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Regulated Expression of Human α- and β-Globin Genes in Transient Heterokaryons

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We have examined the expression of human α- and β-like globin genes in transient heterokaryons formed by fusion of human nonerythroid cells with terminally differentiating mouse erythroleukemia (MEL) cells or with a MEL cell variant (GM979) in which the endogenous mouse embryonic β-globin genes are activated. In both the parental MEL cells and the heterokaryons, the α-globin genes were activated at least 12 h earlier than the embryonic, fetal, and adult β-globin genes. These results suggest that kinetic differences in the activation of α- and β-like globin genes are not simply the result of different rates of accumulation of erythroid-specific regulatory factors but may reflect differences in the mechanisms governing the transcriptional activation of these genes during erythroid cell differentiation. In mouse GM979 × human nonerythroid heterokaryons, the human embryonic β-globin gene was activated, consistent with our previous demonstration that erythroid cells contain stage-specific *trans*-acting regulators of globin gene expression. Moreover, a dramatic increase in the ratio of human fetal to adult β-globin transcription was observed compared with that seen in MEL-human nonerythroid hybrids. This ratio change may reflect competition between the fetal and adult β-globin genes for productive interactions with erythroid cell-specific regulatory elements. Finally, we demonstrate that the behavior of naturally occurring mutations that lead to aberrant hemoglobin switching in humans also leads to aberrant expression in transient heterokaryons. Therefore, erythroid cells must contain *trans*-acting factors that interact with mutated regulatory elements to induce high-level expression of the human fetal globin genes.

Distinct members of the human α- and β-globin gene families are expressed at different times during development. This phenomenon is known as hemoglobin switching and occurs at two stages in humans: the embryonic-to-fetal switch occurs very early in gestation and involves a change in expression of both the α- and β-globin clusters, while the fetal-to-adult switch involves only the β-cluster and occurs around the time of birth (for a review, see reference 33). We have previously shown that globin genes in nonerythroid cells can be activated in a tissue- and stage-specific manner in transient heterokaryons in which the nuclei do not fuse (2). Although the activation of adult globin genes was reported in some somatic cell hybridization studies (reviewed in reference 2), de novo activation of fetal or embryonic globin gene expression could not be detected. Because the nuclei in transient heterokaryons (short-term hybrids) do not fuse, activation of globin gene expression must occur in *trans*. This approach therefore provides a direct assay for tissue- and stage-specific *trans*-acting regulators. Our earlier heterokaryon studies demonstrated that globin genes are not irreversibly repressed in nonerythroid cells and that erythroid cell-specific and developmental stage-specific expression is mediated by tissue- and stage-specific *trans*-acting factors (2). However, they did not address the question of coordinate regulation of different members of the globin gene families or whether the observed *trans* activation operated through *cis*-acting regulatory elements known to be important in vivo.

Although the mammalian α- and β-globin genes are coordinately expressed in fully differentiated erythroid cells, a number of striking differences have been observed in their structures (reviewed in references 8, 19, 20, and 33), patterns of replication (12, 22), and transcriptional behavior in terminally differentiating mouse erythroleukemia (MEL) cells (28, 30), transient expression systems and stably transformed cell lines (e.g., see references 1, 7, 8, 15, 38, 40, and 41), and transgenic mice (e.g., see references 3, 18, 25, 26, 37, and 39). In this report, we show that human β-like globins representing all three major developmental stages (embryonic, fetal, and adult) are activated later than the human α-globin genes when terminally differentiating adult or embryonic mouse erythroid cells are fused with human nonerythroid cells to form transient heterokaryons. The endogenous mouse α-globin genes are also activated earlier than the embryonic and adult β-globin genes in the parental mouse erythroid cell lines during chemically induced terminal differentiation. We conclude that the differences in kinetics of activation of α- and β-like globin genes cannot be explained simply by differences in the rates of accumulation of transcriptional regulators. Our observations are consistent with suggestions from earlier studies that although the α- and β-globin genes are expressed exclusively in erythroid cells, the mechanisms by which these gene families are regulated are not identical.

We examined the activation of human embryonic, fetal, and adult β-like globin genes following fusion of human nonerythroid cells with MEL cells expressing adult and/or embryonic mouse globins. The human embryonic β-globin gene was activated when human nonerythroid cells were fused with a MEL cell variant (GM979) that expresses mouse β-like globins of all developmental stages. The embryonic human β-globin gene was not activated in adult MEL × human nonerythroid heterokaryons, consistent with our previous demonstration that erythroid cells contain stage-specific *trans*-acting regulators of globin gene expres-

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sion. The human fetal and adult β-like globin genes were also activated in GM979 × human nonerythroid heterokaryons. However, much lower levels of human adult β-globin, compared with human fetal β-globin, were observed than would be expected on the basis of the pattern of expression of the mouse globin genes in the parental GM979 cells. Nearly equal amounts of fetal and adult β-globin RNAs were detected in adult MEL × human HeLa heterokaryons. These differences may reflect competition among the various globin gene promoters for the formation of stable complexes with far-upstream regulatory sequences (4, 11).

We have also examined the expression of naturally occurring *cis*-acting (hereditary persistence of fetal hemoglobin [HPFH]) human mutations which interfere with the normal fetal-to-adult hemoglobin switching mechanism (33). Mutated (HPFH) human fetal globin genes are activated in stable somatic cell hybrids (29). In this report, we show that in transient heterokaryons formed by fusion of MEL cells with cells from several different HPFH cell types, the mutated (HPFH) fetal genes are rapidly activated. These experiments provide direct evidence that erythroid cells must contain *trans*-acting factors which interact with the mutated regulatory elements to induce high-level expression of the fetal globin genes.

MATERIALS AND METHODS

Cell culture and induction. Growth and induction of the 585S subline of MEL cells with dimethyl sulfoxide (DMSO) were done as previously described (2). In an alternative induction protocol, MEL cells were treated with 5 mM *N,N'*-hexamethylene bisacetamide (HMBA). GM979 cells, a MEL cell subline that expresses both adult α- and β- as well as embryonic β-like globins following induction with butyric acid (1 mM) or 2% DMSO (1a, 5), were a kind gift from Marshall Edgell and were maintained in Dulbecco modified Eagles medium (DME) supplemented with 10% fetal calf serum (FCS). HeLa cells were grown in DME-10% FCS. The Epstein-Barr virus-immortalized normal and HPFH human lymphocyte cell lines were generously provided by Thalia Papayannopoulou. The normal skin fibroblasts CCD-187 Sk and CCD-82 Sk were obtained from the American Type Culture Collection (ATCC CRL 1564 and CRL 1527, respectively). CRL 1564 was maintained in DME-10% FCS; CRL 1527 was maintained in minimal essential medium (Eagle) containing nonessential amino acids and 10% FCS. The HPFH fibroblasts (GM01368) were obtained from the NIGMS Human Genetic Mutant Cell Repository (Camden, N.J.) and were maintained in DME supplemented with nonessential amino acids and vitamins and 20% FCS.

Preparation of heterokaryons. Fusion of cells by treatment with polyethylene glycol (PEG) was done as previously described (2). Heterokaryons were maintained in culture for 24 to 48 h following fusion and were then harvested for preparation of RNA.

Preparation of total cellular RNA and quantitative RNase mapping. Preparation and analysis of total cellular RNA from heterokaryons were carried out as detailed previously (2). Probe prepared from pSP6γactin (10) was included as an internal control in some of the RNase mapping experiments. This human probe cross-hybridizes with mouse actin RNA (2, 10). Plasmid SP6βh1 · 3'ΔNA (a gift from Jeanne Magram and Frank Costantini) was linearized by digestion with *Bam*HI; transcription by SP6 polymerase yielded a probe of 324 nucleotides, and mapping of mouse yolk sac or GM979 cell RNAs or RNA from MEL × K562 cell heterokaryons (2) resulted in protection of a fragment of approximately 190 nucleotides. Mouse yolk sac RNAs were generously provided by Jeanne Magram, Marie Trudel, and Frank Costantini, and human cord blood RNA was a gift from Stuart Orkin.

Densitometry of autoradiograms. XAR-5 film was preflashed and exposed at −70°C. Densitometry was performed on the preflashed autoradiograms, using an ISCO UA-5 absorbance/fluorescence monitor and gel scanner, model 1312.

RESULTS

Differences in the kinetics of activation of α- and β-globin genes in MEL cells and in transient heterokaryons. Solution hybridization of globin cDNA with MEL cell mRNA had previously revealed that expression of the endogenous α-globin gene in terminally differentiating MEL cells is induced 12 to 16 h earlier than the endogenous β-globin gene (28). This difference in timing of activation of the two genes can be explained, at least in part, by the more rapid transcription rate of the α-globin gene (30). Using a transient heterokaryon expression assay, we have demonstrated that erythroid- and stage-specific *trans*-acting factors are involved in the regulation of globin genes (2). To investigate the possibility that the activation of human α- and β-globin genes in MEL × human nonerythroid cell heterokaryons is also asynchronous, we first tested the properties of the endogenous genes in our MEL cell subline (2). MEL cells were induced to differentiate by treatment with either DMSO or HMBA, and RNA was purified at various times following addition of the chemical inducing agent. Total cellular RNA was analyzed by quantitative RNase mapping. In each of three experiments performed with both inducing agents, the mouse α-globin gene was activated earlier than the β-globin gene. The time course of activation of these genes is represented graphically in Fig. 1 for a DMSO induction experiment. No significant increase in the basal level of β-globin mRNA was observed until 39 to 48 h postinduction, whereas transcription of the α-globin gene began to increase within 30 to 36 hours. The levels of α- and β-globin mRNAs began to plateau to approximately equal levels by about 84 h postinduction (Fig. 1 and data not shown). The two adult β-globin genes βmaj and βmin were activated with identical kinetics, and their transcripts accumulated to approximately the same levels (data not shown).

To determine whether α- and β-globin genes in the human nuclei of DMSO-induced MEL × human nonerythroid heterokaryons behave like the endogenous genes in mouse erythroid cells, RNA was isolated at various times following PEG-mediated cell fusion. The analysis of MEL × HeLa heterokaryons is shown in Fig. 2A. The human α-globin gene was activated within 12 h, while β-globin mRNA was not detectable until 48 h following fusion (in other experiments, β-globin transcripts were observed within 24 to 36 h, but activation of the β-globin gene always occurred at least 12 h later than the α-globin gene). Similar results were obtained when an Epstein-Barr virus-immortalized B-cell line was fused with MEL cells (not shown). These kinetic differences are even more striking than is apparent from a simple visual inspection of Fig. 2A, because the specific activity (and therefore the sensitivity) of the β-globin probe is greater than that of the α-globin probe (see legend to Fig. 2). Thus, in the presence of high levels of *trans*-acting molecules from terminally differentiating mouse adult erythroid cells, the be-

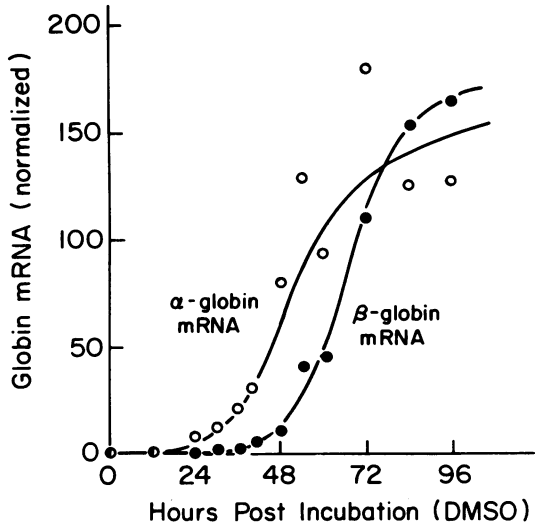

FIG. 1. Activation of α-globin gene expression precedes that of β-globin in terminally differentiating MEL cells. MEL cells were induced by treatment with 2% DMSO. Three micrograms of total cellular RNA prepared from cells at 0, 12, 24, 30, 36, 39, 48, 54, 60, 72, 84, and 96 h after induction was analyzed by quantitative RNase mapping with either the mouse α- or mouse βmaj-globin probe (2). The latter probe generates fragments of different sizes for βmaj (134 nucleotides) and βmin (about 116 nucleotides). The human γ-actin probe, which cross-hybridizes with mouse actin RNA, was included in all sets of hybridizations to control for RNA recovery (the level of actin RNA does not change with the state of differentiation of the MEL cells). The RNA fragments protected by the two globin probes (128 and 134 nucleotides, respectively, for α- and βmaj-globin) were not sufficiently well resolved to permit their simultaneous analysis. Therefore, the levels of α- and (βmaj + βmin)-globin message accumulation were normalized to the internal actin control. In MEL cells induced with 5 mM HMBA, α-globin induction again preceded that of β-globin, but both curves were shifted to the right by 6 to 12 h. All subsequent experiments were performed with DMSO-treated MEL cells.

havior of previously silent human adult globin genes is indistinguishable from that of the mouse genes.

Somewhat surprisingly, the human fetal β-like globin gene (γ) is activated in the nuclei of MEL × HeLa cell heterokaryons (2). With the exception of cells from individuals afflicted with HPFH (see below), we have not observed activation of this gene in the nuclei of any primary or established human cell type tested (including skin, lung, hepatoma, myoblast, and normal lymphocyte cells) following fusion with MEL cells. The activation of the fetal globin gene in MEL × HeLa heterokaryons is therefore likely to reflect a property of the transformed HeLa cell. This anomaly allowed us to examine the activation kinetics of two human β-like globin genes in transient heterokaryons. RNA samples prepared from MEL × HeLa heterokaryons harvested at various times following treatment with PEG were analyzed by using human fetal and adult RNA probes (Fig. 2B). Both genes were activated in synchrony, and this activation occurred later than that of the human α-globin gene.

The MEL cell subline GM979 expresses embryonic (ε and βh1) and adult β-like globins (5; Fig. 3), as well as the fetal/adult α-globin gene (not shown), when induced to differentiate by treatment with DMSO or butyric acid. Expression of the mouse embryonic α-like globin gene ζ is not detectable in these cells (not shown). The kinetics of activation of the β-like globin genes and the adult α-globin gene were examined by RNase mapping of GM979 cell RNA prepared at various times following addition of DMSO or butyric acid to the growth medium. All of the β-like globin genes (ε, βh1, and βmaj/βmin) were activated later than the α-globin gene (not shown). When terminally differentiating GM979 cells were fused with human HeLa or normal lymphocyte cells, activation of human α-globin expression again preceded that of the human β-like globin genes (not shown).

In summary, the activation of the α- and β-globin genes in two different MEL cell sublines (MEL 585S and GM979), and in heterokaryons prepared by fusion of these cells with human nonerythroid cells, is asynchronous: the β-like globin genes are turned on later than the α-globin genes during terminal erythroid differentiation.

Activation of human embryonic and fetal β-like globin genes in transient heterokaryons. The mouse embryonic β-like globin gene ε (see discussion of notation in reference 2) is activated in heterokaryons formed by fusion of human embryonic/fetal erythroid cells (K562) with mouse erythroid or nonerythroid cells (2). A second mouse embryonic β-globin gene, βh1 (23), is also activated in these heterokaryons (not shown). To determine whether expression of the human embryonic β-globin gene (ε) could also be reprogrammed in the presence of the appropriate stage-specific regulatory factors, we analyzed RNA from DMSO-induced GM979 × human nonerythroid cell heterokaryons. Consistent with our previous demonstration that erythroid cells contain developmental stage-specific regulators of globin gene transcription (2), the human embryonic gene ε was activated in GM979 × HeLa heterokaryons (Fig. 4A, lane 6) as well as in GM979 × normal human lymphocyte heterokaryons (Fig. 4B, lane 6). In contrast, the human embryonic β-globin gene was not activated in mouse adult erythroid (MEL) × HeLa (2; Fig. 4B, lane 2) or MEL × normal human lymphocyte (Fig. 4B, lane 4) heterokaryons or in unfused mixtures of cells (Fig. 4A, lane 4; Fig. 4B, lanes 1, 3, and 5). The cloned human fetal globin gene is expressed in the embryonic yolk sac of transgenic mice (4, 6, 11, 38), and we therefore anticipated that the human fetal globin gene in normal lymphocytes would respond to regulatory factors present in GM979 cells. As expected, the human fetal and adult β-globin genes, which are activated in MEL × HeLa heterokaryons (2; Fig. 2B and 4C), are also expressed in GM979 × HeLa (Fig. 4A, lanes 2 and 3) or GM979 × human lymphocyte (Fig. 4E) heterokaryons. The human adult but not the human fetal β-globin gene was activated in heterokaryons formed by fusion of MEL and normal human lymphocytes (Fig. 4D and results presented below). The human embryonic and adult β-globin genes were activated with identical kinetics in the GM979 × human nonerythroid cell heterokaryons (not shown).

The relative accumulation of human β-like globin transcripts in GM979 × human nonerythroid heterokaryons (Fig. 4A, E) does not reflect the levels of endogenous mouse globins in the parental GM979 cells (Fig. 3). The adult mouse β-globin genes are expressed at the highest levels in these cells, while the embryonic gene βh1 (which is most closely related to the human fetal globin genes; 6) is expressed at a much lower level. In contrast, approximately 50 to 100 times more human fetal than adult β-globin mRNA was detected in heterokaryons prepared from GM979 and human nonerythroid cells (Fig. 4A, lanes 2 and 3; Fig. 4E). The levels of adult and fetal β-globin transcripts were nearly equal in MEL × HeLa heterokaryons (Fig. 4C).

Expression in transient heterokaryons of human globin

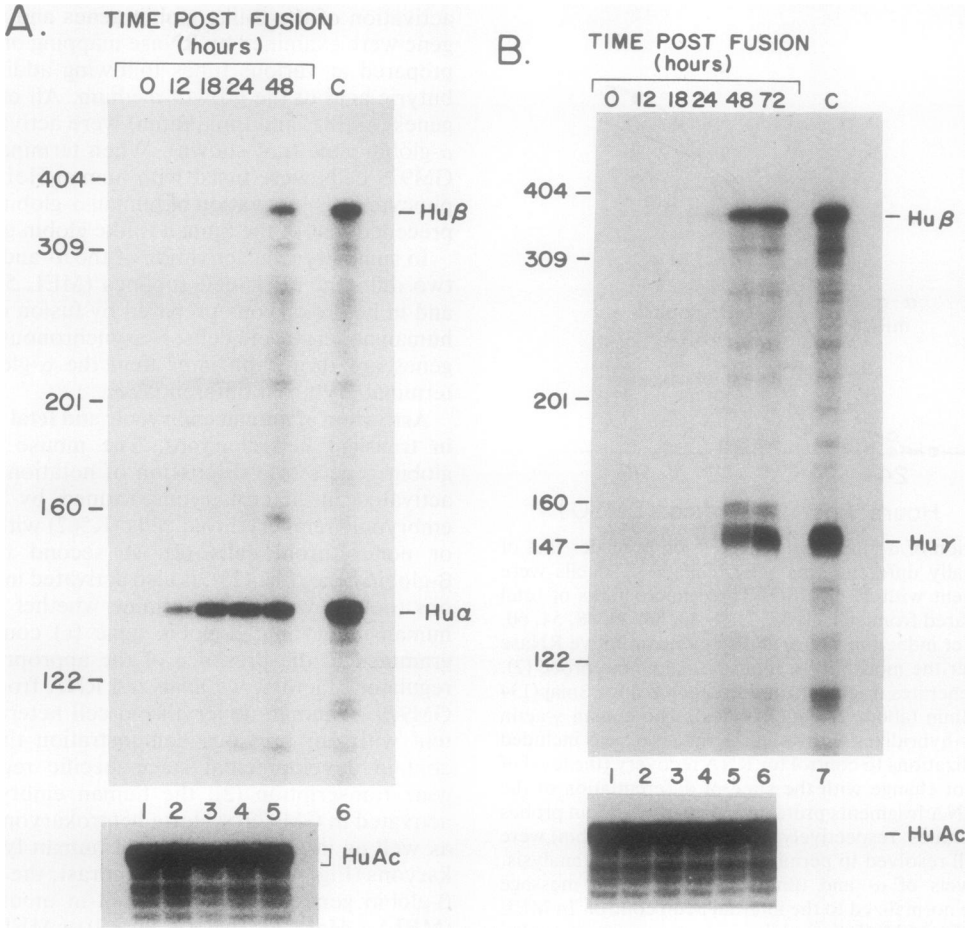

FIG. 2. Kinetics of activation of human α-, β-, and γ-globin genes in MEL × HeLa heterokaryons. (A) Activation of human α-globin precedes that of β-globin in MEL × HeLa heterokaryons. MEL cells were induced by treatment with 2% DMSO for 3 days and then mixed with HeLa cells. Cell fusion was initiated by treatment with PEG. Fifty micrograms of total cellular RNA prepared from unfused mixtures of cells (0 h) or from cells at 12, 18, 24, or 48 h following fusion was analyzed by quantitative RNase mapping with a mixture of the human α- and β-globin probes. Human umbilical cord blood mRNA (0.5 ng) was hybridized with the same probes (C, lane 6). The specific activity (and therefore the sensitivity) of the β-globin probe (25% U, the labeled nucleotide) is greater than that of the α-globin probe (16%), yet activation of the α-globin gene is easily detected much earlier than that of the β-globin gene. The lower panel shows an analysis of the same set of RNA samples (corresponding to lanes 1 to 5), using the human γ-actin (HuAc) probe to control for RNA recovery. (B) Synchronous activation of human fetal (γ) and adult β-globin genes in MEL × HeLa heterokaryons. Fifty micrograms of the RNA samples prepared from DMSO-induced MEL × HeLa heterokaryons at different times following treatment with PEG were analyzed by quantitative RNase mapping with a mixture of the human γ- and β-globin probes. Human umbilical cord blood mRNA (C, lane 7) was hybridized with the same probes. The RNAs analyzed in panels A and B were from independent experiments.

genes containing mutations that affect hemoglobin switching. The human fetal β-globin genes are not activated in MEL × human lymphocyte heterokaryons (Fig. 4D; Fig. 5, lane 2) but are clearly capable of responding to *trans*-acting regulators expressed at an earlier stage of development (Fig. 4E). To determine whether mutated fetal β-globin genes could be activated in cells from individuals who inappropriately express high levels of fetal hemoglobin as adults (a condition known as HPFH; 33), we analyzed RNA from heterokaryons generated by fusion of (adult) MEL cells with HPFH (nondeletion and deletion) lymphocytes. None of these cells expressed fetal globin before fusion (Fig. 5, lanes 1, 3, 5, 7, and 9), but the human fetal globin genes were activated in all of the heterokaryons derived from HPFH lymphocytes (lanes 4, 6, 8, and 10). These mutated genes, then, are capable of responding in *trans* to regulatory factors contributed by the adult erythroid cells.

The adult α- and β-globin genes were activated in heterokaryons prepared by fusion of (adult) MEL cells with normal lymphocytes (Fig. 6, lane 2); with the heterozygous deletion HPFH-2 lymphocytes (lane 4), which contain one normal copy of the adult β-globin gene (not shown); and with the nondeletion (fetal γ-globin promoter point mutation) HPFH lymphocytes (shown for Chinese HPFH; lane 6). Both adult β-globin genes are deleted in the compound HPFH-1/-2 mutant (reference 14 and data not shown), and no adult β-globin expression was detected when the corresponding lymphocytes were fused with MEL cells (data not shown). Thus, in the lymphocytes from a normal individual, stage-specific activation of globin gene expression was observed following fusion with MEL cells. However, this stage specificity was perturbed in the mutant cells, reflecting the failure to complete the fetal-to-adult switch observed in vivo.

Lymphocytes and erythrocytes are both hematopoietic cell types and are thought to arise from a common pluripotent stem cell (reviewed in reference 9). To determine

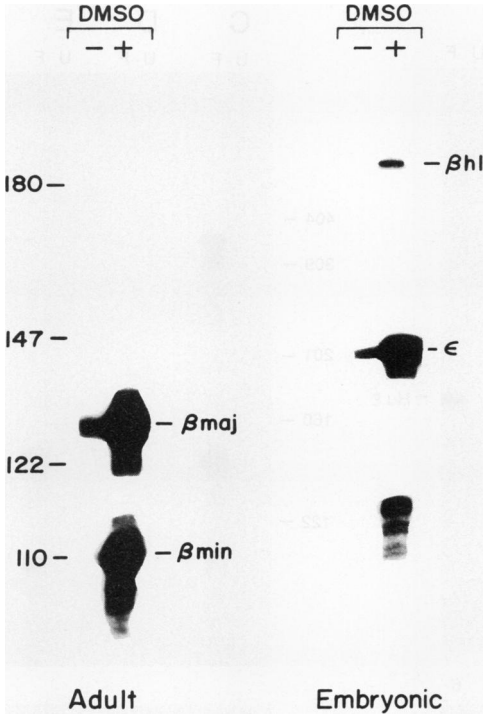

FIG. 3. Induction of mouse β-globin genes in GM979 erythroleukemia cells. GM979 cells were treated with 2% DMSO for 2 days. Ten micrograms of total cellular RNA was analyzed by RNase mapping, using the mouse (adult) βmaj, mouse embryonic ε, and mouse embryonic βh1 probes. Similar results were obtained when the cells were treated with 1 mM butyric acid.

whether mutant fetal globin genes could be activated in the nuclei of nonhematopoietic HPFH cells, we fused MEL cells with skin fibroblasts from an individual with HPFH (Fig. 7). The fetal globin genes were again activated in MEL × HPFH-skin fibroblast heterokaryons (Fig. 7, lane 6) but not in skin cells from normal individuals (lanes 2 and 4).

DISCUSSION

Noncoordinate activation of α- and β-globin gene expression. We examined the kinetics of activation of human α- and β-like globin genes in terminally differentiating MEL cells and in heterokaryons prepared by fusion of MEL cells with human nonerythroid cells. In MEL cells and in a variant line (GM979) that expresses mouse embryonic globin, the α-globin gene is activated earlier than the β-like globin genes. The two embryonic β-like globin genes (ε and βh1) are coordinately activated in differentiating GM979 cells. In MEL or GM979 × human nonerythroid heterokaryons, the human α-globin genes are also activated earlier than the human β-like globin genes. The parental mouse erythroid cells were first treated for several days with chemical inducing agents, at which point they were accumulating approximately equal amounts of α- and β-globin RNA transcripts. Therefore, although all regulatory factors required for full expression of these genes were present in the induced parental mouse erythroid cells at the time of cell fusion, a delay was always observed between the activation of the human α- and β-globin genes.

It is unlikely that differential human α- and β-globin mRNA stability could account for the kinetic differences in accumulation of these transcripts in heterokaryons. Unequal rates of message degradation could account for our results only if human β-globin mRNA were extremely unstable. The half-life of human β-globin mRNA is in fact unusually long (17.5 h; 24).

Our results can be interpreted as reflecting different steps in the activation of α- and β-globin gene expression (as originally proposed by Charnay et al. [8]) or alternatively as the result of different thresholds for activation by one or more erythroid-specific proteins common to the regulation of both families of globin genes. In the latter instance, it would follow that one or more common transcriptional regulators must accumulate (in active form) in erythroid cells during their terminal differentiation. The noncoordinate activation of α- and β-like globin genes in mouse erythroid × human nonerythroid heterokaryons argues against a simple threshold concentration model, however, because both gene families were maximally active in the donor erythroid cells at the time of fusion. We can therefore now rule out the possibility that these kinetic differences simply reflect the time needed to accumulate sufficient levels of the appropriate regulatory molecules. Both the α- and β-globin gene clusters are subject to positive control by sequences located far upstream (16, 20), suggesting that the mechanisms by which these gene families are regulated are likely to be similar. Nevertheless, previously reported differences in the structures, replication patterns, and expression of these genes (see introduction), as well as the noncoordinate activation that we observe in transient heterokaryons, lead us to conclude that some of the molecular events required for activation of α- and β-globin genes must be different. In general, we have observed that both the adult α- and β-globin genes are activated in heterokaryons derived from adult mouse erythroid (MEL) cells and one of several human nonerythroid cells. However, in a few instances, only one of these genes is activated; for example, in MEL × Raji (B-lymphocyte) or MRC-5 (lung fibroblast) heterokaryons, the adult human β- but not the α-globin gene is activated, while in MEL × HepG2 (hepatoma) heterokaryons, the α- but not the adult β-globin gene is activated (1a). These experiments are consistent with a model proposed by Charnay et al. (8) in which the α-globin gene in preerythroid cells is under negative control and the β-globin gene is under both negative and positive control. Thus, a minimum of one (derepression) step would be required for the activation of α-globin gene transcription, while at least two steps (derepression followed by tissue- and/or stage-specific positive activation) would be required for the transcriptional activation of β-globin gene expression. The mechanistic details of this complex regulatory system will require the identification, cloning, and biochemical characterization of the various proteins involved.

If a pathway of regulatory events is induced during MEL cell terminal differentiation, some of these same steps may also be initiated in the nonerythroid nuclei of MEL × human nonerythroid heterokaryons. The lag between activation of human α- and β-like globin genes in these heterokaryons, even when provided with high levels of MEL cell *trans*-acting regulatory molecules at the time of cell fusion, suggests that a series of events may be triggered in the nonerythroid nuclei.

Activation of human embryonic β-globin gene expression in transient heterokaryons. We have shown (2) that a mouse embryonic β-like globin gene (ε) could be activated in heterokaryons formed by fusion of human embryonic/fetal erythroid cells (K562) with mouse erythroid or nonerythroid cells. A second mouse embryonic β-globin gene, βh1 (23), is

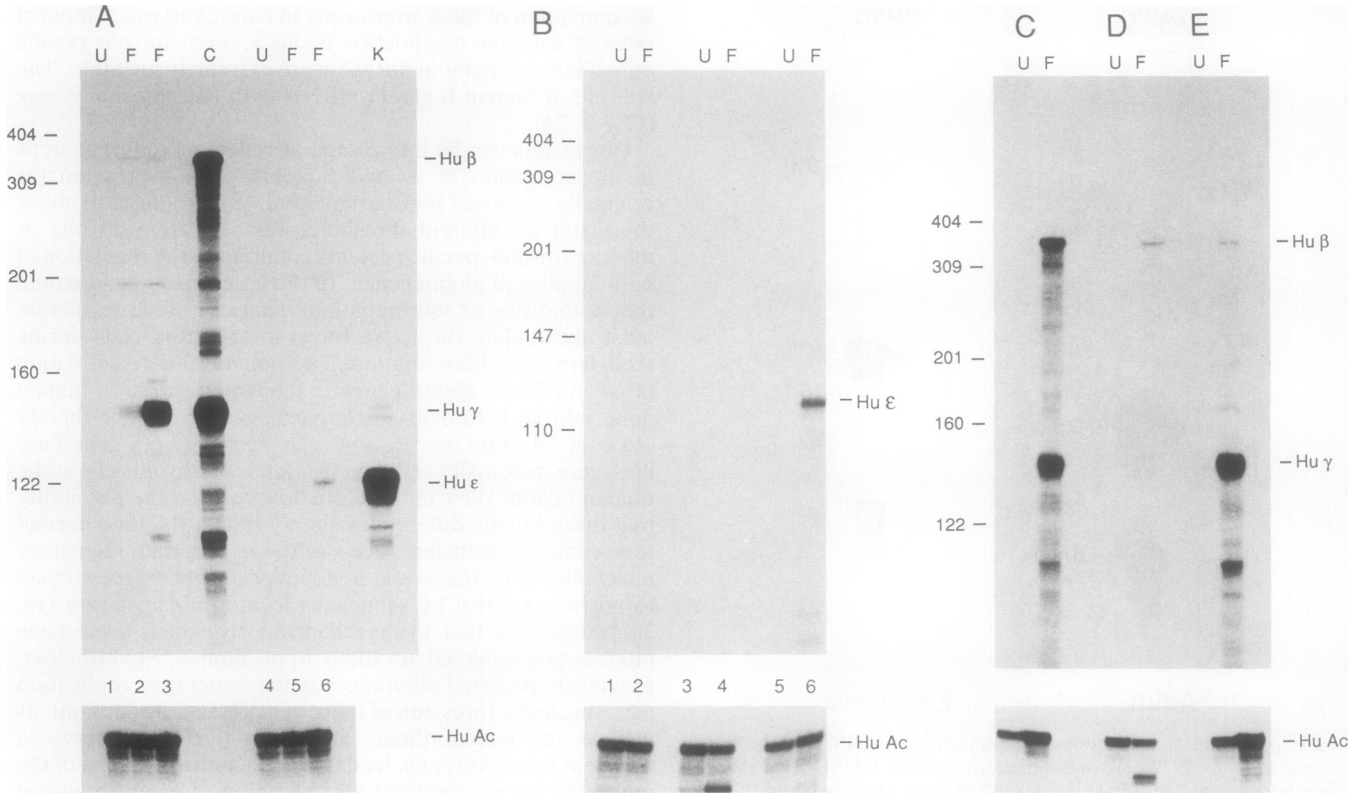

FIG. 4. Activation of human β-globin genes in mouse erythroid × human nonerythroid heterokaryons. (A) Human embryonic, fetal, and adult β-globin genes are activated in GM979 × HeLa cell heterokaryons. In this experiment, GM979 cells were induced with 1% (lanes 2 and 5) or 2% (lanes 1, 3, 4, and 6) DMSO for 2 days prior to fusion to induce expression of mouse embryonic β-globin genes (see Fig. 3). The induced cells were then mixed with HeLa cells. Fifty micrograms of total cellular RNA from unfused (lanes 1 and 4) or fused (lanes 2, 3, 5, and 6) mixtures of cells were analyzed by RNase protection of the ^{32}P-labeled human $^{G}\gamma$- and β-globin (lanes 1 to 3) or human ε-globin (lanes 4 to 6) RNA probes. The lower panel shows an analysis of the same set of RNA samples, using the human γ-actin (Hu Ac) probe to control for RNA recovery. Markers were provided by hybridization of appropriate probes with human umbilical cord blood mRNA (lane C) or RNA from human K562 erythroleukemia cells (lane K). (GM979 cells induced with butyric acid gave similar results.) (B) The human embryonic β-globin gene (ε) is activated in GM979 × normal human lymphocyte heterokaryons. GM979 cells were induced by treatment with butyric acid for 3 days to express embryonic mouse globin genes and were then mixed with normal human lymphocytes. Fifty micrograms of total cellular RNA from unfused (U, lane 5) or fused (F, lane 6) mixtures of cells was analyzed by quantitative RNase mapping, using the probes against the human fetal and adult β-globin mRNAs. The human embryonic β-globin gene (ε) was not activated by fusion of HeLa cells (lane 2) or normal human lymphocytes (lane 4) with MEL cells, which do not express mouse embryonic globin genes. (C) Human fetal and adult β-globin genes are activated in MEL × HeLa cell heterokaryons. MEL cells were induced by treatment with 2% DMSO for 3 days prior to fusion to induce expression of adult mouse globin genes and were then fused with HeLa cells. Fifty micrograms of total cellular RNA from unfused (lanes U) or fused (lanes F) mixtures of cells were hybridized with ^{32}P-labeled human $^{G}\gamma$- and β-globin RNA probes and analyzed by RNase mapping. (D) Human adult but not fetal β-globin is activated in MEL × human normal lymphocyte heterokaryons. MEL cells induced to express adult mouse globin genes were mixed with normal human lymphocytes. Fifty micrograms of total cellular RNA from unfused (lane U) or fused (lane F) mixtures of cells was analyzed by quantitative RNase mapping, using the human fetal and adult β-globin RNA probes. (E) Human fetal and adult β-globin genes are activated in GM979 × normal human lymphocyte heterokaryons. GM979 cells were induced to express embryonic and adult mouse globin genes and were then mixed with normal human lymphocytes. Fifty micrograms of total cellular RNA from unfused (lane U) or fused (lane F) mixtures of cells was analyzed by quantitative RNase mapping, using the probes against the human fetal and adult β-globin mRNAs.

also activated in these heterokaryons (1a). In this report, we have shown that the human embryonic β-globin gene (ε) can also be activated in heterokaryons containing nuclei from a parental mouse erythroid cell variant (GM979) that expresses embryonic mouse β-globin genes. Whereas the absence of both human and mouse embryonic globin gene expression in stable somatic cell hybrids had previously been thought to reflect an irreversible state of repression of these genes in adult erythroid cells (reviewed in reference 2), the results of our heterokaryon studies (2; this report) clearly establish that embryonic genes can be activated in *trans* in the nuclei of adult cell types.

Expression of human fetal and adult β-globin genes in transient heterokaryons. Interestingly, the relative amounts of human fetal (γ) and adult (β) globin mRNAs in GM979 × human nonerythroid heterokaryons does not reflect the relative levels of the corresponding mouse globin transcripts in the parental erythroid cells. Whereas the adult β-globin gene is expressed at very high levels in GM979 cells, the embryonic gene βh1 (which is most closely related to the human fetal β-globin genes; 6) is expressed at much lower levels. In GM979 × human nonerythroid heterokaryons, the fetal β-globin genes are activated much more strongly than the adult β-globin gene. In comparison with human globin gene activation in adult MEL × human nonerythroid heterokaryons, the ratio of fetal to adult transcripts is dramatically

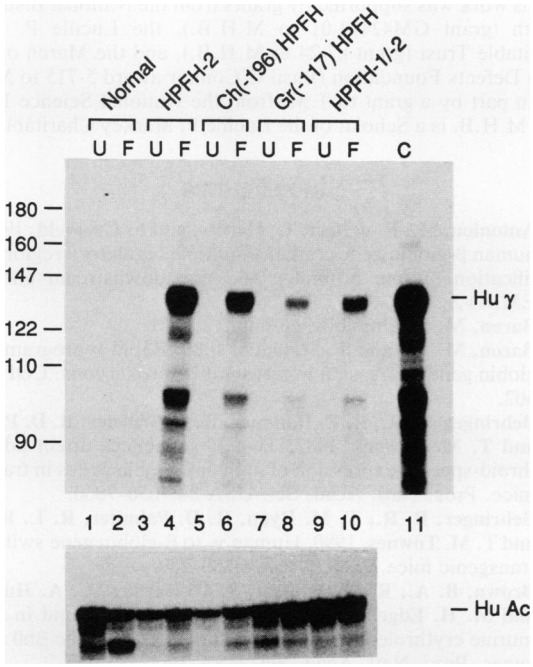

FIG. 5. Activation of human fetal γ-globin genes in MEL × HPFH lymphocyte heterokaryons. MEL cells were induced by treatment with 2% DMSO for 3 days and then mixed with Epstein-Barr virus-immortalized human lymphocytes. Fifty micrograms of total cellular RNA, isolated 48 h later from unfused mixtures of cells (lanes 1, 3, 5, 7, and 9) or from mixtures of cells fused by treatment with PEG (lanes 2, 4, 6, 8, and 10) were analyzed by an RNase protection assay, using a ^{32}P-labeled SP6 RNA probe, corresponding to the 5′ end of the human $^{G}\gamma$-globin gene, which hybridizes to both fetal $^{A}\gamma$- and $^{G}\gamma$-globin mRNAs. The lymphocytes used in this experiment carry normal fetal globin genes (lanes 1 and 2); a heterozygous deletion mutation, HPFH-2 (lanes 3 and 4); the −196 $^{A}\gamma$ Chinese HPFH promoter point mutation (lanes 5 and 6); the −117 $^{A}\gamma$ Greek HPFH promoter point mutation (lanes 7 and 8); and the compound heterozygous deletions HPFH-1 and HPFH-2 (lanes 9 and 10). The lower panel shows an analysis of the same set of RNA samples (corresponding to lanes 1 to 10), using the human γ-actin (Hu Ac) probe to control for RNA recovery. A marker for human fetal globin RNA was prepared by hybridization of the SP6 probe with mRNA from human umbilical cord blood (C, lane 11).

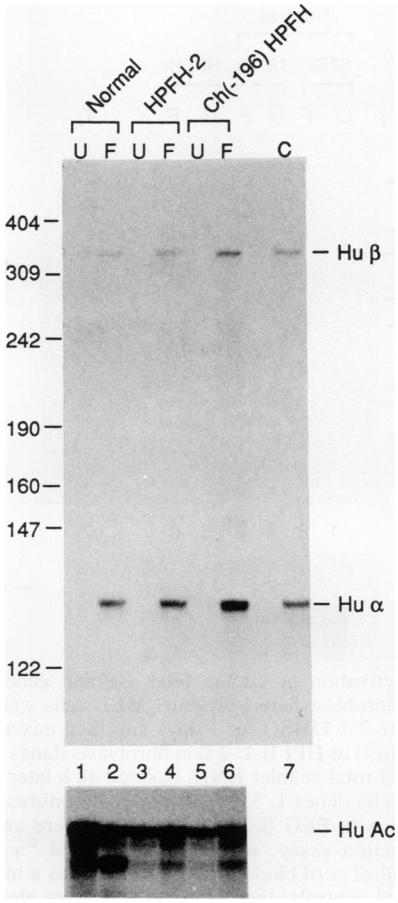

FIG. 6. Activation of human adult globin genes in MEL × normal or HPFH lymphocyte heterokaryons. Cell fusions were performed as described for Fig. 5, and 50 μg of total cellular RNA from unfused (lanes 1, 3, and 5) or fused (lanes 2, 4, and 6) mixtures of cells was analyzed by RNase protection analysis of ^{32}P-labeled SP6 RNA probes corresponding to the 5′ ends of the human α- and β-globin genes (see reference 2). The lymphocytes used in this experiment carry normal fetal globin genes (lanes 1 and 2); a heterozygous deletion mutation, HPFH-2 (lanes 3 and 4); and the −196 $^{A}\gamma$ Chinese HPFH promoter point mutation (lanes 5 and 6). The lower panel shows an analysis of the same set of RNA samples (corresponding to lanes 1 to 6), using the human γ-actin (Hu Ac) probe to control for RNA recovery; these samples are the same as those shown in Fig. 5. Human umbilical cord blood mRNA was used as a marker (C, lane 7). Although in this experiment the level of activation of the human adult β-globin gene was slightly higher in the MEL × Chinese HPFH heterokaryons than in heterokaryons prepared from normal or HPFH-2 lymphocytes, in other experiments the levels were essentially equal.

increased. Unlike the parental MEL cells, GM979 cells contain regulatory factors that are capable of activating mouse and human β-like globin genes of all developmental stages. Therefore, the observed ratios of human fetal and adult globin mRNAs in different types of heterokaryons may reflect competition between the promoters of these genes for productive interactions with regulatory sequences (locus-activating or dominant control region; 16, 31, 32, 36) located far upstream of the entire β-globin locus. As suggested by the developmentally regulated expression of physically linked human fetal and adult β-globin genes in transgenic mice (4, 11), these interactions may be mediated by stage-specific regulatory proteins (2) associated with the promoters of individual globin genes, resulting in the fetal-to-adult hemoglobin switch. The definitive determination of whether individual nuclei within heterokaryons can express both fetal and adult β-globins will require the use of a single-cell assay.

Activation of mutant human fetal globin genes in transient heterokaryons. Our earlier studies demonstrated that embryonic and fetal globin genes are not irreversibly repressed in the nuclei of adult cells, whether erythroid or nonerythroid, but can be rapidly activated in the presence of the appropriate tissue- and stage-specific *trans*-acting molecules (2). We have now extended these observations to a set of naturally occurring developmental mutations in humans in which the later (fetal-to-adult) switch in globin gene expression is never resolved. Individuals carrying these *cis*-acting point mutations upstream of the γ-globin genes or deletion mutations elsewhere in the β-globin locus (33) produce inappropriately high levels of fetal hemoglobin as adults. These mutated fetal globin genes are activated in MEL × HPFH heterokaryons but not in heterokaryons prepared by fusion of MEL cells

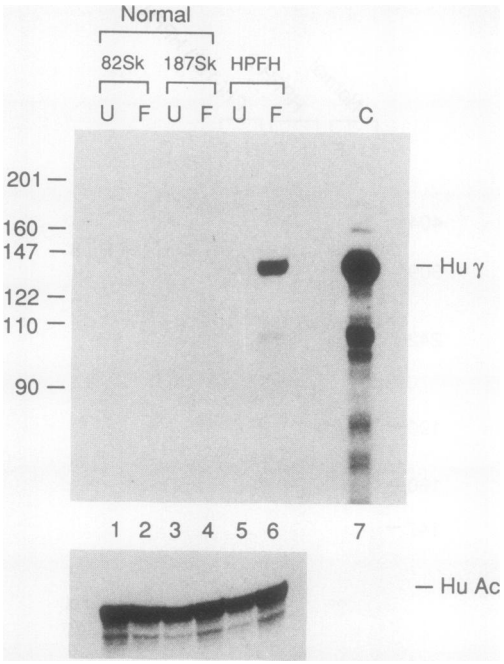

FIG. 7. Activation of human fetal γ-globin genes in MEL × HPFH skin fibroblast heterokaryons. MEL cells were induced by treatment with 2% DMSO for 3 days and then mixed with normal skin (lanes 1 to 4) or HPFH-1/-2 skin fibroblasts (lanes 5 and 6). Fifty micrograms of total cellular RNA, isolated 48 h later from unfused mixtures of cells (lanes 1, 3, and 5) or from mixtures of cells fused by treatment with PEG (lanes 2, 4, and 6) were analyzed by an RNase protection assay, using the ^{32}P-labeled $^{G}\gamma$-globin probe. Human umbilical cord blood mRNA was used as a marker (C, lane 7). As internal controls, these same RNAs were also analyzed by using a human γ-actin probe (Hu Ac; lower panel).

with normal human cell types. Therefore, *cis*-acting mutations that lead to aberrant hemoglobin switching during human development also result in aberrant globin gene expression in these transient heterokaryons.

The molecular mechanisms underlying these disorders of fetal globin gene regulation are thought to be different for deletion and nondeletion HPFH mutations (33) but are likely to involve changes in the interactions of regulatory proteins with various *cis*-acting sequence elements. Indeed, a number of such changes have been observed in association with several nondeletion (point) HPFH mutations, although none of these has been definitively implicated in the regulation of human fetal globin gene expression, whether normal or aberrant (for example, see references 17, 27, 34, and 35). The fetal globin gene activation observed in our transient heterokaryons is only observed in the presence of the appropriate *trans*-acting regulatory molecules. The experiments reported here therefore confirm and extend a previous somatic cell hybridization study (29) by providing direct functional evidence for changes in the interactions of these mutated human fetal globin genes with regulatory proteins in erythroid cells.

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