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Promoter Dependence of Enhancer Activity

PATRICIA E. BERG,* ZVEZDANA POPOVIC,† AND W. FRENCH ANDERSON

Laboratory of Molecular Hematology, National Heart, Lung and Blood Institute, Bethesda, Maryland 20205

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The interaction of enhancers with different promoters was studied by measuring the influence of two enhancers (from simian virus 40 and from Harvey sarcoma virus) on the activity of expression vectors that are identical except for their promoter region. The promoters examined were from the simian virus 40 early region, with or without its own 72-base-pair repeat, and the mouse β^{major}-globin gene. It is clear that the promoter acted upon strongly influences the level of activity of an enhancer.

It is known that DNA sequences called enhancers can activate certain cloned genes (1, 2, 7, 9, 15–17, 21, 26). A number of enhancers have been identified, both of viral and cellular origin (1, 7, 12, 15, 21, 22, 24–26). Enhancers act in *cis* in an orientation-independent manner (10, 26, 30). Factors known to influence enhancer activity include the cell line the enhancer is in and, in some cases, its position relative to the promoter being tested (1, 4, 6, 10, 20, 26). It is not known what role, if any, the promoter plays in this process, i.e., are enhancer effects the same on different promoters? To test this question, we examined the effect of two well-characterized enhancers, the simian virus 40 (SV40) 72-base-pair (bp) repeat and the Harvey sarcoma virus (HaSV) 73-bp repeat, on the SV40 early promoter with its own enhancer deleted (SV40d) and the mouse β^{major}-globin promoter by using constructions identical except for the promoter being tested. The promoter in each case was fused to an easily assayable gene, *Escherichia coli galK*, so that galactokinase activity could be used to measure promoter function.

The basic plasmid used for these constructions was pSVK100, described in detail elsewhere (27). It contains the SV40 early promoter driving the *E. coli galK* gene (Fig. 1). The 72-bp repeat (enhancer) was cleaved from pSVK100 by digestion with *Pvu*II and *Sph*I, which deletes 142 bp, leaving the terminal 21 bp of one enhancer (26). This deletion is sufficient to eliminate detectable enhancer activity (11, 14, 26). The resultant DNA was ligated to give pZP1 (Fig. 1). The exact deletion size was confirmed by DNA sequencing (data not shown). pZP1, then, contains the SV40 early promoter with no enhancer (SV40d). The HaSV enhancer was added to give pZP2, whereas the SV40 enhancer was added to pZP1 at the *Bam*HI site, ca. 2 kilobase pairs from its location in pSVK100, giving pZP1-*gpt*. The final plasmid, pZP2-*gpt*, contains both enhancers and was constructed by inserting the *Bam*HI fragment just described into pZP2, which already has the HaSV enhancer. A schematic of these plasmids is shown in Fig. 2A. Figure 2B shows the parallel constructions with the mouse β^{major}-globin promoter. The following plasmids are identical except for their promoter: pZP1 and pPB12; pZP2 and pPB12H; pZP1-*gpt* and pPB22; pZP2-*gpt* and pPB22H. Details of the construction of pPB12, pPB12H, pPB22, and pPB22H have been reported (4).

Do enhancers have the same relative effect on different promoters? This question was approached by comparing galactokinase levels in the plasmids containing the SV40 or HaSV enhancer in combination with the SV40d or β-globin promoter in CV-1 (monkey) cells, L (mouse) cells, and Chinese hamster cells (Tables 1 and 2). Transfections and galactokinase assays were as described previously (3, 4). A comparison of the ratios of the galactokinase observed in the SV40-enhanced plasmid to that in the unenhanced plasmid in CV-1 cells for the two promoters indicated a large difference in enhancement, i.e., the SV40 enhancer activates the β-globin promoter much more than the SV40d promoter (a ratio of 13 for the β-globin promoter and 1.3 for the SV40d promoter). The SV40 enhancer was 60% stronger with the β-globin promoter than with the SV40d promoter in L cells, whereas in R1610 cells, the SV40 enhancer was fivefold stronger with the β-globin promoter. The situation was similar for the HaSV enhancer; in all cases it was more effective on the β-globin promoter than on the SV40d promoter. Thus, the β-globin promoter was able to be enhanced 9.3-fold more than the SV40d promoter. The SV40d promoter has been shown by others to be enhanceable (13, 19, 20, 29), so the lack of activation we observed is significant.

The effect of deleting the SV40 enhancer and replacing it in a different position is shown in Table 3. The pZP1/pSVK100 galactokinase ratio indicates the effect of deleting the enhancer (pZP1) compared with having it in its natural position adjacent to the promoter (pSVK100). The pZP1-*gpt*/pSVK100 galactokinase ratio indicated the activity of the two enhancer positions since pZP1-*gpt* contains the SV40 enhancer 2 kb from the promoter. In CV-1 cells, the pZP1/pSVK100 galactokinase ratio was 0.10, meaning that deletion of the enhancer caused a 90% loss of galactokinase activity. Replacing it 2 kb from the promoter (pZP1-*gpt*/pSVK100) gave a ratio of 0.13 or 13% activity. Therefore, adding the enhancer, but at a distance from the promoter, only increased galactokinase by 30%. Similar calculations were performed for L cells and R1610 cells. Deletion of the enhancer caused a 31% loss of activity in L cells and a 77% loss in R1610 cells, and replacing it downstream did not result in a significant increase in promoter function in rodent cells. From these experiments and others (26), it is clear that the SV40 early promoter is very sensitive to enhancer position. Perhaps position effects are not as significant for the β-globin promoter; this could explain why this promoter was more readily activated in our constructions.

The relative strengths of the SV40d and mouse β-globin

* Corresponding author.
† Present address: Institute for Biological Research, 29, Novembra 142, Belgrade 11,000, Yugoslavia.

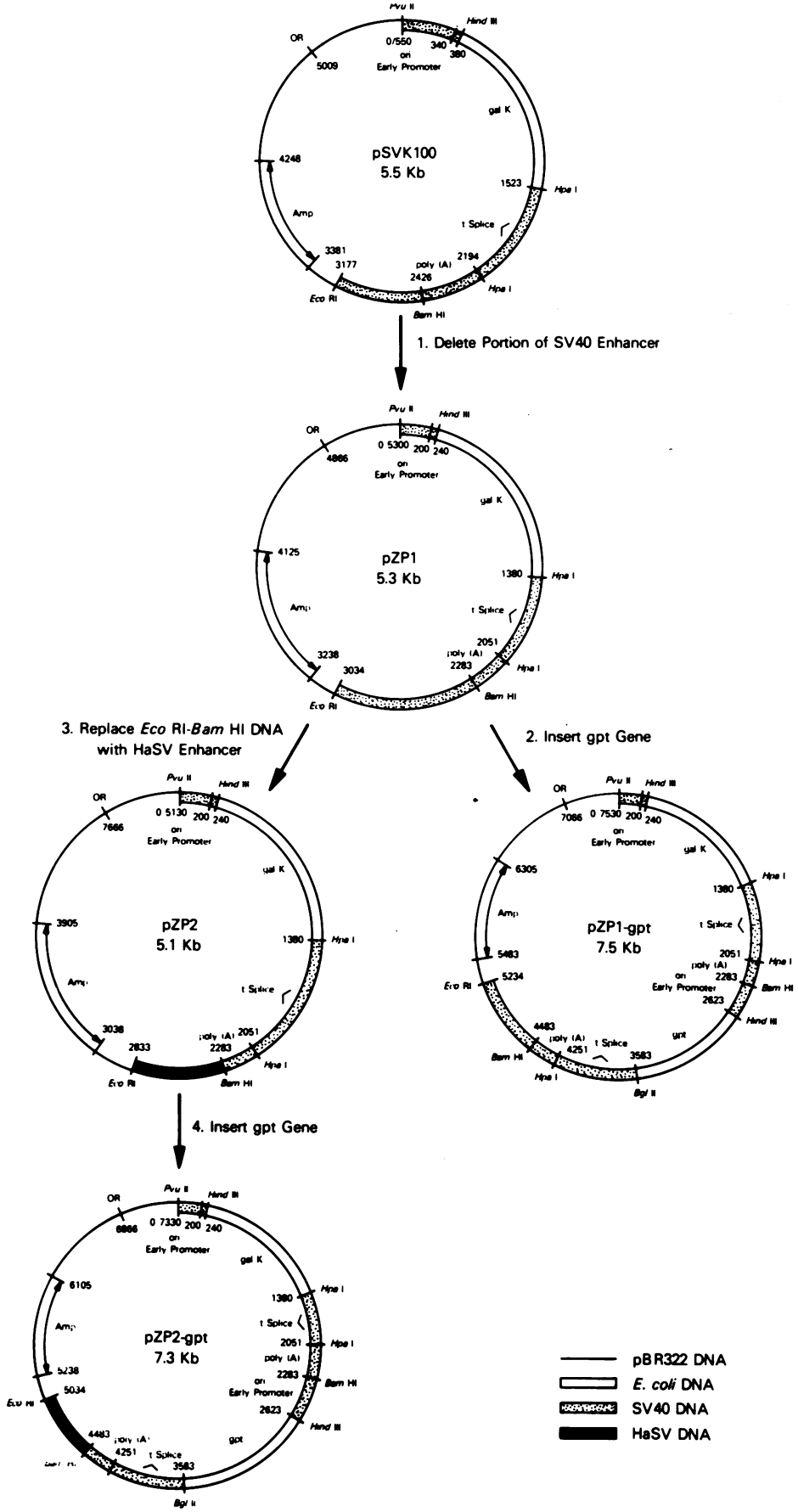

FIG. 1. Construction of plasmids containing enhancers. Step 1, pSVK100 was cleaved with *Sph*I and *Pvu*II to delete the SV40 enhancer, giving pZP1. Step 2, The SV40 enhancer, which is on a 2.2-kb *Bam*HI fragment containing the SV40 early promoter fused to the *E. coli gpt* gene, was inserted into the single *Bam*HI site of pZP1 to make pZP1-*gpt*. Step 3, The 750-base-pair *Eco*RI-*Bam*HI DNA in pZP1 was replaced with the 550-base-pair *Eco*RI-*Bam*HI fragment containing the HaSV enhancer (the adjacent promoter of the HaSV long terminal repeat is not on this fragment). This plasmid is pZP2. Step 4, pZP2-*gpt* was constructed by adding the 2.2-kb *Bam*HI fragment with the SV40 enhancers to pZP2.

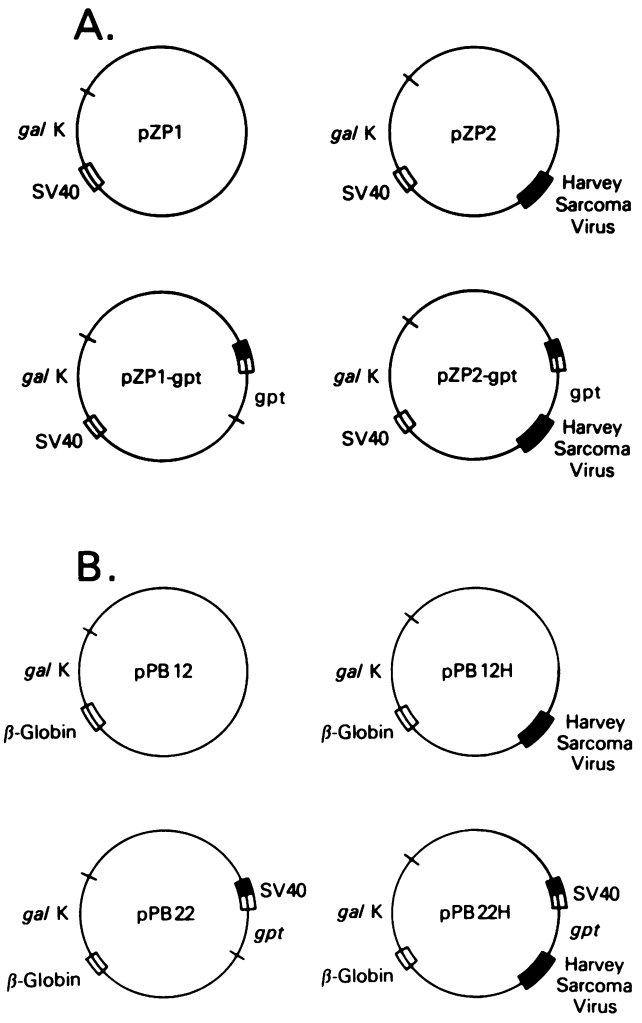

FIG. 2. Schematic diagram of plasmids. Open boxes indicate promoters and closed boxes indicate enhancers. (A) SV40 promoters. (B) β-Globin promoters.

TABLE 1. Galactokinase activities

Promoter	Plasmid	Galactokinase activity[a] in:		
		CV-1	L	R1610
SV40	pSVK100	171	12,600	10,500
	pZP1	18.9	8,670	2,350
	pZP1-*gpt*	22.7	8,830	2,410
	pZP2	5.3	12,050	3,250
	pZP2-*gpt*	23.0	11,900	4,580
β-Globin	pPB12	3.6	3,490	625
	pPB12H	12.0	5,890	3,840
	pPB22	47.9	5,630	3,150
	pPB22H	77.7	10,050	3,410

[a] Galactokinase activities are in arbitrary units for CV-1 cells and in counts per minute per microgram of protein for L and R1610 cells. A portion of the data for the mouse β^{major}-globin promoter are from Berg et al. (3, 4). Each value is the average of four or more independent assays.

TABLE 2. Enhancer effects on the SV40d and mouse β-globin promoters*

Promoter	Cell line	Galactokinase ratio[a]	
		SV40 enhanced/ unenhanced	HaSV enhanced/ unenhanced
SV40d	CV-1	1.3	0.29
	L	1.0	1.4
	R1610	1.0	1.4
β-Globin	CV-1	13	3.3
	L	1.6	1.7
	R1610	5.0	6.1

[a] Calculated from the data in Table 1.

TABLE 3. Effect of enhancer position on SV40 promoter activity

Cell line	Galactokinase activity ratio		% Change
	pZP1/ pSVK100	pZP1-*gpt*/ pSVK100	
CV-1	0.10	0.13	30
L	0.69	0.70	1
R1610	0.23	0.23	0

promoters were compared by using two plasmids with no enhancers, pPB12 (β-globin promoter) and pZP1 (SV40d promoter). The SV40d promoter was 5.0-fold stronger than the β-globin promoter (18.9 versus 3.6) in CV-1 cells, 2.5-fold stronger in L cells (8,670 versus 3,490), and 3.8-fold stronger in R1610 cells (2,350 versus 625) (see Table 1). One could speculate that this may reflect the biological roles of these two promoters: the SV40 early promoter is competing with other cellular promoters for RNA polymerase II after viral infection, whereas the β-globin promoter is active in an erythroid differentiated cell that is specialized for hemoglobin production. However, there is no way at present to know the in vivo activity of the β-globin promoter since it may be further activated by enhancers.

Species specificity of enhancers observed by us (4) and others (6, 20) was confirmed for both promoters. The relative enhancer effects were indicated by galactokinase ratios in CV-1, L, and R1610 cells (Table 4). It was clear that the SV40 enhancer is more active than the HaSV enhancer in CV-1 cells for both promoters, and the HaSV enhancer is more effective than the SV40 enhancer in rodent cells (L and

TABLE 4. Cell type dependence of enhancer effects

Promoter	Cell line	SV40/ HaSV enhancer ratio[a]
SV40 (primate)	CV-1 (monkey)	4.3
	L (mouse)	0.73
	R1610 (Chinese hamster)	0.74
β-Globin (mouse)	CV-1 (monkey)	4.0
	L (mouse)	0.81
	R1610 (Chinese hamster)	0.92

[a] Calculated from the data in Table 1.

TABLE 5. Transformation frequency in R1610 cells

Promoter	Plasmid	No. of colonies per 10^6 cells
SV40	pZP1	320
	pSVK100	2,105
	pZP1-*gpt*	697
β-Globin[a]	pPB12	51
	pPB12H	460
	pPB22	571

[a] Data are from Berg and Anderson (3).

TABLE 6. Transformation frequency and galactokinase activity ratios

Ratio of plasmids:	Transformation frequency ratio	Galactokinase activity ratio
pPB12H/pPB12	9.0	6.1
pPB22/pPB12	11.2	5.0
pSVK100/pZP1	6.6	4.4
pZP1-*gpt*/pZP1	2.2	1.0

R1610 cells). Therefore, species specificity of enhancer activity appears to be promoter independent. In addition, there was no detectable difference between these ratios in L and R1610 cells, even though the galactokinase levels were different in these cell lines (Table 1). It would be interesting to compare this enhancer ratio in other cell lines to determine whether it is consistently either near 4.0 for cells in which the SV40 enhancer is more active or between 0.70 and 0.90 for cells in which the HaSV enhancer is more active.

The presence of an enhancer on a plasmid can increase transformation frequency by as much as 100-fold (5, 8, 18, 23, 31), probably due to an increase in enzyme activity (3, 22). In particular, there is a direct correlation between the galactokinase level and the frequency of $GalK^+$ stable transformants in R1610 (3). Three of the plasmids described here, pSVK100, pZP1 and pZP1-*gpt*, were used to transform R1610, which is *galK*$^-$, with selection for stable transformants as described (3, 28) (Table 5). Data from previous experiments with pPB12, pPB12H, and pPB22 are included for comparison (3). The transformation assay is compared with the galactokinase enzyme assay for R1610 cells in Table 6. The effect of an enhancer is shown by comparing either the number of colonies (transformation assay) or the galactokinase level of a plasmid containing an enhancer to that in a plasmid with no enhancer. In each case the transformation frequency appeared to be a more sensitive indicator of enhancer activity than enzyme activity. There could be a number of explanations for this observation. Although Luciw et al. (22) showed that the number of cells which integrated plasmid DNA after microinjection is independent of the presence of an enhancer, the factors determining integration after calcium phosphate-mediated DNA transfer may not be enhancer independent. Another possibility is that enhancers may allow earlier expression of the *galK* gene so that more cells are able to survive the challenge with galactose.

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LITERATURE CITED

1. **Banerji, J., S. Rusconi, and W. Schaffner.** 1981. Expression of a β-globin gene is enhanced by remote SV40 DNA sequences. Cell **27**:299–308.
2. **Benoist, C., and P. Chambon.** 1981. In vivo sequence requirements of the SV40 early promoter region. Nature (London) **290**:304–310.
3. **Berg, P. E., and W. F. Anderson.** 1984. Correlation of gene expression and transformation frequency with the presence of an enhancing sequence in the transforming DNA. Mol. Cell. Biol. **4**:368–370.
4. **Berg, P. E., J.-K. Yu, Z. Popovic, D. Schumperli, H. Johansen, M. Rosenberg, and W. F. Anderson.** 1983. Differential activation of the mouse β-globin promoter by enhancers. Mol. Cell. Biol. **3**:1246–1254.
5. **Capecchi, M. R.** 1980. High efficiency transformation by direct microinjection of DNA into cultured mammalian cells. Cell **22**:479–488.
6. **de Villiers, J., J. Olson, C. Tyndall, and W. Schaffner.** 1982. Transcriptional "enhancers" from SV40 and polyoma virus show a cell type preference. Nucleic Acids Res. **10**:7965–7976.
7. **de Villiers, J., and W. Schaffner.** 1981. A small segment of polyoma virus DNA enhances the expression of a cloned β-globin gene over a distance of 1400 base pairs. Nucleic Acids Res. **9**:6251–6264.
8. **Folger, K. R., E. A. Wong, G. Wahl, and M. R. Capecchi.** 1982. Patterns of integration of DNA microinjected into cultured mammalian cells: evidence for homologous recombination between injected DNA molecules. Mol. Cell. Biol. **2**:1372–1387.
9. **Fromm, M., and P. Berg.** 1982. Deletion mapping of DNA regions required for SV40 early region promoter function *in vivo*. J. Mol. Appl. Genet. **1**:457–481.
10. **Fromm, M., and P. Berg.** 1983. Simian virus 40 early- and late-region promoter functions are enhanced by the 72-base-pair repeat inserted at distant locations and inverted orientations. Mol. Cell. Biol. **3**:991–999.
11. **Fromm, M., and P. Berg.** 1982. Deletion mapping of DNA regions required for SV40 early region promoter function *in vivo*. J. Mol. Appl. Genet. **1**:457–481.
12. **Gillies, S. D., S. L. Morrison, V. T. Oi, and S. Tonegawa.** 1983. A tissue-specific transcription enhancer element is located in the major intron of a rearranged immunoglobulin heavy chain gene. Cell **33**:717–728.
13. **Green, M. R., R. Treisman, and T. Maniatis.** 1983. Transcriptional activation of cloned human β-globin genes by viral immediate-early gene products. Cell **35**:137–148.
14. **Gruss, P.** 1984. Magic enhancers? DNA **3**:1–5.
15. **Gruss, P., R. Dhar, and G. Khoury.** 1981. Simian virus 40 tandem repeated sequences as an element of the early promoter. Proc. Natl. Acad. Sci. U.S.A. **781**:943–947.
16. **Humphries, R. K., T. Ley, P. Turner, A. D. Moulton, and A. W. Nienhuis.** 1982. Differences in human α-, β- and γ-globin gene expression in monkey kidney cells. Cell **30**:173–183.
17. **Khoury, G., and P. Gruss.** 1983. Enhancer elements. Cell **33**:313–314.
18. **Kriegler, M., and M. Botchan.** 1983. Enhanced transformation by a simian virus 40 recombinant virus containing a Harvey murine sarcoma virus long terminal repeat. Mol. Cell. Biol. **3**:325–339.
19. **Laimois, L., P. Gruss, R. Pozzatti, and G. Khoury.** 1984. Characterization of enhancer elements in the long terminal repeat of Moloney murine sarcoma virus. J. Virol. **49**:183–189.
20. **Laimins, L. A., G. Khoury, C. Gorman, B. Howard, and P. Gruss.** 1982. Host-specific activation of transcription by tandem repeats from simian virus 40 and Maloney murine sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **79**:6453–6457.
21. **Levinson, B., G. Khoury, G. Vande Woude, and P. Gruss.** 1982. Activation of SV40 genome by 72 base pair tandem repeats of Maloney sarcoma virus. Nature (London) **295**:568–572.
22. **Luciw, P. A., J. M. Bishop, H. E. Varmus, and M. R. Capecchi.** 1983. Location and function of retroviral and SV40 sequences that enhance biochemical transformation after microinjection of DNA. Cell **33**:705–716.
23. **Luskey, M., L. Berg, and M. Botchan.** 1982. Enhancement of *tK* transformation by sequences of bovine papilloma virus. p. 99–107. *In* Y. Gluzman (ed.), Eukaryotic viral vectors. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

24. **Luskey, M., L. Berg, H. Weiher, and M. Botchan.** 1983. Bovine papilloma virus contains an activator of gene expression at the distal end of the early transcription unit. Mol. Cell. Biol. **3**:1108–1122.
25. **Mercola, M., X.-F. Wang, J. Olsen, and K. Calame.** 1983. Transcriptional enhancer elements in the mouse immunoglobulin heavy chain locus. Science **221**:663–665.
26. **Moreau, P., R. Hen, B. Wasylyk, R. Everett, M. P. Gaub, and P. Chambon.** 1981. The SV40 72 base pair repeat has a striking effect on gene expression both in SV40 and other chimeric recombinants. Nucleic Acids Res. **9**:6047–6068.
27. **Schumperli, D., B. H. Howard, and M. Rosenberg.** 1982. Efficient expression of *Escherichia coli* galactokinase gene in mammalian cells. Proc. Natl. Acad. Sci. U.S.A. **79**:257–261.
28. **Thirion, J.-P., D. Banville, and H. Noel.** 1976. Galactokinase mutants of Chinese hamster somatic cells resistant to 2-deoxygalactase. Genetics **83**:137–147.
29. **Treisman, R., M. R. Green, and T. Maniatis.** 1983. *cis* and *trans* activation of globin gene transcription in transient assay. Proc. Natl. Acad. Sci. U.S.A. **80**:7428–7432.
30. **Wasylyk, B., C. Wasylyk, P. Augereau, and P. Chambon.** 1983. The SV40 72 bp repeat preferentially potentiates transcription starting from proximal natural or substitute promoter elements. Cell **32**:503–514.
31. **Yamaizumi, M., A. L. Horwich, and F. H. Ruddle.** 1983. Expression and stabilization of microinjected plasmids containing the herpes simplex virus thymidine kinase gene and polyoma virus DNA in mouse cells. Mol. Cell. Biol. **3**:511–522.