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THE 5'-FLANKING SEQUENCES OF HUMAN GLOBIN GENES CONTRIBUTE TO TISSUE SPECIFIC EXPRESSION

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Summary: The specificity of expression of the isolated 5'-flanking sequences of the human β- and ε-globin genes was examined in the K562 human erythroleukemia cell line, the murine erythroleukemia (MEL) cell, and in nonerythroid cell lines CV-1, HeLa-S3, and WI-38. Globin flanking sequences were active only in the erythroid K562 and MEL cell lines. Furthermore, the upstream sequence of the adult, β-globin gene was active in MEL cells which express adult globin and not in K562 cells which express embryonic and fetal globins suggesting that tissue specificity resides in these upstream sequences. No expression was observed in K562 cells which have an embryonic-fetal hemoglobin phenotype.

Although regulatory regions involved in tissue specific expression have been localized to the upstream flanking sequences and to intragenic sequences for several genes (1-7), little is known about the location of such sequences for the human globin genes. To determine whether such regulatory sequences may flank the human globin genes, we isolated the upstream flanking sequences of the human β- and the human ε-globin genes and studied the specificity of expression in erythroid and nonerythroid cell lines. To monitor expression we fused the globin upstream segments to the bacterial marker gene chloramphenicol acetyl transferase (CAT) and measured CAT enzyme activity (8) following introduction of the hybrid genes into each cell type. Our studies were performed in the absence of exogenous viral enhancers which can alter the expression of complete globin genes and presumably may have transcriptional specificities unrelated to globin genes (9).

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Materials and Methods

Cell Culture. K562 cells were grown attached loosely to plastic Primaria™ (Falcon) flasks in RPMI 1640 medium with 10% fetal calf serum, 10mM Hepes, penicillin and streptomycin. Cells were induced with hemin as described (10). Murine erythroleukemia (MEL) cells, HeLa-S3 cells, CV-1 cells, and WI-38 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum, penicillin and streptomycin.

Transfection. K562 cells were transfected as previously described (11). MEL cells were similarly transfected with the exception that after washing, cells were replated in DMEM with 15% fetal calf serum and 2% dimethyl sulfoxide (DMSO) and harvested after 72 hours. All other cells were transfected, subjected to glycerol shock after 4 hours, washed, covered with fresh medium, and harvested at 40 to 48 hours.

Hybrid Genes. Hybrid genes were constructed by inserting the globin promoters into the Hind III site upstream of the CAT gene in pSVOCAT. pSVOCAT, constructed from pSV2CAT by deleting all of the SV40 early promoter sequences, contains no eucaryotic promoter adjacent to the CAT gene (8). The globin promoter-CAT hybrid genes are represented diagrammatically in Fig. 1. Details of the construction have been published elsewhere (11).

CAT ASSAY. CAT assays were performed by incubating cellular extracts with ^{14}C-chloramphenicol (New England Nuclear) and acetyl CoA in a manner similar to that previously described (8). Within each cell type CAT activity for each plasmid was normalized by protein concentration. Linearity of the assay was tested up to 18 hours using extracts of K562 cells (induced with hemin as well as not) transfected with the hybrid CAT genes. Prior to incubation with substrate, extracts from MEL cells were heated to 65°C for 10 minutes to destroy an interfering activity. Transfections with no DNA (mock transfections) were performed for each cell type to quantitate any background. To quantitate the level of nonspecific expression from the parent plasmid, CAT expression was measured in each cell type following transfection with pSVOCAT. Transfections with pRSVCAT were performed as a positive control for expression in each cell type. Expression from the globin-CAT hybrid genes, pSVOCAT, and pRSVCAT was screened in each cell type

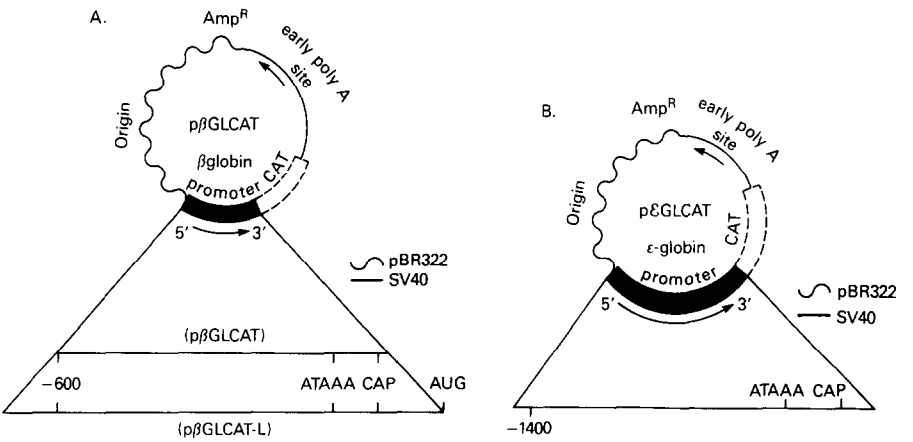

Figure 1. Hybrid Globin-CAT Genes. β-globin and ε-globin 5'flanking sequences were introduced into the Hind III site of pSVOCAT. The 5'-flanking sequence of the β-globin gene was fused to the CAT gene either in the untranslated region of β-globin at +20 bp from the CAP site (pβGLCAT) or at one bp 3' to the initiator codon of the β-globin gene (pβGLCAT-L). The ε-globin gene was fused to the CAT gene in the untranslated region of the ε-globin gene at +19 bp from the ε-globin CAP site (pεGLCAT).

using CAT assays incubated 2 to 4 hours. In cell types where expression from globin-CAT hybrid genes was observed, incubations up to 18 hours were performed to better distinguish any possible differences in expression between globin/CAT hybrid genes.

Results and Discussion

The globin-CAT hybrid genes were introduced into erythroid as well as into nonerythroid cell lines. Two erythroid lines were employed--the human erythroleukemic cell line, K562, and the murine erythroleukemia (MEL) cell line. K562 cells have an embryonic-fetal globin phenotype and do not express the adult, β-globin gene (10,12,13). Work by us and others (14-16) previously demonstrated that the absence of β-globin expression in K562 cells results not from a structural alteration in the K562 β-globin gene, but rather from the particular environment of trans-acting factors within the K562 cell. On the other hand, MEL cells, induced with DMSO, express exogenous adult globin genes as well as the endogenous murine globin genes (17,18).

CAT enzyme activity following transfection of these two erythroid lines is shown in Table I. In K562 cells the level of CAT expression from either of the two plasmids containing β-globin 5'-flanking sequences did not differ significantly from that of pSVOCAT. Two different β-globin-CAT hybrid genes were used to reduce the likelihood that a construction artifact might yield a false negative result. That pβGLCAT can be expressed is shown by our studies in MEL cells Fig. 2 and discussed below. In contrast, expression of pεGLCAT was sixteen-fold higher than that of pSVOCAT. Thus, the pattern of expression of the 5'-flanking sequences of the ε- and β-globin genes in K562 cells is similar to that observed for the endogenous ε- and β-globin genes (10). Hemin, which has been shown to enhance expression of the active globin genes in K562 cells (10,12,13) had no significant effect on the expression of any of the transfected hybrid globin-CAT genes introduced into K562 cells (data not shown).

Within MEL cells pβGLCAT and pεGLCAT produced comparable levels of CAT enzyme activity (Table I). However, expression of pβGLCAT was more variable than that of pεGLCAT. The pattern of expression of the upstream globin

Table I

CELLS	PLASMID	%CONVERSION
K562	pSVOCAT	0.2 ± 0.1
	pβGLCAT	0.7 ± 0.4
	pβGLCAT-L	0.4 ± 0.4
	pεGLCAT	3.2 ± 0.5
	pRSVCAT	100.
MEL	pSVOCAT	1.9 ± 1.9
	pβGLCAT	9.3 ± 6.5
	pεGLCAT	10.2 ± 2.8
	pRSVCAT	100.
CV-1	pSVOCAT	3.1 ± 0.6
	pβGLCAT	0.5 ± 0.3
	pεGLCAT	1.1 ± 0.2
	pRSVCAT	100.
HeLa-S3	pSVOCAT	*
	pβGLCAT	*
	pεGLCAT	*
	pRSVCAT	100.
WI-38	pSVOCAT	*
	pβGLCAT	*
	pεGLCAT	*
	pRSVCAT	100.

Expression of hybrid-CAT genes in erythroid and nonerythroid cells. CAT enzyme activity is reported as the percent conversion of ^{14}C-chloramphenicol to ^{14}C-chloramphenicol acetate. Each value represents the mean ± S.E. (standard error) of 3 to 8 experiments and is expressed relative to the activity of pRSVCAT.

* = not detectable (<0.2% above mock).

sequences (each hybrid gene was active in MEL cells) resembles that observed when cosmids containing the complete β- or ε-globin genes are introduced into MEL cells (19).

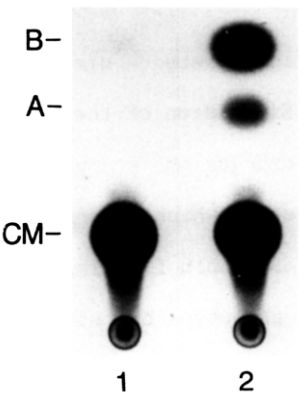

Figure 2. CAT Activity in MEL Cells. Extracts containing 500ug of protein from MEL cells transfected with either pSVOCAT (lane 1) or pβGLCAT (lane 2) were incubated with ^{14}C-chloramphenicol and acetyl coenzyme A for 18 hours. The acetylated products (A,B,C) were separated from unreacted chloramphenicol (CM) by thin layer chromatography; subsequently, the plates were exposed to film.

Activity of the β- and ε-globin flanking sequences introduced into nonerythroid cell lines is shown in Table 1. In CV-1 cells, an adult African green monkey kidney cell line, expression from each of the globin-CAT hybrid genes was less than that observed for the control plasmid, pSVOCAT. Similarly, in HeLa-S3 cells (adult human cervical carcinoma cells) and WI-38 (human embryonic fibroblasts), no expression from any plasmid other than pRSVCAT was detected.

In the absence of exogenous enhancers or viral immediate-early gene products the complete β- and ε-globin genes are correctly expressed in erythroid but not in nonerythroid cells (9,17,20,21). Our experiments provide evidence that the tissue specific expression of globin genes can be conferred by the upstream elements alone. As such, our results are similar to those involving the insulin, chymotrypsin (4), rat elastase I (5), and mouse major histocompatibility genes (7) in which the upstream flanking sequences were shown to confer tissue specific expression. However, such findings do not exclude a possible role for downstream elements in tissue specific expression. Tissue specific cellular enhancers have been identified within the immunoglobulin genes (1-3). But the immunoglobulin V_H promoter in the absence of the intragenic enhancer can direct not only tissue specificity but cell type specificity within the B cell lineage (6). The intragenic enhancer did not show a similar cell type specificity (6). Our results offer evidence for cell type specific expression directed by the upstream sequence of the human β-globin gene. Expression of the β-globin-CAT hybrid gene was observed in MEL cells (which express the endogenous adult murine globin genes) (18) and not in K562 cells (which express embryonic and fetal globin genes) (10,12,13). Thus, the β-globin flanking sequence was (appropriately) active in cells with an adult phenotype but not in cells with an embryonic-fetal phenotype. It is difficult to extend such analysis to the ε-globin-CAT gene without a detailed understanding of the transcriptional state of the endogenous murine embryonic genes in this MEL cell line. With the recent demonstration that the Mouse Bh1 gene codes for the z chain of

embryonic hemoglobin, certain MEL cells have been shown to express murine embryonic genes as well (22).

In conclusion, our experiments provide evidence that globin flanking sequences can restrict expression to cell lines of the appropriate tissue type and the β-globin flanking sequences may play a role in limiting expression to erythroid cells with an adult developmental phenotype.

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