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DNA Sequences Involved in Transcriptional Regulation of the Mouse β-Globin Promoter in Murine Erythroleukemia Cells

ALISON COWIE AND RICHARD M. MYERS*

Department of Physiology, University of California, 513 Parnassus, San Francisco, California 94143

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We have developed a transient assay in murine erythroleukemia (MEL) cells to analyze the *cis*-acting sequence requirements for transcriptional regulation of the mouse β-major-globin promoter. From deletion analysis, a fragment of the promoter region, from −106 to +26 relative to the RNA cap site, was found to be sufficient for regulated transcription in MEL cells following induction of differentiation by dimethyl sulfoxide. Single-base mutational analysis of this 132-base-pair promoter fragment identified three sequence elements required for transcription in MEL cells. These are the ATATAA sequence at −31 to −26, the CCAATC sequence between −77 and −72, and the GCCACACCC sequence between −95 and −87. In addition, we found a requirement for sequences adjacent to the CCAAT and ATATAA consensus motifs. Point mutations within the promoter did not abolish transcriptional regulation following induction of differentiation by dimethyl sulfoxide. However, mutations that resulted in reduced transcription levels in uninduced MEL cells gave similarly decreased levels in induced MEL cells.

The adult β-globin genes exhibit tissue-specific and developmentally specific regulation, being expressed only in adult erythroid cells in the final stages of differentiation. A prerequisite towards understanding the mechanisms of β-globin expression is the identification of *cis*-acting DNA sequences involved in the regulation of transcription of the gene in erythroid cells. Murine erythroleukemia (MEL) cells offer a number of advantages as a model system for the study of adult globin regulation. These established cell lines, derived from adult hematopoietic precursor cells transformed with the Friend virus complex (14), are easily propagated in culture and will undergo terminal differentiation in response to a number of different inducing agents such as dimethyl sulfoxide (DMSO), hexamethylenebisacetamide (HMBA), and hemin, closely mimicking normal events of hematopoiesis (for a review, see reference 25). Among the many morphological and biochemical changes that occur during this induced differentiation of MEL cells is a 10- to 50-fold increase in the level of β-globin RNA. When cloned mouse, rabbit, or human β-globin genes are introduced into MEL cells by DNA-mediated gene transfer, they are regulated in a manner similar to that of the endogenous globin genes (3, 28, 39, 40). This increase in the amount of β-globin RNA is due in part to an increase in the rate of transcription (20, 40) and in part to an increase in RNA stability (37).

Studies of hybrid mouse and human β-globin genes have suggested the existence of multiple control elements within and adjacent to the gene involved in this increase in RNA synthesis following induction of differentiation. It was demonstrated for mouse and human β-globin that sequences 5′ to the gene, including the promoter and upstream region, and sequences 3′ to the initiation codon, including the coding region and 3′-flanking sequences, were independently able to bring about the increase in specific RNA in response to induction (5, 32, 40). These earlier studies were carried out by introducing cloned DNAs into MEL cells to generate stable cell lines following selection for a dominant genetic marker. It was found that in many but not all of the resulting cell lines, the introduced gene was coregulated with the endogenous gene. However, the response to induction and the level of transcription from the exogenous genes varied widely between different clonal isolates. Because of this variability in expression and regulation, which is presumably due to effects from the surrounding chromatin at the site of integration, a detailed analysis of the sequences required for transcription and induction of β-globin in MEL cells has so far not been possible.

The *cis*-acting DNA sequences required for transcription of the mouse and rabbit β-globin promoters in nonerythroid cells such as HeLa cells and 3T6 cells have been extensively studied (4, 10, 18, 19, 30). Unlike the studies with MEL cells that used long-term assays, expression from DNAs introduced into HeLa cells and 3T6 cells is analyzed transiently, thereby avoiding some of the problems of variability associated with long-term assays. These studies identified sequence elements involved in the constitutive, nonregulated transcription of the β-globin promoters in nonerythroid cells. However, the relevance of these sequence elements to regulated β-globin transcription in erythroid cells remained unknown.

To circumvent the problems of long-term assays, we have developed a transient assay in MEL cells. Here we analyze only those sequences 5′ to the gene, to avoid potential interference from possible regulatory elements within the gene, linking the mouse β-globin promoter to the mouse metallothionein (MT) gene. Deletion analysis of the mouse β-globin promoter revealed that sufficient information to bring about the increase in RNA level, in response to induction of differentiation by DMSO, lies within −106 to +26 base pairs (bp) of the mRNA cap site. We have undertaken a detailed analysis of this 132-bp promoter fragment by using point mutations to identify those 5′ sequences involved in transcription and the induction response.

MATERIALS AND METHODS

Plasmid constructions. A 2.2-kilobase-pair genomic DNA fragment containing the coding sequence of the mouse metallothionein-1 gene (16) was inserted into pSP73 (23) between the *Bgl*II and *Eco*RI sites. Mouse β-globin pro-

* Corresponding author.

moter fragments were fused to the mouse MT gene at the *Bgl*II site at +65 in the MT sequence to generate the plasmids pβMTΔ106, pβMTΔ300, and pβMTΔ1200. A 300-bp *Pst*I-to-*Sac*I fragment from the Friend spleen focus-forming virus ($SFFV_A$) long terminal repeat (LTR) containing the putative enhancer element (6) was inserted 3′ of the MT sequence to give the plasmids pβMTF, pβMTΔ300F, and pβMTΔ1200F. pΔSVneoF was constructed by deleting the *Pvu*II-to-*Sph*I fragment containing the 72-bp repeats of the simian virus 40 (SV40) enhancer from pSV2neo (35) and adding the Friend enhancer fragment at the 3′ end of the *neo* gene. Previously generated point mutants (30) were subcloned into pβMTF by exchanging the wild-type *Cla*I-to-*Bgl*II promoter fragment with the equivalent fragment from the mutants. pβneoF was constructed by replacing the *Bgl*II-to-*Eco*RI MT gene fragment in pβMTF with the *Bgl*II-to-*Eco*RI neo/SV40polyA site fragment from pSV2neo (35). All enzymes were purchased from New England BioLabs, Inc., and used as recommended. Plasmids were propagated in *Escherichia coli* HB101, and plasmid DNA was prepared by a modification of the alkaline lysis method (36).

Cells. MEL cells (aprt$^-$; 8) were grown in Dulbecco modified Eagle medium (DME) supplemented with 10% fetal calf serum (Hyclone). Cells were split 24 h prior to transfection and replated onto 60-mm Primaria dishes (Becton Dickinson Labware). MEL cells, which normally grow in suspension, adhere to these dishes, which are chemically treated so that the net electrostatic charge of the plastic is positive, thus promoting adherence of nonfibroblast cell types. DEAE-dextran-mediated transfection was carried out as follows: 2 μg of supercoiled plasmid DNA in 0.5 ml of 0.5 mg/ml DEAE-dextran (Sigma Chemical Co.) in Tris-buffered saline (9) was added to the cells for 40 min at room temperature. The DNA was removed, and the cells were washed with Tris-buffered saline and then treated with 0.1 mM chloroquine (Sigma) in DME plus 10% fetal calf serum for 4 h at 37°C (2.5 ml per plate). After the chloroquine was removed, the cells to be induced were allowed to recover for 1 h before addition of DMSO to 2% to the culture medium. Cytoplasmic RNA was made from the cells 44 to 48 hr posttransfection. Transfection of DNAs for long-term assays was by the calcium phosphate coprecipitation method (38). For the selection, G418 (Geneticin; GIBCO Laboratories) was added to the culture medium at 0.5 mg/ml.

RNA analysis. Cytoplasmic RNA was isolated by the method of Favaloro et al. (13). Specific RNAs were determined by S1 analysis by using 5′-end-labeled, single-stranded DNA probes (22). Probes were made by primer extension from specific oligonucleotides on single-stranded plasmid DNAs. The oligonucleotides were first labeled with polynucleotide kinase (New England BioLabs) and [γ-^{32}P]ATP (>7,000 Ci/mmol; ICN Pharmaceuticals Inc.) and then annealed to a single-stranded plasmid (29) containing the sense strand of the DNAs used in the transfections and elongated by addition of deoxynucleoside triphosphates (dNTPs; Pharmacia) and Klenow polymerase (New England BioLabs). The newly synthesized double-stranded region was cut with a single-site restriction enzyme (in this case, *Cla*I), and the labeled single-strand probe fragment was separated from the vector strand by denaturing acrylamide gel electrophoresis and recovered by crush-soak elution (26). For each analysis, 30 μg of RNA was hybridized at 30°C overnight with 0.01 pmol of probe. S1 digestion was carried out at 10°C for 2 h with 400 U of S1 nuclease (Sigma) per ml as previously described (13). The samples were fractionated on 12% polyacrylamide–50% urea denaturing gels (24). Specific bands were quantified by excision from the gel and Cerenkov counting.

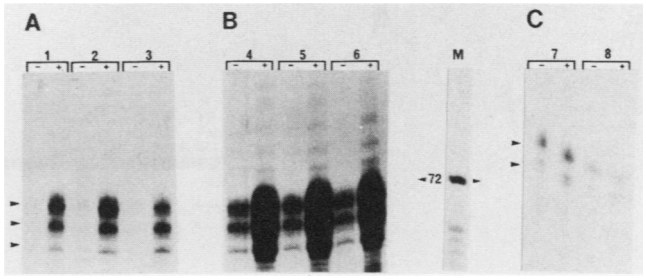

FIG. 1. S1 analysis of cytoplasmic RNA from promoter deletions in uninduced (−) and induced (+) MEL cells. (A) Lanes 1, Plasmid pβMTΔ106; lanes 2, pβMTΔ300; lanes 3, pβMTΔ1200. (B) Lanes 4, pβMTF (Δ106 construct) after addition of the Friend virus enhancer; lanes 5, pβMTΔ300F; lanes 6, pβMTΔ1200F. (C) Lanes 7 and 8, ΔSVneoF, two independent transfections. ►, Protected fragment; the number of protected fragments observed is determined by the S1 digestion conditions. M, Size marker.

RESULTS

Sequences 5′ of the coding region of the mouse and human β-globin genes have been shown to be sufficient to bring about at least some of the increase in RNA observed in response to induction of differentiation of MEL cells by DMSO or HMBA (5, 32, 40). Furthermore, Rutherford and Nienhuis (32) demonstrated that sequences of the human β-globin promoter from −385 to +34 from the RNA cap site were sufficient for expression and regulation in MEL cells in long-term assays. To determine the minimum 5′ sequence of the mouse β-globin promoter required for the induction response in MEL cells, we constructed three deletions of the 5′-flanking region of the promoter, linked at position +26 of the globin gene to position +65 of the mouse metallothionein (MT) gene. These are the plasmids pβMTΔ1200, pβMTΔ300, and pβMTΔ106, where the number indicates the length of 5′ sequence present relative to the RNA cap site at +1. When introduced into MEL cells in a transient assay, the three plasmids behaved similarly, producing equivalent amounts of RNA initiating from the β-globin promoter, as measured by quantitative S1 analysis. They were all positively regulated after induction with DMSO for 44 h, again producing equivalent amounts of RNA (Fig. 1A). These same deletion mutants were introduced into MEL cells together with the neomycin resistance gene under the control of the herpes simplex virus thymidine kinase promoter to establish long-term cell lines following selection with G418. The results obtained from S1 analysis of cytoplasmic RNA from several pools of colonies from each DNA were the same as those seen in the transient assay in that all three deletions were positively regulated after induction and gave similar levels of RNA (data not shown). This suggests that the only 5′ sequences required for the induction response lie between −106 and +26 bp from the RNA cap site. The amount of RNA transcribed from the endogenous MT gene is unaltered during MEL cell induction, whereas the level of transcription from the endogenous β-globin gene increases (data not shown).

In the transient assay, the amount of RNA from the transfected genes observed after induction was relatively high; however, the level before induction was barely detectable (Fig. 1A, compare lanes − and +). To analyze muta-

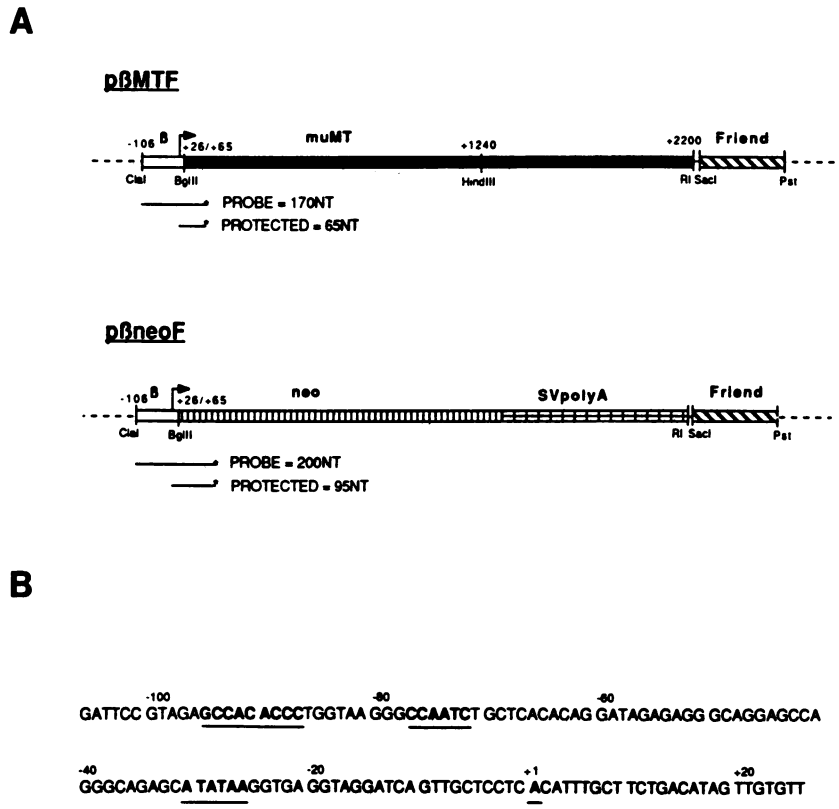

FIG. 2. Plasmids used for the mutational analysis. (A) pβMTF wild-type and pβneoF reference plasmids; the positions of S1 probes and the sizes of the protected fragments are shown. β, 132-bp mouse β-major-globin promoter fragment; muMT, genomic DNA fragment containing the mouse metallothionein gene; neo, neomycin resistance gene; SVpolyA, the SV40 poly(A) addition site; Friend, the 300-bp fragment from the Friend virus LTR. (B) Sequence of the 132-bp mouse β-major-globin promoter. Sequence elements required for transcription in HeLa cells are indicated in underlined boldface type.

tions within the promoter, it was necessary to increase the amount of RNA initiating from the mouse β-globin promoter. For this purpose, a fragment from the Friend virus LTR containing a putative enhancer element (6) known to be active in erythroid cells (2) was cloned into the deletion mutant constructs at the 3′ end of the MT gene to give the plasmids pβMTΔ1200F, pβMTΔ300F, and pβMTF (pβMTF, the Δ106 construct, is shown in Fig. 2A). The results from transient assay of the three promoter deletions with the added Friend enhancer are shown in Fig. 1B. The level of transcription from the mouse β-globin promoter is increased 10- to 20-fold; however, the response to induction remains unaltered. To determine whether the Friend enhancer was contributing to the induction response, it was cloned into a derivative of pSV2neo (35), in which a fragment containing the 72-bp repeats of the SV40 enhancer had been deleted and functionally replaced by the Friend enhancer. The SV40 promoter transcribes poorly in these cells. However, the amount of RNA observed from this plasmid is clearly the same in both uninduced and induced cells (Fig. 1C); with no enhancer or with the SV40 enhancer, no RNA was detected (data not shown).

To analyze further the sequence requirements for transcription of the mouse β-globin promoter in erythroid cells, we transferred single-base mutations into the plasmid carrying the mouse MT gene and the Friend enhancer (pβMTF; Fig. 2A) by a simple exchange of the wild-type promoter fragment for the corresponding mutant fragment. As an internal control for wild-type β-globin promoter function, the plasmid pβneoF, in which the wild-type mouse β-globin promoter is linked to the neo/SV40polyA fragment from pSV2neo (35) and the same Friend enhancer fragment (Fig. 2A), was included in all transfections. The amount of correctly initiated RNA from the transfected DNAs was determined by quantitative S1 analysis. The positions and sizes of the probes used are shown schematically in Fig. 2A. The wild-type sequence of the 132-bp mouse β-globin promoter fragment used in these plasmids is given in Fig. 2B, with the sequence elements previously shown to be required for maximum transcription in HeLa cells indicated in underlined boldface type (30). Cytoplasmic RNA was isolated 48 h after transfection from uninduced cells or from cells to which DMSO had been added 4 to 6 h after addition of the DNA. The amount of correctly initiated RNA from both the βMT and βneo plasmids was determined by S1 analysis followed by separation on denaturing polyacrylamide gels (Fig. 3). The amount of RNA represented by each band was quantified by excision of the gel piece and Cerenkov counting. The βMT test signal for each sample was normalized to the βneo internal control (ref), and the relative transcription level (RTL), compared to the wild type, for the uninduced and induced signals for each mutant was determined. The results from three or four independent transfection experiments were averaged and are listed in Table 1. A graphic representation of the RTL values obtained for all of the mutations tested is shown in Fig. 4.

From the mutations tested, three sequence elements were demonstrated to affect transcription in both uninduced and

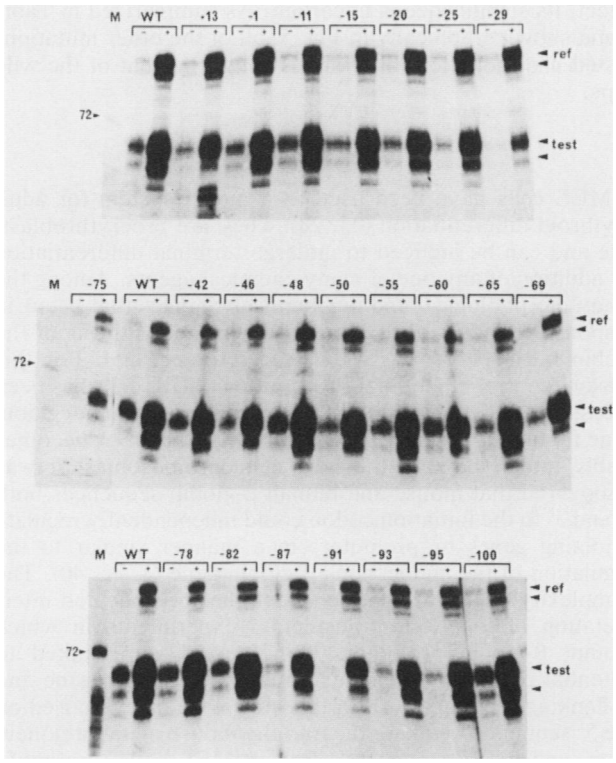

FIG. 3. S1 analysis of cytoplasmic RNA from wild-type and mutant promoter plasmids, in uninduced (−) and induced (+) MEL cells. Numbers above the paired lanes refer to the position of the mutation in the promoter region, relative to the cap site at +1. ►. Protected fragment; ref, βneo; test, βMT. The number of protected fragments observed is determined by the S1 digestion conditions. M, Size marker.

induced MEL cells. One element identified lies between positions −95 and −87 relative to the RNA cap site at +1. Mutations within this region decreased transcription levels in uninduced cells to between 50% and 20% of the wild-type level. In induced cells, the effects of the individual mutations

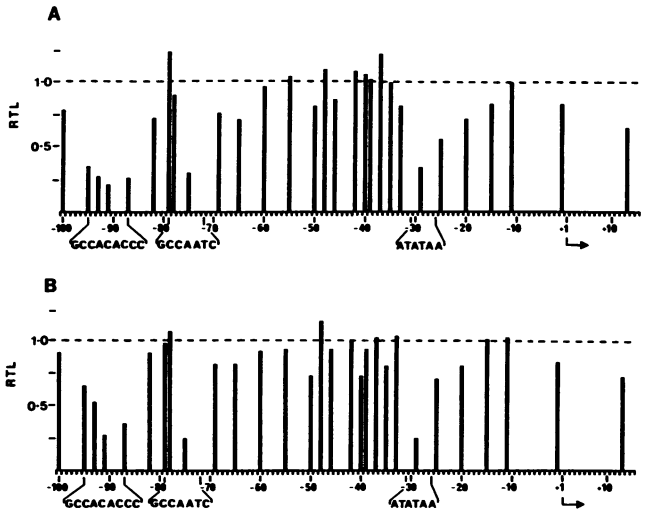

FIG. 4. RTL of promoter mutations, with respect to position in the sequence, in uninduced MEL cells (panel A) and induced MEL cells (panel B). Wild-type value = 1.0.

TABLE 1. Summary of promoter mutations

DNA position	Mutation	RTL[a]		Induction ratio[b]
		Uninduced	Induced	
WT[c]	None	1.0	1.0	100
+13	G to A	0.63	0.71	113
−1	C to T	0.81	0.82	104
−11	A to G	0.97	1.01	108
−15	G to A	0.81	1.0	126
−20	G to A	0.7	0.8	119
−25	G to T	0.55	0.7	118
−29	T to G	0.33	0.24	73
−33	G to T	0.8	1.03	131
−35	G to A	0.97	0.8	94
−37	C to A	1.19	1.01	114
−39	G to A	1.0	0.93	80
−40	C to A	1.04	0.72	56
−42	G to A	1.06	0.99	100
−46	G to A	0.85	0.93	117
−48	A to T	1.07	1.14	111
−50	G to A	0.8	0.72	90
−55	A to G	1.03	0.93	88
−60	G to A	0.95	0.91	97
−65	C to A	0.69	0.81	114
−69	C to A	0.75	0.81	119
−75	A to G	0.29	0.25	95
−78	G to A	0.88	1.06	114
−79	G to A	1.22	0.98	80
−82	A to G	0.71	0.9	126
−87	C to T	0.25	0.36	136
−91	C to A	0.2	0.27	138
−93	C to T	0.27	0.52	179
−95	G to A	0.34	0.64	192
−100	G to A	0.77	0.9	113

[a] RTL calculated as (cpm of mutant ÷ cpm of βneo reference)/(cpm of wild type ÷ cpm of βneo reference), where cpm is counts per minute.
[b] Induction ratio calculated as (cpm of βMT induced ÷ cpm of βMT uninduced)/(cpm of βneo reference induced ÷ cpm of βneo reference uninduced) expressed as a percentage of the wild-type value.
[c] WT, Wild type.

were somewhat less; for example, RNA levels from the −93 mutation were reduced to about 25% of the wild-type level in uninduced cells but to only about 50% in induced cells (Fig. 3, −93 lanes; Table 1). These mutations all lie within the sequence GCCACACCC, shown previously to be required for transcription of the mouse β-globin promoter in HeLa cells (4, 30) and the rabbit β-globin promoter in HeLa cells and 3T6 cells (10, 18).

The second element was identified around position −75. A mutation at −75 caused a decrease in transcription to approximately 20% of the wild-type level in both uninduced and induced cells (Fig. 3, −75 lanes; Table 1). This mutation lies within the sequence CCAATC, an element also shown to be required for transcription of the mouse β-globin promoter in HeLa cells (4, 30) and the rabbit β-globin promoter in HeLa cells and 3T6 cells (10, 19). Mutations in the sequence adjacent to this CCAATC motif, at positions −69 and −65, reduced the transcription level to about 70% of the wild-type level in uninduced cells and 80% of the wild-type level in induced cells (Fig. 3, −69 and −65 lanes; Table 1). Although the CCAATC sequence was shown to be required for transcription of the mouse β-globin promoter in HeLa cells, no effect of adjoining downstream sequences was observed when analyzed by point mutations (30). Interestingly, two G-to-A point mutations at positions −78 and −79 increased β-globin transcription in HeLa cells by three- to fourfold (30), whereas in MEL cells both mutations retained wild-type activity (Fig. 3, −78 lanes; Table 1).

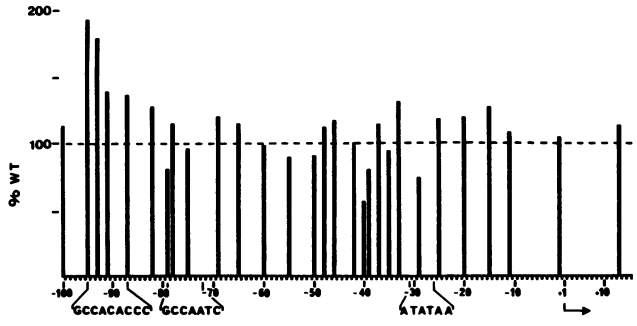

FIG. 5. Induction ratios for promoter mutations with respect to position in the sequence. Wild-type (WT) value = 100.

The third sequence element was identified by a mutation at position −29, within the ATATAA sequence. Again, this sequence was shown to be required for transcription of the mouse β-globin promoter in HeLa cells (4, 30) and the rabbit β-globin promoter in HeLa cells and 3T6 cells (10, 19). This mutation resulted in transcription levels approximately 20% of wild type in both uninduced and induced MEL cells (Fig. 3, −29 lanes; Table 1). This was also the case in HeLa cells (30). In MEL cells, mutations downstream of the ATATAA sequence, at −25, −20 and +13, reduced transcription by 30% to 50%, whereas these mutations resulted in wild-type levels in HeLa cells (30). A mutation at position −37, which had resulted in a 50% reduction in transcription in HeLa cells (30), remained at the wild-type level in MEL cells (Table 1).

To determine whether possible effects of negative elements were being masked by the presence of the Friend enhancer, several of the mutations that retained wild-type, or close to wild-type, activity were analyzed in the absence of the Friend virus enhancer fragment. All of the mutations tested in this way resulted in relative RNA levels similar to that with the Friend enhancer present. The absolute amounts of RNA transcribed were, however, greatly reduced (data not shown). Therefore, we conclude that the Friend enhancer is not affecting regulation of the β-globin promoter, and regulation does not involve derepression of negative elements.

Comparison of lanes − and + in Fig. 3 reveals that none of the mutations tested eliminated the response to induction of differentiation by DMSO. Those mutations that resulted in decreased transcription in uninduced cells also showed decreased transcription in induced cells. However, by comparing panels A and B in Fig. 4 it can be seen that the mutations between −95 and −87 had significantly higher RTL values in induced MEL cells than in uninduced cells. To illustrate changes in the extent of induction for the various mutations, the ratio between the amount of induced RNA and uninduced RNA, normalized to the ratio obtained from the cotransfected βneo signals, was calculated for both wild-type and mutant promoters. In Table 1, this induction ratio is expressed as a percentage of the wild-type value for each of the mutations tested. The values for the induction ratio obtained for the mutations between −95 and −87 were significantly higher than the wild-type value, up to 200% (Fig. 5; Table 1). An induction ratio significantly lower than the wild-type value was observed only with the mutation at position −40. For this mutant, the increase in transcription after induction was less than that observed for the wild-type promoter; the induction ratio was only 50%. Because this is the only mutation within the promoter region to show this effect, its significance is uncertain. As summarized in Table 1 and shown graphically in Fig. 5, all of the other mutations tested had induction ratio values similar to that of the wild type.

DISCUSSION

MEL cells have been used as a model system for adult erythroid differentiation (25, 28). They are proerythroblast-like and can be induced to undergo terminal differentiation by addition of any one of many chemical agents. Among the many morphological and biochemical changes that occur in response to induction is an increase in the synthesis of the globin mRNAs (for a review, see reference 25). For this reason, control of globin transcription in MEL cells has been the subject of much study. By introducing cloned globin gene fragments into MEL cells such that the DNA becomes stably integrated into the host cell chromosomes, it was discovered that mouse and human β-globin sequences both 5′ and 3′ to the initiation codon could independently regulate adjoining genes or promoters in a manner similar to the regulation of the endogenous β-globin gene (4, 32, 40). The complexity of this regulation system has complicated interpretation of long-term transfection experiments in which mutant β-globin promoters being tested were linked to potential regulatory elements within the β-globin gene and 3′-flanking sequences (4). In this study, we have focused on the 5′ sequences, linking them to the mouse metallothionein gene, and used a transient assay in MEL cells to identify sequences within the promoter required for transcription in uninduced and induced cells and sequences involved in the induction response.

By deletion analysis of the mouse β-globin promoter in transient assay with and without the Friend enhancer (Fig. 1) and also in long-term assay in the absence of the enhancer (data not shown), we determined that all 5′ sequences required for expression and regulation lie between −106 to +26 relative to the mRNA cap site. This result is at variance with that obtained by Antoniou et al. (1) with the human β-globin promoter in MEL cells. They identified a sequence at −160 required for regulation after induction. Although there is considerable homology between the mouse and human β-globin genes (12), it appears from our study that there may be a difference in requirements for regulation. Alternatively, the discrepancy between the two results may be due to differences in the parent MEL cell lines used or plasmid constructs and transfection methods used.

We found three sequence elements, within the −106 to +26 β-globin promoter fragment, required for maximum transcription in both uninduced and induced MEL cells: the GCCACACCC sequence at position −95 to −87, the CCAATC element at position −77 to −72, and the ATATAA box at −31 to −26. These sequence elements have been previously determined to be required for transcription from the mouse β-globin promoter in HeLa cells (4, 30) and the rabbit β-globin promoter in HeLa cells and 3T6 cells (10, 18, 19). However, comparison of the sequence requirements of the mouse β-globin promoter in HeLa cells and MEL cells revealed significant differences. Most striking was the difference in the effect of point mutations at −78 and −79, adjacent to the CCAAT box. In HeLa cells, these mutations caused a three- to fourfold increase in the level of RNA observed (30); in MEL cells, they remained at wild-type level. In contrast, point mutations at −69 and −65 reduced transcription in MEL cells but not in HeLa cells (29). These differences around the CCAAT consensus sequence may

reflect a difference in CAAT-binding proteins in MEL cells and HeLa cells, suggesting a possible role for this sequence in the differential expression of β-globin in the two cell types. Comparison of the sequences of different globin genes from mouse, rabbit, goat, and human reveals homology within each globin family between the sequences immediately 5′ and 3′ of the CCAAT core element. For example, the β-globins have (A/G)GCCAATCT(A/G)C, the γ-globins have GACCAATAG(C/T)C, and the α-globins have AGC CAATGA (12). Mutations at the 5′ G residue of the human γ-globin CCAAT box are implicated in the Greek hereditary persistence of fetal hemoglobin syndrome, suggesting a possible role for the sequence in developmental regulation of that gene (7, 15). This observation is in accordance with several recent reports indicating the existence of multiple CAAT factors present in different cell types and acting on different promoters (11, 17, 21, 27).

Another sequence-dependent difference between transcriptional effects in MEL and HeLa cells was observed within the sequence immediately downstream of the ATA TAA consensus at −30. Only mutations within the ATA TAA sequence affected transcription in HeLa cells (4, 30) whereas in MEL cells, mutations outside of this region at −25 and −20 were reduced in transcriptional activity by 30% to 50%. Similarily a mutation at +13 gave reduced RNA levels only in MEL cells. One possible explanation which would account for these differences is the existence of multiple cell-type-specific factors interacting at the TATA consensus sequence that involve adjacent downstream sequences for full recognition. DNase I footprint analysis of TATA binding proteins isolated from *Drosophila* and HeLa cells revealed that these proteins protected an extensive region from DNase I digestion downstream of the TATA core binding motif (31, 33).

Which sequences within the promoter are involved in the induction response remains unclear. All of the point mutations tested responded with an increase in RNA following induction of differentiation with DMSO. Mutations in the CCAAT and ATA sequences gave correspondingly low levels before and after induction, i.e., similar RTL values. This resulted in the ratio between the uninduced RNA and the induced RNA level being the same as for the wild-type promoter (Table 1; Fig. 5). However, there were subtle differences in the behavior of some of the mutant promoters after induction. For some of the plasmids bearing mutations within the GCCACACCC sequence, the RTL in induced MEL cells was significantly higher than that in uninduced cells (−95, −93, −91 in Table 1 and Fig. 4). Therefore, the value for the induction ratio was significantly higher than for the wild-type promoter (Table 1; Fig. 5). A reduced requirement for this sequence, and presumably the factor that binds to it, after induction of differentiation is a possible explanation for this difference between the uninduced and induced cells. Alternatively, induction may stimulate production of another transcription factor(s) which interacts with this sequence but with different recognition requirements, or the factor acting at this sequence may be modified in some way after induction so that it recognizes the sequence differently.

There are several possible reasons as to why none of the promoter mutations tested eliminated the induction response. It is possible that the critical sequence was missed because not every nucleotide within the promoter sequence was analyzed. We consider this unlikely because mutations every 2 to 5 bases between positions −100 and −11 were tested. Only two mutations between −11 and +26 were analyzed, but both lay in known conserved sequences of the promoter region (12). The mutant promoters analyzed contained only single-base mutations. This may not have led to sufficient disruption of any putative control element to permit detection. However, we found that single-base changes in the GCCACACCC, CCAATC, and ATA sequences were sufficient to detectably disrupt function. Alternatively, we may have been unable to identify a specific sequence responsible for the induction response because it overlaps one or more of the three elements identified as being necessary for transcription.

MEL cells, when passaged in culture, undergo spontaneous induction at a low frequency. It is not known whether the low level of endogenous β-globin transcription observed in uninduced MEL cells is due to an actual low level in all cells of the population or to a high level in a small percentage of cells that have undergone or are undergoing spontaneous induction. Our results suggest that if a transfected plasmid carrying a β-globin promoter is capable of transcription, even at a reduced rate, then the level of transcription will increase upon induction. In addition, studies of the chromatin structure around the endogenous β-globin gene in MEL cells have demonstrated the appearance after induction of a DNase I hypersensitive site (34). This site appears immediately 5′ of the gene in the promoter region between positions −200 and −50 (34). This observation suggested the possibility of the existence of specific protein factors interacting with the β-globin promoter after the onset of induction. Our inability to identify a specific sequence element separate from those required for transcription may indicate that they are one and the same. The increase in transcription and the appearance of the DNase I hypersensitive site, observed after induction, may be because of an increase in the availability and binding of erythroid cell-specific transcription factors interacting at the GCCACACCC, CCAAT, and/or ATATAA sequences of the β-globin promoter.

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