During negative regulation of the human papillomavirus-16 E6 promoter, the viral E2 protein can displace Sp1 from a proximal promoter element

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ABSTRACT

The principal early promoter of human papillomaviruses (HPVs), designated P97 in the case of HPV-16, contains four characteristically aligned cis-responsive elements, namely one binding site for Sp1, two for the viral E2 proteins, and the TATA box. The Sp1 binding site is needed to mediate activation of P97 by the remote epithelial-specific enhancer, and the two E2 binding sites contribute to a negative feedback-loop of viral gene expression. The Sp1 consensus motif and the TATA-box distal E2 binding site are spaced in all genital papillomaviruses by a single nucleotide. We show here that at physiological concentrations, the binding of E2 proteins and Sp1 are mutually exclusive events, since a bandshift analysis with nuclear extracts from BO13, a mouse cell line transformed by BPV-1, showed only the E2 or the Sp1 bandshift, but no complex indicative of the concomitant binding of both factors. Increasing concentrations of in vitro translated E2 protein compete efficiently with the Sp1 factor for binding to an oligonucleotide containing both binding sites. Interference between Sp1 and E2 protein binding is apparently relevant for P97 repression in vivo, since a mutational analysis revealed that both E2 binding sites are necessary for negative transcriptional regulation: Alone, neither the distal site, where E2 protein can induce Sp1 displacement, nor the proximal site, where E2 protein interferes with formation and function of the pre-initiation complex, have a significant effect, but two functional E2 binding sites lead to repression of P97.

INTRODUCTION

Human papillomavirus-16 (HPV-16) is the most frequently encountered papillomaviruses associated with benign and malignant neoplasias of the cervix uteri (1). The virus is believed to be causally involved in the etiology of this disease, since its genome or that of related papillomaviruses is present in most cervical lesions (2, 3), and since the protein products of three viral genes, E5, E6, and E7 modify cellular homeostasis by molecular mechanisms that are similar to those of other DNA tumor viruses (4, 5, 6, 7).

Transcriptional feedback regulation in papillomaviruses follows at least two alternative schemes: Bovine papillomavirus type 1 (BPV-1) makes three different products of the viral E2 gene: A protein that corresponds to the full length E2 open reading frame contains a DNA binding as well as a transcription activation domain. In contrast, two short derivatives of the E2 gene contain only the DNA binding domain (8, 9). Twelve E2 binding sites of BPV-1, which have the palindromic sequence 5'-ACC-N6GGT-3' are in an enhancer configuration in relation to the E6 promoter, namely remote to the 5'-side of this promoter. Binding of the large E2 protein to these sites leads to transcriptional enhancement of the BPV-1 E6 promoter, while this effect is annihilated by the short E2 proteins.

In genital human papillomaviruses, the product(s) of the E2 gene are not yet precisely known. However, the locations of the E2 binding sites are distinct and suggestive of regulatory mechanisms different from those in BPV-1: The long control region (LCR) upstream of the E6 promoter contains only four E2 binding sites. Two elements with unknown function flank an epithelial specific enhancer, while the other two are located at the 3' end of the LCR, close to the E6 promoter (10). This promoter, termed P97 in HPV-16 leads to transcripts that initiate 7 bp upstream of the E6 gene (11). The cis-responsive elements of this E6 promoter are precisely conserved in at least eight genital human papillomaviruses and in a Rhesus Monkey papillomavirus (Fig.1), although, these viruses diverge strongly in most other sequence details. The TATA-box of these nine E6 promoters is centered 29 bp upstream of the transcription start and it is spaced by two or three basepairs from the 3'-end of two binding sites for the viral E2 proteins. These two sites are two, three, or four basepairs apart from one another. To the 5'-site, the distal E2 binding site is in HPV-16, 11 and 16 exactly one basepair apart from a degenerated binding site for the cellular transcription factor Sp1 (12), and sequence homologies are indicative of the same element in all related papillomaviruses.

Functional studies have shown, that in HPV-16, this Sp1 binding site is required (12) to mediate transcriptional stimulation by the epithelial specific enhancer (13—15). On the other side, in HPV-16 and HPV-18, the E2 binding sites are required for down-regulation of the the E6 promoter (12, 16, 17). Cellular and viral factors therefore are able to fine-tune viral transcription according to physiological signals and the state of viral gene expression.
Conservation of these promoter sequences suggests that identity and alignment of these four transcription factor binding sites serves a precise mechanism of negative control of transcription. In principle, transcriptional repression of eukaryotic promoters could be brought about by three different mechanism (for reviews see 18, 19). Firstly, the repressor may bind to specific sites, that overlap with the binding sites of transcriptional activators or the basal transcription complex, thereby competitively displacing these factors. Secondly, the repressor may bind elsewhere, and may interfere with the function of the transcriptional activators or the basal transcription complex without displacing them from the DNA. This mechanism has been referred to as quenching or silencing. Thirdly, the negatively regulating factor may not bind the DNA at all, but may form complexes with factors that are in solution or bound to the DNA, thereby interfering with the process of transcription initiation. Alternatives of this latter mechanism had been described as squelching or surface saturation (20, 21).

In this paper we demonstrate that the binding of Sp1 and E2 protein to P97 sequences of HPV-16 is mutually exclusive in vitro. We propose that competitive displacement of Sp1 is involved in P97 regulation, since we determined that repression of P97 in vivo strictly requires both flanking E2 binding sites, and a recently published interference between E2 protein bound to the proximal target site and the establishment of the basal transcription complex (22) alone does not seem to suffice to bring about negative regulation.

MATERIALS AND METHODS

Cell lines

Mouse c127 fibroblasts and their ID13 derivates, which contain episomally replicating bovine papillomavirus genomes (23), and the human carcinoma cell lines HeLa, SiHa, and CaSkI (24) have been cultured in our lab for several years.

Synthetic oligonucleotides

Oligonucleotides were synthesized on a Pharmacia gene assembler and purified by gel electrophoresis. For bandshift experiments, the two oligos 5'-CTAAGGGCTTATGGGAAATCCGGTTG-3' (Sp1 and E2 binding sites shown italicized) and 5'-TCAAGCAACCGATTTCGGTTAGCCCTTAGAGCT-3' were hybridized, 32P-end-labelled with Klenow DNA polymerase resulting in a 33 bp double stranded DNA, with the flanking Sp1 and the distal E2 binding sites between the HPV-16 genomic positions 28 to 46 (28). 15 to 20 fmol (or 5000 cpm) of this preparation were incubated with 1.2 to 5 µg of nuclear extracts or 1 to 8 µl of in vitro translated E2 protein in a buffer at final concentrations of 30 mM KCl, 3 mM spermidine, 0.1% NP-40, 0.1 mM EDTA, 0.5 mM DTT, and 4 mM MgCl2. After 20 min incubation on ice, the binding reaction was separated on a native 5 % polyacrylamide gel in 1x Tris-glycine buffer at 150 V/20 mA for 2 h at 4°C. 500 ng of poly d(I-C) was used as unspecific carrier, and for specific competition, a 500 fold molar excess of oligonucleotides with the sequence given above, but as Sp1 mutation the upper strand sequence 5'-CTAGTTTAGTAAACCAGAAATCCGGTG-3' (mutated Sp1 and E2 binding sites italicized). To study binding of E2 protein to two flanking E2 binding sites, we used the wild-type oligonucleotide (upper strand, Sal I ends) 5'-TCGACACCCGAATTCGGTTGAAACCAGAAACCCGAAA-

CCGGTG-3', and a mutant oligo with one unfunctional and one functional binding site (replacement of the italicized 2 Gs by 2 As).

In vitro transcription and translation

For in vitro transcription and translation, the BPV-1 E2 gene was subcloned from pC59 (25) in form of a BamHI fragment into the vector pSP64, downstream of the SP6 promoter (26). After cleavage of this construct downstream of the E2 gene, capped RNA was generated by transcription of 2 µg of DNA in a 50 µl reaction volume containing 40 mM Tris-HCl pH7.5, 6 mM MgCl2, 2 mM spermidine, 0.1 mg/ml bovine serum albumin, 10 mM DTT, 1.0 mM each of ATP, UTP, and CTP, 0.66 mM GTP, 0.5 mM me7G-(5')ppp(5')AG cap (Pharmacia), 20 units of RNasin (Boehringer) and 40 units of SP6 RNA polymerase (Boehringer). The reaction was incubated at 37°C for 60 minutes. 10 units of DNasel were added and incubated for a further 10 minutes. The RNA was extracted with phenol/chloroform and ether and precipitated with ethanol. E2 protein was translated in vitro in a reaction volume of 50 µl with 2 µl of in vitro translated transcript, 20 nM amino acid mix (40 nM methionine) in rabbit reticulocyte lysate as described by the manufacturer (Promega). In control experiments, the translation product was examined after incorporation of radioactive methionine by gel electrophoresis (data not shown).

Plasmids for in vivo transcription

The basic vectors OVEC1, and OVEC-REF have been published (27). The construct SVeHPvpOVEC contained in the Sal I/Pst I digested OVEC vector a 79 bp oligonucleotide with the sequence: 5'-TGCAACCCGGTTATAAACTAGGCTTAAAGGAAATCAGCAGAAC-3' which includes the genomic positions 16 to 80 of HPV-16 (28), plus a 172 bp SV40 fragment containing the two 72 bp repeats (previously subcloned from pSV2CAT into pUC19), in the Sal I site of SVeHPvpOVEC (12). In both cases the β-globin TATA-box was replaced by the HPV-16 TATA-box with unaltered spacing to the β-globin transcription start site. The constructs SV3, SV5, SV6 were derived from the above SVeHPvpOVEC but with either the promoter proximal, distal or both proximal and distal E2 binding sites respectively mutated by cloning of oligonucleotide which differed in sequence from those above. The SV3 mutation was 5'-ACCGAAACCaaT-3',
the SV5 mutation was 5'-ACCGAAATCaaT-3', and the SV6 mutation was a combination of both. For details, see Fig. 2. To transiently express BPV-1 E2 or HPV-16 E2 in contransfection experiments, the plasmids pc59 or p859 respectively were used, which were a kind gift from P. M. Howley. To monitor positive regulation by E2 protein, we cloned the sequence 5'-ACCGAAATCGGTTGAACCGAAACCGGT-3' with Sal 1 protruding ends into pBLCAT2 (ref. 34).

Transfection procedures
HeLa cells were grown in minimal essential medium with 10% fetal calf serum to less than 80% confluency, trypsinized, and washed twice with phosphate-buffered saline. 3x10^6 cells in 600 μl phosphate-buffered saline were mixed with 15 μg DNA in 200 μl phosphate-buffered saline and left for 10 min on ice before electroporation with a Bio-Rad Gene Pulser with capacitance extender at 960 μF and 250 volts. After electroporation, the cells were plated into 30 ml medium in 150 mm dishes and harvesting for RNA analysis after 48 h.

RNA extraction and analysis
Transfected cells were washed three times with phosphate-buffered saline before 5 ml guanidinium-mix (50% w/v guanidinium-thiocyanat, 50 mM Tris-HCl pH 7.5, 20 mM EDTA, 2% N-laurylsarcosine, 150 mM β-mercaptoethanol) per 150 mm dish was added. The cell suspension was mixed with 2 g CsCl and layered on top of a 4 ml cushion of 5.7 M CsCl and spun in a SW41Ti rotor at 30,000 rpm for 22 h. The RNA pellet was dissolved in 400 μl H₂O, DNase 1 digested, phenol/chloroform extracted and precipitated with ethanol. After photometric determination of the RNA, 10 μg of RNA was used to hybridize a α-32P-UTP labelled SP6-polymerase synthesized RNA probe, covering the sequence of OVEC 1 from position -37 (Sal 1 site) to postion +179 (Taq1 site). Hybridization and subsequent steps were performed as previously described (29). The design of probe and test vectors was such that the HPV-16 or β-globin promoter gave an RNA signal of 179 nucleotides, whereas the transcript of the reference vectors after processing with the same probe gave a signal at 160 nucleotides. CAT transcripts originating from pBLCAT2-E2 were processed to give a 210-nucleotide signal by use of an SP6-CAT vector containing the inverted Eco RI-Pvu II fragment from pBLCAT2 (12).

RESULTS
Bandshift experiments identify alternative complexes between Sp1 or E2 protein and HPV-16 promoter sequences
Fig. 1 illustrates the promoter sequences of genital human papillomaviruses, which are negatively regulated by a protein product of the viral E2 gene, as shown for HPV-16, and 18 (13,16,17). The most stringently maintained alignment among these highly conserved promoter elements is the 1 bp spacing between the Sp1 proximal element and the promoter-distal E2 binding site. To study the interactions between the HPV-16 promoter and the corresponding transcription factors, we performed bandshift experiments with an oligonucleotide that represented these two cis-responsive elements (for sequence details see methods section) and nuclear extracts of ID13 cells.

ID13 cells are mouse fibroblasts with episomally replicating BPV-1 genomes. These cells were chosen since they are the only readily available cell line that stably supports the early gene expression of any papillomavirus with a requirement both for the cellular Sp1 and viral E2 transcription factors (12, 17). Consequently, nuclear extracts from ID13 cells will represent in vitro concentrations of both factors similar to the situation in vivo. Cell lines with human papillomaviruses that are derived from cervical cancer cells are believed to contain either sequence interrupted HPV-genomes deficient of E2 expression (30) or mutated genomes that may express unfunctional shortened forms of E2 (31,32), while recently described cells with episomal HPV-16 genomes have not yet analyzed for expression of early viral gene functions (33).

Fig. 3 shows five complexes that are formed between ID13 nuclear extracts and an oligonucleotide with the HPV-16 Sp1 and the distal E2 binding site (slot 3). All five complexes were competed by a homologous oligo (slot 4), while an oligo with a mutated Sp1 site competed only the lower two bands (slot 5), and an oligo with a mutated E2 binding site only the upper three bands (slot 6), and no competition is observed with an oligo with mutations in both sites (slot 7). Similar selective competitions were also observed with oligos representing only Sp1 and E2 motifs (slot 8 to 10). Only the upper three bands, but not the lower two were visible in a bandshift with nuclear extracts from c127 cells, the predecessors of ID13, which do not contain BPV-1.
We interpret these data to mean that the upper three bands are complexes with Sp1, and the lower two bands with E2 proteins. The observation of several E2 complexes was anticipated, since the BPV-1 E2 open reading frame is used to make a large E2 protein with a molecular weight of 48 kD and two small E2 proteins with molecular weights of 32 and 28 kD (8,9). With the help of antisera, we have previously identified two and sometimes three Sp1 bands in a similar position in human cells (13) which may be the product of post-translational modification of Sp1 (34). In those published experiments, as well as in those that led to Fig. 2 and 3, only the upper band (Sp1(A)) was strong and reproducibly seen, while bands B and C were inapparent at lower concentrations of nuclear extracts.

None of the experiments that led to Fig. 3 showed any complex that indicated concomitant binding of Sp1 and E2 proteins. We interpret the absence of such a super-shift as an indication of mutual exclusion of DNA binding due to steric hindrance of Sp1 and E2 proteins binding to the two recognition motifs.

**In vitro translated E2 protein displaces the Sp1 transcription factor from HPV-16 promoter sequences**

To further study the possibility of mutual exclusion of Sp1 and E2 factor binding to the HPV-16 promoter, we performed a bandshift of the Sp1/E2 fragment with c127 nuclear extracts in the presence of increasing concentrations of in vitro translated BPV-1 E2 protein. Fig. 4 shows that the strong band A of the Sp1 complex is decreasing in intensity by approximately one order of magnitude during the addition of an increasing amount of E2 protein (slot 2 to 7). The comparison of slot 5 and 8 shows that upon competition with an oligo with a functional E2 but mutated Sp1 binding site, the E2 bandshift is eliminated, while an intensity of the Sp1 bandshift corresponding to that in the absence of E2 protein is restored. Correspondingly, competition with a Sp1 oligo eliminates the Sp1 band and increases the E2 shift (slot 9). The accumulation of labelled material that did not enter the gel with increasing E2 protein concentration (slot 4 to 7 and 9) we interpret as aggregates with E2 complexes that do not contain Sp1. This interpretation is suggested by the comparability of these complexes with the E2 oligo (slot 8) but not with the Sp1 oligo (slot 9). We conclude from this experiment that E2 protein competitively displaces Sp1 from the adjacent binding site.

**Requirement of both E2 binding sites for negative regulation of P97 suggests Sp1 displacement in vivo**

In BPV-1, E2 binding sites are in a distal upstream position to most viral promoters. These sites activate viral promoters through binding of the large E2 protein, which bears a DNA binding as well as a transcription activation domain (8), while two short E2 proteins (9), which have only the DNA-binding domain, annihilate this effect, probably through competition with the large E2 protein for DNA binding. In HPV-16 and 18, in contrast, even the large BPV-1 E2 protein represses the P97 promoter (13, 16, 17).

To study this mechanism in more detail, we quantitated RNA initiation at the β-globin promoter in HeLa cells with OVEC vectors, that contained oligonucleotides comprising the promoter sequences of HPV-16, and mutants of alternative E2 binding sites. OVEC vectors have been constructed as tools to have a defined transcription unit of the human beta-globin gene under control of its TATA-box (27) with upstream cloning sites for the insertion of additional promoter and enhancer elements. The plasmid SVE-MHPBp (12) contains an oligonucleotide constructed according to the natural sequences of the HPV-16 promoter controlling transcription of the β-globin reporter gene. This transcription unit is activated by the SV40 enhancer, which has been chosen, since it is not unspecifically influenced by E2 proteins as it is the case for the HPV-16 enhancer (13, 35, and our unpublished observations). The plasmids SV3, SV5, and SV6 are sequence related constructs which have, however, mutation either in the promoter distal (SV3), or proximal (SV5), or both (SV6) E2 binding sites.

![Figure 3](image-url) Bandshift of an oligonucleotide comprising the HPV-16 E6 promoter distal E2 binding site and the flanking Sp1 element in the absence (c127 cells) and presence (ID13 cells) of BPV-1 E2 proteins. Bands A, B and C identify Sp1 complexes (12), the appearance of band 6 depended on the very high protein concentrations (5 μg/ml) in this experiment. These three bands can be competed with oligos with Sp1 binding sites (slot 4, 6, 9). Bands E and F, which appear with ID13 but not with c127 nuclear extracts, identify E2 complexes, since they are competed only by oligo with E2 binding sites (slot 4, 5, 8). For slot 9 and 10, competition was done with oligonucleotides that represented either the two flanking E2 binding sites of HPV-16 P97, or the Sp1 binding site fp2u of this promoter (12).

![Figure 4](image-url) Disappearance of Sp1 complexes under increased concentrations of in vitro translated E2 proteins. To avoid unspecific effects due to contents of the reticulocyte lysate, slots 3 to 7 were adjusted accordingly. For further details see the result section.
Fig. 5 (slot 2 and 3) shows that the P97 construct (β-globin transcript signal) is down-regulated by approximately a factor 10 under the influence of the BPV-E2 expression vector pC59 (25). In contrast, all three mutants show a complete and equal lack of down-regulation (compare slot 9 with slot 10, 11, 12), and a promoter strength equal to that in the absence of E2 protein (compare slot 5 to seven with 10 to 12). We conclude from these data that none of the two E2 binding sites alone is able to down-regulate P97, and that synergism between the two sites is necessary for repression.

Slot 2 and 3 and 5 to 12 of Fig. 5 contain results from two cotransfected transcription vectors, which give rise to the REF-transcript to monitor equal transfection efficiency and the tk-CAT transcript to monitor efficient expression of BPV-E2. This latter transcript stems from a vector which contains E2 binding sites in enhancer configuration to the herpes-simple-virus-thymidine-kinase promoter. Comparison of slot 2 with 3 and of slots 5 to 7 with 10 to 12 shows the efficient transcriptional activation of this promoter due to these cis-responsive elements in the presence of the large BPV-1 E2 protein. Interestingly, slot 8 shows that the HPV-16 E2 protein expressed from the vector p859 (25) is not able to give such a transcriptional stimulation, while it down-regulates SVeHPVP in a manner similar to the BPV-1 E2 protein. From these data it can be speculated that the HPV-16 E2 protein misses or has a reduced transcriptional activation function in comparison with the BPV-1 E2 protein. Such a speculation may be supported by the fact that HPV-16 does not seem to have two flanking E2 binding sites in enhancer configuration to any known promoter, a necessary prerequisite for enhancer function in BPV-1 (for a review, see 36). This interpretation is limited, however, by the present lack of knowledge of the nature of the HPV-16 E2 proteins.

The BPV-1 E2 protein does not bind cooperatively to the two E2 target sites in the HPV-16 promoter

The strict sequence conservation of two E2 protein target sites at the E6 promoters of genital papillomaviruses strongly suggests a functional requirement for both sites. One of these possible functions could be to facilitate displacement of flanking factors by binding to the two E2 binding sites in a cooperative manner. Cooperative binding of E2 proteins has been observed (37, 38), but was not seen in another study (39).

Fig. 6 shows the outcome of a bandshift experiment with one oligonucleotide with two flanking E2 binding sites that mimic HPV-16 promoter sequences, and another oligo where two point mutations eliminated the distal E2 recognition sequence. The latter sequence led to one complex only, most likely representing the binding of one E2 protein dimer to the single palindromic binding site (slot 1—3) while the wild-type sequence showed an additional complex of slower migration, most likely originating from the binding of two E2 protein dimers to both flanking target sites (slot 1—3). The observation of this low mobility complex shows that both E2 binding sites can be occupied at the same time, but its weaker intensity in comparison to the complex with only one bound E2 dimer does not suggest that this latter one creates a propensity for cooperative binding of a second E2 dimer. Additional competition experiments with unlabelled oligos, performed at a 1:1 ratio rather than with an excess of competing oligo, also do not indicate particularly strong affinity of the wild-type competitor. We conclude from these experiments that two E2 dimers do not bind cooperatively to the target site arrangement of the HPV-16 promoter, although both E2 sites are needed for repression in a functionally synergistic manner.

**DISCUSSION**

In contrast to BPV-1, whose E6 promoter is stimulated by viral E2 transcription factors, activation of the E6 promoters of genital papillomaviruses is critically dependent on an epithelial specific enhancer that is recognized by cellular transcription factors (10,

Figure 5. Cooperativity between two E2 binding sites is required for negative regulation of the HPV-16 E6 promoter. Negative regulation by the BPV-1 E2 protein (compare slot 2 with 3 and 9) and the HPV-16 E2 protein (slot 8) is released by mutating either the promoter distal (slot 10) or proximal (slot 11), or both (slot 12) E2 binding sites. Comparison of slots 5 to 7 (absence of E2 protein) with 10 to 11 (presence of BPV-1 E2 protein) shows that BPV-1 E2 protein has no effect at all on these promoters once either binding site is mutated. The REF-transcript monitors equal transfection efficiency, the tk-CAT transcript the transcription activation function of the BPV-1 E2 protein on a vector with E2 binding sites in enhancer configuration.

Figure 6. Simultaneous establishment of two BPV-1 E2 protein dimer complexes on an oligonucleotide with both E2 binding sites of HPV-16 P97 (slot 3) and elimination of the double complex by mutation of one E2 binding site (slot 4). The experiment was done with in vitro transcribed and translated BPV-1 E2 protein. Competitions at a 1:1 ratio with oligos that either had one (slot 1 and 6) or both E2 binding sites (slot 2 and 5) show little competition with the first and same but not extensive competition by the latter sequence. This observation and the low ratio between the upper and the lower complex (slot 1—3) argues against cooperative binding. Band × is derived from incomplete in vitro translation products (data not shown).
the BPV-1 biology. For example, the BPV-1 promoter P7185, the displacement of cellular transcription factors is not alien to prerequisit for this binding competition. The space between the Sp1 site and the TATA box is filled by two 12 bp binding sites for the viral E2 proteins. This strict space between the Spl site and the TATA box is filled by the stimulation by this epithelial specific enhancer (12). This Spl proximal promoter element is strictly required to mediate repression. Our data suggest that displacement of Spl is one facet of this regulatory process, in addition to a similar influence with establishment and function of the basal transcription complex as of this regulatory process, in addition to a similar influence with a similar E2 enhancer function. This question may also shed light on the fact that the Spl binding sites of all HPV-16 sites of negative regulation may further raise the question of whether, in HPV-16 transcriptional regulation, there may be a strict prerequisite for this binding competition.

It has been previously shown that the large E2 protein of BPV-1 requires tandem repeated binding sites for strong enhancer activation. The usage of the only tandemly repeated E2 binding sites of HPV-16 for negative regulation may further raise the question of whether, in HPV-16 transcriptional regulation, there is any need for a similar E2 enhancer function. This question is made pertinent by our observation that a HPV-16 E2 expression vector, unlike a BPV-1 E2 expression vector, does not bring about transcriptional activation of an E2 enhancer construct (Fig. 5, slot 8/9) to the same extent as a BPV-1 E2 protein as observed previously by Cripe et al (46). Consequently, the E2 transcription regulation system may have diversified quite considerably from that of the BPV-1 E2 control mechanism. However, negative regulation, even by large E2 proteins, through the displacement of cellular transcription factors is not alien to the BPV-1 biology. For example, the BPV-1 promoter P7185, whose function is not yet understood, is activated by a cellular transcription factor which may be Sp1. Furthermore, the function of this promoter is annihilated as a result of competitive binding of E2 proteins to a flanking binding site (41, 42).

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