PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Human <latex>α</latex>- and <latex>β</latex>-Globin Gene Transcription in Mouse Erythroleukaemia Cells

P. Charnay, R. Treisman, Pamela Mellon, M. Chao, R. Axel and T. Maniatis

Phil. Trans. R. Soc. Lond. B 1984 **307**, 261-270
doi: 10.1098/rstb.1984.0126

Phil. Trans. R. Soc. Lond. B **307**, 261–270 (1984) [261]
Printed in Great Britain

Human α- and β-globin gene transcription in mouse erythroleukaemia cells

By P. Charnay[1], R. Treisman[1], Pamela Mellon[1], M. Chao[2], R. Axel[2] and T. Maniatis[1]

[1] *Harvard University, Department of Biochemistry and Molecular Biology, 7 Divinity Avenue, Cambridge, Massachusetts* 02138, *U.S.A.*
[2] *Institute of Cancer Research, Columbia University, College of Physicians and Surgeons, 701 W 168th Street, New York, N.Y.* 10032, *U.S.A.*

Human β-globin genes introduced into mouse erythroleukaemia (MEL) cells by DNA co-transformation are correctly regulated when erythroid cell differentiation is induced by dimethylsulphoxide (DMSO). In contrast, cloned human α-globin genes are efficiently transcribed in MEL cells before induction, and no increase in the level α-globin mRNA is observed when the cells differentiate. These observations suggest that the mechanisms by which α- and β-globin genes are activated during erythroid cell differentiation are fundamentally different. Analysis of the transcription of hybrid human α–β-globin genes in MEL cells revealed that the sequences responsible for differences in transcription of the intact α- and β-globin genes are located on the 3′ side of the mRNA capping site of the two genes, suggesting that *cis*-acting regulatory sequences are located within the structural genes.

Introduction

Human α- and β-globin gene expression is activated during adult erythroid cell differentiation (see Weatherall & Clegg 1981, for review). One approach to the study of the mechanisms of α- and β-globin gene regulation in adult erythroid cells is to introduce cloned globin genes into mouse erythroleukaemia (MEL) cells, which can be induced to differentiate in culture (Spandidos & Paul 1982; Chao *et al.* 1983; Wright *et al.* 1983). MEL cells are adult haematopoietic precursors transformed by the Friend virus complex. The transformation results in a block in differentiation at a relatively late stage in erythropoiesis (see Marks & Rifkind 1978, for review). Treatment of MEL cells with dimethylsulphoxide (DMSO) or any of a number of other chemical inducers circumvents this block and results in a series of biochemical and morphological changes that mimic events in normal erythroid cell differentiation (Friend *et al.* 1971; Reuben *et al.* 1976; Marks & Rifkind 1978). Among the changes observed is a dramatic increase in the rate of transcription of the adult α- and β-globin genes, resulting in increased levels of the globin mRNA and proteins (Marks & Rifkind 1978; Hofer *et al.* 1982; Chao *et al.* 1983).

When the human β-globin gene cluster is introduced into MEL cells on intact chromosomes by cell fusion, the human β-globin gene is co-regulated with the endogenous mouse globin genes (Willing *et al.* 1979; Pyati *et al.* 1980). If human or mouse β-globin genes are removed from their normal chromosomal contexts by gene cloning and introduced into MEL cells by DNA cotransformation procedures, they are also appropriately regulated (Chao *et al.* 1983; Wright

et al. 1983). These results indicate that the β-globin gene fragments used in the DNA transformation experiments include sequences required in *cis* for specific transcriptional activation of β-globin genes during erythroid cell differentiation.

Previous studies demonstrated that human α-globin genes introduced into MEL cells on intact chromosomes by cell fusion are expressed at high levels in induced cells (Deisseroth & Hendrick 1978; Deisseroth *et al.* 1980). We extended the analysis of these cell hybrids and demonstrated that the human embryonic and adult α-like globin genes are appropriately regulated during MEL cell differentiation (Charnay *et al.* 1984). We also examined the expression of cloned human α-globin genes introduced into MEL cells by DNA transformation. We found that, unlike the human β-globin gene which is regulated in most lines (Chao *et al.* 1983; Wright *et al.* 1983), the human α-globin gene is appropriately regulated in only one out of 36 lines studied. Furthermore, when both human α- and β-globin genes are introduced into MEL cells on the same plasmid, the β-globin gene is inducible while the α-globin gene is not, and the level of human α-globin mRNA before induction is greater than the level of human β-globin mRNA after DMSO treatment. To localize the DNA sequences responsible for these differences in expression, hybrid α–β-globin genes were constructed and analysed in MEL cells. Our results suggest that the differences in α- and β-globin gene expression are determined by sequences within the structural genes.

Results

To determine whether cloned human α-globin genes are appropriately regulated when introduced into MEL cells by the method of DNA-mediated gene transfer, we used the calcium phosphate co-precipitation technique to co-transform a derivative of an aprt-MEL cell line (Deisseroth & Hendrick 1978) with the cloned hamster aprt gene and a variety of plasmids containing human α-globin genes. Unexpectedly, we did not observe regulated transcription of the cloned human α-globin genes in 35 out of 36 MEL cell lines examined, even though 70% of the lines produce detectable levels of α1-globin gene transcripts before cell differentiation (Charnay *et al.* 1984). This is in sharp contrast with the results obtained with hybrid MEL cells containing the human chromosome 16, where both of the adult human adult α-globin genes are correctly regulated (Deisseroth & Hendrick 1978; Charnay *et al.* 1984).

We considered two possible explanations for this observation. First, it is possible that the exogenous human α-globin gene is expressed at low levels in uninduced MEL cells and is not responsive to DMSO treatment in its new environment. Alternatively, the gene may be fully activated in the transformed cell even before DMSO treatment, such that MEL cell differentiation leads to no further increase in the level of exogenous α-globin gene expression. To distinguish between these two possibilities, we quantitated the relative levels of human α- and β-globin RNAs in transformed MEL cells before and after DMSO treatment. To accomplish this, we examined pools of large numbers of independently derived transformed MEL cell colonies generated by using a modification of a protoplast fusion protocol (Schaffner 1980; Sandri-Goldin *et al.* 1981). As a much higher transformation efficiency is obtained with this procedure compared with the calcium phosphate precipitation method, we were able to generate pools of 50–100 independent clones of cells transformed with either the human α- or both the human α- and β-globin genes.

Pools of independent clones were generated by protoplast fusion with bacteria harbouring the plasmid pAPRTα or pAPRTαβ (figure 1*a*, *b*). These plasmids carry the hamster aprt gene

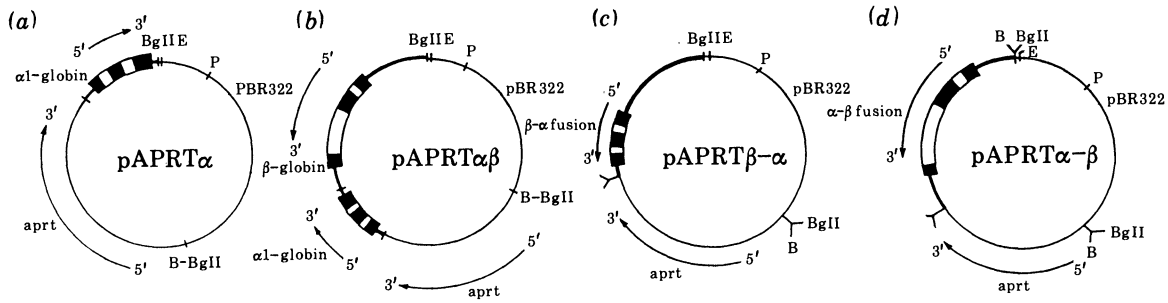

FIGURE 1. Structure of plasmids used in MEL cell co-transformation experiments, (*a*) and (*b*). pAPRTα and pAPRTαβ were constructed by cloning *Nae*I−*Bgl*II fragments carrying respectively the human α1-globin gene and the human α1- and β-globin genes into the vector pAPRT*Bgl* carrying the hamster aprt gene (P. Charnay, unpublished); N, *Nae*I (destroyed); X, *Xba*I (destroyed). (*c*) Plasmid pAPRTβ/α, containing a *Bgl*II+*Pst*I fragment carrying the β–α-fusion gene inserted between the *Bgl*II and *Xba*I sites of plasmid pAPRT*Bgl*. The *Pst*I and *Xba*I sites were destroyed in the construction. (*d*) pAPRTα/β, containing a *Bam*HI+*Pst*I fragment carrying the α–β-fusion gene inserted between the *Bgl*II and *Xba*I sites of plasmid pAPRT*Bgl*, both of which were destroyed in the construction.

and either the human α1-globin gene (p*APRT*α) or both the human α1- and β-globin genes (p*APRT*αβ). RNA was extracted from each pool of cells before and after DMSO treatment and analysed with human α- and β-globin complementary RNA probes (figure 2). As in the case of cell lines generated by the calcium phosphate co-precipitation technique, the level of human β-globin mRNA increases by a factor of 11 upon DMSO treatment, whereas the level of human α1-globin mRNA remains constant or increases very slightly. The ratio of the signals obtained with α1- and β-globin probes is 5:1 in induced cell RNA; after correcting for the specific activities of the protected α- and β-globin RNA fragments, we estimate that the ratio of levels of human α- and β-globin RNA in the induced transformants is about 12:1. Thus, this analysis indicates that the level of human α-globin RNA before cell differentiation is even higher than that of human β-globin RNA after differentiation. By comparison with a control hybridization to RNA from human cord blood, we estimate that human α1-globin mRNA represents 0.3–0.4 % of cellular poly A+ RNA in the two pools examined. In conclusion, these results are consistent with the possibility that the cloned human α-globin gene is fully activated in uninduced MEL cells and no further increase in transcription is possible after DMSO treatment.

Analysis of hybrid α–β*-globin gene transcription in* MEL *cells*

To identify the sequences responsible for the different transcriptional behaviour of cloned human α- and β-globin genes in MEL cells, we constructed hybrid genes in which the 5′ flanking sequences of the human α1- or β-globin genes are fused to the β- or α1-globin structural genes, respectively. These hybrid genes were subcloned into a plasmid carrying the hamster aprt gene to create plasmids pAPRTβ/α and pAPRTα/β (figure 1*c*, *d*). Plasmid pAPRTβ/α contains a β–α fusion gene comprising β-globin sequences from −1700 to +49 joined to α-globin sequences from +37 to +944. The fusion point between α- and β-globin sequences is at the translational initiation codon, and the 5′ ends of the β–α fusion gene transcripts map at the β-globin mRNA capping site. Plasmid pAPRTα/β contains an α–β fusion gene comprising α1-globin sequences from −118 to −12 joined via a synthetic DNA linker to β-globin sequences from −6 to +2163. The spacing between the α1-globin gene TATA homology and the β-globin mRNA capping site is maintained, and transcripts of this gene are therefore initiated at the β-globin mRNA capping site.

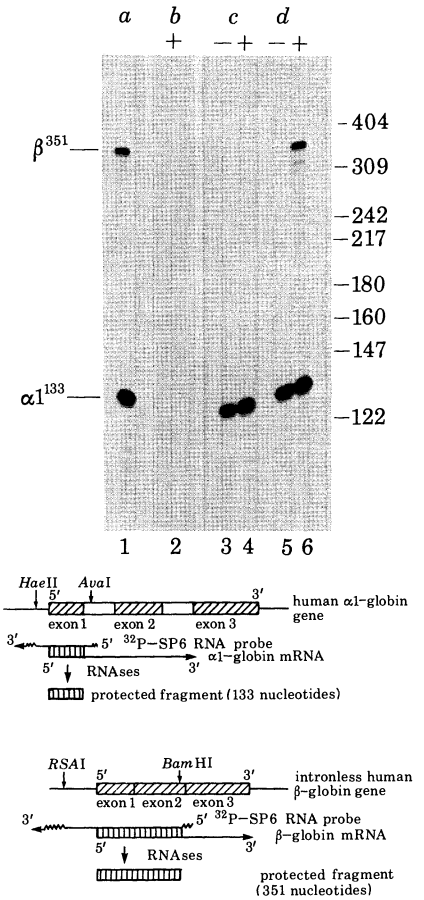

FIGURE 2. Comparative analysis of human α- and β-globin gene transcription in pools of MEL cell colonies. Pools of 50–100 independent MEL cell lines transformed with the plasmid pAPRTα or pAPRTαβ were grown for 4 days in the absence (−) or in presence (+) of 2% DMSO. 10 μg of cytoplasmic RNA was hybridized to an excess of both of the human α- and β-globin complementary-strand ^{32}P-SP6 RNA probes, and digested with RNases. The protected RNA fragments were fractionated by electrophoresis on 6% polyacrylamide-urea sequencing gel. Hybridization of the β-globin probe with polyA+RNA from human cord blood (5 ng) protects a 351 nucleotide long fragment (lane a). Hybridization of the α-globin probe with polyA+RNA from human cord blood protects a 133 nucleotide long fragment (lane a). Lane b corresponds to cytoplasmic RNA from DMSO-induced MEL cells not transformed with human globin gene. Lanes c correspond to RNA from a pool of MEL cells clones transformed with the plasmid pAPRTα. Lanes d correspond to RNA from a pool of MEL cells clones transformed with the plasmid pAPRTαβ. Exposure times were 3 h for lane a and 15 h for lanes b, c and d. Reprinted from *Cell*.

We used the protoplast fusion technique to transform MEL cells to the aprt$^+$ phenotype with these hybrid gene plasmids, and established pools of *ca.* 200 independent colonies for transcription analysis. Figure 3*a* shows an S1 nuclease mapping analysis of RNA from different pools of colonies transformed with the β–α gene before and after DMSO treatment. As a control, a probe for the 5′ ends of the endogenous mouse β-major globin gene transcripts was included in the hybridization reaction. As in the case of the intact human α-globin gene, the β–α fusion gene was transcribed before DMSO treatment and no increase in mRNA level was observed upon induction. In contrast, the transcription of the endogenous mouse β-major globin gene was markedly induced.

Analysis of MEL cells containing the hybrid α–β globin fusion gene is shown in figure 3*b*.

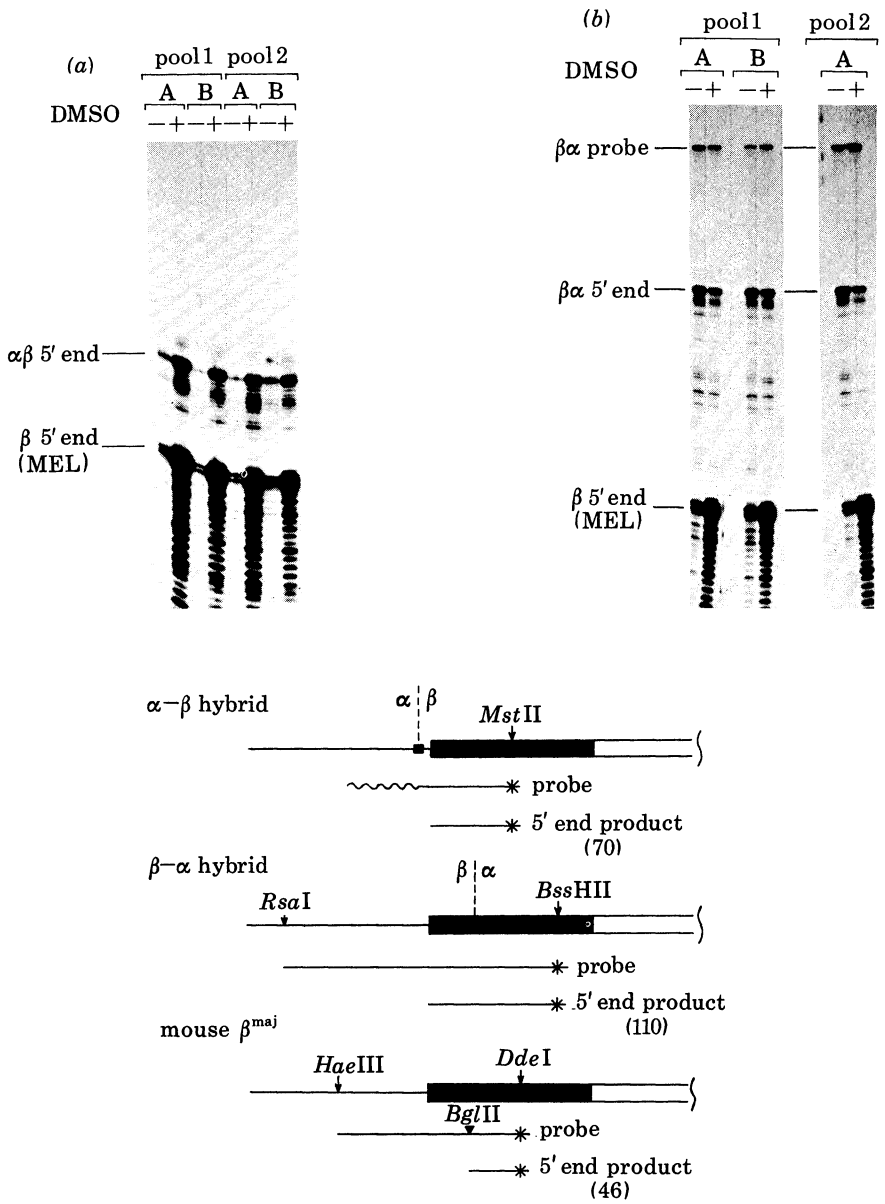

FIGURE 3. Analysis of RNA from MEL cells transformed with hybrid α–β-globin genes. (*a*) Transcription analysis of the β–α-fusion gene. Plasmid pAPRTβ/α was used to generate two separate pools of *ca.* 200 clones of aprt$^+$ MEL cells and two separate DMSO inductions were performed with pool 1 and one with pool 2. 20 μg of total cellular RNA isolated from DMSO treated (−) or untreated (+) cells was analysed by nuclease S1 mapping with single stranded 5′ end ^{32}P-labelled probes. The identities of the various nuclease resistant products are indicated. (*b*) Transcription analysis of the α–β fusion gene. Plasmid pAPRTα/β was used to generate pools of aprt$^+$ MEL cells, which were analysed as described in (*a*) except that two DMSO inductions were done with each pool of clones. (*c*) Schematic representation of the probes used for the analysis. The different genes are shown, with flanking sequences indicated by thin lines, exons by solid blocks and introns as open blocks. The junction between α- and β-globin sequences in the hybrid genes is indicated by a dashed line, and nucleotides derived from the synthetic DNA linker by a solid block. The hybridization probe used for each gene is shown below the diagram, together with the S1 nuclease product expected for correctly initiated RNA. The probes used were as follows: for the α–β fusion gene, a human β-globin gene *Hae*III-**Mst*II fragment (nucleotides −76 to +70; the wavy line indicates that this probe is not complementary to the α-globin 5′ flanking sequences); for the β–α fusion gene, *Rsa*I-**Bss*HII (nucleotides −127 to +110); for the mouse β-major globin gene, *Hae*III to **Dde*I (nucleotides −76 to +76); this probe contains a *Bgl*II octameric linker (solid triangle) inserted at nucleotide +28, causing all mouse β-major globin transcripts to generate a 48 nucleotide nuclease S1 resistant fragment. Reprinted from *Cell*.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Two independent pools of transformed cells were analysed by the S1 nuclease mapping procedure with a human β-globin probe and a probe for the endogenous mouse β-major globin gene. As with the intact β-globin gene (Chao *et al.* 1983) a significant increase in the level of the α–β fusion gene RNA was observed after DMSO treatment, with a concomitant increase in the level of mouse β-major globin RNA (figure 3*b*). An increase in the amount of RNA initiated upstream of the α/β fusion gene capping site was also observed, as revealed by S1 nuclease resistant DNA fragments that map the point of sequence divergence between the DNA probe and the fusion gene template (figure 3*b*).

In conclusion, we find that both the intact α-globin gene, and the hybrid β–α globin genes are transcribed before MEL cell differentiation, and no increase in the mRNA levels is observed after DMSO treatment. In contrast, both the intact human β-globin gene and the hybrid α/β globin genes are transcribed at a low level before induction and the level of mRNA increases 5- to 20-fold after DMSO treatment.

Discussion

Differences in human α- and β-globin gene expression

Human α-like and β-like globin genes comprise a small gene family whose members are expressed at different times during embryonic and foetal development, and during adult life. Although the levels of α-like and β-like globin gene products are approximately equal at each stage of development, the temporal pattern of expression of the adult α- and β-globin genes is quite different. For example, the adult α-globin genes are fully active in both foetal and adult erythroid cells, but the adult β-globin genes are not fully active until after birth. The timing of α- and β-globin gene activation during adult erythroid cell differentiation also appears to be different. For example, the endogenous mouse α-globin genes are turned on approximately 8 h earlier than the β-globin genes during DMSO-induced MEL cell differentiation (Nudel *et al.* 1977). These observations and the data presented here suggest that the mechanisms by which adult α- and β-globin genes are turned on in erythroid cells may be fundamentally different.

We have found significant differences in the transcription of cloned human α- and β-globin genes when they are introduced into erythroid and non-erythroid cells in culture. Although both α- and β-globin genes are appropriately regulated when introduced into MEL cells on intact chromosomes by cell fusion (Deisseroth & Hendrick 1978; Willing *et al.* 1979; Pyati *et al.* 1980; figure 1), we find that the expression of cloned human α-globin genes is not subject to regulation when the genes are introduced into the same cells by DNA co-transformation. In contrast, the human β-globin gene is regulated when introduced into MEL cells by DNA co-transformation procedures (Chao *et al.* 1983; Wright *et al.* 1983). The possibility that this difference in cloned α- and β-globin gene transcription in MEL cells is due to a systematic difference in the establishment of transformed cell lines, is ruled out by the observation that in cloned MEL cell lines and in pools of colonies transformed with a plasmid carrying both the human α- and β-globin genes, the human β-globin is inducible, while α-globin gene expression is not regulated. Furthermore, the level of human α-globin mRNA in uninduced MEL cells is about 100 times the level of human β-globin mRNA. This suggests that the lack of appropriate regulation of the cloned human α-globin gene during MEL cell differentiation is due to the fact that the gene is already fully active in uninduced MEL cells.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

In addition to the observed differences in human α- and β-globin gene transcription in MEL cells, these genes also differ significantly in their transcriptional properties when introduced into non-erythroid cells in culture. For example, the human α-globin gene is readily transcribed when transfected into HeLa cells in transient expression assays, but β-globin transcription requires linkage to a viral transcription enhancer sequence (Mellon *et al.* 1981; Humphries *et al.* 1982; Treisman *et al.* 1983). Moreover, α-globin gene transcription is stimulated only 5- to 10-fold by the SV40 enhancer (Treisman *et al.* 1983), while this enhancer increases the level of β-globin transcription by at least 100-fold in transient expression assays (Banerji *et al.* 1981; Treisman *et al.* 1983). Finally, we have shown that the level of human α1-globin mRNA is more than 100-fold higher than that of β-globin mRNA in pools of mouse L cell colonies stably transformed with a plasmid carrying the human α- and β-globin genes (Charnay *et al.* 1984). These results are summarized in table 1. The α-globin gene is active in a variety of non-erythroid cells, and in uninduced MEL cells; while the β-globin gene is inactive in non-erythroid cells in the absence of a linked viral transcription enhancer sequence. The β-globin gene is inactive in MEL cells unless the cells are induced to differentiate.

Table 1. Expression of globin genes in erythroid and non-erythroid cells

(No expression or a low level of expression of the gene is referred as (−) and a relatively high level as (+). Transfected gene data correspond to transient expression in HeLa cells and to stable transformation in mouse L cells and MEL cells. The relative levels of expression of the β–α- and α–β-hybrid genes in MEL cells were not determined. n.d. not done. The table is reprinted from *Cell*.)

	HeLa cells	L cells	uninduced MEL cells	induced MEL cells
endogenous α-globin gene	−†	−‡	−	+
endogenous β-globin gene	−†	−‡	−	+
transfected human α-globin gene	+	+	+	+
transfected human β-globin gene	−	−	−	+
transfected human β–α hybrid gene	n.d.	n.d.	+	+
transfected human α–β hybrid gene	n.d.	n.d.	−	+

† R. Treisman & M. Green, unpublished results.
‡ Humphries *et al.* (1976).

DNA *sequences responsible for differences in human* α- *and* β-*globin gene transcription in* MEL *cells*

We examined the transcription of hybrid human α–β-globin genes in MEL cells to locate the DNA sequences responsible for differences in their expression during MEL cell differentiation. These experiments suggest that the observed differences are a consequence of differences in regulatory sequences located within the α- and β-globin structural genes. Both the intact β-globin gene and the hybrid α–β-globin gene are expressed at a low level in uninduced MEL cells, and DMSO treatment leads to a 5- to 20-fold increase in the level of mRNA. In contrast, both the intact α-globin gene and the hybrid β–α-globin gene are expressed in uninduced MEL cells, and no increase in the level of expression of these genes is observed upon DMSO treatment. Thus, constitutive expression of the α-globin gene appears to be determined by sequences located within the structural gene since the α-globin promoter does not confer constitutive expression to the β-globin structural gene in the α–β-globin hybrid gene.

The observation that transcription of both the intact β-globin gene and the α–β-hybrid globin gene is inducible in MEL cells suggests that regulatory sequences necessary for appropriate

control of the β-globin gene may also be located within the structural gene. This possibility is consistent with recent studies which demonstrate that a hybrid gene consisting of 5′ flanking sequences from a mouse H2 class I histocompatibility gene fused to the human β-globin structural gene is inducible in MEL cells (Wright *et al.*, this symposium). Our data do not address the question of whether the α- and β-globin gene promoters also contain regulatory sequences. For example, both the α- and β-globin gene promoters might contain regulatory sequences, but their function might be masked by dominant regulatory sequences within the two structural genes. Wright and collaborators have also shown that a hybrid gene consisting of the human β-globin gene promoter fused to a mouse H2 class I histocompatibility gene is inducible in MEL cells (Wright *et al.*, this symposium). Thus, there may be a minimum of two different *cis*-acting sequences that influence the expression of the human β-globin gene in MEL cells.

A possible mechanism for differential expression of cloned human α- *and* β-*globin genes in* MEL *cells*

As shown in table 1, the cloned human α-globin gene is expressed at high levels in non-erythroid cells and in MEL cells before induction. In contrast, the cloned human β-globin gene is expressed at low levels in non-erythroid cells and in uninduced MEL cells, but induction of MEL cell differentiation leads to a significant increase in its expression. On the basis of these observations, we propose that the endogenous α-globin gene in erythroid cells is under negative control while the endogenous β-globin gene in these cells is under both negative and positive control. According to this model, a minimum of one step, which we will refer to as derepression, is required for the activation of human α-globin gene transcription, while a minimum of two steps, derepression followed by cell-specific positive activation may be required for β-globin gene transcription. The derepression step with both genes could be due to an alteration in chromatin structure, the inactivation of a repressor, or to changes in the state of DNA methylation. An alteration in chromatin structure is suggested by the observation that the induction of MEL cell differentiation by DMSO is associated with the appearance of new DNAse I hypersensitive sites in the 5′ flanking DNA and within the second intervening sequence of the endogenous β-major globin gene (Hofer *et al.* 1982; Sheffery *et al.* 1982). Although a causal relationship between the appearance of a DNAse I hypersensitive site and globin gene activation has not been established, such chromatin changes have been shown to coincide with gene activation during chicken development (Groudine *et al.* 1981) and to precede maximal β-globin gene expression in humans (Groudine *et al.* 1983). Changes in chromatin structure appear to be necessary but not sufficient for gene activity, since they are retained by chicken β-globin genes that have been activated and then turned off (Groudine & Weintraub 1982). Cloned genes that are stably introduced into fibroblasts in culture appear to adopt an altered chromatin configuration (Weintraub 1983). Furthermore, our preliminary studies of cloned mouse β-globin genes introduced into MEL cells indicate that a DNAse I hypersensitive site is present 5′ to the exogenous gene both before and after induction with DMSO, while this site is detected in the endogenous gene only after induction. The altered chromatin structure of the transfected gene may result in an increased accessibility of the gene to the cellular transcriptional machinery relative to its endogenous counterpart. This possibility is suggested by the observation that the rat α2μ-globulin and the human growth hormone genes introduced into mouse L-cells by co-transformation are both active and remain responsive to glucocorticoid stimulation while the endogenous counterpart genes remain inactive (Kurtz 1981; Robins *et al.* 1982). Moreover,

a cloned human β-globin gene introduced into HeLa cells in transient expression experiments is transcriptionally activated by viral immediate–early gene products but the endogenous globin genes in the same cells remain inactive (Green *et al.* 1983; Treisman *et al.* 1983).

We propose that both α- and β-globin genes introduced into MEL cells by DNA transfer are in a chromatin configuration that is required for their efficient expression. However, the β-globin gene, unlike the α-globin gene, requires an additional, erythroid cell-specific factor for maximal expression. This factor would appear only after induction of MEL cell differentiation by DMSO. This hypothesis would be consistent with the observation that the cloned α-globin gene is efficiently expressed in non-erythroid cells and in uninduced MEL cells. This model does not preclude the possibility that a positive activator is required for α-globin transcription, but merely that such an activator is not erythroid cell-specific, since it would be present in a variety of cell types including the MEL cell before induction. In the case of the endogenous α- and β-globin genes in MEL cells, or of the human globin genes introduced into MEL cells on intact chromosomes by cell fusion, both genes would be in an inactive chromatin configuration before induction. MEL cell differentiation would lead to derepression followed by positive activation. In the case of the α-globin gene, the positive activation would not be required or be accomplished by a factor present before induction, while the β-globin gene would be activated by a factor that appears after induction.

In summary, the data presented in this paper suggest that even though the adult human α- and β-globin genes are expressed exclusively in erythroid cells, the mechanisms by which they are regulated are different. The two genes clearly evolved from a single ancestral gene by duplication, but they have been diverging from each other for approximately 500 million years (Efstratiadis *et al.* 1980). Moreover, the α- and β-globin genes are located within gene clusters that have independently evolved, as indicated by differences in the number and times of divergence of genes within each cluster (see Proudfoot *et al.* 1982, for discussion). Given these quite distinct evolutionary histories, it is possible that the α- and β-globin genes acquired different control mechanisms during evolution.

P. C. was supported by fellowships from the European Molecular Biology Organization and the Fogarty International Center of the N.I.H. R.T. and P.M. were supported by Special Fellowships from the Leukemia Society of America, and M. C. was an American Cancer Society Postdoctoral Fellow. This work was funded by grants from the National Science Foundation and the National Institutes of Health to R.A. and T.M.

References

Banerji, J., Rusconi, S. & Schaffner, W. 1981 Expression of β-globin gene is enhanced by remote SV40 DNA sequences. *Cell* **27**, 299–308.

Chao, M. V., Mellon, P., Charnay, P., Maniatis, T. & Axel, R. 1983 The regulated expression of β-globin genes introduced into mouse erythroleukemia cells. *Cell* **32**, 483–493.

Charnay, P., Treisman, R., Mellon, P., Chao, M., Axel, R. & Maniatis, T. 1984 Differences in human α- and β-globin gene expression in mouse erythroleukemia cells: the role of intragenic sequences. *Cell*. **38**, 251–263.

Deisseroth, A., Bode, U., Fontana, J. & Hendrick, D. 1980 Expression of human α-globin genes in hybrid mouse erythroleukemia cells depends on differentiated state of human donor cell. *Nature, Lond.* **285**, 36–38.

Deisseroth, A. & Hendrick, D. 1978 Human α-globin gene expression following chromosomal dependent gene transfer into mouse erythroleukemia cells. *Cell* **15**, 55–63.

Efstratiadis, A., Posakony, J. W., Maniatis, T., Lawn, R. M., O'Connell, C., Spritz, R. A., DeRiel, J. K., Forget, B. G., Weissman, S. M., Slightom, J. L., Blech, A. E., Smithies, O., Baralle, F. E., Shoulders, C. C. & Proudfoot, N. J. 1980 The structure and evolution of the β-globin gene family. *Cell* **21**, 653–668.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Friend, C., Scher, W., Holland, J. G. & Sato, T. 1971 Hemoglobin synthesis in murine induced leukemic cells *in vitro*: stimulation of erythroid differentiation by dimethylsulfoxide. *Proc. natn. Acad. Sci. U.S.A.* **68**, 378–382.

Gillies, S. D., Morrison, S. L., Oi, V. T. & Tonegawa, S. 1983 A tissue-specific transcription enhancer element is located in the major intron of a rearranged immunoglobulin heavy chain gene. *Cell* **33**, 717–728.

Green, M. R., Treisman, R. & Maniatis, T. 1983 Transcriptional activation of cloned human β-globin genes by viral immediate–early gene products. *Cell* **35**, 137–148.

Groudine, M., Kohwi-Shigematsu, T., Gelinas, R., Stamatoyannopoulos, G. & Papayannopoulou, T. 1983 Human fetal to adult hemoglobin switching: changes in chromatin structure of the β-globin locus. *Proc. natn. Acad. Sci. U.S.A.* **80**, 7551–7555.

Groudine, M., Peretz, M. & Weintraub, H. 1981 Transcriptional regulation of hemoglobin switching in chicken embryos. *Molec. Cell Biol.* **1**, 281–288.

Groudine, M. & Weintraub, H. 1981 Activation of globin genes during chicken development. *Cell* **24**, 393–401.

Groudine, M. & Weintraub, H. 1982 Propagation of globin DNase I hypersensitive sites in absence of factors required for induction: a possible mechanism for determination. *Cell* **30**, 131–139.

Hofer, E., Hofer-Warbinek, R. & Darnell, J. E. 1982 Globin RNA transcription: a possible termination site and demonstration of transcription control correlated with altered chromatin structure. *Cell* **29**, 887–893.

Humphries, R. K., Ley, T., Turner, P., Moulton, A. D. & Nienhuis, A. W. 1982 Differences in human α-, β- and δ-globin gene expression in monkey kidney cells. *Cell* **30**, 173–183.

Kurtz, D. T. 1981 Hormonal inducibility of α2μ globulin genes in transfected mouse cells. *Nature, Lond.* **291**, 629–631.

Lauer, J., Shen, C.-K. J. & Maniatis, T. 1980 The chromosomal arrangement of human α-like globin genes: sequence homology and α-globin gene deletion. *Cell* **20**, 119–130.

Little, P., Treisman, R., Beirut, L., Seed, B. & Maniatis, T. 1983 Plasmid vectors for the rapid isolation and transcriptional analysis of human β-globin gene alleles. *Molec. Biol. Med.* **1**, 473–488.

Marks, P. A. & Rifkind, R. A. 1978 Erythroleukemic differentiation. *A. Rev. Biochem.* **47**, 419–426.

Mellon, P. M., Parker, V., Gluzman, Y. & Maniatis, T. 1981 Identification of DNA sequences required for transcription of the human α1-globin gene using a new SV40 host-vector system. *Cell* **27**, 279–288.

Michelson, A. M. & Orkin, S. H. 1980 The 3′ untranslated regions of the duplicated human α-globin genes are unexpectedly divergent. *Cell* **22**, 371–377.

Nishioka, Y. & Leder, P. 1979 The complete sequence of a chromosomal mouse α-globin gene reveals elements conserved throughout vertebrate evolution. *Cell* **18**, 875–882.

Nudel, U., Salmon, J., Fibach, E., Terada, M., Rifkind, R., Marks, P. A. & Bank, A. 1977 Accumulation of α- and β-globin messenger RNAs in mouse erythroleukemia cells. *Cell* **12**, 463–469.

Proudfoot, N. J., Gil, A. & Maniatis, T. 1982 The structure of the human zeta-globin gene and a closely linked, nearly identical pseudogene. *Cell* **31**, 553–563.

Pyati, J., Kucherlapati, R. S. & Skoultchi, A. 1980 Activation of human β-globin genes from non-erythroid cells by fusion with murine erythroleukemia cells. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4337–4341.

Reuben, R. C., Wife, R. L., Breslow, R., Rifkind, R. A. & Marks, P. A. 1976 A new group of potent inducers of differentiation in murine erythroleukemia cells. *Proc. natn. Acad. Sci. U.S.A.* **73**, 862–866.

Robins, D. M., Peak, I., Seeburg, P. H. & Axel, R. 1982 Regulated expression of human growth hormone genes in mouse cells. *Cell* **29**, 623–631.

Sandri-Goldin, R. M., Goldin, A. M., Levine, M. & Glorioso, J. C. 1981 High frequency transfer of cloned Herpes Simplex virus type 1 sequences to mammalian cells by protoplast fusion. *Molec. Cell. Biol.* **1**, 743–752.

Schaffner, W. 1980 Direct transfer of cloned genes from bacteria to mammalian cells. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2163–2167.

Seed, B. 1983 Purification of genomic sequences from bacteriophage libraries by recombination and selection *in vivo*. *Nucl. Acids Res.* **11**, 2427–2443.

Sheffery, M., Rifkind, R. A. & Marks, P. A. 1982 Murine erythroleukemia cells differentiation: DNaseI hypersensitivity and DNA methylation near the globin genes. *Proc. natn. Acad. Sci. U.S.A.* **79**, 1620–1627.

Spandidos, D. A. & Paul, J. 1982 Transfer of human globin genes to erythroleukemic mouse cells. *EMBO J.* **1**, 15–20.

Treisman, R., Green, M. R. & Maniatis, T. 1983 *Cis*- and *trans*-activation of globin gene transcription in transient assays. *Proc. natn. Acad. Sci. U.S.A.* **80**, 7428–7432.

Weatherall, D. J. & Clegg, J. B. 1981 *The thalassaemia syndromes*. London: Blackwell Scientific Publications.

Weintraub, H. 1983 A dominant role for DNA secondary structure in forming hypersensitive structures in chromatin. *Cell* **32**, 1191–1203.

Willing, M. C., Nienhuis, A. W. & Anderson, W. F. 1979 Selective activation of human β- but not γ-globin gene in human fibroblast and mouse erythroleukaemic cell hybrids. *Nature, Lond.* **277**, 534–538.

Wright, S., deBoer, E., Grosveld, F. G. & Flavell, R. A. 1983 Regulated expression of the human β-globin gene family in murine erythroleukaemia cells. *Nature, Lond.* **305**, 333–336.

Zinn, K., DiMaio, D. & Maniatis, T. 1983 Identification of two distinct regulatory regions adjacent to the human β-interferon gene. *Cell* **34**, 865–879.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

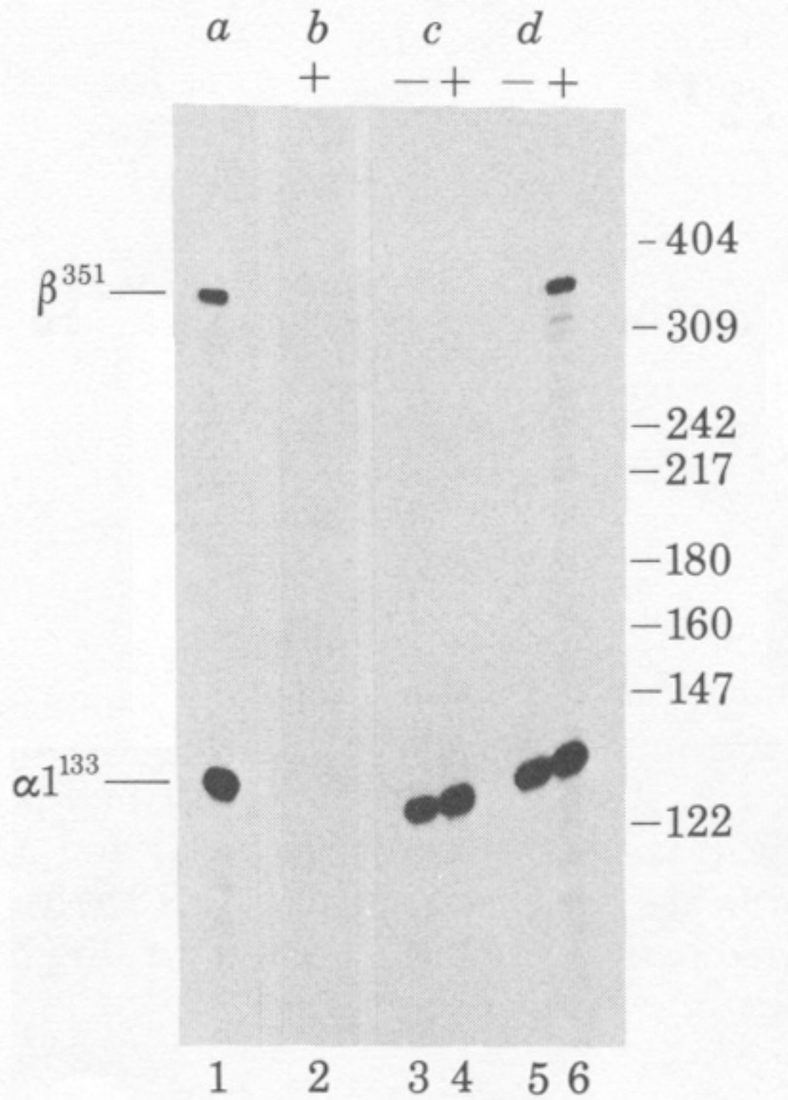

FIGURE 2. Comparative analysis of human α- and β-globin gene transcription in pools of MEL cell colonies. Pools of 50–100 independent MEL cell lines transformed with the plasmid pAPRTα or pAPRTαβ were grown for 4 days in the absence (−) or in presence (+) of 2% DMSO. 10 μg of cytoplasmic RNA was hybridized to an excess of both of the human α- and β-globin complementary-strand ^{32}P-SP6 RNA probes, and digested with RNases. The protected RNA fragments were fractionated by electrophoresis on 6% polyacrylamide-urea sequencing gel. Hybridization of the β-globin probe with polyA+RNA from human cord blood (5 ng) protects a 351 nucleotide long fragment (lane a). Hybridization of the α-globin probe with polyA+RNA from human cord blood protects a 133 nucleotide long fragment (lane a). Lane b corresponds to cytoplasmic RNA from DMSO-induced MEL cells not transformed with human globin gene. Lanes c correspond to RNA from a pool of MEL cells clones transformed with the plasmid pAPRTα. Lanes d correspond to RNA from a pool of MEL cells clones transformed with the plasmid pAPRTαβ. Exposure times were 3 h for lane a and 15 h for lanes b, c and d. Reprinted from *Cell*.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

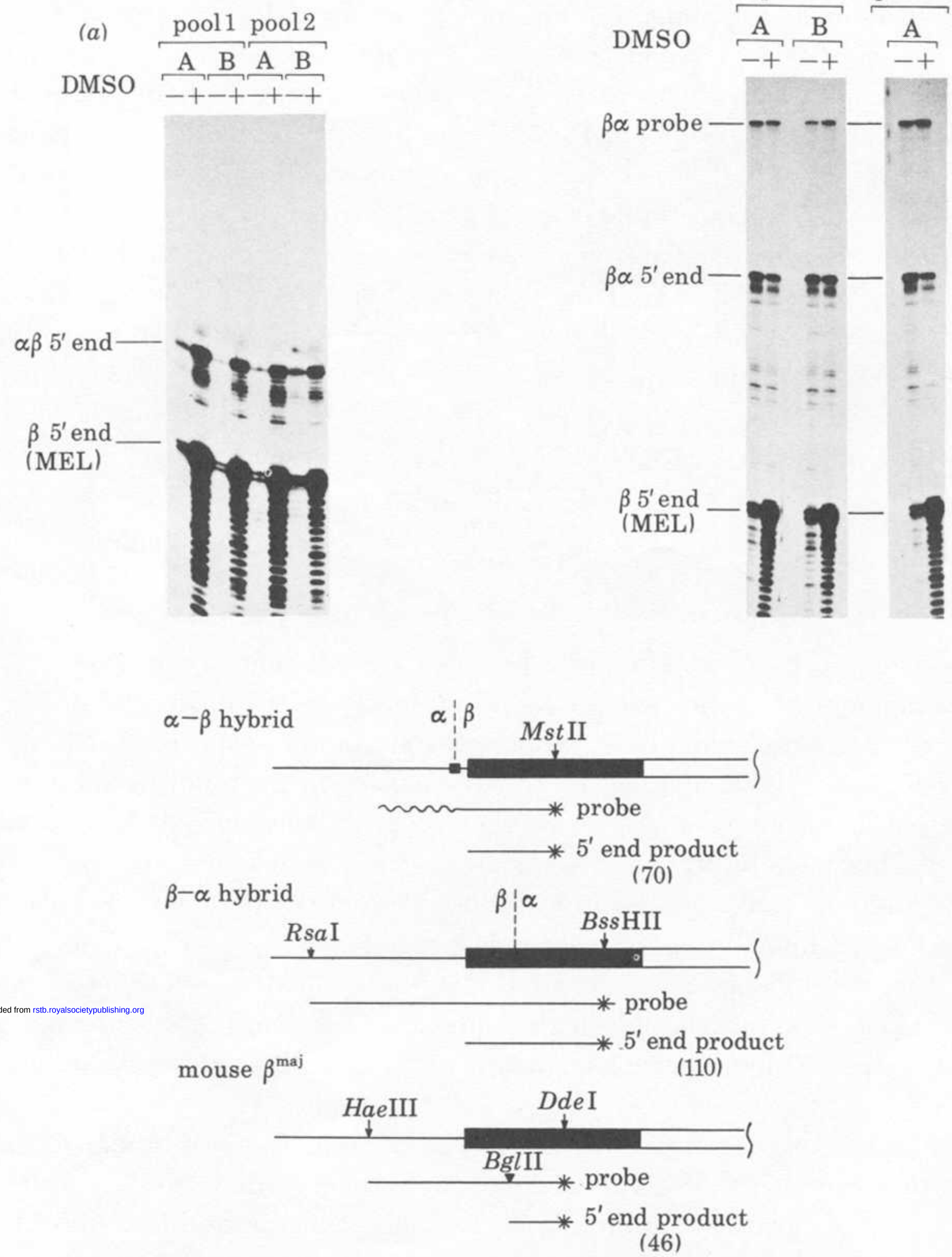

FIGURE 3. Analysis of RNA from MEL cells transformed with hybrid α–β-globin genes. (*a*) Transcription analysis of the β–α-fusion gene. Plasmid pAPRTβ/α was used to generate two separate pools of *ca.* 200 clones of aprt$^+$ MEL cells and two separate DMSO inductions were performed with pool 1 and one with pool 2. 20 μg of total cellular RNA isolated from DMSO treated (−) or untreated (+) cells was analysed by nuclease S1 mapping with single stranded 5′ end ^{32}P-labelled probes. The identities of the various nuclease resistant products are indicated. (*b*) Transcription analysis of the α–β fusion gene. Plasmid pAPRTα/β was used to generate pools of aprt$^+$ MEL cells, which were analysed as described in (*a*) except that two DMSO inductions were done with each pool of clones. (*c*) Schematic representation of the probes used for the analysis. The different genes are shown, with flanking sequences indicated by thin lines, exons by solid blocks and introns as open blocks. The junction between α- and β-globin sequences in the hybrid genes is indicated by a dashed line, and nucleotides derived from the synthetic DNA linker by a solid block. The hybridization probe used for each gene is shown below the diagram, together with the S1 nuclease product expected for correctly initiated RNA. The probes used were as follows: for the α–β fusion gene, a human β-globin gene *Hae*III-**Mst*II fragment (nucleotides −76 to +70; the wavy line indicates that this probe is not complementary to the α-globin 5′ flanking sequences); for the β–α fusion gene, *Rsa*I-**Bss*HII (nucleotides −127 to +110); for the mouse β-major globin gene, *Hae*III to **Dde*I (nucleotides −76 to +76); this probe contains a *Bgl*II octameric linker (solid triangle) inserted at nucleotide +28, causing all mouse β-major globin transcripts to generate a 48 nucleotide nuclease S1 resistant fragment. Reprinted from *Cell*.