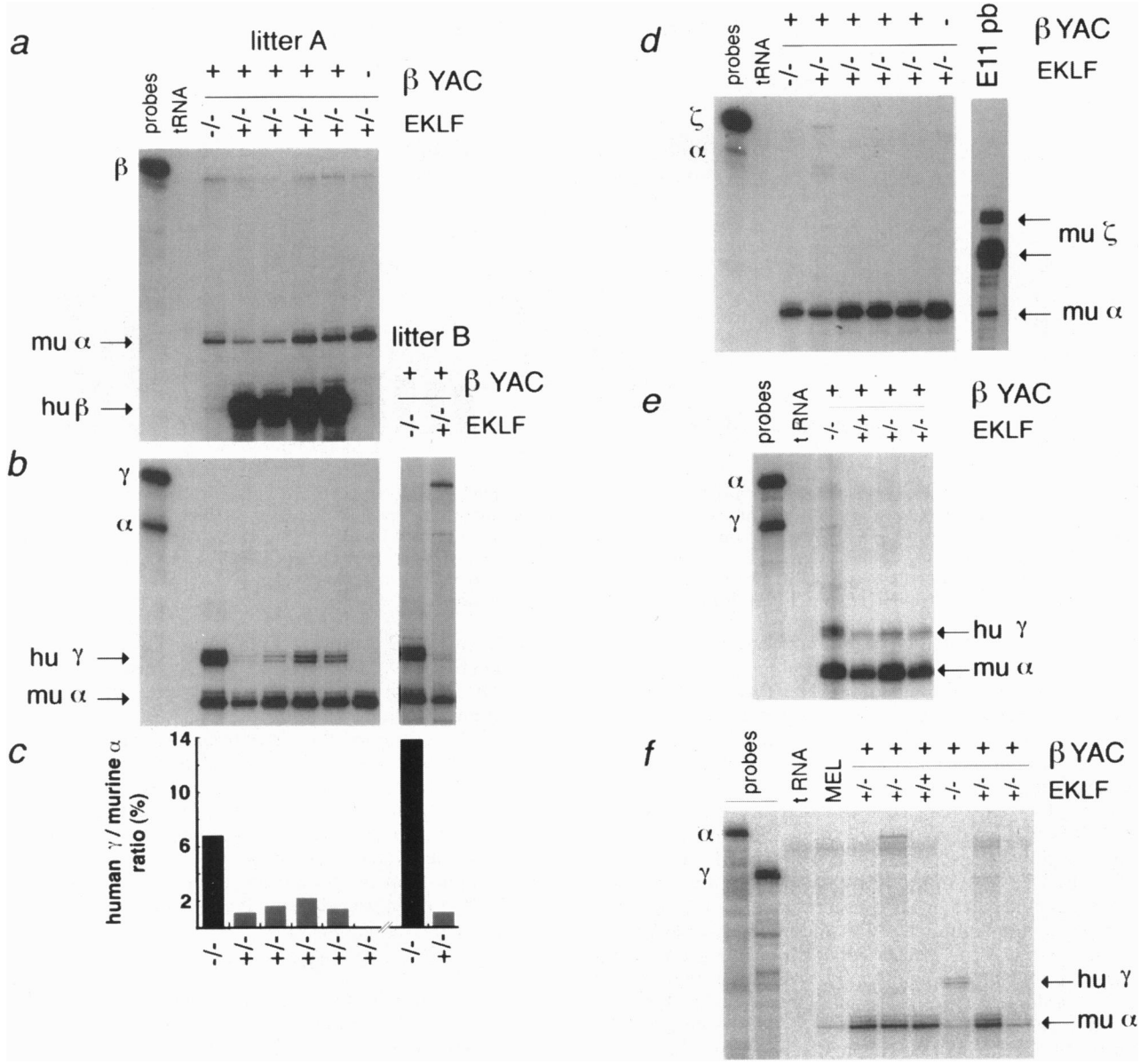

FIG. 1. Persistent γ-globin expression in EKLF$^{-/-}$ fetal liver erythroid cells. (*a–d*) RNase protection analyses for human β-globin relative to mouse α-globin (*a*), human γ-globin relative to mouse α-globin (*b* and *c*), and mouse ζ (embryonic) globin relative to mouse α-globin (*d*) transcripts in E15 fetal liver RNA samples. Undigested probes and tRNA controls were loaded in lanes 1 and 2. Total RNA (500 ng) was loaded in the remaining lanes. RNA from E11 embryonic red cells was loaded in the far right hand lane of *d* as a positive control for ζ-globin transcripts. The EKLF genotype and presence of the β-YAC transgene, as determined by Southern blot analysis, is indicated above each lane. (*c*) A graphic representation of the PhosphorImager-analyzed data from *b*. A correction to account for the 10-fold greater specific activity of the human globin riboprobes with respect to the mouse α-globin riboprobe has been incorporated into the calculated ratios of globin transcripts. (*e* and *f*) RNase protection assays for human γ-globin and mouse α-globin transcripts in erythroid cells derived from bulk culture of E13 fetal liver cells (*e*) or pools of 10–20 individually isolated BFU-e from E11 yolk sac-derived definitive progenitor cells (*f*).

mouse (but not the human) (4, 25). To exclude this possibility, we assayed mouse embryonic ζ-globin in the same samples. As shown in Fig. 1*d*, ζ-globin expression was not detected in fetal liver preparations at E15. To confirm that elevated γ-globin levels resulted from the absence of EKLF in definitive erythroid cells, we also expanded cells from definitive E13 fetal liver progenitors *in vitro* with kit ligand and erythropoietin. Embryonic cells, if present at this stage, fail to divide, and are rapidly lost upon culture. Human γ-globin transcripts were ≈5-fold more abundant in EKLF$^{-/-}$ versus EKLF$^{+/-}$ cell cultures, as compared with mouse α-globin (Fig. 1*e*). Thus, contamination with embryonic cells expressing the γ-globin gene fails to account for the increased γ/α ratio in EKLF$^{-/-}$, β-YAC^{+} erythroid cells.

Elevated γ-Globin Gene Expression Is Not Secondary to Anemic Stress. We also considered the possibility that anemic stress in EKLF$^{-/-}$ embryos might contribute to impaired γ-globin silencing, since acute erythropoietic stress in adult humans transiently reactivates low levels of γ-globin gene expression (reviewed in refs. 26 and 27). To address this possibility, adult erythroid colonies were expanded from the yolk sacs of E11 EKLF$^{-/-}$ mice and their littermates (28). At this stage, EKLF$^{-/-}$ embryos are not anemic and cannot be distinguished by inspection from EKLF$^{+/-}$ littermates (16). γ-Globin transcripts were examined from pools of 10–20 individual colonies. As shown in Fig. 1*f*, γ-globin transcripts were elevated only in the absence of EKLF (Fig. 1*f*, lane 8). Thus, increased γ-globin expression is a direct consequence of

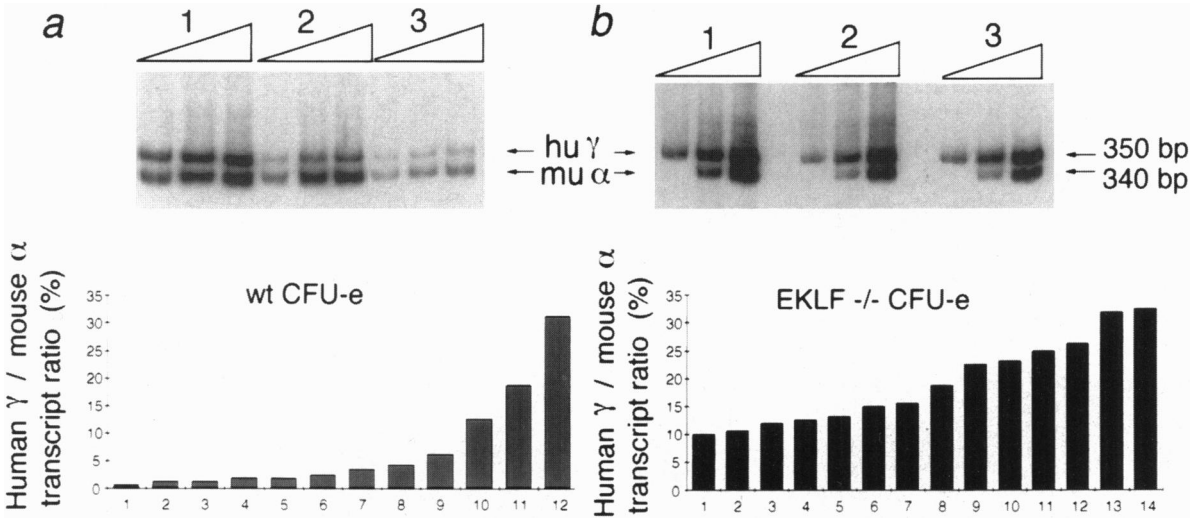

FIG. 2. Most EKLF$^{-/-}$ fetal liver-derived erythroid colonies are reprogrammed to γ-globin gene expression. RNA derived from individual CFU-E from fetal liver cells harvested at E13 was subjected to RT-PCR analysis with primers specific for murine α- and human γ-globin transcripts. PCR products in the linear range of amplification from three representative EKLF$^{+/-}$ (*a*) and EKLF$^{-/-}$ (*b*) CFU-E are shown. The expected sizes of the human γ- and mouse α-globin PCR products are indicated by arrows. The triangles represent increasing PCR cycles (two further cycles per lane). Below each autoradiograph is a graphical representation of the quantitated ratios of human γ- to mouse α-globin PCR products derived from analysis of 12 EKLF$^{+/-}$ and 14 EKLF$^{-/-}$ CFU-E. Results have been arranged in ascending order of γ-globin expression levels.

the absence of EKLF rather than a consequence of anemic stress.

EKLF$^{-/-}$ Erythroid Progenitor Cells Have an Increased Probability of Executing a γ-Globin Expression Program. To examine the role of EKLF in the establishment of the globin gene expression program at the time the γ-globin to β-globin gene switch is in progress, individual erythroid colonies derived from E13 fetal liver cells (CFU-E) were subjected to reverse RT-PCR analysis to quantitate the ratio of human γ/mouse α-globin transcripts (Fig. 2). In 14 of 15 EKLF$^{-/-}$ and 12 of 15 EKLF$^{+/-}$ colonies, RNA of adequate integrity was recovered as evidenced by the presence of detectable α-globin transcripts. Semiquantitative PCR simultaneously performed in the linear range for murine α- and human γ-globin transcripts (see *Materials and Methods*) revealed that all 14 EKLF$^{-/-}$ colonies expressed γ-globin at >10% of mouse α-globin levels (overall range, 10–35%). In contrast, the majority of EKLF$^{+/-}$ colonies expressed γ-globin at low levels, although 3 of 12 expressed γ-globin at a level overlapping that seen in EKLF$^{-/-}$ colonies. Therefore, in the absence of EKLF, a higher proportion of erythroid colonies at E13 exhibit a program of high γ-globin expression.

We also examined the distribution of γ-globin protein and mRNA at the individual cell and colony level. As shown by immunofluorescence (Fig. 3), a higher percentage of E14 fetal liver cells from EKLF$^{-/-}$ (≈30%) versus EKLF$^{+/-}$ (≈8%) embryos stained positively for γ-globin protein. Therefore, not only was the level of γ-globin expression increased in erythroid populations, but the proportion of erythroid cells expressing γ-globin protein was also greater in EKLF$^{-/-}$ embryos.

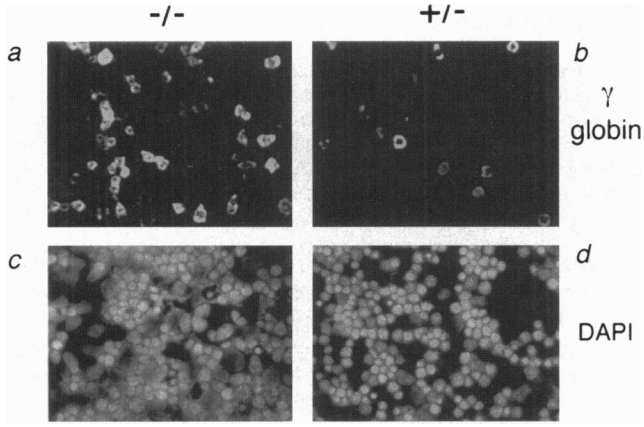

FIG. 3. Increased fetal (γ) globin protein in EKLF$^{-/-}$ erythroid cells. Immunofluorescence of E14 fetal liver (*a* and *b*) cytocentrifuge preparations after staining with a monoclonal antibody (9C3) raised against human HbF ($\alpha_2\gamma_2$). A high percentage of EKLF$^{-/-}$ erythroid cells (*a*) express detectable amounts of human γ-globin, whereas few EKLF$^{+/-}$ erythroid cells (*b*) do so. (*c* and *d*) Immunofluorescence of the same fields as shown in *a* and *b* after staining with 0.001% 4′6-diamidino-2-phenylindole (DAPI) HCl. (×600.)

DISCUSSION

In transgenic mice, the human β-globin gene can be transcribed at all developmental stages, but its expression in embryonic cells is silenced competitively by the upstream ε- and γ-genes (8, 9, 29). Although down-regulation of the γ-gene at the adult stage is initiated in the absence of a linked β-globin gene (6), the relative contribution of such autonomous control versus competition by a β-globin gene to the silencing of γ-globin gene is unresolved. Impaired silencing of the γ-globin gene in adult erythroid cells lacking EKLF provides strong evidence that competition by an active β-globin gene for a common regulatory component, such as the LCR, plays an important role in the γ- to β-globin switch. Although we consider it very unlikely, it is formally possible that EKLF could regulate an adult-stage repressor of γ-gene expression and thereby silence the γ-gene independent of competition by an active β-globin gene. The consistent increased γ-globin expression observed in patients heterozygous for β-promoter deletions (19) or CACC box mutations (18) argues in favor of a cis-acting mechanism for impaired γ-silencing. Since EKLF is present and active at both embryonic and adult stages (16, 30), we presume that absence of β-globin expression at the embryonic stage reflects more effective competition of the ε- and γ-globin genes for the LCR at early times by virtue of their proximity to the LCR and/or a regulatory milieu favoring their expression (8, 13, 25).

Several possibilities may account for nonrescue of EKLF$^{-/-}$ embryos by the β-YAC transgene. Excess α-globin chain toxicity may not be alleviated due either to insufficient human

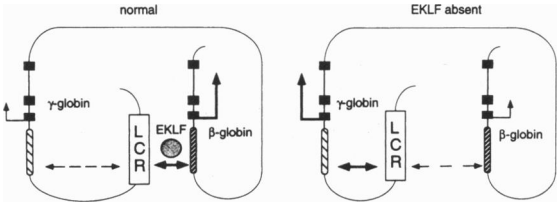

FIG. 4. Proposed mechanism by which EKLF facilitates γ-gene silencing. In late fetal and adult erythroid cells, EKLF (shaded circle) bound to the β-globin promoter (hatched box) mediates protein–protein interactions with the LCR. The affinity of this interaction is sufficiently strong as to be favored over association of the γ-globin promoter (thin striped box) with the LCR. In the absence of EKLF, the affinity of the LCR-β-promoter interaction is weak, resulting in enhanced probability of a productive LCR-γ-promoter interaction.

γ-globin chain accumulation or to inefficient assembly of human γ-globin and mouse α-globin into hemoglobin tetramers. In addition, other putative EKLF target genes may be missing in EKLF$^{-/-}$ embryos and contribute the hemolytic anemia. Since we have observed readily detectable hybrid (huγ_2–muα_2) hemoglobin (A.C.P. and K. Peterson, unpublished results) in EKLF$^{-/-}$ erythroid cells derived from breeding with a transgenic strain of mice expressing high levels of human Aγ-globin (31), we currently favor this latter possibility.

Heterogeneity in the level of γ-globin gene expression in individual erythroid colonies is consistent with stochastic models in which it is envisioned that the LCR may interact in an alternate fashion with downstream γ or β-globin genes in individual cells over time (32). Accordingly, the probability of γ- versus β-gene expression would depend on the specific affinities of the interactions between the γ- and β- globin promoters and the LCR. The respective affinities would reflect the milieu of nuclear regulatory proteins within the cell. Persistence of a program of γ-globin gene expression in the majority of EKLF$^{-/-}$ colonies suggests that EKLF may serve a critical role in mediating communication between the β-globin promoter and the LCR (Fig. 4). Most simply, communication may be achieved through physical interactions with other nuclear proteins, such as the erythroid factor GATA-1, bound to the LCR (33) or a non-DNA binding adapter molecule(s) that links EKLF bound to the β-promoter to LCR-bound protein(s). The indirect role by which EKLF facilitates γ-globin gene silencing may provide a general mechanism for developmental control within the β-globin locus. For example, transcription factors that interact specifically with the ε- and γ-globin genes may stabilize their interaction with the LCR and thereby serve to repress β-expression at early developmental stages. Accordingly, γ- to β-globin switching is likely to reflect the cumulative interactions of trans-acting factors with the LCR and regulatory sequences throughout the human β-globin locus, rather than the effect of a single stage-specific regulatory factor.

We thank Dr. T. Campbell of Isolabs for providing fluorescein isothiocyanate-conjugated monoclonal antibodies specific for human HbF, and members of the Orkin laboratory for their suggestions. A.C.P. is a Traveling Fellow of the Lucille P. Markey Foundation. S.H.O. is an Investigator of the Howard Hughes Medical Institute.

1. Hanscombe, O., Vial, M., Kaeda, J., Luzzatto, L., Greaves, D. R. & Grosveld, F. (1989) *Genes Dev.* **3,** 1572–1581.
2. Orkin, S. H. (1995) *Eur. J. Biochem.* **231,** 271–281.
3. Choi, O.-R. & Engel, J. D. (1988) *Cell* **55,** 17–26.
4. Chada, K., Magram, J. & Costantini, F. (1986) *Nature (London)* **319,** 685–689.
5. Townes, T. M., Lingrel, J. B., Chen, H. Y., Brinster, R. L. & Palmiter, R. D. (1985) *EMBO* **4,** 1715–1723.
6. Dillon, N. & Grosveld, F. (1991) *Nature (London)* **350,** 252–254.
7. Behringer, R. R., Ryan, T. M., Palmiter, R. D., Brinster, R. L. & Townes, T. M. (1990) *Genes Dev.* **4,** 380–389.
8. Enver, T., Raich, N., Ebens, A. J., Papayannopoulou, T., Costantini, F. & Stamatoyannopoulos, G. (1990) *Nature (London)* **344,** 309–313.
9. Lloyd, J. A., Krakowsky, J. M., Crable, S. C. & Lingrel, J. B. (1992) *Mol. Cell. Biol.* **12,** 1561–1567.
10. Gaensler, K. M. L., Kitamura, M. & Kan, Y. W. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 11381–11385.
11. Peterson, K. R., Clegg, C. H., Huxley, C., Josephson, B. M., Haugen, H. S., Furukawa, T. & Stamatoyannopoulos, G. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 7593–7597.
12. Jane, S. M., Ney, P. A., Vanin, E. F., Gumucio, D. L. & Nienhuis, A. W. (1992) *EMBO J.* **11,** 2961–2969.
13. Jane, S. M., Nienhuis, A. W. & Cunningham, J. M. (1995) *EMBO J.* **14,** 97–105.
14. Miller, I. J. & Bieker, J. J. (1993) *Mol. Cell. Biol.* **13,** 2776–2786.
15. Feng, W. C., Southwood, C. M. & Bieker, J. J. (1994) *J. Biol. Chem.* **269,** 1493–1500.
16. Perkins, A. C., Sharpe, A. H. & Orkin, S. H. (1995) *Nature (London)* **375,** 318–322.
17. Nuez, B., Michalovich, D., Bygrave, A., Ploemacher, R. & Gorsveld, F. (1995) *Nature (London)* **375,** 316–318.
18. Gonzalez-Redondo, J. M., Stoming, T. A., Lanclos, K. D., Gu, Y. C., Kutlar, A., Kutlar, F., Nakatsuji, T., Deng, B., Han, I. S., McKie, V. C. & Huisman, T. H. J. (1988) *Blood* **72,** 1007–1014.
19. Dimovski, A. J., Divorky, V., Adekile, A. D., Baysal, E., Wilson, J. B., Prior, J. F., Raven, J. L. & Huisman, T. H. J. (1994) *Blood* **83,** 822–827.
20. Ryan, T. M., Behringer, R. R., Martin, N. C., Townes, T. M., Palmiter, R. D. & Brinster, R. L. (1989) *Genes Dev.* **3,** 314–23.
21. Kieran, M. W., Perkins, A. C., Orkin, S. H. & Zon, L. I. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 9126–9131.
22. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
23. Baron, M. H. & Maniatis, T. (1986) *Cell* **46,** 591–602.
24. Weiss, M. J., Keller, G. & Orkin, S. H. (1994) *Genes Dev.* **8,** 1184–1197.
25. Strouboulis, J., Dillon, N. & Grosveld, F. (1992) *Genes Dev.* **6,** 1857–1864.
26. Stamatoyannopoulos, G., Nienhaus, A. W., Majerus, P. W. & Varmus, H. V. (1994) *The Molecular Basis of Blood Diseases* (Saunders, Philadelphia), 2nd Ed., p. 127–132.
27. Ley, T. J. (1991) *Blood* **77,** 1146–1152.
28. Wong, P. M., Clarke, B. J., Carr, D. H. & Chui, D. H. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2952–2956.
29. Hanscombe, O., Whyatt, D., Fraser, P., Yannoutsos, N., Greaves, D., Dillon, N. & Grosveld, F. (1991) *Genes Dev.* **5,** 1387–1394.
30. Southwood, C. M., Downs, K. M. & Bieker, J. J. (1996) *Dev. Dyn.* **206,** 248–259.
31. Stamatoyannopoulos, G., Josephson, B., Zhang, J.-W. & Li, Q. (1993) *Mol. Cell. Biol.* **13,** 7636–7644.
32. Wijgerde, M., Grosveld, F. & Fraser, P. (1995) *Nature (London)* **377,** 209–213.
33. Merika, M. & Orkin, S. H. (1995) *Mol. Cell. Biol.* **15,** 2437–2447.