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Differential Activation of the Mouse β-Globin Promoter by Enhancers

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A series of plasmids was constructed to study the effect of two enhancers, the simian virus 40 72-base-pair repeat and the Harvey sarcoma virus 73-base-pair repeat, on the mouse β^{maj}-globin promoter. These plasmids contain the mouse β^{maj}-globin promoter linked to the *Escherichia coli galK* gene, thus allowing galactokinase enzyme activity to be used as a measure of promoter function. In CV-1 (primate) cells, it was found that an enhancer is required for optimal promoter activity and that the simian virus 40 (primate) enhancer increases galactokinase fourfold more than the Harvey sarcoma virus (mouse) enhancer. In L (mouse) cells, however, the Harvey sarcoma virus enhancer is 1.3-fold stronger than the simian virus 40 enhancer. These data support the hypothesis that enhancer activity can be species specific. Furthermore, when both enhancers are present on the same plasmid, their effect is additive on the β-globin promoter whether the plasmid is in CV-1 cells or L cells.

The mouse β-globin gene serves as a valuable model for the study of gene expression in mammals. We wished to develop expression vectors which would allow us to optimize the expression of cloned mouse β-globin genes that are transferred into mouse tissue culture cells or into intact animals. Important elements that have been shown to stimulate the expression of certain mammalian genes have recently been discovered. These are *cis* DNA sequences called enhancers (1, 2, 4–7, 11–14). As a first step toward obtaining regulated globin gene expression in mice, we examined the effect of two different enhancing sequences, the monkey simian virus 40 (SV40) 72-base-pair (bp) repeat and the mouse Harvey sarcoma virus (HaSV) 73-bp repeat, on the expression of a cloned mouse β^{maj}-globin promoter in both monkey (CV-1) cells and mouse (L) cells.

Specifically, we asked several questions. (i) Studies have been performed with the SV40 enhancer with its natural promoter, the SV40 early promoter (2, 4, 6, 11, 13). Does the SV40 enhancer have a similar effect on the mouse β-globin promoter? (ii) The SV40 and the murine Moloney sarcoma enhancer act differently on the SV40 early promoter (11), and the SV40 and polyoma enhancers show cell-specific stimulation of the SV40 and rabbit β-globin promoters (4). Is this cell (species)-specific effect a general one? (iii) Do two different enhancers have an additive effect on promoter activity?

To answer these questions, we constructed a series of plasmids containing the mouse β^{maj}-globin promoter linked to the *Escherichia coli* galactokinase gene (*galK*). This allows us to measure galactokinase (Gal K) levels as a function of the activity of the β-globin promoter. Some constructs also contain a second assayable *E. coli* gene, *gpt*, which is driven by the SV40 early promoter, giving an internal control for transformation frequency. These constructions are based on the system described by Schumperli et al. (16). The SV40 72-bp repeat and the HaSV 73-bp repeat were added singly and in combination to these constructs. The Gal K and *gpt* enzyme activities were then measured in CV-1 cells and L cells.

MATERIALS AND METHODS

Isolation of plasmid DNA and DNA fragments. Plasmid DNA was prepared as described previously (3). It was banded twice in cesium chloride-ethidium bromide gradients before use. DNA fragments were purified by electroelution from an agarose or polyacrylamide gel (150 V for 2 to 3 h) and a subsequent ethanol precipitation.

Restriction enzymes. Restriction enzymes were obtained from New England Biolabs or Bethesda Research Laboratories and used according to the manufacturer's directions.

Bacterial transformations. The bacterial recipient in transformations was strain HB101 (r_k^- m_k^- *recA*). The method used was as described previously (18).

Mammalian cell transfections. CV-1 and L cells were

grown in improved modified Eagle medium supplemented with 10% (vol/vol) fetal calf serum and were split to a density of 5×10^5 cells per 100-mm^2 dish the day before transfection. DNA concentrations were tested to determine the highest efficiency of transformation. It was found that 5 μg of plasmid DNA and 15 μg of carrier (L cell) DNA was optimal. The method used was that of Wigler et al. (19) except that the DNA remained on the cells for 24 h. Cells were harvested 48 h after the DNA was removed. Each transformation was repeated three or more times.

Enzyme assays. (i) CV-1 cells. Starch gel electrophoresis was used as described by Schumperli et al. (16). Starch was from Electrostarch, Madison, Wis. Since each lot varies, the best conditions for the properties of band separation were determined for lot 392 and found to be 15% starch. Gels were run at 125 V overnight. The assay for Gal K was performed in 300 mM Tris-hydrochloride (pH 8.0)–30 mM ATP–38 mM $MgCl_2$–25 μCi of [^{14}C]galactose (49.4 mCi/mmol; Amersham Corp., Arlington Heights, Ill.). The xanthine guanine phosphoribosyltransferase (XGPRT) reaction mixture included 95 mM Tris-hydrochloride (pH 7.5), 9.5 mM $MgCl_2$, 0.7 mM phosphoribosyl pyrophosphate, and 25 μCi of [^{14}C]guanine (54 mCi/mmol; ICN Pharmaceuticals, Inc., Irvine, Calif.). The gel was made in 1.2 mM citric acid–6.07 mM K_2HPO_4–0.5 mM dithiothreitol. The running buffer was 27 mM citric acid and 167 mM K_2HPO_4. The gel was sliced horizontally after electrophoresis, and the Gal K assay was performed on one half, the XGPRT assay on the other half. Analysis of autoradiograms was performed with a Zeineh scanning densitometer (LKB Instruments, Inc., Rockville, Md.).

(ii) L cells. A filter assay was performed to determine Gal K and XGPRT activities (H. Johansen and M. Rosenberg, manuscript in preparation). L cells were disrupted by three cycles of freezing and thawing in 0.25 M Tris-hydrochloride (pH 7.8). The reaction mixture for Gal K included 100 mM Tris-hydrochloride (pH 7.9), 1.5 mM $MgCl_2$, 1.6 mM ATP, 1 mM dithiothreitol, 4 mM NaF, and 3.2 μCi of [^{14}C]galactose (49.4 mCi/mmol; Amersham Corp.). Incubation was for 60 min at 37°C (Fig. 1). The reaction mixture was spotted on Whatman DE81 filters, which were washed and counted. The XGPRT reaction mixture included 50 mM Tris-hydrochloride (pH 7.4), 15 mM $MgCl_2$, 2 mM phosphoribosyl pyrophosphate, 2.4 mM bovine serum albumin, 3.2 mM NaF, and 0.5 μCi of [^{14}C]xanthine (41 mCi/mmol; ICN Pharmaceuticals, Inc.). Incubation was at 37°C for 45 min (Fig. 2). The reaction mixture was spotted on polyethyleneimine paper (Schleicher & Schuell Co., Keene, N.H.), which was washed and counted. The radioactivity for both Gal K and XGPRT was standardized against the protein concentration of each extract. Protein determinations were performed by using the Bio-Rad (Bio-Rad Laboratories, Richmond, Calif.) protein assay.

RESULTS

Isolation of β^{maj}-globin promoter. Plasmid pPK288 (Fig. 3A) contains the *Eco*RI to *Bam*HI segment of the mouse β^{maj}-globin gene (P. Kretschmer, unpublished data). This fragment of the β^{maj}-globin gene was subcloned from a bacteriophage λ recombinant containing the entire gene (17). The *Hin*cII restriction map of pPK288 (Fig. 3A) indicates four restriction sites, two of which are near the mouse β^{maj}-globin promoter. Since the β^{maj}-globin gene has been sequenced (10), including DNA 235 bp 5′ to the

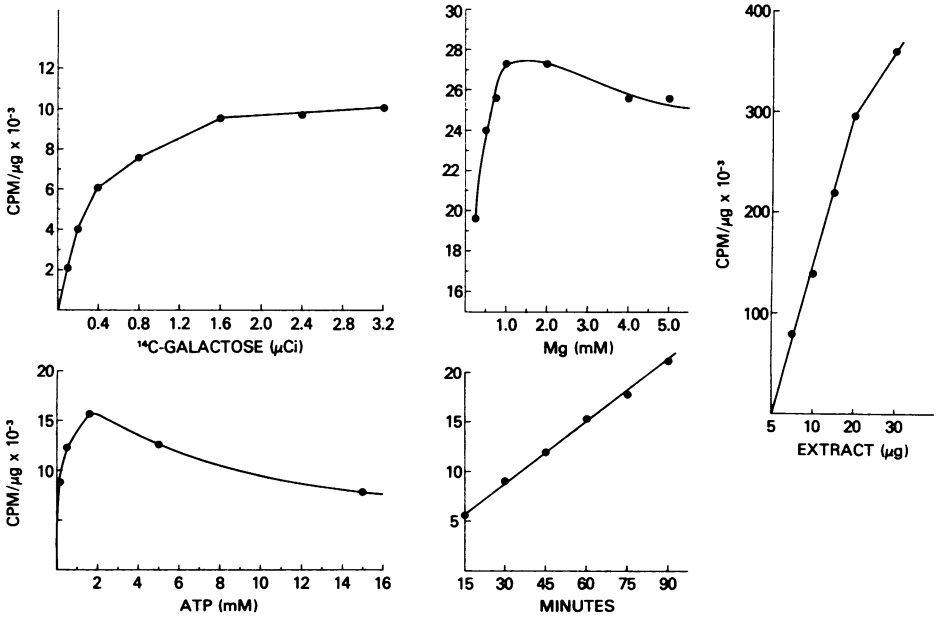

FIG. 1. Characterization of the galactokinase assay (see text).

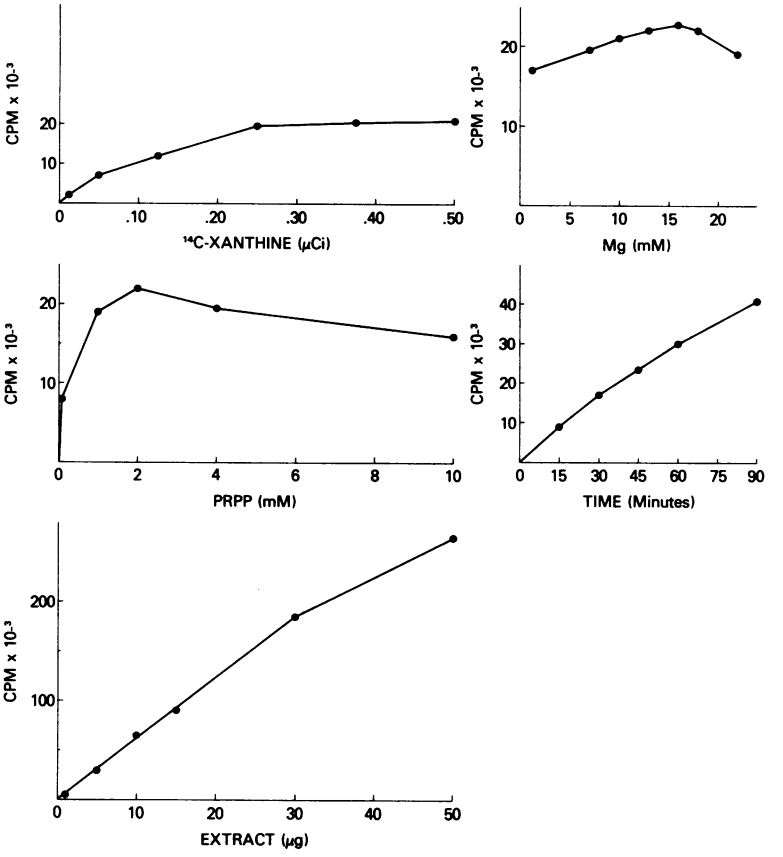

FIG. 2. Characterization of the XGPRT assay (see text).

start site of translation, the exact cleavage site of *Hinc*II is known. There is a *Hinc*II site that is 26 bases 3′ to the cap site and 26 bases 5′ to the initiation codon ATG. Cleavage at this site yields DNA with the appropriate signals for transcription, the CCAAT box, the ATA box, and the cap site, yet eliminates the entire coding region of the β-globin gene, including the translational start site. Cleavage at the next available upstream *Hinc*II site yields a DNA fragment of 550 bp which then includes the β^{maj}-globin promoter and approximately 450 bp 5′ to this region (Fig. 3B). This *Hinc*II fragment was used in the constructions described below.

Construction of plasmids containing enhancer elements. The β^{maj}-globin promoter fragment was first cloned into pSVK104, a derivative of pSVK100 (M. Reff and M. Rosenberg, unpublished data), which includes the *E. coli galK* gene and a DNA segment from SV40 containing the t splice and polyadenylation signal necessary for processing eucaryotic mRNA, thus allowing *galK* expression in a variety of mammalian cell systems (16). pSVK104, like all pSVK derivatives, also carries the *Eco*RI to *Pvu*II segment of pBR322 and can therefore replicate in *E. coli* and confer ampicillin resistance on its host (Fig. 4). In pSVK104, the *galK* gene is fused to the SV40 early promoter, but this promoter can be deleted by *Pvu*II plus *Sma*I cleavage; thus, another promoter can be inserted on any blunt-end fragment. This cleavage product is shown in Fig. 4. The *Hinc*II fragment containing the β^{maj}-globin promoter and 5′ flanking region was cloned into the *Pvu*II and *Sma*I sites of this vector. The orientation of the promoter was determined by *Hin*F and *Hae*III digestions of the plasmids with analysis on polyacrylamide gels (data not shown). The resulting plasmid, pPB12, is shown in Fig. 4. This plasmid is the basis for all subsequent constructions.

Next, a 2.2-kilobase *Bam*HI fragment was cloned into the single *Bam*HI site in pPB12. This fragment contains the *E. coli gpt* gene driven by the SV40 early promoter (15). Addition of this DNA gives an internal standard for transformation frequency; furthermore, it contains the SV40 72-bp repeat. The plasmid containing the

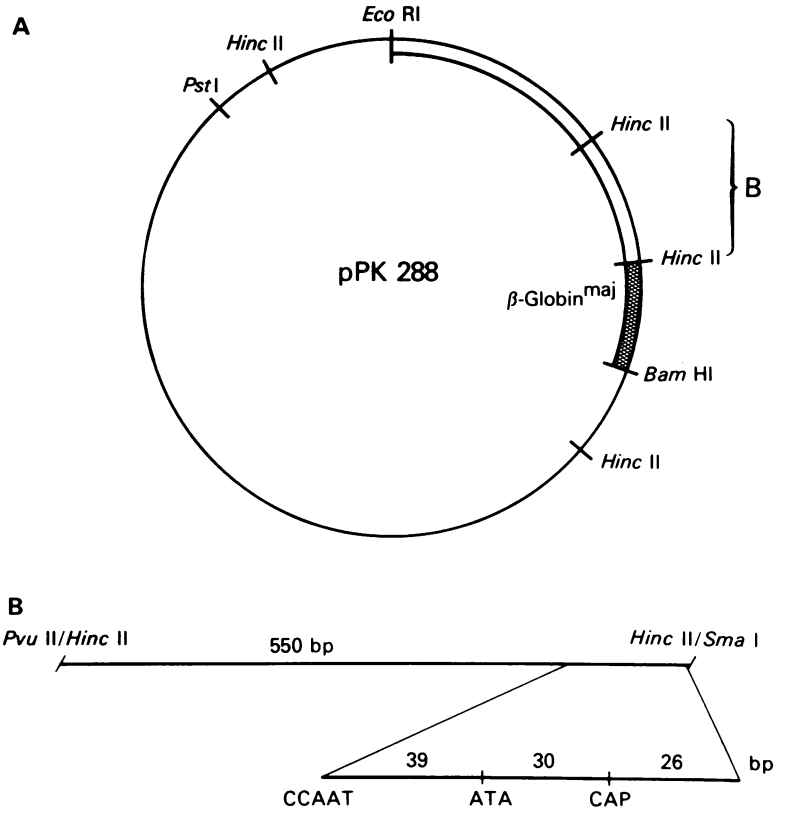

FIG. 3. Isolation of the mouse β^{maj}-globin promoter. (A) *Hinc*II restriction map of pPK288. The shaded portion indicates the mouse β^{maj}-globin structural gene. The *Hinc*II fragment, indicated by a brace and labeled B, is shown below. (B) The mouse β^{maj}-globin promoter on a 550-bp *Hinc*II DNA fragment. The elements known to be necessary for transcription are indicated: the CCAAT box and the ATA box, as well as the cap site.

gpt DNA is called pPB22 (Fig. 4). Eucaryotic cells transformed with pPB22, therefore, can express both the *galK* and *gpt* genes.

The second enhancer element added to pPB12 was the 73-bp repeat from HaSV (M. Ostrowski and G. Hager, manuscript in preparation). The repeat is on a 550-bp *Eco*RI-*Bam*HI fragment which is part of the long terminal repeat but does not contain promoter activity. The *Eco*RI-*Bam*HI region of pPB12 was excised and replaced with the HaSV enhancer fragment to give pPB12H (Fig. 4).

Finally, a plasmid was constructed containing both enhancer elements. This plasmid, pPB22H, was constructed from pPB22 by replacing the *Eco*RI-*Bam*HI fragment with the HaSV enhancer fragment.

A schematic diagram of all these constructions is shown in Fig. 5. The enhancer elements are indicated by black boxes.

Comparisons of enhancers on β-globin promoter activity. Starch gel electrophoresis was used to assay both Gal K and XGPRT levels on the same samples from CV-1 cells. These gels are known to be useful in the separation of isozymes, and it has been demonstrated in CV-1 cells that the endogenous Gal K and hypoxanthine phosphoribosyltransferase (HPRT) enzymes separate well from the plasmid-encoded bacterial counterparts (Fig. 6) (16). Autoradiograms of starch gels were traced and quantitated. For L cells, the separation of endogenous Gal K and HPRT enzymes is very poor (P. Berg, unpublished data). Instead, a filter assay was used for both Gal K and XGPRT (see above). The labeled precursor used for XGPRT is [^{14}C]xanthine, since the *E. coli* enzyme can use xanthine as a substrate, whereas HPRT cannot. Therefore, there is no detectable background from L cells. For the Gal K assay, endogenous Gal K was measured in L cells treated only with carrier DNA, and this value was subtracted from the Gal K enzyme level in transformed cells. Data are expressed as counts per minute per microgram of protein.

Two methods of comparing the data from the constructions were used. For pPB22 and pPB22H, which both contain the *gpt* gene, ratios

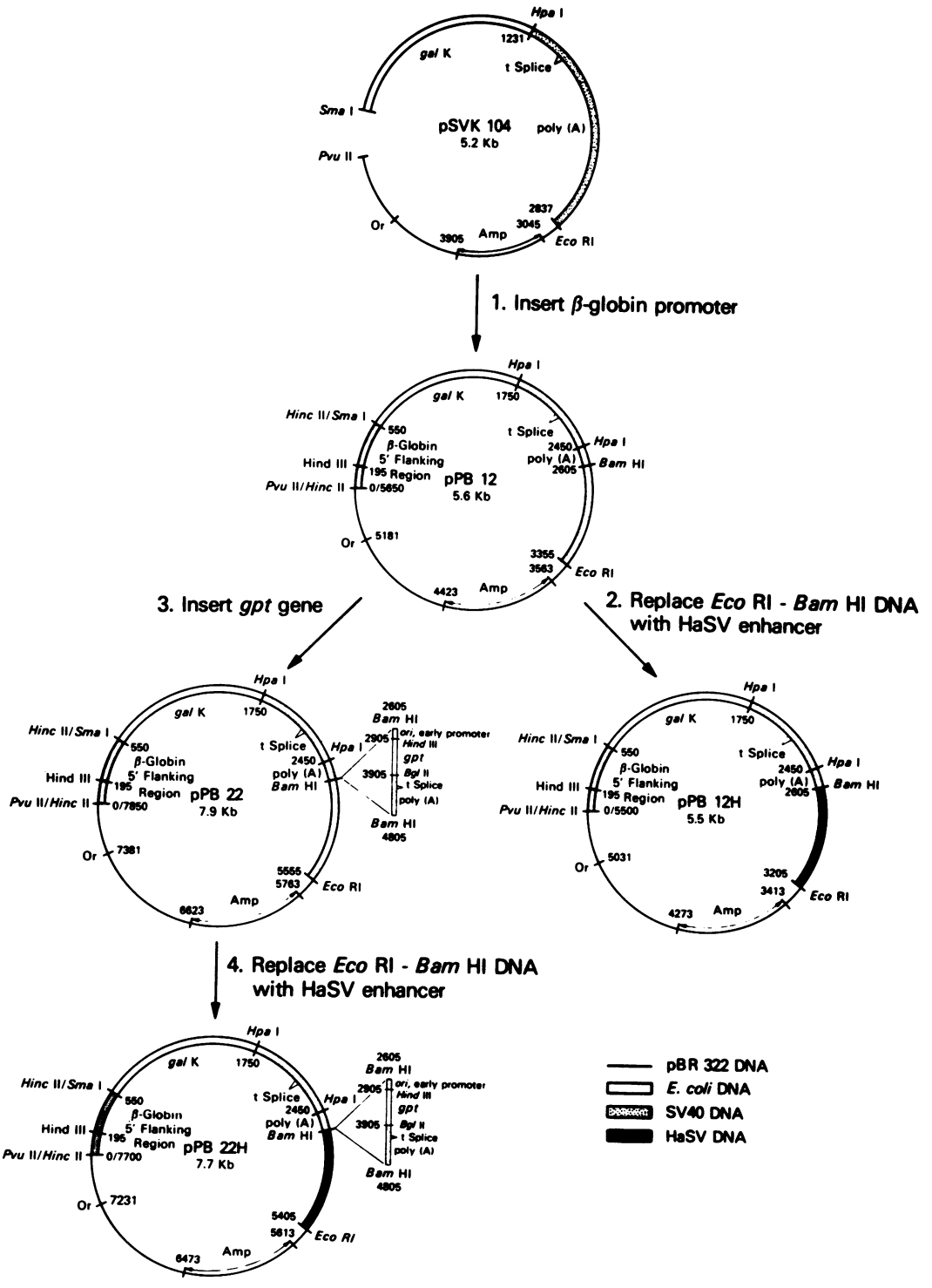

FIG. 4. Construction of plasmids containing enhancers. pSVK104 is shown after cleavage with *Pvu*II and *Sma*I to remove the SV40 promoter. Step 1: The mouse β^{maj}-globin promoter on a *Hin*cII fragment was ligated into the *Pvu*II and *Sma*I sites on pSVK104. Step 2: The 750-bp *Eco*RI-*Bam*HI SV40 DNA of pPB12 was replaced with the 550-bp *Eco*RI-*Bam*HI HaSV enhancer DNA. Step 3: The *E. coli gpt* gene on a 2.2-kilobase *Bam*HI fragment was inserted into the single *Bam*HI site on pPB12. Step 4: The 750-bp *Eco*RI-*Bam*HI SV40 DNA of pPB22 was replaced with the 550-bp HaSV enhancer DNA.

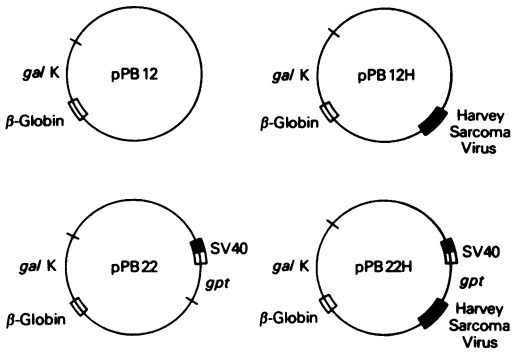

FIG. 5. Schematic diagram of the plasmids used in the assays. Open boxes indicate promoters, and closed boxes indicate enhancers.

TABLE 1. Levels of Gal K and XGPRT enzyme activities

Cell type	Plasmid	Normalized Gal K[a]	Normalized XGRPT[a]
CV-1	pPB12	3.6	—
	pPB12H	12.0	—
	pPB22	47.9	31.8
	pPB22H	77.7	27.5
L	pPB12	7,618	—
	pPB12H	12,613	—
	pPB22	9,824	48,719
	pPB22H	21,561	60,412

[a] Data were normalized as described in the text. The values for CV-1 cells are in arbitrary units, and the values for L cells are expressed in counts per minute per microgram. Each number is the average of three or more independent assays.

of plasmid-coded Gal K to XGPRT were calculated. Since pPB12 and pPB12H do not contain the *gpt* gene, a normalized Gal K enzyme value for the plasmid-coded enzyme was determined. This normalized value was found by measuring endogenous Gal K enzyme activity, setting that value equal to 100 for CV-1 cells or 50,000 cpm/μg for L cells, and then adjusting the plasmid Gal K. The XGPRT data can be similarly normalized. Gal K-to-XGPRT ratios calculated with or without normalization gave similar results (Table 1). In CV-1 cells, there is a low but reproducible level of plasmid-determined Gal K, even with no enhancer (pPB12), which increases to 12.0 in the presence of the HaSV enhancer (pPB12), 47.9 in the presence of the SV40 enhancer (pPB22), and 77.7 when both enhancers are on the plasmid (pPB22H).

The effect of enhancers on the β-globin promoter in L cells is also shown in Table 1. Addition of the HaSV enhancers (pPB12H) increases the Gal K activity from 7,618 to 12,613 cpm/μg, whereas the SV40 enhancer only increases the value to 9,824 cpm/μg. Both enhancers (pPB22H) give the highest Gal K level, 21,561 cpm/μg.

Table 2 shows the ratios of normalized plas-

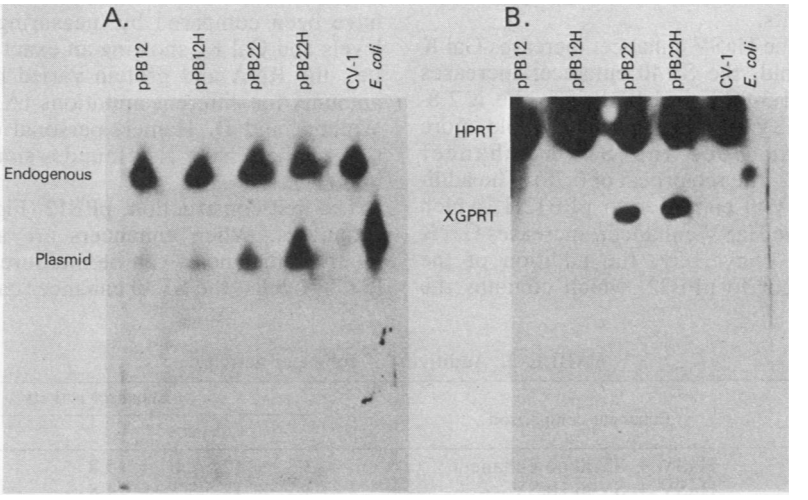

FIG. 6. Starch gel electrophoresis of Gal K and XGPRT. CV-1 cells were transformed with the appropriate DNA, as described in the text, and lysed (10). Extracts from 5×10^6 cells were loaded per well. (A) Assay for Gal K. The endogenous and plasmid-coded Gal K are indicated. Controls were untreated CV-1 cells and an *E. coli* extract which contains *E. coli* Gal K and XGPRT. (B) Assay for XGPRT. The endogenous HPRT and the plasmid-coded XGPRT are indicated. The samples are identical to those in (A), since the gel was sliced horizontally after electrophoresis and before the assays.

TABLE 2. Comparison of Gal K levels produced by different plasmids

Plasmids compared	Gal K ratio of cell type:		Gal K/XGPRT ratio of cell type:	
	CV-1	L	CV-1	L
pPB12H/pPB12	3.3	1.66	ND[a]	ND
pPB22/pPB12	12.5	1.29	ND	ND
pPB22H/pPB12	20.0	2.83	ND	ND
pPB22/pPB12H	4.0	0.78	ND	ND
pPB22H/pPB12H	6.7	1.71	ND	ND
pPB22H/pPB22	1.6	2.19	1.85	1.78

[a] ND, Not determined.

mid Gal K enzyme values for the SV40 and HaSV enhancers in both cell types. The relative strengths of the enhancers are very different in CV-1 cells and L cells. In CV-1 cells, the low level of *galK* expression in pPB12 is increased 3.3-fold by the addition of the HaSV enhancer, 12.5-fold by the SV40 enhancer, and 20-fold when both enhancers are present. The SV40 enhancer in pPB22 gives four times more Gal K than the HaSV enhancer in pPB12H, whereas both enhancers together give 6.7-fold more Gal K than the HaSV enhancer alone, but only 1.6-fold more Gal K than the SV40 enhancer alone. If the pPB22H/pPB22 ratio is calculated from the Gal K/XGPRT enzyme activity ratios for these two plasmids, the value is 1.8, as compared with the value of 1.6 obtained by using normalized Gal K enzyme values. These numbers are in good agreement, indicating that normalization of the data for plasmids without the *gpt* gene gives accurate results.

In L cells, the HaSV enhancer increases Gal K activity 1.7-fold, the SV40 enhancer increases this activity 1.3-fold, and both increase it 2.8-fold. The HaSV enhancer gives 1.3-fold more activity than does the SV40 enhancer (pPB12/pPB22, the reciprocal of 0.78). The addition of the SV40 enhancer to pPB12H, which already has the HaSV enhancer, increases Gal K 1.7-fold and, conversely, the addition of the HaSV enhancer to pPB22, which contains the SV40 enhancer, increases Gal K 2.2-fold. A comparison of the Gal K/XGPRT ratio (1.8) for pPB22H/pPB22 with the Gal K ratio (2.2-fold) indicates fairly good agreement.

The effect of the two enhancers is approximately additive (Table 3). In CV-1 cells, the SV40 enhancer alone increases expression 12.5-fold; the HaSV enhancer alone increases expression 3.3-fold, and the two together increase expression 20-fold. Both enhancers (pPB22H) result in a 6.7-fold increase over the HaSV enhancer alone (pPB12H) and a 1.6-fold increase over the SV40 enhancer alone (pPB22). This compares well with calculated values of 4.8- and 1.3-fold, respectively. Similar calculations can be made for enhancer additivity in L cells (Table 3). There, too, the calculated and observed values are in good agreement.

DISCUSSION

The two main findings of the work presented here are, first, that in CV-1 (primate) cells the SV40 (primate) enhancer is significantly stronger than the HaSV (mouse) enhancer in its action on the mouse β^{maj}-globin promoter, whereas the opposite enhancer strength is seen in L (mouse) cells, and, second, that the effect of both enhancers when present on the same DNA molecule is additive.

Enhancer strength was determined by using various plasmid constructions in which Gal K was assayed (Fig. 4). It was assumed that the level of protein is a direct reflection of the mRNA level, since different promoter mutations have been compared by measuring both RNA levels and Gal K, showing an exact correlation; i.e., the RNA and protein varied by the same amounts for different mutations (A. Carter, M. Walling, and D. Hamer, personal communication). Others have also found a similar correlation (11).

The first construction, pPB12 (Fig. 4), has no enhancers. When enhancers are added, their relative efficiencies can be measured (Table 2). In CV-1 cells, the SV40 enhancer causes a 12.5-

TABLE 3. Additivity of enhancer activity

Cell type	Enhancer comparison	Enhancer activity[a]	
		Calculated	Observed
CV-1	HaSV + SV40/no enhancer	3.3 + 12.5/1.0 = 15.8	20.0
	HaSV + SV40/HaSV	3.3 + 12.5/3.3 = 4.8	6.7
	HaSV + SV40/SV40	3.3 + 12.5/12.5 = 1.3	1.6
L	HaSV + SV40/no enhancer	1.66 + 1.29/1.0 = 2.95	2.83
	HaSV + SV40/HaSV	1.66 + 1.29/1.66 = 1.78	1.71
	HaSV + SV40/SV40	1.66 + 1.29/1.29 = 2.29	2.19

[a] The values are taken from Table 1.

fold increase in the amount of Gal K compared with the plasmid with no enhancer (pPB22 compared with pPB12), whereas the HaSV enhancer causes a 3.3-fold increase (pPB12H compared with pPB12). This means that the SV40 enhancer is four times more active than the HaSV enhancer under these conditions. In L cells, the highest Gal K level is seen when both enhancers are present. This value, however, is only 2.8-fold higher than that for L cells containing pPB12. The HaSV enhancer is stronger than the SV40 enhancer but gives only slightly more Gal K than the SV40 enhancer. The maximum enhancer strength in CV-1 cells (pPB22H) is almost 10 times higher than the maximum strength in L cells. Possible reasons for this are discussed below.

To correct for differences in transformation frequency, which may vary severalfold, some of these constructions include a second gene which serves as a reference or internal standard. Two of the plasmids used in these assays, pPB22 and pPB22H, contain the *gpt* gene. In these cases the ratio of Gal K to XGPRT enzyme activity can be measured. However, since the SV40 DNA used as a promoter for *gpt* also contains the SV40 enhancer, *gpt* was not used for all constructions. It was found that by performing three or more independent transformations, the data could be averaged, and the results were similar to the Gal K/XGPRT enzyme ratio (Table 2; 1.6 compared with 1.8, and 2.19 compared with 1.78).

Although enhancers greatly increase the activity of the β^{maj}-globin promoter, there is a significant level of Gal K, even in cells containing pPB12 (Table 1). This is similar to the results of Humphries et al. (7), who examined the effect of the SV40 enhancer on a cloned human β-globin gene in COS cells (CV-1 cells containing a defective, integrated SV40 which synthesizes T antigen but does not replicate). They found a significant level of transcription of the β-globin gene without the SV40 72-bp repeat, but most transcripts were incorrectly initiated. Addition of the SV40 enhancer allowed increased and accurate transcription. In contrast, in HeLa cells very low levels of transcription of cloned rabbit or human β-globin gene were detected without the SV40 enhancer (1, 14). The reason for these differences in promoter activity without enhancers may be that the copy number of plasmids introduced into COS cells was high, since the plasmids contained the SV40 origin of replication and so could replicate (7). Thus, even a low level of transcription from each plasmid might yield a high total activity.

Enhancers may interact with host cell factors to cause species specificity of enhancer function. Polyoma virus, which has an enhancing sequence (5), is normally unable to grow in undifferentiated embryonal carcinoma cells, but mutants which can grow in these cells are altered in the DNA of the enhancing region (8, 9). These data have been interpreted to suggest that there are host factors which do not recognize the wild-type virus but do recognize the mutant. A different line of evidence comes from Laimins et al. (11), who studied the effect of two different enhancers in CV-1 cells and L cells on the SV40 early promoter, using plasmids in which the SV40 promoter was fused to the chloramphenicol acetyltransferase (CAT) gene. They looked at the SV40 enhancer and the Moloney murine sarcoma virus (MuSV) enhancer. MuSV is structurally related to HaSV, and in fact the sequences of the enhancers are identical (M. Ostrowski and G. Hager, manuscript in preparation). Laimins et al. found that the SV40 enhancer is about 6-fold stronger than the MuSV enhancer in monkey CV-1 cells but that the MuSV enhancer is about 2.5-fold stronger in mouse cells (11). In addition, de Villiers et al. (4) observed that the polyoma (mouse) enhancer is more active in mouse cells than the SV40 enhancer for both a polyoma and a rabbit β-globin promoter, but the reverse is true in primate cells. Our data also support this model, since in our constructions the SV40 enhancer is stronger than the HaSV enhancer in CV-1 cells, but the HaSV enhancer is stronger than the SV40 enhancer in L cells. These data all fit the model of species specificity, since monkey CV-1 cells are permissive for SV40, but not HaSV, MuSV, or polyoma virus, whereas mouse L cells are permissive for HaSV, MuSV, and polyoma virus, but not SV40.

It is interesting to compare the differences in enhancer strength in CV-1 cells and L cells (Table 2). For example, the SV40 enhancer is 4-fold stronger than the HaSV enhancer in CV-1 cells, but the HaSV enhancer is only 1.3-fold stronger than the SV40 enhancer in L cells. One possible reason for this difference is that the mouse β-globin promoter is more active in mouse cells than in monkey cells due to a host factor(s) which activates the promoter. An alternative explanation is that enhancers are not as active in L cells. Laimins et al. (11) observed that in L cells the MuSV enhancer is about 2.5-fold stronger than the SV40 enhancer with their SV40 promoter constructions; de Villiers et al. (4) showed that in L cells the polyoma enhancer is about 1.6-fold stronger than the SV40 enhancer using a polyoma promoter. Therefore, it appears that a mouse-specific enhancer can function at a higher level than it did in our experiments. In agreement with our data, de Villiers et al. (4) observed almost no enhancement of the rabbit β-globin gene in L cells by a

polyoma enhancer, although they saw four- to sixfold stimulation of that promoter by the SV40 enhancer in CV-1 cells. This implies that it is the difference in promoters (SV40 or polyoma compared with mouse or rabbit β-globin) which caused the lower enhancer effect in L cells.

One can compare the effect of the same two enhancers on different promoters by using our β-globin promoter constructs and the SV40 promoter-CAT constructs of Laimins et al. (11). Only a general comparison can be made since the constructions are different; ideally, one would like to compare plasmids which are identical except for the promoter. In CV-1 cells after 20 min of incubation, Laimins et al. (11) detected a 17-fold increase in CAT activity when the SV40 enhancer was present and a 3-fold increase in CAT expression due to the MuSV enhancer. In the constructions described here (Table 2), the SV40 enhancer increased expression 12.5-fold compared with 3.3-fold for the HaSV enhancer. These data are similar and, alone, might lead one to believe enhancer strength is promoter independent. However, as discussed above, the L-cell data show that this is apparently not the case. It therefore appears that the effect of an enhancer depends not only on the species but also on the promoter being acted upon.

When both enhancers are present, their effects are additive in both CV-1 cells and L cells (Table 3). Moreau et al. (13) compared the effect of one or two SV40 enhancers (four 72-bp repeats) in CV-1 cells on activation of the adenovirus 2 major late promoter linked to the structural gene for the SV40 T antigen. The level of expression of T antigen was approximately doubled in the presence of two enhancers. It thus appears that enhancer activity is additive whether both enhancers are of the same (SV40) or different (SV40 and HaSV) types. Furthermore, their constructions and ours were very different in that the SV40 and HaSV enhancers were separated by approximately 2,000 bp in our construction (pPB22H, Fig. 4), whereas their two SV40 enhancers were in tandem. Finally, the additivity of these two enhancers appears to be independent of the species.

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