Identification of trans-acting factors or drugs capable of reacti-
vating γ-globin gene expression is complicated by the lack of suita-
cble cell lines. Human K562 cells co-express ε- and γ-globin but not β-globin; transgenic mouse erythroleukemia 585 cells express pre-
dominantly human β-globin but also γ-globin; and transgenic murine GM979 cells co-express human γ-and β-globin. Human β-globin locus yeast artificial chromosome transgenic mice display correct developmental regulation of β-like globin gene expression. We rationalized that cells established from the adult bone marrow of these mice might express exclusively β-globin and therefore could be employed to select or screen inducers of γ-globin expres-
sion. A thrombopoietin receptor derivative that brings the prolifer-
avative status of primary mouse bone marrow cells under control of a 
chemical inducer of dimerization was employed to institute and 
maintain these cell populations. Human β-globin was expressed, but γ-globin was not; a similar expression pattern was observed in 
cells derived from fetal liver. γ-Globin expression was induced upon 
leukemia, in cells derived from −117 Greek hered-
tary persistence of fetal hemoglobin human β-globin locus yeast artificial chromosome (β-YAC), mice, showing that the hereditary persistence of fetal hemoglobin (HPFH) phenotype was maintained in 
these cells or was reactivated by an artificial zinc finger–γ-globin trans-
scription factor and the previously identified fetal globin trans-
activators fetal Krüppel-like factor (FKLF) and fetal globin-increas-
ing factor (FGIF). These cells may be useful for identifying tran-
scription factors that reactivate γ-globin synthesis or screening γ-globin inducers for the treatment of sickle cell disease or β-thalassemia.

Members of the human β-like globin family are developmentally 
regulated. The genes are arrayed in the order in which they are 
expressed during development, 5′-ε-γ-δ-β-3′. During primitive 
erthropoiesis, the embryonic ε-globin gene is expressed in nucleated, 
yolk sac-derived erythroid cells. Later, during fetal definitive erythro-
poiesis, the tandem fetal γ-globin genes are expressed in enucleated erythroid cells of the fetal liver. Finally, the β-globin gene, and to a much lesser extent, the δ-globin gene, are expressed initially in the liver during fetal definitive erythropoiesis and ultimately in bone marrow-derived erythroid cells during adult definitive erythropoiesis. The temporally 
regulated expression of the β-like globin genes provides a paradigm for 
developmental control in mammalian cells. Transgenic mice have been 
instrumental to the identification of many cis-acting elements and 
trans-acting factors necessary for normal developmental control. The 
individual globin genes, including their gene-proximal regulatory 
sequences, are variably expressed in transgenic mice. However, when 
linked to the locus control region (LCR),2 a powerful regulatory motif 
consisting of five DNase I-hypersensitive sites located upstream of the 
e-globin gene, high level, copy number-dependent, integration site-in-
dependent expression is achieved. Correct human hemoglobin switching 
can be largely mimicked in transgenic mice containing a human 
β-globin locus yeast artificial chromosome (β-YAC, Ref. 1). Although 
mice do not have a fetal stage of globin gene expression, the human 
γ-globin genes are expressed in the fetal liver, similar to humans. 

An improved understanding of globin gene regulation is clinically 
relevant since the beneficial effects of elevated fetal hemoglobin levels 
(HbF, αβγδ) in patients with sickle cell anemia and β-thalassemia are 
well documented (2). Considerable attention has been directed at iden-
tifying Krüppel-like factors (KLFs) that specifically transactivate γ-glo-
bin gene expression as a potential approach to gene therapy (3, 4). Addi-
tionally, many investigations have focused on identifying new 
pharmacological compounds that are capable of inducing γ-globin pro-
duction (5). However, the identification of γ-globin inducers, either 
proteins or drugs, has been hampered by the lack of suitable in vitro 
model systems for selection of activators or for screening chemical 
compounds.

A number of in vitro models for evaluating putative γ-globin inducers 
have been reported. Cultures of primary adult human erythroid progeni-
tors capitalize on the significant levels of γ-globin that are detected in 
these cells. Cultures of primary human burst-forming units-erythroid,

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* This work was supported by National Institutes of Health Grants DK61804, HL67336, and DK53510 and by a Lila & Madison Self Faculty Scholarship (to K. R. P.), National Institutes of Health Grant DK61803 (to C. F. B.), and National Institutes of Health Grants DK52997 and HS57350 (to C. A. B.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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2 The abbreviations used are: LCR, locus control region; β-YAC, human β-globin locus yeast artificial chromosome; KLF, Krüppel-like factor; FKLF, fetal KLF; FGIF, fetal glo-
bin-increasing factor; MEL, mouse erythroleukemia cells; BNC, bone marrow cells; CID, chemical inducer of dimerization; mpl, thrombopoietin receptor; BSA, bovine serum albumin; HPFH, hereditary persistence of fetal hemoglobin; 5-aza, 5-azacyti-
dine; RT, reverse transcriptase; PBS, phosphate-buffered saline. NF-E4, nuclear factor
ergythroid 4.
either in clonogenic assays (6) or in suspension (7), have been used to evaluate putative γ-globin inducers. However, these assays cannot be standardized; thus, they are difficult to use for large scale screening. Although greater levels of standardization can be achieved with established erythroid cell lines, none of the human erythroleukemia lines display a normal adult pattern of globin gene expression. K562 cells, commonly used for this purpose, predominantly express γ-globin, variable levels of ε-globin, and no β-globin. Therefore, an increase in γ-globin gene transcription in K562 cells may be a consequence of promoting globin gene expression generally rather than via a mechanism that preferentially activates γ-globin gene expression. Mouse erythroleukemia (MEL) cells display an adult pattern of globin gene expression; however, as noted above, mice lack a fetal globin gene. Initial attempts to develop MEL cells suitable for screening γ-globin inducers involved the generation of stably transfected cells incorporating various DNA fragments containing the human γ-globin gene, but transgenic cell lines generated in this manner failed to demonstrate proper regulation of the introduced γ-globin gene (8). Similarly, γ-globin gene expression in MEL cells containing a human β-globin locus YAC transgene was not correctly regulated (9, 10). In MEL 585 cells, β-globin gene expression predominates, although some γ-globin gene product is observed, whereas in GM1979 cells, γ-globin and β-globin are co-expressed. Finally, developmentally stage appropriate expression of human globin genes is achieved over time in hybrids generated from MEL cells fused to human fetal liver cells (HFE-MEL hybrids) or in MEL cells fused to human lymphoblasts (11). However, the degree of completion of switching is variable, and the cells eventually display the globin expression pattern of the terminal MEL cells used in the initial fusion.

None of the aforementioned cell systems completely mirror adult erythropoiesis; that is, γ-globin expression is markedly higher than normal adult human physiological levels, even in those cell lines in which β-globin is the major species synthesized. Thus, these models can only be used to screen for enhancement of already existent low level γ-globin gene expression rather than to select for activation of a silent γ-globin gene. We reasoned that immortalized cells derived from the bone marrow of β-YAC transgenic mice might express exclusively β-globin since the human pattern of globin gene synthesis was recapitated in these mice. β-YAC bone marrow cells (BMCs) were established using an artificial proliferation signal comprised of the thrombopoietin (mpl) signaling domain fused to FKBP binding domains responsive to a chemical inducer of dimerization (CID; Refs. 12–14). In the presence of a CID, homodimers are generated, and the resultant growth signal maintains the BMC population indefinitely as long as the CID is present. These cells exclusively expressed human β-globin, but γ-globin expression can be reactivated by various treatments or through the presence of a hereditary persistence of fetal hemoglobin (HPFH) mutation in the β-globin gene. Therefore, these CID-dependent, multipotential β-YAC BMCs provided a model system in which γ-globin protein transactivators can be selected and pharmacologic inducers of HbF can be definitively screened.

EXPERIMENTAL PROCEDURES

Transgenic Mice—Generation of β-YAC transgenic mice was described previously (1). Ppo-155 β-YAC transgenic mouse line 1, containing the 155-kb β-YAC (15), or −117 β-YAC transgenic mice, containing the 248-kb −117 γ−/− Greek HPFH β-YAC (16), were the sources of bone marrow or fetal liver used to establish CID-dependent cell populations.

Derivation of Drug-dependent, Multipotential Cells and Cell Culture—CID-dependent cells were derived as described previously (14, 17). Briefly, 5-fluorouracil (150 mg/kg) was injected intraperitoneally into 155-kb wild-type β-YAC transgenic mice. After 48 h, marrow cells were collected and cultured for 48 h in Dulbecco’s modified Eagle’s medium containing 16% fetal calf serum (HyClone; Logan, UT), 5% mouse interleukin-3 supplement (BD Biosciences), recombinant human interleukin-6 (100 ng/ml), and recombinant mouse stem cell factor (50 ng/ml; Chemicon, Temecula, CA) at 37 °C in 5% CO2. After prestimulation, cells were transferred onto irradiated (1,500 centigrays) GPE+86 producer cells containing an F36V-modified FKBP12 derivative fused to the intracellular portion of the thrombopoietin receptor mpl. Two F36Vmpl vectors were used, one containing a green fluorescent protein marker downstream of an internal ribosomal entry site (17) or a neomycin reporter expressed from a separate phosphoglycerate kinase promoter (18). Transductions were carried out using the same growth factors as for prestimulation with the addition of Polybrene (8 μg/ml; Sigma). After 48 h, cells were washed and cultured in the presence of AP20187 dimerizer (100 nmol/liter; Ariad Pharmaceuticals, Cambridge, MA) in Iscove’s modified Dulbecco’s medium containing 10% fetal calf serum, penicillin, and streptomycin. CID-dependent BMCs were similarly established from 248-kb −117 Greek HPFH β-YAC transgenic mice. This approach was also used to establish drug-dependent cells from transgenic murine fetal liver (day 12 post-conception). Livers were dissected from day 12 fetuses, and single cell liver suspensions were prepared as described (18).

Stable Transfection of CID-dependent Wild-type β-YAC BMCs with pcDNA3.1/Hygro gg1-VP64—A 0.8-kb Apal-HindIII (New England Bio- labs, Beverly, MA) fragment was isolated from pcDNA-gg1-VP64-HA (19) and ligated into Apal-HindIII-cut and phosphatased (calf intestinal alkaline phosphatase, Promega, Madison, WI) pcDNA3.1/Hygro (+) (Invitrogen) to produce pcDNA3.1/Hygro gg1-VP64 so that transfected cells could be selected for hygromycin resistance. CID-dependent cells derived from β-YAC mice (3.5 × 106) were washed in PBS and resuspended in 0.8 ml of Dulbecco’s modified Eagle’s medium. During the transfection, both cells and the DNA/Lipofectamine (Invitrogen) mix were maintained in Dulbecco’s modified Eagle’s medium. The DNA/Lipofectamine mix was prepared according to the manufacturer’s instructions using 4 μg of plasmid DNA in 10 μl of Lipofectamine; this mix was incubated for 45 min. Cells were added to the DNA/Lipofectamine, and the transfection mixture was incubated for 6 h at 37 °C in 5% CO2 before adding 3 ml of Iscove’s modified Dulbecco’s medium containing 10% heat-inactivated fetal bovine serum, 2 mM l-glutamine, 1 mM sodium pyruvate, 1× non-essential amino acids, and 10 mM HEPES supplemented with CID and 0.1 ml of extra fetal bovine serum. After overnight incubation, cells were centrifuged (200 × g, room temperature) and resuspended in Iscove’s modified Dulbecco’s medium supplemented with CID and 200 μg/ml hygromycin. After selection, clones were generated by limiting dilution and were screened by Southern blot hybridization analysis (data not shown).

Enforced Expression of Potential Fetal Globin Transactivators in CID-dependent Wild-type β-YAC BMCs—Enforced erythroid-specific expression of cDNAs may be obtained by cloning them into the unique BglII restriction enzyme site of a derivative of our expression vector, pμ’LCR-β pr-BglII-B int2-ehn (20, 21). Full-length cDNAs of human NF-E4 (550 bp; Ref. 22), FKLF (H KLF11, 1.6 kb; Ref. 3), or FGIF (750 bp; Ref. 23) were generated by RT-PCR using the reaction conditions described below from total RNA isolated from K562 cells with forward and reverse primers containing a BglII site to allow ligation of the cDNA into the vector. Primer sequences were: NF-E4, 5’-GATACATAAATAA-
Characterization of AP20187-dependent Cells—Bone marrow- and fetal liver-derived cells were expanded and harvested at various times during culture for analysis of murine and human globin gene expression by RNase protection (24), RT-PCR, or antibody staining (21) to detect globin chains. For antibody staining, cells were washed with PBS in 15-ml conical tubes and then fixed in 1 ml of freshly prepared 5% paraformaldehyde/PBS, pH 7.2, for 1 h at 37 °C. After the addition of 10 ml of PBS/0.1% BSA (PBS/BSA), cells were centrifuged at 200 × g for 5 min, resuspended in 0.5 ml of methanol, and incubated at room temperature for 5 min. 10 ml of PBS/BSA were added; cells were centrifuged and washed once more in PBS/BSA. Cells were resuspended in 100 μl of PBS/BSA containing 0.1% Triton X-100 (PBT) and incubated for 30 min at room temperature with 1 μg of primary mouse anti-human γ-globin chain antibody (Cortex catalog number CR8115M1, San Leandro, CA) diluted in PBT. Cells were washed in the same manner as for the primary antibody. This wash was repeated twice more. Cells were resuspended in 100 μl of PBT and incubated with 100 μl of secondary fluorescein isothiocyanate-labeled goat anti-mouse IgG antibody (Jackson ImmunoResearch Laboratories, catalog number 115-095-146, West Grove, PA) diluted 1:750 in PBT at room temperature for 30 min. Cells were washed in the same manner as for the primary antibody. Pellets were resuspended in 50 μl of PBS; 5-μl aliquots were applied to slides. Control samples were prepared similarly except that the fix only control

FIGURE 1. A, human β-globin transcripts are detected in CID-dependent cells derived from 135-kb wild-type β-YAC transgenic mice, and CID-dependent cells derived from 248-kb 117 Greek HPFH β-YAC transgenic mice exhibit HPFH. The autoradiograph shows results of RNase protection analysis. Sample sources are illustrated above the panel; numbers indicate samples collected from more than one cell population or animal. Protected fragments are shown to the right of the panel; pBR322 MspI molecular mass markers (MW markers) are shown to the left. γ(γ + β) quantitative values are the averages of two separately established cell populations; if more than two populations were analyzed, standard deviations are also shown. Hu β, human β-globin (205 bp); Hu γ, human γ-globin (170 bp); Mo α, mouse α-globin (128 bp); wt β-YAC, wild-type β-YAC transgenic mice; FL, fetal liver; BI, blood. B, fluorescent antibody staining of human γ-globin protein chains in CID-dependent wild-type or 117 HPFH β-YAC BMCs. γ-Globin chains are detected in 117 HPFH β-YAC CID-dependent BMCs but not in wild-type β-YAC BMCs. Upper, non-transgenic BMCs. Middle, wild-type β-YAC BMCs. Lower, 117 HPFH β-YAC BMCs. Background staining in the upper and middle panels is due to fixation and nonspecific staining with the secondary antibody. After background correction, 30% of the cells in the lower panel are positive for γ-globin chain staining.
had no antibody added, and the secondary antibody control had no primary antibody added. Results were observed with a Nikon E800 microscope at 200 magnification using an EF-4 fluorescein isothiocyanate Hq filter set. Results were documented using a Photometrics Cool Snap ES camera and MetaMorph software. Software was set to automatically correct for nonspecific background using an image of the green filter set. Results were observed with a Nikon E800 microscope at 200 magnification using an EF-4 fluorescein isothiocyanate Hq filter set. Results were documented using a Photometrics Cool Snap ES camera and MetaMorph software. Software was set to automatically correct for nonspecific background using an image of the green filter set.

Analysis of Globin and Transcription Factor mRNA Levels Using RT-PCR—Total cDNAs were synthesized from RNA isolated from β-YAC bone marrow or fetal liver cells using an oligo(dT) primer (Promega, Madison, WI) and Superscript II reverse transcriptase (Invitrogen). 1 μg of total RNA was combined with 0.5 μg of oligo(dT) in a total volume of 11 μl and preheated at 70 °C for 10 min. The reaction mixture was then cooled rapidly on ice followed by the addition of 4 μl of 5× first strand buffer, 2 μl of 0.1 M dithiothreitol, 1 μl of 10 mM dNTP mix, and 1 μl of RNasin. The reaction mixture was heated to 42 °C for 2 min, at which point 1 μl of Superscript II RT was added, and the reaction was incubated at 42 °C for 50 min. The RT enzyme was heat-inactivated by incubation at 70 °C.

For globin gene products, PCR was performed using the following three sets of primers in a single reaction: mouse α-globin, 5′-GATTCT-GACAGACTCAGGAAGAAAC-3′ (forward), 5′-CCTTTCAGG-GCTTCAGCTCCATAT-3′ (reverse); human γ-globin, 5′-GAC-CGTTTTGGAATCCATTTTC-3′ (forward), 5′-TATTTGCTTGCA-GAATAAGCC-3′ (reverse); and human β-globin, 5′-ACACAAGCT-GTTTCAGCTAGCACACTCAACCTC-3′ (forward), 5′-GGTTGCCCATAAAC-AGCATCAGGAGT-3′ (reverse). Each reaction contained 5 μl of 10× NH4 buffer, 2 μl of 50 mM MgCl2, 0.5 μl of 25 mM dNTP mix, 25 pmol of forward primers, 25 pmol of reverse primers, 2 μl of cDNA, and 1 unit of Bioline Taq polymerase (Bioline, Randolph, MA). PCR was carried out with initial denaturation at 95 °C for 7 min, 25 cycles of 1 min steps at 95, 58, and 72 °C followed by a final extension at 72 °C for 10 min. All globin primers were designed to cross an exon so that RNA-templated reverse-transcribed DNA amplification products could be distinguished from gene amplification products.

Similar reaction conditions were used for FGIF and NF-E4 mRNAs; primer sets were the same as used to generate the full-length cDNA clones described above. RT-PCR for gg1-VP64 mRNA was performed as described, except that the first PCR was omitted (19). All of the PCR reactions to detect expression were semiquantitative.

RESULTS AND DISCUSSION

We have previously described a system that allows multipotential cell lines to be established from murine bone marrow. This system uses a
recombinant retroviral vector to introduce a gene encoding a conditional signaling molecule into mouse BMCs followed by activation of the signaling molecule using a small molecule drug called a chemical inducer of dimerization (25). A CID-activated derivative of the thrombopoietin receptor, mpl, induces transduced mouse BMCs to expand dramatically in culture. Cells generated in this manner adopt predominantly megakaryocytic features but also include multipotent progenitors capable of generating monocytes, neutrophils, and erythroid cells, but not B or T lymphoid cells, upon the addition of the appropriate growth factors (17, 26). Cultures can be maintained for longer than a year, and cell growth remains strictly dependent upon the continued presence of the CID. Our initial studies demonstrated that cells generated in this manner expressed adult mouse α-, βmin-, and βmin-globin mRNAs that could be readily detected by RNase protection assays (data not shown).

To test whether the human β-globin locus would be appropriately regulated in cells derived from the bone marrow of adult mice, we generated F36Vmpl-transduced cells from transgenic mice containing a 155-kb mpl-transduced cells from transgenic mice containing a F36Vmpl-transduced cells from transgenic mice containing a regulated F36Vmpl expression cassette. These cells were confirmed by fluorescent anti-α-globin antibody staining (Fig. 1A). The higher level of human β-globin relative to mouse α-globin was attributed to a significant reduction in mouse α-globin levels in the cells when compared with the β-YAC mice. A parallel decrease in mouse β-globin mRNA was also observed (data not shown). These findings also indicated a lack of environmental influence on the pattern of β-like globin gene expression, suggesting that the mechanisms regulating β-globin expression are cell-autonomous.

To confirm that the pattern of globin gene expression observed in β-YAC mice is maintained in derivative cell lines, we performed two additional studies. First, we examined the pattern of globin gene expression in cells derived from transgenic mice containing a 248-kb β-YAC. In humans, a point mutation (G to A transition) at position −117 relative to the mRNA start site of the β-globin gene causes the Greek form of hereditary persistence of fetal hemoglobin (2). Previous studies showed that −117 A→G β globin transgenic mice maintained human γ-globin expression into adult life (16). Similarly, three independent cell populations derived from the marrow of these mice expressed significant levels of γ-globin in addition to β-globin (Fig. 1A). The presence of human γ-globin chains in these cells was confirmed by fluorescent anti-γ-globin antibody staining (Fig. 1B). Although γ-globin chains were detected, no hemoglobin was formed in these multipotent cells as assessed by benzidine staining.

As a second test of whether the pattern of β-like globin gene expression in β-YAC mice was reflected in the derived cells, we generated cell populations from the fetal livers of post-gestation day 12 wild-type β-YAC mice, which expressed significant levels of γ-globin. In three independently derived cell populations, human β-globin mRNA was detected by RT-PCR whereas γ-globin mRNA was not expressed (data not shown). These results were consistent with either a switch from γ- to β-globin gene expression prior to or during establishment of cell culture, or alternatively, that fetal liver cells capable of extensive growth in the presence of F36Vmpl signaling are committed to the generation of erythroid progeny that express solely β-globin.

Reactivating γ-globin gene expression may be beneficial for patients with sickle cell anemia. BMCs derived from β-YAC mice may be useful for screening putative γ-globin inducers. Since 5-azacytidine (5-aza), an inhibitor of DNA methylation, is a strong inducer of γ-globin gene expression (27, 28), we tested whether 5-aza could activate γ-globin transcription in wild-type β-YAC BMCs. As shown in Fig. 2A, 5-aza induced significant levels of γ-globin transcription, thereby establishing that γ-globin gene expression can be induced in these cells. However, γ-globin expression was not induced by a number of other compounds, including α-aminobutyric acid, sodium butyrate, valproic acid, or trichostatin A (data not shown).

Next we tested an artificial transcription factor for its ability to induce γ-globin gene expression in wild-type β-YAC BMCs. We employed a synthetic construct, gg1-VP64, in which a zinc finger DNA binding domain, designed to target the region proximal to the −117 position of the γ-globin promoter, was linked to the VP64 transcriptional activator domain (19). This transcription factor has been shown to interact directly with the γ-globin promoter and to up-regulate γ-globin gene expression in K562 cells (19). A pool and five independent clones of β-YAC cells stably expressing gg1-VP64 displayed detectable levels of γ-globin mRNA by RT-PCR (Fig. 2B). The weak induction of γ-globin observed may be due to the relatively low expression of gg1-VP64 from the CMV promoter, which is inefficient in these cells.

Finally, the ability of previously identified fetal globin transcription factors to activate γ-globin gene expression was assessed. Full-length cDNAs for each factor were placed under control of a human β-globin locus LCR/β-globin gene promoter cassette in a construct previously shown to confer erythroid-specific gene expression upon linked cDNAs (20, 21). FGIF, FKLF, and gg1-VP64 under control of LCR β-globin

FIGURE 3. A, fetal globin transcriptional activators induce γ-globin gene expression in CID-dependent wild-type β-YAC (wt β-YAC) BMC pools. Linearized puLCR/β-pr-cDNA-β int2-enh constructs were lipofected into cells. Sample names and full-length cDNAs tested are shown above the image. Expression of globin mRNAs in the pools was assayed by RT-PCR. Labeling is as for Fig. 2B. Hu β, 212 bp; Hu γ, 165 bp; Mo α, 122 bp; gg1-VP64, 300 bp. β expression of transcription factors was assessed by RT-PCR. Sample names are shown above the image. (−), non-transfected CID-dependent wild-type β-YAC BMCs; (+), CID-dependent wild-type β-YAC BMCs transfected with indicated cDNA clone. Molecular mass markers are shown on the left side of the image, and sizes of RT-PCR fragments are displayed on the right side. FGIF, 750 bp; NF-E4, 600 bp; gg1-VP64, 300 bp.
promoter sequences reactivated γ-globin gene transcription in the wild-type CID-dependent β-YAC BMCs (Fig. 3A), whereas NF-E4 did not. Expression of all the transactivator cDNAs was detected at the RNA level in the BMCs, except for FKLF, which was not tested (Fig. 3B). The inability of NF-E4 to induce γ-globin gene expression in these cells was consistent with previous data demonstrating that it does not reactivate fetal globin synthesis in adult transgenic mice (29).

CID-dependent β-YAC cells may be useful primarily for identifying transcription factors that reactivate γ-globin gene expression, and to a lesser extent, for screening some chemical inducers of γ-globin expression for the treatment of sickle cell disease or β-thalassemia. γ-Globin transcription went from a completely repressed state to detectable mRNA levels in all instances in which expression was observed. This qualitative γ-globin “off-on” switch is unique to these cells; in other established cell lines, only a change in constitutive expression may be measured. Although γ-globin was activated by 5-azacytidine (which affects methylation) or by an HPFH mutation, treatments that affect acetylation (butyric acid, etc.) did not induce γ-globin synthesis, suggesting that these cells do not completely mirror the erythroid phenotype. Alternately, regulation of human γ-globin in mice may be fundamentally different than it is in humans, and these cell populations reveal that difference. However, gg1-VP64, FKLF, and FGIF activation, coupled with the HPFH results, clearly demonstrated that these cells offer an ideal system to clone and characterize transcriptional activators that act directly upon the γ-globin gene promoters.

Acknowledgment—We thank Lesya Zelenchuk for animal husbandry related to the β-YAC transgenic animals utilized in this study.

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