Journal of Cellular Biochemistry 42:101–110 (1990)

Modulation of HPV18 and BPV1 Transcription in Human Keratinocytes by Simian Virus 40 Large T Antigen and Adenovirus Type 5 E1A Antigen

Bruno A. Bernard, Catherine Bailly, Marie-Cecile Lenoir, and Michel Y. Darmon

Cell Biology Department (C.I.R.D.), Sophia Antipolis, 06565 Valbonne, France

Transcription of early open reading frames initiated from the long control region (LCR) of HPV18 and BPV1 is known to be modulated by homologous and heterologous papillomavirus E2 gene products. Using CAT constructs transfected into normal human keratinocytes, we show that SV40 large T antigen activates transcription from the LCR of both viruses, whereas Ad5-E1a antigen represses transcription from the HPV18-LCR but activates transcription from BPV1-LCR. Experiments using constructs containing subfragments of the HPV18-LCR cloned in enhancer configuration ahead of the SV40 early promoter or the HSV1-Tk promoter suggest that the effect of Ad5-E1a antigen on HPV18 transcription is probably due to a repression of the enhancer function of the LCR. The mechanism of transcription stimulation by SV40 large T antigen is less clear. The 230 bp Rsa1-Rsa1 central domain of the HPV18-LCR seems involved both in transcriptional stimulation by SV40 large T antigen and transcriptional inhibition by adenovirus E1a antigen.

Key words: papillomavirus, transcription, activation, inhibition, SV40 large T antigen, Ad5-E1a antigen

Papillomaviruses are small epitheliotropic DNA viruses that replicate as extra-chromosomal plasmids in infected cells [1]. They are responsible for a variety of benign skin and mucocutaneous tumors such as warts and condylomas [2]. More recently, several papillomaviruses were identified as etiological agents of genital tract dysplasias and carcinomas [3]. Most of the papillomaviruses contain eight early (E) and 2 late (L) open reading frames (ORF) and a non-coding long control region (LCR) located between the end of the L1 ORF and the beginning of the E6 ORF.

Because of the absence of in vitro culture system for human papillomaviruses, our understanding of the regulation of their gene expression relies almost exclusively on the study of the transcriptional activity of the LCR when transfected into various cell types. In most cases, LCRs were cloned in front of a reporter gene such as the bacterial chloramphenicol acetyl transferase (CAT) and their modulation by transeffectors

Received March 7, 1989; accepted October 11, 1989.

© 1990 Wiley-Liss, Inc.

analyzed. Using this approach, the product of the E2 ORF was shown to be a very efficient modulator of transcription initiated from the BPV1 and HPV11, 16, and 18 LCRs [4–9]. It has also been reported that SV40 large T (SV40-LT) antigen and adenovirus type 5 E1a antigen (Ad5-E1a antigen) were able, respectively, to activate and inhibit transcription from the HPV18-LCR [10], at least in transformed epithelial cells such as HeLa and SW13. The transcriptional activation by SV40-LT antigen was suspected to be indirect because of the absence of consensus large T binding site in the HPV18-LCR sequence. It was thus hypothesized that the effect of large T antigen was due to its interaction with a host factor specific for transformed cells [10]. In the present report, we show that SV40-LT antigen behaves also as a strong transcriptional activator of both BPV1 and HPV18-LCRs in normal cells such as human epidermal keratinocytes. The Ad5-E1a antigen effect was presumed to occur in the transformed cell lines by inhibition of enhancer function of the LCRs [10]. We show in this report that in normal human keratinocytes Ad5-E1a antigen also inhibits transcription from HPV18-LCR, but that, on the contrary, it stimulates transcription from BPV1-LCR.

MATERIALS AND METHODS

Cell Culture

Interfollicular human keratinocytes were isolated from human breast skin [11] and subcultured according to Rheinwald and Green [12] in the presence of a mitomycin-treated NIH 3T3 feeder layer.

Plasmids (See Table I)

PSVECAT and PDSVECAT plasmids containing either the complete SV40 early promoter with its enhancer or the same region deleted of the enhancer sequence (72 bp

TABLE I. Plasmids Used

Name	Enhancer	Promoter	Gene
pro BPV	BPV1-LCR (Dde1-Dde1)	BPV1 (E6)	CAT
pH18CAT	HPV18-LCR (BamH1-BamH1)	HPV18 (E6)	CAT
dl400	HPV18-LCR (Bgl1-BamH1)	HPV18 (E6)	CAT
p18-1 SVECAT p18-2 SVECAT	HPV18-LCR (BamH1-BamH1)	SVE	CAT
TKM 3.2; TKM 3.3	HPV18-LCR (Rsa1-BamH1 proximal	TK	CAT
TKM 5′; TKM 3′	HPV18-LCR (Rsa1-Rsa1)	TK	CAT
TKM 6; TKM 12	HPV18-LCR (BamH1-Rsa1 distal)	TK	CAT
pC59	72 bp repeat	SVE	E2-BPV1
pSVECAT	72 bp repeat	SVE	CAT
pE1a	Complete transcription unit of AD5-E1a		Ad5-E1a
pMK 16 SV	Complete sequence of SV40 with a short deletion in the origin of replication		SV40 large T

repeat) positioned 5′ to the CAT coding sequence have already been described [13] as pE1a and pMK16SV plasmids [10]. Figure 1 shows the map of HPV18-LCR with the restriction sites used in the present study. The plasmids containing the HPV18-LCR cloned either in promoter configuration (pH18CAT) or in enhancer configuration (p18-1D and p18-2DSVECAT) have already been described [14], as well as the dl400 plasmid that was derived from pHH18CAT plasmid by a 5′ deletion to the Bgl1 site, 400 nucleotides upstream of the ATG of E6. The distal BamH1-Rsa1 fragment (nt 6930–7509), the central Rsa1-Rsa1 fragment (nt 7510–7738), and the proximal Rsa1-BamH1 fragment (nt 7739–124) were first cloned in the polylinker of pSP64 before being cloned 5′ to the herpes simplex virus thymidine kinase tk promoter (at position −109) in TK-CAT expression plasmids in both orientations [15]. ProBPV plasmid contains the entire BPV1-LCR (Dde1-Dde1 fragment of 753 bp), cloned 5′ of the CAT gene in pSB1 plasmid [10,13]. Table I summarizes the different plasmids used in the present study.

Transfection and CAT Assays

Transfections of secondary cultures of human keratinocytes were performed, in duplicate, by the polybrene procedure [16] with a total of 10–20 μg of plasmid DNA per 100 mm dish. In cotransfection experiments, the total amount of DNA was kept constant by adding pSVneo plasmid DNA. After a 6 h incubation in the presence of polybrene (30 μg/ml) and plasmid DNA, the keratinocytes were shocked with 30% DMSO for 5 min, washed twice with PBS, and refed with fresh medium. Seventy-two hours after transfection, cells were harvested and lysed by sonication and three cycles of freezing and thawing. CAT assays were performed on 80 μl of the cleared lysates. The reaction was carried out at 37°C for 3 h, fresh acetyl coenzyme A being added every 45 min. Results were expressed as percentage of chloramphenicol conversion.

RESULTS

Effect of SV40-LT- and Ad5-E1a Antigens on HPV18 Transcription

To investigate the effects of SV40-LT- and Ad5-E1a antigens on HPV18 transcription in human keratinocytes, the construct containing the HPV18-LCR cloned in enhancer-promoter configuration upstream of the CAT gene (pH18CAT) was cotransfected (10 μg) with increasing amounts, from 1 to 10 μg, of a plasmid expressing SV40-LT antigen (pMK16SV) (Fig. 2A), a plasmid expressing Ad5-E1a antigen

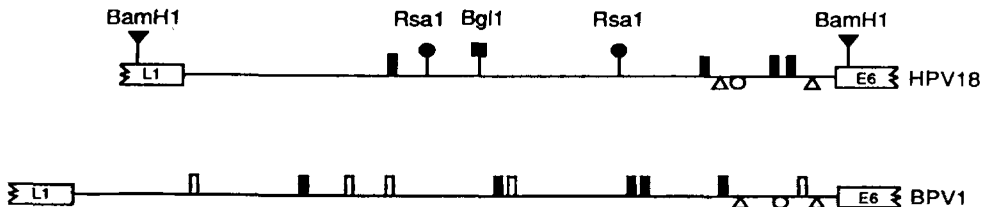
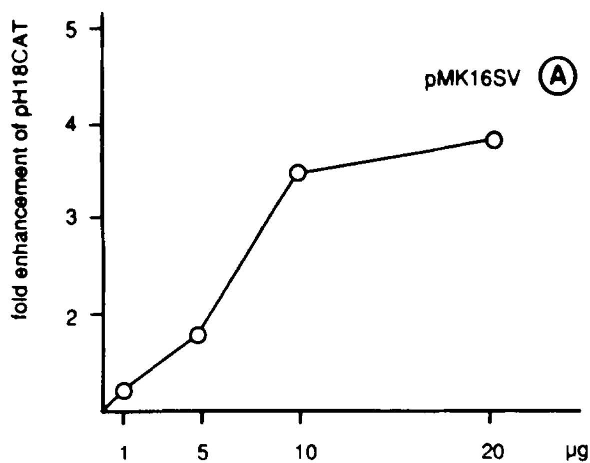
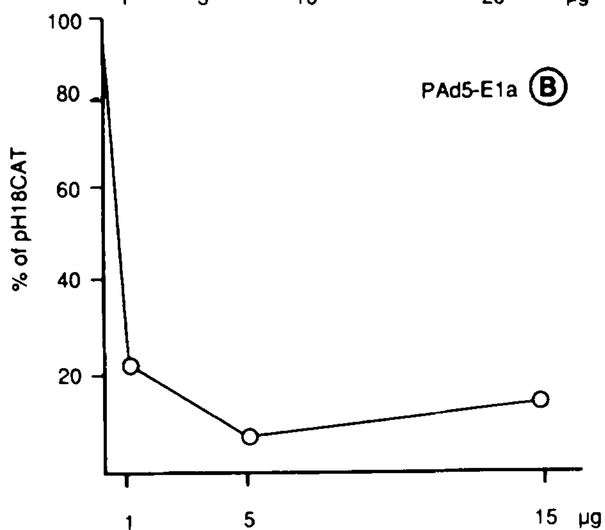
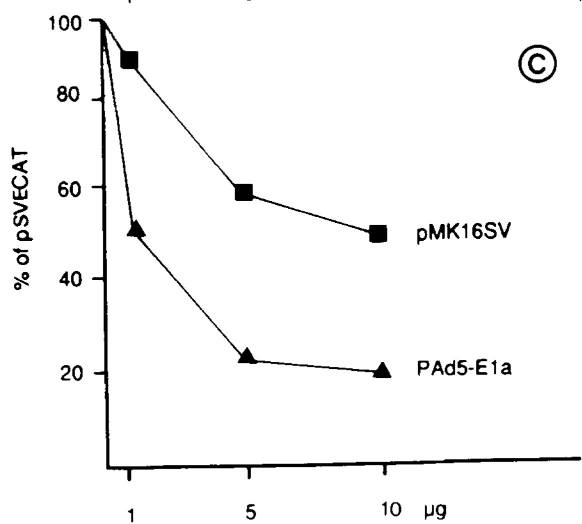


Fig. 1. Schematic representation of HPV18- and BPV1-LCRs. The HPV18- and BPV1-LCRs are located between the stop codon of L1 ORF and the translation initiation codon of E6 ORF. Restriction sites are indicated as well as putative CAAT box (O) and TATA box (△). Filled and empty bars indicate ACCGN4CGGT and ACCN6GGT E2 binding sites, respectively.


fold enhancement of pH18CAT
5
4
3
2
pMK16SV
A
1
5
10
20
µg


% of pH18CAT
100
80
60
40
20
PAd5-E1a
B
1
5
15
µg


% of pSVECAT
100
80
60
40
20
C
pMK16SV
PAd5-E1a
1
5
10
µg

(pE1a) (Fig. 2B), or a control plasmid expressing the neo gene (pSVneo). In the last case, the total amount of DNA was kept constant by adding pBR 322. CAT expression from pH18CAT was stimulated by SV40-LT, but was repressed by Ad5-E1a antigen, in both cases in a dose-dependent manner. No effect was noted when pH18CAT plasmid was cotransfected with increasing amounts of pSVneo plasmid (results not shown). The stimulating effect observed in the presence of pMK16SV was specific for pH18CAT since in human keratinocytes the CAT activity of the control pSVECAT plasmid (10 μg) decreased in a dose-dependent manner (Fig. 2C), as expected from previous works [17,18]. The inhibitory effect of E1a antigen was, however, also noticed with pSVECAT in cotransfection experiments with pE1a plasmid (Fig. 2C).

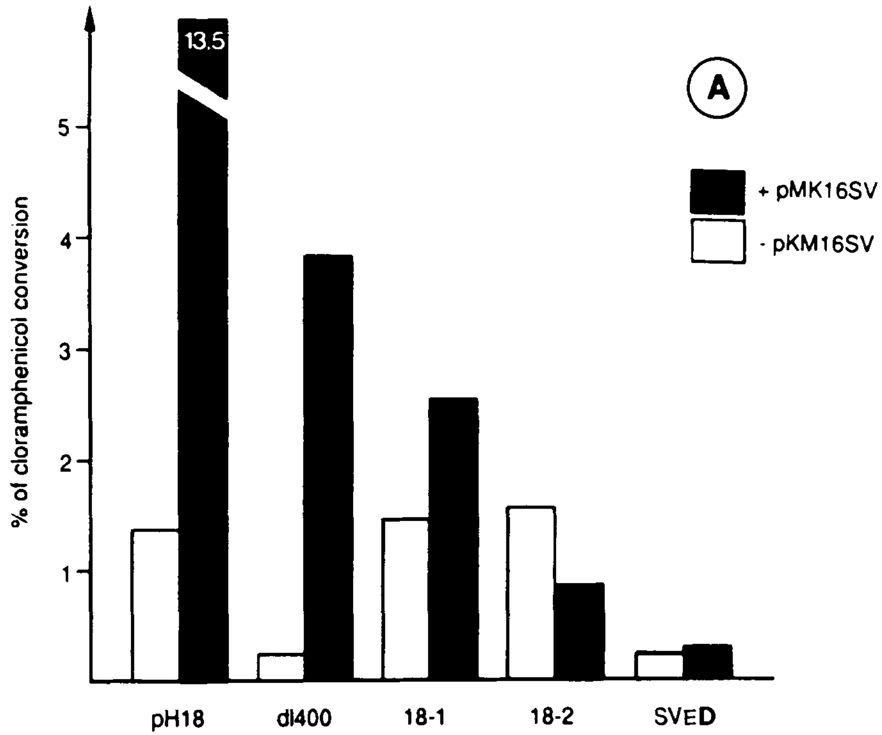
Characterization of the Effects of SV40-LT and Ad5-E1a Antigens on HPV18 Transcription

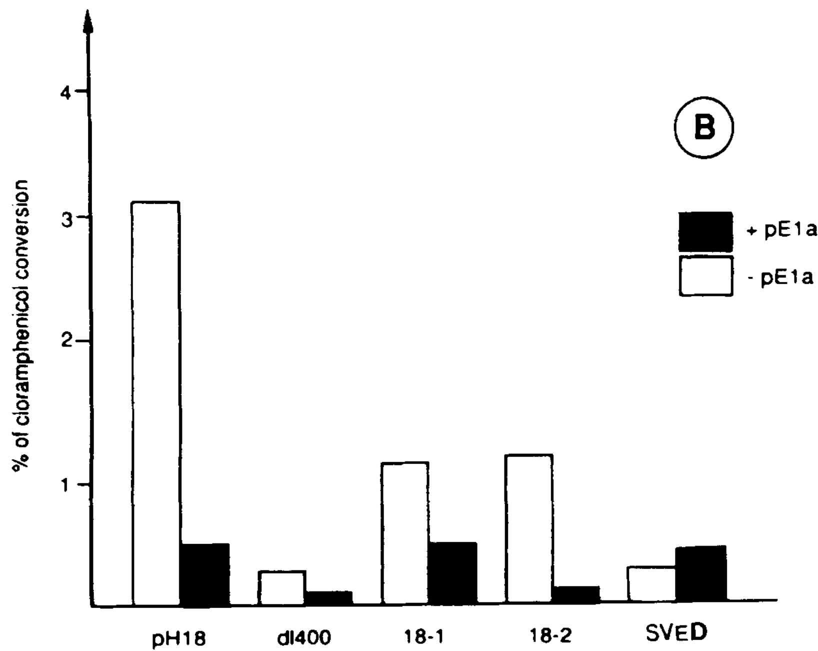
To better characterize the effects of SV40-LT and Ad5-E1a antigens on HPV18 transcription and to localize possible responsive domains in HPV18-LCR, constructs containing either the intact HPV18-LCR (pH18CAT) or the HPV18-LCR deleted of its 400 more upstream bp (dl400; deleted up to the Bgl1 site) (see Fig. 1) cloned in a promoter configuration upstream of the CAT gene, were cotransfected in human keratinocytes, with pMK16SV, pE1a, or pSVneo plasmid (10 μg each) (Fig. 3).

It is noteworthy (Fig. 3A) that even though the 400 bp deletion resulted in a fivefold decrease in promoting activity compared to pH18CAT, the stimulating effect of SV40-LT antigen was maintained. These results suggest either that SV40-LT responsive domains are located between the Bgl1 and 3′-BamH1 sites of HPV18-LCR (see Fig. 1) or that SV40-LT antigen acts directly at the level of the promoter. This latter hypothesis is strengthened by the fact that the stimulating effect of SV40-LT antigen was not seen on the SV40 early promoter deleted of its 72 bp repeats (SVED) or when the HPV18-LCR (18-1D and 18-2D) was cloned in an enhancer configuration ahead of it (Fig. 3A).

On the other hand, the repressing effect of Ad5-E1a was observed with both types of constructs (Fig. 3B) in promoter (pH18CAT and dl400) and enhancer configuration (18-1D and 18-2DSVECAT). It had no effect on pSVEDCAT construct, which contained only the enhancer deleted SV40 promoter. This suggests that, contrary to SV40-LT antigen that seems to activate the promoter activity of HPV18-LCR (Fig. 3A), Ad5-E1a antigen represses enhancer-induced stimulation of transcription, as previously observed for Ad2-E1a antigen on SV40, polyoma virus, and adenovirus-2 E1a enhancers [19]. This repression probably involves an interaction between a *trans*-acting factor(s) and enhancer elements located 3′ to the Bgl1 restriction site of HPV18-LCR, since it was still detectable in the absence of the upstream BamH1-Bgl1 fragment (Fig. 3B).

Fig. 2. Effect of SV40-LT antigen and Ad5-E1a antigen on HPV18 transcription. Secondary cultures of human keratinocytes were cotransfected with increasing amounts of pMK16SV (**A**) or pE1a (**B**) plasmid, from 1 to 10 μg, together with 10 μg of pH18CAT plasmid. In control experiments (**C**), keratinocytes were cotransfected with pSVECAT plasmid (10 μg) with increasing amounts of either pMK16SV (■) or pE1a (▲) plasmids, from 1 to 10 μg. Experiments were performed in duplicate with an experimental variation lower than 10%. Values correspond to the mean of stimulation factor (A) or percentage of inhibition (B,C) of CAT activity, compared to that obtained in cotransfection experiments with pSVneo plasmid.


13.5
A
+ pMK16SV
- pKM16SV
% of cloramphenicol conversion
5
4
3
2
1
pH18
dl400
18-1
18-2
SVED


B
+ pE1a
- pE1a
% of cloramphenicol conversion
4
3
2
1
pH18
dl400
18-1
18-2
SVED

To further analyze these effects, constructs containing the 390 bp distal Rsa1-Rsa1 fragment (TkM 400/6 and TkM 400/12), the 230 bp central Rsa1-Rsa1 fragment (TkM 3′ and TkM 5′), or the 240 bp proximal Rsa1-BamH1 (TkM 3.2 and TkM 3.3) fragment cloned in TKM plasmid [15] in enhancer configuration upstream of the enhancerless tk promoter of herpes simplex virus were cotransfected in human keratinocytes with 10 μg of pMK16SV plasmid (Fig. 4A) or pE1a plasmid (Fig. 4B). Although an average of threefold stimulation of CAT activity was noticed in the presence of SV40-LT antigen (Fig. 4A) even in the case of the enhancerless TKM plasmid, the stimulation of TkM 5′ was constantly at least sevenfold (depending on the experiment), resulting in a 2.5 relative increase. In the case of cotransfection with pE1a plasmid (Fig. 4B), the CAT activity of all TK-CAT constructs, included that of the enhancerless TKM plasmid, was increased by an average of 3.4-fold, except that of TKM 5′ and 3′ plasmids, which was repressed by an average of one-fourth, resulting in a 4.5-fold relative decrease.

Specificity of the Modulation of HPV18-transcription by SV40-LT and Ad5-E1Aa Antigens

To determine whether the effects of SV40-LT- and Ad5-E1a antigens on HPV18 transcription were specific of this virus type, experiments similar to those reported above were performed with a construct that contained the BPV1-LCR (proBPV) cloned in a enhancer-promoter configuration upstream of the CAT gene (Fig. 5). When it was cotransfected (10 μg) with increasing amounts, from 1 to 10 μg, of either pMK16SV or pE1a plasmid, CAT expression was stimulated in both cases in a dose-dependent manner.

DISCUSSION

The present results show that SV40-LT and Ad5-E1a antigens can alter the promoter activity of HPV18- and BPV1-LCRs. Though SV40-LT antigen stimulates transcription from both LCRs, Ad5-E1a antigen represses transcription from HPV18-LCR but activates the BPV1-LCR. This supports the notion that even though papillomaviruses share common general genome organization and function, the fine tuning of transcriptional activity can differ from one virus type to another in a given cell type, namely, the human keratinocyte.

The transcriptional activation observed with SV40-LT antigen seems specific, since, in parallel experiments, transcription from pSVECAT is repressed. Although the mechanism by which SV40-LT antigen stimulates transcription is not clear, LT antigen may indirectly activate BPV1 and HPV18 promoters by binding or modifying a cellular protein, as previously suggested for SV40 late-promoter activation [20] and cause a

Fig. 3. Characterization of the effect of SV40-LT and Ad5-E1a antigens on HPV18 transcription. Constructs containing the HPV18-LCR (pH18CAT) or the HPV18-LCR deleted of its 400 more distal domain (dl400, deleted to the Bgl1 site) cloned in promoter configuration upstream of the CAT gene were cotransfected in human keratinocytes (10 μg of either plasmid) together with pMK16SV (A), pE1a (B), or pSVneo plasmid (10 μg each). Similar cotransfection experiments were performed with constructs containing either the enhancerless SV40 early promoter (SVED) or the HPV18-LCR cloned in enhancer configuration (18-1 and 18-2) upstream of it. Experiments were performed in duplicate, and the values correspond to the mean of chloramphenicol conversion.

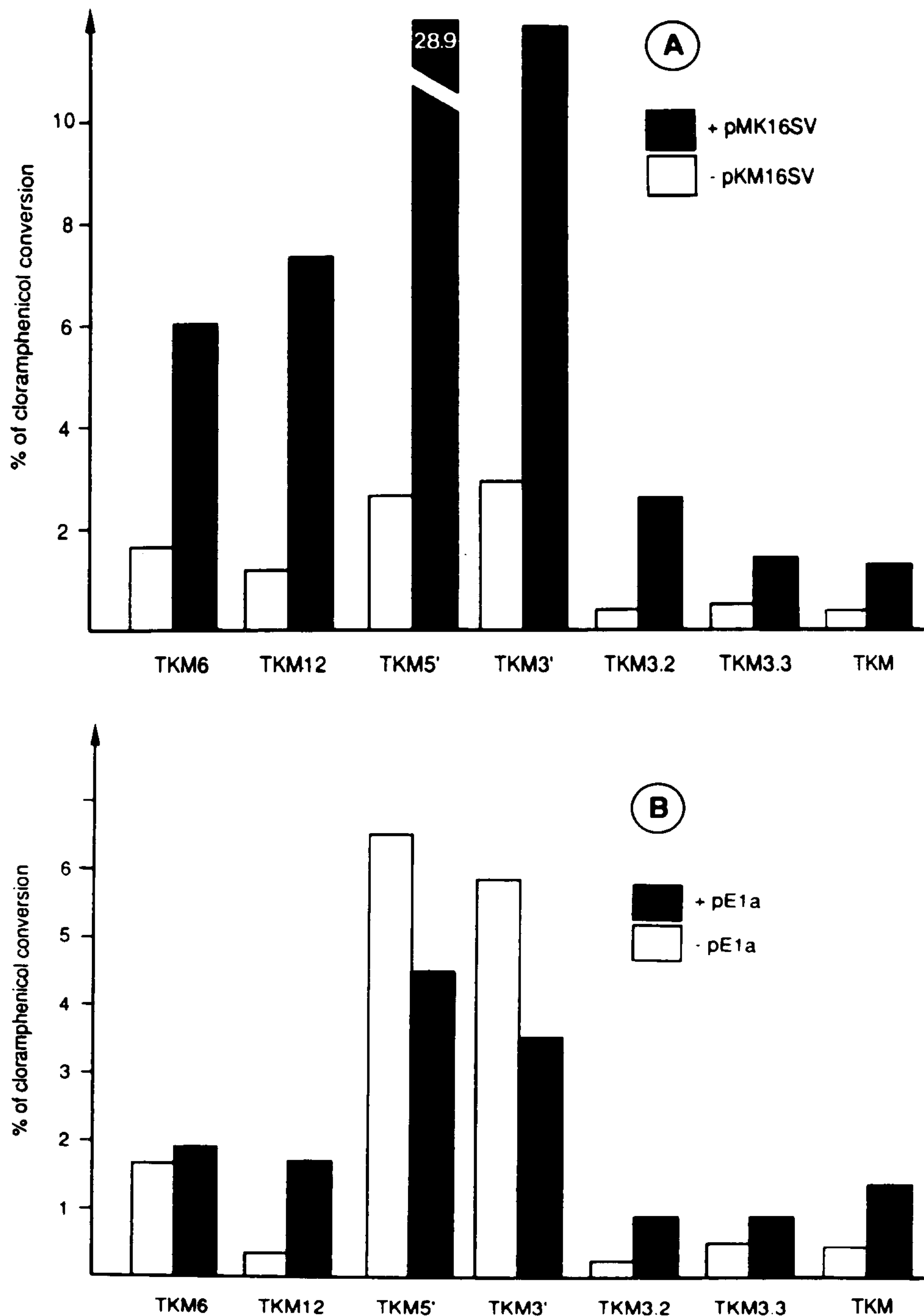


Fig. 4. Effect of SV40-LT and Ad5-E1a antigens on HPV18 enhancer. In this representative experiment, constructs containing the enhancerless tk promoter (TKM), in front of which was cloned in enhancer configuration the distal (TKM 6 and 12), the central (TKM 5′ and 3′), or the proximal (TKM 3.2 and 3.3) domain of HPV18-LCR, were cotransfected (10 μg each) in human keratinocytes together with 10 μg of either pMK16 SV (**A**) or pE1a plasmid (**B**). Values given represent the mean of percentages of chloramphenicol conversion.

change in the host cell transcription apparatus that would facilitate promoter activation. The fact that expression of an active SV40-LT antigen leads to changes in the expression of cellular genes that, like the HPV18-LCR [10], do not contain antigen binding sites [21] support this interpretation. Such cellular factor(s), however, is certainly not, as previously suggested [10], specific for the transformed phenotype of the transfected cells since our experiments were performed with normal human keratinocytes. Although SV40-LT antigen may thus activate HPV18 promoter, it is noteworthy that although transcription from plasmids 18-1 and 18-2DSVECAT is not stimulated in the presence of SV40-LT antigen, that of TKM 5' plasmid undergoes a 2.5 relative increase. This could be explained by the fact that 1) the 230 bp Rsa1-Rsa1 central fragment of HPV18-LCR, previously identified as a constitutive enhancer [22], contains an AP1 binding element [15] and 2) the stability of AP1 DNA-binding is known to be increased in the presence of SV40-LT antigen [23].

In the case of E1a antigen, it is known that the E1a gene products can either inhibit [19] or activate transcription [24,25]. Repression probably involves an interaction between enhancer elements and a *trans*-acting factor [19], whereas activation seems to be mediated through the transcriptional complex [24,25]. The repression of HPV18-transcription very likely occurs by inhibition of enhancer function since it is observed with constructs bearing the HPV18-LCR cloned either in promoter or enhancer configuration. More precisely, the central constitutive enhancer (see Fig. 4) might be the primary target for this repression.

On the other hand, the stimulating effect of Ad5-E1a on the proBPV construct could be interpreted as the result of the interaction of the E1a antigen with a cellular factor that otherwise would repress the activity of proBPV, as suggested by the absence

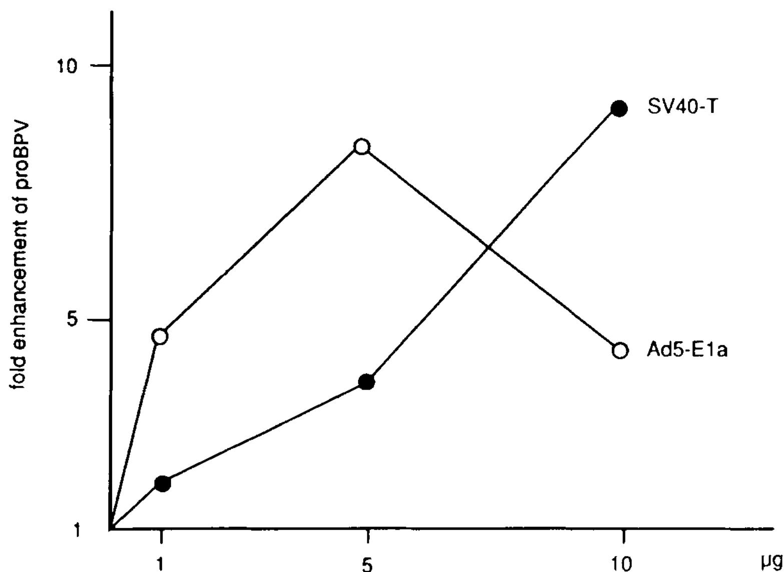


Fig. 5. Effect of SV40-LT antigen or adenovirus type 5 E1a antigen on BPV1 transcription. The construct containing the BPV1-LCR (proBPV) cloned in enhancer-promoter configuration upstream of the CAT gene was cotransfected (10 μg) with increasing amounts, from 1 to 10 μg, of either pMK16SV, pE1a, or pSVneo plasmids. Values given represent the stimulation of CAT expression, compared to that observed in the presence of pSVneo plasmid, and correspond to the mean of three independent experiments, performed in duplicate.

of any basal transcriptional activity from this construct. Similar interpretation could be given to the effect of SV40-LT antigen on proBPV transcription. Since both adenovirus E1a [26] and SV40-LT antigens [27] have been recently shown to bind to the retinoblastoma gene product, one might speculate that the absence of spontaneous activity of proBPV construct is the result of the interaction of the RB gene product with the BPV1-LCR, this interaction being hampered by Ad5-E1a or SV40-T antigen. Although a transcription-regulatory role has not yet been demonstrated for RB gene product, it is suggested by the fact that 1) RB is a nuclear-localized phosphoprotein able to bind DNA-cellulose [28] and 2) RB contains a putative metal binding site found in several transcription factors [29].

ACKNOWLEDGMENTS

We wish to thank F. Thierry, M. Yaniv, and P. Howley for the gift of essential plasmids.

REFERENCES

1. Law MF, Lowy DR, Dvoretsky I, Howley P: Proc Natl Acad Sci USA 78:2727–2731, 1981.
2. Pfister H: Rev Physiol Biochem Pharmacol 99:111–182, 1984.
3. zur Hausen H, Gissmann L, Schlehofer: Prog Med Virol 30:170–188, 1985.
4. Androphy EJ, Lowy DR, Schiller JT: Nature 325:70–73, 1987.
5. Hirochika H, Broker TR, Chow L: J Virol 61:2599–2606, 1987.
6. Lambert P, Spalholz B, Howley P: Cell 50:69–78, 1987.
7. Moskaluk CJ, Bastia D: Proc Natl Acad Sci USA 84:1215–1218, 1987.
8. Phelps WC, Howley P: J Virol 61:1630–1638, 1987.
9. Spalholz B, Yang YC, Howley P: Cell 42:183–191, 1985.
10. Thierry F, Heard JM, Dartmann K, Yaniv M: J Virol 61:134–142, 1987.
11. Regnier M, Prunieras M, Woodley D: Front Matrix Biol 9:4–35, 1975.
12. Rheinwald JG, Green H: Cell 6:331–344, 1975.
13. Herbomel P, Bourachot B, Yaniv M: Cell 39:653–662, 1984.
14. Thierry F, Yaniv M: EMBO J 6:3391–3397, 1987.
15. Garcia-Carranca A, Thierry F, Yaniv M: J Virol 62:4321–4330, 1988.
16. Farr A, McAteer JA, Roman A: Cancer Cells 5:171–177, 1987.
17. Hansen U, Tenen DG, Livingston DM, Sharp PA: Cell 27:603–612, 1981.
18. Rio D, Robbins A, Myers R, Tjian R: Proc Natl Acad Sci USA 77:5706–5710, 1980.
19. Borrelli E, Hen R, Chambon P: Nature 312:608–612, 1984.
20. Keller JM, Alvine JC: Mol Cell Biol 5:1859–1869, 1985.
21. Scott MRD, Westphal KH, Rigby PWJ: Cell 34:557–567, 1983.
22. Thierry F, Garcia-Carranca A, Yaniv M: Cancer Cells 5:23–32, 1987.
23. Gallo GJ, Gilinger G, Alwine JC: Mol Cell Biol 8:1648–1656, 1988.
24. Lillie JW, Green MR: Nature 338:39–44, 1989.
25. Williams GT, McClanahan TK, Morimoto RI: Mol Cell Biol 9:2574–2587, 1989.
26. Whyte P, Buchkovich KJ, Horowitz JM, Friend SH, Raybuck M, Weinberg RA, Harlow E: Nature 334:124–129, 1988.
27. DeCaprio JA, Ludlow JW, Figge J, Shew JY, Huang CM, Lee WH, Marsilio E, Paucha E, Livingston DM: Cell 54:275–283, 1988.
28. Lee WH, Shew JY, Hong FD, Sery TW, Donoso LA, Young LG, Bookstein R, Lee EYHP: Nature 329:642–645, 1987.
29. Lee WH, Bookstein R, Hong FD, Young LJ, Shew JY, Lee EYHP: Science 235:1394–1399, 1987.