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The LCR-like α-globin positive regulatory element functions as an enhancer in transiently transfected cells during erythroid differentiation

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ABSTRACT

A positive regulatory element (PRE) similar to the locus control region (LCR) of the human β-globin gene cluster has recently been identified 40 kb upstream of the human ζ-globin mRNA cap site (Higgs D.R. W.G. Wood, A.P. Jarman, J.Sharpe, J. Lida, I.M. Pretorius, and H. Ayyub. 1990). We investigated the influence of the αPRE on human α-globin promoter activity in transiently transfected cells. The introduction of the αPRE into α-globin promoter/CAT expression constructs increased α-globin promoter activity by 15 – 30 fold in a human erythroid cell line (Putko) as well as in mouse erythroleukemia cells (MELCs) induced with hexamethylene bisacetamide (HMBA). When these constructs were introduced into uninduced MELCs or HeLa cells, only a 2 – 3 fold increase in α-globin promoter activity was observed. Deletion of 600 bp of α-globin 5′ flanking sequences containing six putative SP1-binding sites had no significant effect on levels of α-globin promoter enhancement by the αPRE. We further demonstrated that the αPRE and HS2 of the β-LCR could similarly enhance transcriptional activity of the SV40 early promoter in HMBA induced MELCs. Finally, we showed that α-globin promoter activity in the presence of the αPRE increased with continued HMBA exposure and was coincident with transcriptional activation of endogenous globin genes.

INTRODUCTION

The human α-globin cluster consists of three functional genes arranged 5′-ζ2−α2-α1−3′ at the tip of chromosome 16p (see Figure 1A). The human β-globin cluster consists of five functional genes arranged 5′-ε-Gγ-Aγ-δ-β-3′ on chromosome 11p. The genes within both loci are regulated in a tissue and developmental stage specific manner. This complex regulation is partly dependent on sequences within the promoter and intragenic elements within or directly flanking the individual globin genes. Transgenic mice have provided a useful system for studies on the regulation of human globin genes. A 4 kb fragment containing the entire human β-globin gene is correctly regulated in a tissue and developmental stage specific manner in transgenic mice (1−3). However, the level of expression of the introduced gene is only up to 5% of that seen from the endogenous globin genes and is dependent on the site of integration into the host genome. In addition, transgenic mice containing a fragment that includes the entire human α-globin gene show no expression of the introduced gene (4−6). When the α-and β-globin genes on intact chromosomes 16 and 11 are transferred to human×MELC hybrids, their levels of expression approximate that of the endogenous mouse globin genes (7−10). These results imply that additional cis-regulatory sequences remote from the structural genes are necessary for high level, position-independent expression of globin genes.

Studies have shown that such regulatory sequences are present 5−20 kb upstream the human β-cluster (11,12). These sequences, collectively referred to as the locus control region (LCR), can direct high levels of tissue specific expression of the β- and α-globin genes in a copy-dependent and position-independent manner in transgenic mice (4,6,12,13). Recently, an LCR-like α-positive regulatory element (αPRE) located far upstream the α-cluster has been identified (see Figure 1A). Like the β-LCR, this element is capable of directing high level expression of the human α-globin gene in stable erythroid cell lines and transgenic mice (8). Both the β-LCR and the αPRE are characterized by the presence of erythroid specific DNAse 1 hypersensitive sites. These sites are present at all stages of erythroid development examined and occur 5−20 kb upstream the human β-locus in the β-LCR and 30−40kb upstream the α-locus in the αPRE. Such tissue specific DNase I hypersensitive sites generally map closely to the binding sites for transacting factors (14) and may play a crucial role in β-LCR/αPRE function.

Experiments on DNase I hypersensitive site 2 (HS2) of the β-LCR have shown that it can function as an enhancer in transiently transfected erythroid cell lines (15,16). Upon hemin induced differentiation, the level of HS2 enhancer function in transiently transfected K562 cells increased by 10 fold (15). Dissection of the HS2 fragment showed that a double consensus sequence for Jun/Fos family of DNA binding factor proteins is crucial for HS2 enhancer function in transient and stable assays (15,17,18). Induction of this enhancer may therefore play a role in the increase of globin gene expression that characterizes erythroid differentiation.

We wished to further characterize the function of the αPRE by determining whether it could enhance human α-globin expression in transient assays. In this report, we demonstrate that the αPRE can enhance transcriptional activity of the human α-

globin promoter and the SV40 early promoter in transient assays. Enhancement by the αPRE is erythroid specific and dependent upon erythroid differentiation. We propose that activation of αPRE enhancing potential during erythroid differentiation may play an important role in the increased expression of α-globin mRNA that characterizes erythroid development.

MATERIAL AND METHODS

DNA constructs

All constructs employed in this study are depicted in Figure 1. CAT constructs αCAT and αΔCAT were provided by E. Whitelaw (19). These plasmids contain the human 1-globin promoter from the Sma I site at −750 (αCAT) or Hinf1 site at −110 (αΔCAT) to +20 (cap site 1) cloned into the CAT expression vector pSV0CAT (20) at a Bgl II site.

The plasmid αCATE provided by E.Whitelaw (19) contains the SV40 enhancer cloned into the BamH I site 3′ to the polyadenylation site in the plasmid αCAT.

The plasmid αCAT* and αΔCAT* are made up of a 4.0 kb Hind III, BamH I linkered fragment containing the α-positive regulatory element (see Figure 1A) kindly provided by D. Higgs (21) cloned in the BamH 1 site (genomic and anti-genomic relative to CAT) of αCAT and αΔCAT.

The plasmid pSVOCAT* and pSV1CAT* contain the αPRE inserted into the BamH I site (genomic and anti-genomic relative to CAT) of the plasmid pSVOCAT and pSV1CAT (20).

The plasmid pSV1HS2 is made up of a 1.5kb BamH I linkered Not 1-BstE II fragment containing hypersensitive 2 of the β-LCR cloned into the BamH I site of pSV1CAT.

The plasmid αpSVod provided by E.Whitelaw (19) is made up of a 1.6kb Pst I fragment containing the human α1-globin gene inserted between the Pvu I and Pst I sites of the expression vector pSVod (22).

The plasmid αpSVod* contains a blunt ended 4.0 kb Hind III αPRE fragment inserted into the Sca I site of αpSVod in genomic and anti-genomic opposite orientations relative to the α-globin gene.

The plasmid pIRV (23) containing the β-galactosidase gene linked to the rat β-actin promoter served as a co-transfection control.

Tissue culture and cell transfection

Putko and MELCs 707 were maintained in RPMI 1640 supplemented with 10% (Putko) or 20% (MELCs) fetal calf serum, 100 μg/ml penicillin and 100 U/ml streptomycin. HeLa cells were grown in DMEM containing 10% fetal calf serum, 2 mM glutamine, 100 μg/ml penicillin and 100 U/ml streptomycin.

Putko and MELCs cells were transfected by electroporation. Cells were grown to a density of approximately 1.0×10^6/ml, washed with phosphate buffered saline (PBS) and cell pellets resuspended in 0.8 ml of electroporation buffer (25 mM HEPES [N-2 hydroxyethylpiperazine-N′-2-ethanesulfonic acid], pH 7.1, 140 mM NaCl, 0.75 mM Na_2HP0_4, 0.5% polyethylene glycol 6000), 15−25 μg of test DNA and 15 μg of pIRV DNA. Cells were placed on ice for 10 minutes and the mixture was electroporated with a BioRad Gene Pulser at 1.75 Kv, 25 μF (Putko) or 0.3 Kv, 960 μF (MELCs). After electroporation, cells were transferred to 25 ml of pre-warmed media and incubated for 48−60 hours at 37° C in 5% CO_2. To induce differentiation of MELCs, HMBA (Sigma) was added to the medium following transfection at a final concentration of 5 mM. HeLa cells were transfected by calcium phosphate co-precipitation technique according to Proudfoot et al. (22).

Assay for β-galactosidase and CAT activity

To assay for β-galactosidase and chloramphenicol acetyltransferase (CAT) activity, cells were harvested 48−60 hrs after transfection and washed with PBS. The pelleted cells were sonicated in 100 ul of 0.25 M Tris hydrochloride (pH 7.5) and spun in an Eppendorf microfuge for 10 min. β-galactosidase assays were performed according to Herbomel et al. (24) and protein concentration of lysates determined according to Bradford (25). Lysates corresponding to equal amounts of β-galactosidase activity were heated to 65°C for 5 minutes and used in a standard CAT assay (20). CAT assays were quantitated by cutting the chloramphenicol and 3-acetyl-chloramphenicol spots from TLC plates and counting them in a liquid scintillation counter. The% conversion figures so obtained were then divided by the% conversion of the background control plasmid pSVOCAT to give relative CAT activities. Experiments were performed a minimum of 2−3 times for each CAT construct.

RNA isolation and primer extension

Total cytoplasmic RNA was prepared by NP-40 lysis in the presence of vanadyl ribonucleoside complex as previously described (26). For primer extension analysis, an end labeled oligonucleotide primer complementary to the sequence TCGG-

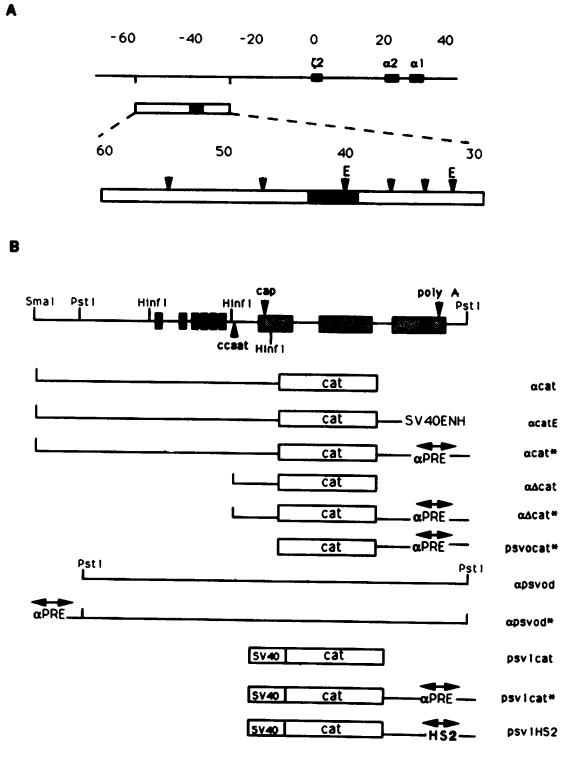

Figure 1. (A) Line diagram of the human α-globin locus showing the relative positions of erythroid (E) and non-erythroid hypersensitive sites (↓) and the individual globin genes. Darkened box represents 4.0 kb αPRE fragment employed in all DNA constructs. (B) Line diagram showing important features of the human α-globin gene and its flanking DNA. Grey boxes represent exons and black boxes represent SP1 consensus sequences. Below this diagram are the plasmid constructs employed in this study.

CGCGCACGCTGGCGAG within exon-1 of the human α-globin gene was synthesized. The primer was 5′ end labeled with [γ-^{32}P] and annealed to 20 μg samples of total cytoplasmic RNA and extended with 20 U of AMV reverse transcriptase (26). Samples of the reaction product were run on 6% polyacrylamide denaturing gels.

RESULTS

Transcriptional enhancement of α-globin promoter by the αPRE in an erythroid cell line

Whitelaw et al. (19) showed that in a non-replicating plasmid, human α-globin promoter activity was enhancer dependent in transiently transfected erythroid and non-erythroid cell lines. We wished to determine whether the αPRE could act as an enhancer in a non-replicating plasmid and activate transcription from the α-globin promoter in transiently transfected erythroid cells. The construct αCAT with or without the αPRE was electroporated into Putko cells (a derivative of K562 cells known to produce significant amounts of human α-globin mRNA) (27). In these constructs, the CAT gene is placed under control of the α-globin promoter (see Figure 1B). Putko cells were also transfected with the construct αCATE (αCAT containing the SV40 enhancer) serving as a positive control for α-globin promoter activation (19). After 48 hours, CAT activity in extracts from transfected cells was measured. Variations in cell density and transfection efficiency were controlled by protein assays and co-transfection with the β-actin/β-galactosidase plasmid pIRV (see materials and methods).

Figure 2A and 2B shows a representative example of CAT assay and quantitation of CAT activities, respectively. In the absence of an enhancer, no significant α-globin promoter activity was detected in a non-replicating system consistent with Whitelaw et al. (19). When the αPRE was inserted at the 3′end of the CAT gene in the construct αCAT (αCAT*), a significant increase in CAT activity was observed. αCAT containing the αPRE in a genomic position relative to the CAT gene showed a greater than 25 fold increase in CAT activity compared to cells transfected with αCAT in the absence of the αPRE. When the αPRE was inserted in αCAT in the anti-genomic position relative to the CAT gene, up to a 15 fold increase in CAT was observed. The presence of the SV40 enhancer in the construct αCAT (αCATE) increased

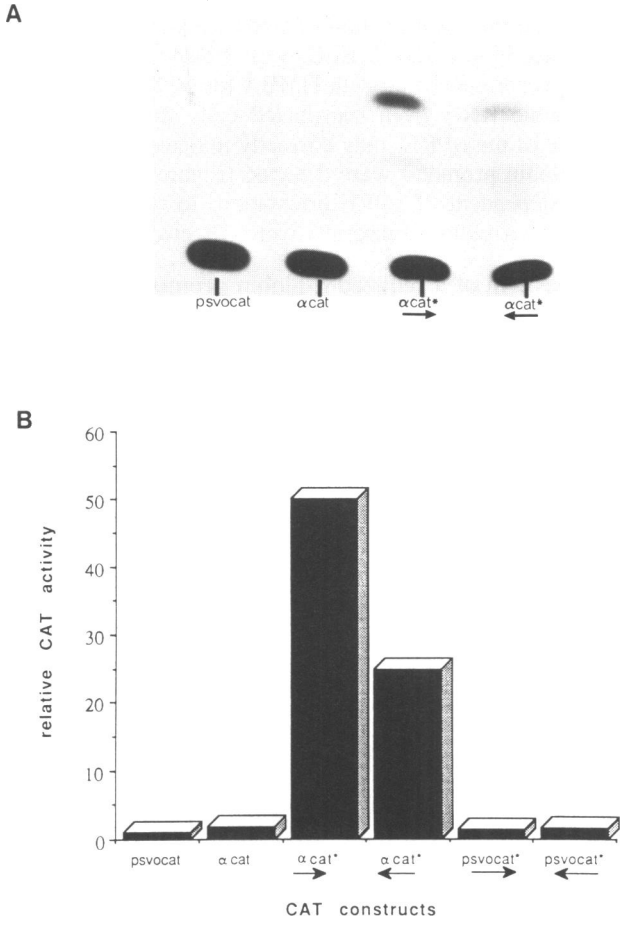

Figure 2. (A) Representative CAT activity of cell lysates from Putko cells transfected with pSVOCAT, αCAT, and αCAT*. → denotes αPRE is in genomic position relative to CAT gene, while ← denotes αPRE is in anti-genomic position relative to CAT gene. (B) Quantitation of CAT activity from Putko cells transfected with the above constructs and pSVOCAT*. Relative CAT activity was determined by comparing CAT activity of a given construct to that of the promoterless CAT plasmid pSVOCAT (see material and methods). Data represents an average of three reproducible, independent experiments.

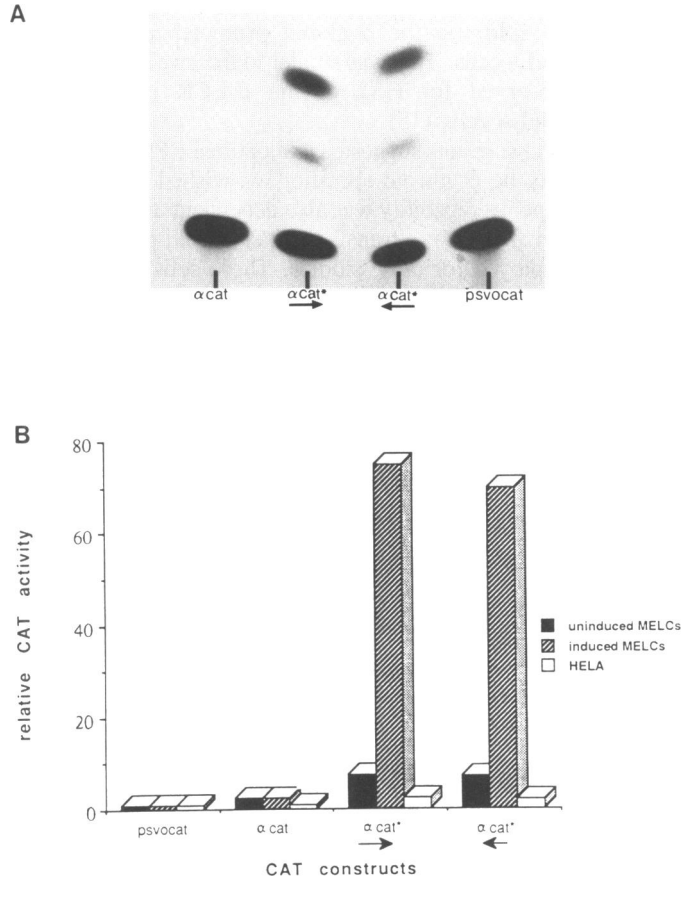

Figure 3. (A) Representative CAT assay of cell lysates from HMBA induced MELCs transfected with pSVOCAT, αCAT, and αCAT* (αPRE genomic → and anti-genomic ← relative to CAT). Following electroporation, MELCs were treated with 5mM HMBA for 60 hours prior to lysate preparation. (B) Quantitation of CAT activity from HeLa, HMBA induced and uninduced and MELCs transfected with the above constructs. Relative CAT activity was determined by comparing CAT activity of a given construct to that of the promoterless CAT plasmid pSVOCAT (see material and methods). Data represents an average of three reproducible, independent experiments.

α-globin promoter activity by over 100 fold consistent with Whitelaw et al. (19) (data not shown).

It was possible that increased CAT activity from constructs containing the αPRE was due to promoter activity arising from within the αPRE. To study this question, we inserted the αPRE in both orientations into the BamH 1 site of the promoterless plasmid pSVOCAT (pSVOCAT*) (see figure 1B) and assayed for CAT activity from Putko cells transfected with these construct. No significant difference in CAT activity between pSVOCAT* and pSVOCAT transfected cells was observed. These results suggested that the αPRE can act as an enhancer in transient assays and increase α-globin promoter activity in an orientation independent manner.

Tissue and erythroid developmental specificity of α-globin promoter activation by the αPRE

Since αPRE function is erythroid specific in transgenic mice (8), we wished to determine whether enhancement of the α-globin promoter by the αPRE in transiently transfected cells was also erythroid specific. HeLa cells were transfected with the previously employed constructs by calcium phosphate precipitation. Approximately 48 hours after transfection, CAT activity from cells was assayed. Quantitation of CAT activity can be seen in Figure 3B. Data showed that the αPRE had only slight enhancing effects (2−3 fold) on the α-globin promoter in transiently transfected HeLa cells. This was similar to the level of enhancing capability observed for HS2 of the β-LCR in transiently transfected HeLa cells (15).

Since the above results suggested that optimal αPRE enhancing capability may be erythroid specific, we wished to determine whether enhancing capability was also dependent upon erythroid differentiation. MELCs, a transformed erythroid precursor cell line were employed for these studies. These cells appear to be blocked in development approximately at the stage of the colony-forming cell but can be induced to differentiate with HMBA (28−30). After 48−60 hrs of exposure to HMBA, commitment to terminal erythroid differentiation in these cells becomes inducer independent. During this period, a 10−30 fold increase in the level of globin mRNA can be observed due primarily, to an increase in globin gene transcription (28−29).

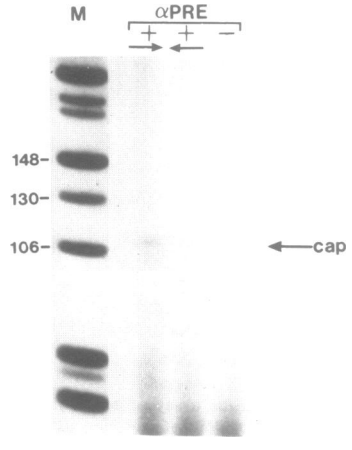

Figure 4. 5′ end primer extension analysis of cytoplasmic mRNA from HMBA induced MELCs transfected with pSVod in the presence (+) or absence (−) of the αPRE (αPRE in genomic → or anti-genomic ← relative to α-globin gene). 20 μg of RNA for each analysis was utilized. Molecular weight markers (M) are shown in left most lane.

MELCs were transfected by electroporation with α-globin promoter/CAT constructs and placed in fresh medium with or without HMBA. After 60 hours, MELCs were harvested for β-galactosidase and CAT assays. Figure 3A is a representative CAT assay from induced cells and figure 3B is quantitation of CAT activities. In the absence of the αPRE (αCAT), low levels of α-globin promoter activity are detected in induced cells (2−3 fold over promoterless CAT plasmid pSVOCAT). In the presence of the αPRE (αCAT*) a 25−30 fold increase in α-globin promoter activity compared to cells transfected with αCAT was observed. The levels of enhancement appeared to be independent of αPRE orientation in αCAT.

In uninduced MELCs, the levels of CAT activity from αCAT transfected cells was identical to that found in induced cells. In the presence of the αPRE, a 3 fold increase in CAT activity was observed. These data suggested that enhancing capability of the αPRE in transient assays was not only tissue specific but dependent upon erythroid differentiation.

In order to rule out the possibility that the increase in CAT activity upon HMBA induction resulted from promoter activity within the αPRE, primer extension experiments were performed. For these experiments the plasmid pSVod* was employed. This plasmid was constructed by placing the αPRE (in opposite orientations) close to the 5′ end of the α-globin gene in order to facilitate the identification of transcripts initiated within the αPRE (see Figure 1B). MELCs were transfected with pSVod* or pSVod and incubated with HMBA for 60 hours. Analysis of cytoplasmic RNA from transfected cells showed that in the presence of the αPRE, only correctly initiated transcripts from the α-globin promoter were detected (Figure 4). These results were independent of αPRE orientation. In the absence of the αPRE, no α-globin transcripts were detected.

Enhancement of a reduced α-globin promoter by the αPRE

The DNA at the 5′end of the human α-globin gene is highly GC rich and contains six sequences that putatively bind the DNA transcription factor SP1 (19). SP1 is known to play an important

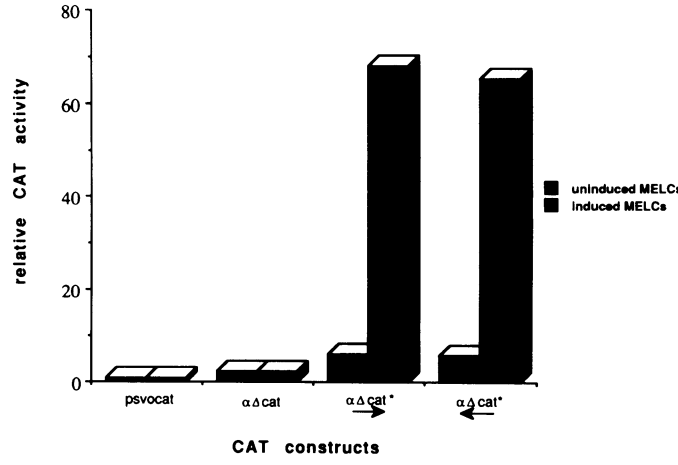

Figure 5. Quantitation of CAT activity from HMBA induced and uninduced MELCs transfected with pSVOCAT, αΔCAT and αΔCAT* (αPRE genomic → and anti-genomic ← relative to CAT). Relative CAT activity determined by comparing CAT activity from cells transfected with a given construct to CAT activity from cells transfected with the promoterless construct pSVOCAT (see material and methods). Data represents an average of three reproducible, independent experiments.

role in the transcriptional activity of a large number of genes (31). It has been observed that deletion of these binding sites decreased SV40 enhanced α-globin promoter activity by up to 3 fold (19). We were interested in determining whether these SP1 binding sites or additional sequences in the α-globin 5′ flanking region were important for enhancement of the α-gobin promoter by the αPRE. For these experiments, the construct αΔCAT was employed. This plasmid contained a reduced α-globin promoter (5′end of construct contains 35 bp DNA flanking the CCAAT box) in which 600 bp of GC rich 5′flanking region (including all SP1 binding sites) were deleted (see Figure 1B). Quantitation of CAT activity can be seen in Figure 5. When the αPRE was inserted into this vector and transfected into MELCs, data showed that the α-PRE enhanced the reduced α-globin promoter to the same degree as the full α-promoter constructs in HMBA induced MELCs. In uninduced MELCs, enhancement by the αPRE was similar to that seen with uninduced cells transfected with CAT*. These experiments suggested that enhancement of the α-globin promoter by the αPRE was not mediated through SP1 binding sites or additional sequences in the 5′ α-globin flanking region but by elements (possibly CCAAT and/or TATA box) within a 150 bp reduced α-globin promoter.

Enhancement of a heterologous promoter by the αPRE upon HMBA induced erythroid differentiation

We were interested in determining if the αPRE could enhance a heterologous promoter upon erythroid differentiation in MELCs. The construct pSV1CAT* was employed in this experiment. This plasmid was constructed by inserting the αPRE into the enhancer trap plasmid pSV1CAT, 3′ of the CAT gene. pSV1CAT contains the SV40 early promoter without its enhancer, driving CAT expression (see Figure 1B). Quantitation of CAT activity for these experiments is in Figure 6. In induced and uninduced MELCs, CAT activity from pSV1CAT did not significantly differ and was between 2−3 fold above the promoterless CAT vector pSVOCAT. Insertion of αPRE to pSV1CAT in either orientation increased SV40 promoter activity by an additional 2−3 fold in uninduced cells, while in induced cells, αPRE increases SV40 promoter activity up to 20 fold over pSV1CAT.

HS2 of the β-LCR has been shown to contain a powerful enhancer whose activity increases upon hemin induction in K562 cells (15,17). We were interested in comparing the enhancing potential of HS2 with the αPRE upon HMBA induced erythroid differentiation of MELCs. We therefore constructed the plasmid pSV1HS2. This construct contains HS2 of the β-LCR inserted at the 3′ end of the CAT gene (see Figure 1B). In uninduced MELCs, HS2 had a modest enhancing effect of 2−3 fold above pSV1CAT transfected cells. Upon HMBA induction, HS2 increased SV40 promoter activity by up to 20 fold above pSV1CAT in an orientation independent manner (see Figure 6). These data suggested that both HS2 and the αPRE were functionally similar in our transient assay system and induced expression of a heterologous non-globin promoter upon erythroid differentiation.

Time course on HMBA activation of αPRE enhancing potential

Within 12−18 hours exposure of MELCs to HMBA, detectable increases in endogenous α-globin gene transcription can be observed. After 48 hours of exposure to HMBA, globin gene transcription has increased a total of 10−30 fold (28,29).

We wished to study the time course of α-globin promoter activation by the αPRE in the presence of HMBA. If maximal

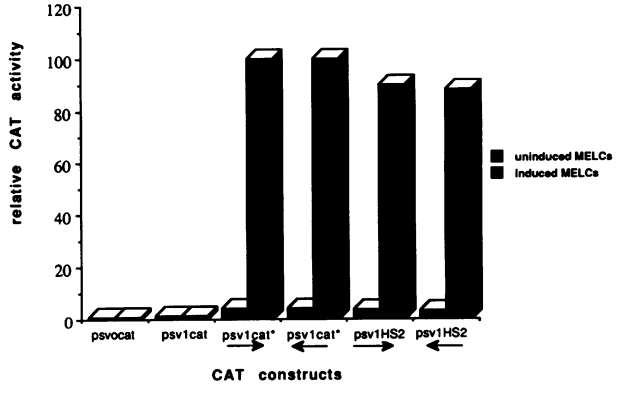

Figure 6. Quantitation of CAT activity from HMBA induced and uninduced MELCs transfected with pSVOCAT, pSV1CAT, pSV1CAT* (αPRE in genomic → and anti-genomic ← relative to CAT) and pSV1HS2 (containing HS2 of β-LCR). Relative CAT activity was determined by comparing CAT activity from a given construct to CAT activity from the promoterless plasmid pSVOCAT (see material and methods). Data represents an average of three reproducible, independent experiments.

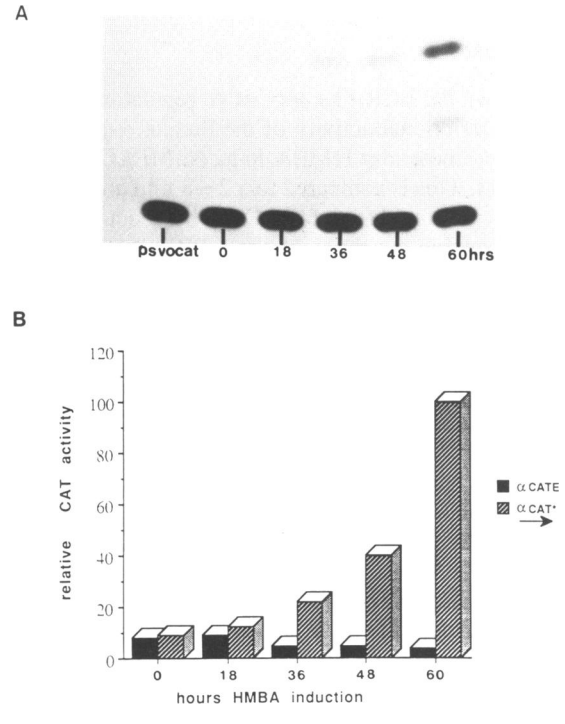

Figure 7. (A) CAT assay showing time course of α-globin promoter activation by the αPRE with continued HMBA exposure. MELCs were transfected with αCAT* (αPRE in genomic position relative to CAT gene) or pSVOCAT. Following electroporation, cells transfected with αCAT* were divided and exposed for 0,18,36,48 or 60 hours to HMBA. CAT assays were performed on equal amounts of protein from cell lysates. (B) Quantitation of CAT activity from cells transfected with αCAT* and αCATE (αCAT containing the SV40 enhancer) with increasing HMBA exposure. Relative CAT activity was determined by comparing CAT activity of transfected cells incubated for varying periods of time with HMBA to cells transfected with the promoterless plasmid pSVOCAT. Data represents an average of two reproducible, independent experiments.

αPRE enhancer function requires erythroid differentiation, CAT activity from αPRE/α−globin constructs should increase with continued exposure of MELCs to HMBA. MELCs were transfected with αCAT* and split into 5 separate flasks. 5 mM HMBA was added to each flask 0, 12, 24 or 42 hours post-transfection. One flask received no HMBA. In order to demonstrate that increases in α-globin promoter activity were specific to constructs containing the αPRE, MELCs were transfected with αCATE (Figure 1B) and cultured with HMBA as above. Since SV40 enhancer function is not dependent upon erythroid differentiation, CAT activity from cells transfected with this construct should remain constant with continued HMBA exposure. 60 hours after transfection, cells were harvested and CAT assays performed on equal amounts of protein extracts from cells. Representative CAT assays and quantitation of data can be seen in figure 7A and 7B.

When cells transfected with αCATE were exposed to HMBA for increasing periods of time, α-globin promoter activity remained fairly constant, although a slight decrease was noted after 36 hrs of HMBA exposure. In the presence of the αPRE, however, α-globin promoter activity progressively increased until a greater than 10 fold enhancement of promoter activity was observed between 48−60 hrs of HMBA induction. Analysis of RNA from these cells showed similar increases in endogenous mouse α-globin mRNA with continued HMBA exposure (data not shown). These data suggested that αPRE enhancing potential was positively correlated with erythroid differentiation of MELCs in culture.

DISCUSSION

We have shown the LCR-like α-positive regulatory element can increase transcriptional activity of the human α-globin and the SV40 early promoters in HMBA induced MELCs by as much as 25−30 fold. This is compared to a 2−3 fold enhancing effect in Hela cells or uninduced MELCs. These data suggest that maximal enhancing potential of the αPRE is not only tissue specific, but may require erythroid differentiation. Similar studies on the β-LCR have shown that the enhancing potential of HS2 in transiently transfected K562 cells increased by as much as 10 fold upon hemin induced erythroid differentiation (15). In our assay system, the αPRE and HS2 have similar enhancing potential both prior to and after induction of erythroid differentiation by HMBA in MELCs. These data suggest that the αPRE and HS2 of the β-LCR share similar functions and that induction of their enhancing potential may play an important role in the coordinated increase in globin gene expression seen during erythroid differentiation.

We have shown that in transient assays, the αPRE can induce a non-globin gene (SV40) in a tissue specific manner. This characteristic is shared by HS2 of the β-LCR (16). In addition, the herpes simplex virus (HSV) thymidine kinase (TK) (13) and murine Thy1 promoters (32) have been shown to become inducible in MELCs in the presence of the β-LCR. Higgs et al. (8) and Jarman et al. (21) reported that MELCs transfected with α-globin/αPRE neo constructs had significantly higher numbers of colonies upon G418 selection than those transfected with α-globin/neo constructs without the αPRE. It is likely that the SV40 and TK promoter driving neo resistance in these constructs was enhanced by the presence of the αPRE. However, we do not know whether the element in the αPRE acting as an enhancer in our transient assays is responsible either alone, or in conjunction with additional sequences for this effect.

Antoniou and Grosveld (33) showed that the β-LCR can induce high level expression from a promoter containing only a TATA box in combination with a CCAAT or CAC box. We demonstrated that the level of the α-globin promoter enhancement by the αPRE was not effected by the removal of 600 bp of 5′ flanking sequences containing 6 SP1 binding sites. We therefore hypothesize that the αPRE is capable of interacting with the CCAAT and/or TATA in our transient assay system.

When MELCs in culture are treated with HMBA, detectable increases in globin gene transcription occur as early as 12−18 hours. After 48 hours of exposure to inducer, a 10−30 fold overall increase in endogenous globin gene transcription occurs (28,29). Our data show that a detectable increase in human α-globin promoter activity, in the presence of the αPRE, occurs within 18 hours exposure to HMBA. α-Globin promoter activity progressively increases until maximal activity is reached between 48−60 hours exposure to HMBA. These data suggests that the level of enhancing potential of the αPRE in MELCs is positively correlated with increasing erythroid differentiation of MELCs in culture. Therefore, despite the presence of DNase I hypersensitive sites in the αPRE in uninduced MELCs (8) at least some aspects of its functioning may be dependent upon erythroid differentiation.

We have demonstrated that in the absence of the αPRE, no increased α-globin promoter activity is observed upon HMBA induction. Campbell et al. (34) showed that HMBA induced the expression of a transiently transfected human α-globin gene up to 20 fold in the absence of the αPRE. However, the increase in α-globin transcription occurred and disappeared long before detectable increases in endogenous α-globin gene transcription was observed. In the presence of the α-PRE however, we have observed progressive increases in α-globin promoter activity detectable after 18 hours of HMBA exposure. Following 48−60 hours of exposure to HMBA, up to 30 fold enhancement of the−globin promoter is achieved. This pattern is more characteristic of endogenous α-globin induction than the previous mentioned study. Our data therefore suggest that induction of the α-globin gene can only be understood by studying the interaction between the αPRE and the α-globin gene promoter during erythroid differentiation.

Ney et al. (15) showed that two tandem AP1 consensus sequences in HS2 are responsible for its ability to act as an inducible enhancer in transient assay. These sequences were shown to bind the transcription factor NF-E2. Recent data has shown the presence of two AP1 consensus sequences separated by 25 bp of DNA in the αPRE (21). It remains to be determined whether these sequences are responsible for the αPRE's ability to function as an inducible enhancer in our experimental system. The assay system employed in this study may prove useful in identifying erythroid specific enhancing elements within the αPRE and examining their interaction with the α-globin promoter. These studies may help us to more clearly understand the mechanism(s) responsible for the coordinated increase of α and β-globin gene expression during terminal erythroid differentiation.

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