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# Transcription of the transforming genes of the oncogenic human papillomavirus-16 is stimulated by tumor promotors through AP1 binding sites

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## ABSTRACT

**The promoter P97 of human papillomavirus-16 (HPV-16) gives rise to transcripts that encode the principal transforming genes of the virus, E6 and E7. The activity of P97 is regulated by a cell-type-specific enhancer, as well as by glucocorticoids and progesterone. We show here, that in CaSki cells, which contain HPV-16 genomes, P97 is also inducible by phorbol esters. Functional analysis of restriction fragments and oligonucleotides of the viral enhancer localizes two phorbol ester response elements on two transcription factor binding sites termed fp4e and fp9e. Sequence comparison, footprint analysis and bandshift competition of the cloned motifs suggest that both fp4e and fp9e are bound by the transcription factor AP1. These AP1 binding sites in HPV-16 and other papillomaviruses may provide a link between cellular oncogenes like jun, fos and possibly ras, whose transcription stimulating activity may lead to an elevated expression of the viral transforming genes E6 and E7.**

## INTRODUCTION

Human papillomavirus-16 and 18 (HPV-16 and HPV-18) are considered to play a central role in the etiology of cervical and other genital tumors (1). Arguments for their tumorigenicity stem from the functional analysis of the products of the open reading frames E6 and E7 which induce focus formation of rodent fibroblasts (2), modify epithelial differentiation (3–5), induce immortalization (6), modulate the activity of transcriptional elements (7), and bind the retinoblastoma gene product (8).

In HPV-16, these two transforming genes are transcribed from the promoter P97, whose activity is modulated by a complex network of transcriptional elements, located in the long control region (LCR) of the viral genome: A cell-type-specific enhancer (9,10) which is centered 350 bp upstream of P97, stimulates P97 activity through binding sites for various transcription factors including 7 that are recognized by nuclear factor 1 (NF1) (11,12). Another element in this region mediates regulation of P97 by glucocorticoids or progesterone. This glucocorticoid/progesterone response element (GRE/PRE) can function independently from or in cooperation with the enhancer (13). P97 activity is further regulated through two binding sites for the viral E2 protein, a papillomavirus specific feedback regulation system (10,14). We have previously mapped in a 850 bp segment of the papillomavirus genome which constitutes the long control region (LCR), 23 footprints. Out of 9 footprints that are present in the DNA fragment that functions as a cell-type-specific enhancer, seven are bound by a NF1 like factor (12). Another element, fp4e, seems to bind several factors in an overlapping fashion which include the glucocorticoid and progesterone receptor (13). Binding of one of these factors can be competed by an oligonucleotide representing fp9e (11). The two sequence motifs, fp4e and fp9e are related to the heptamer 5'TGACTCA-3' the preferred binding site of the AP1 transcription factor (15,16).

AP1 has been identified as a factor that mediates transcriptional induction by phorbol esters. Phorbol esters mimic the biochemical properties of diacylglycerol, a second messenger induced by certain polypeptide hormones and growth factors leading to the induction of protein kinase C (PKC). PKC phosphorylates unknown target proteins, a process ultimately resulting in the stimulation of AP1 dependent promoters (17). Interestingly, AP1 was shown to be a heterodimer of the gene products of the cellular proto-oncogenes c-jun and c-fos (for a recent review see 18).

This paper describes the stimulation of the P97 promoter of HPV-16 by phorbol esters as well as the identification of two sequences within fp4e and fp9e as bona fide AP1 sites. These two sequence elements are able to stimulate linked promoters under the influence of phorbol esters. Our data suggest that the enhancer/promoter system of HPV-16 is placed at an interesting functional link between the proto-oncogenes c-jun and c-fos and the transcription of the viral genes E6 and E7 which by themselves have transforming and transcription stimulatory properties.

## MATERIALS AND METHODS

### Cell culture and transfection

The cell lines CaSki (19) and HeLa were grown in minimal essential medium supplemented with 10% or 5% (HeLa) fetal calf serum. For transfection experiments, cells were washed twice

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with phosphate buffered saline, 5μg DNA were added to a total volume of 2 ml of 0.1% DEAE dextran and incubated for 5 hr, followed by incubation for 24 h in minimal essential medium containing 0.5% fetal calf serum. For induction by phorbol esters, 100 ng/ml of 12-O-Tetradecanoyl-phorbol-13 acetate (TPA) was added for 12 h (CAT assays) or 3 h (RNA analysis). To determine chloramphenicol acetyltransferase (CAT) (20) activity, cell extracts were prepared by freeze-thawing and CAT activity assays were performed using 25 μg of protein with 1 h incubation at 37°C. CAT activity was determined as percent conversion of chloramphenicol and is given in picomoles converted per minute per milligram of protein. Each value represents the average of duplicate transfections, and each transfection has been done at least three times without aberrant results.

### RNA isolation and primer extension

Total RNA was isolated from CaSki cells using the guanidinium isothiocyanate method with a subsequent CsCl centrifugation step (21). Primer extension reactions were performed using a 40-nucleotide-long synthetic primer, complementary to the HPV-16 E6/E7 mRNA (22) from position 5'589 to 3'-550, and 15 μg of RNA. After hybridization at 55°C, we used for the primer extension reaction, avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim). The reaction products, products of Sanger sequencing reactions and $^{32}$P-labeled DNA marker fragments were separated on an 8% sequencing gel.

### Synthetic oligonucleotides and plasmids

Oligonucleotides were synthesized on a Pharmacia gene assembler and were used directly for cloning or purified by gel electrophoresis for primer extension and band shift competition experiments.

To test phorbol ester stimulated CAT expression, restriction fragments or oligonucleotides were inserted either into pBLCAT2 (23) or into ptkCATdH/N (24). This second vector is identical to pBLCAT2 but has a deletion from the NdeI to the HindIII sites removing prokaryotic sequences with fortuitous binding sites for eukaryotic transcription factors. In our hands, both basic vectors did not show any phorbol ester inducibility. In several cases, homologous constructs were made with either basic vector, which did not result in functional differences. pBLCAT2 derived constructs used in this paper are pHPV16-LCR1 (9,13, previously called pHPV16-00.1), pHPV16-DraI-232, pHPV16-DraI/HhaI-152, pHPV16-HhaI/DraI-80 (see Fig.2, all plasmids published in 11), pHPV16-RsaI-112 (carrying in the SalI site of pBLCAT2 a HPV-16 RsaI fragment representing the genomic positions 7532 to 7643) (25), pHPV16-fp4e-71 (carrying in the XbaI site of pBLCAT2 an oligonucleotide representing HPV-16 genomic positions 7611 to 7676 completely encompassing fp4e and fp5e) and pHPV16-GRE (13), (representing 15 bp HPV-16 sequence, a segment of fp4e that binds the glucocorticoid/progesterone receptor in the center of fp4e and includes an AP1 motif). Derived from ptkCATdH/N, through insertion of oligonucleotides into the XbaI site, are: pHPV16-fp4el-15 (representing an AP1 motif from HPV-16, positions 7630 to 7638 in fp4e different from the AP1 motif in pHPV16-GRE), pHPV16−4el-15-5x (the same oligonucleotide inserted as a 5 mer), pHPV16-fp4e-32 (HPV-16 position 7630 to 7655, the central part of fp4e including the GRE/PRE as well as both AP1 motifs), and pHPV16-fp9e-28 (fp9e of HPV-16 with the genomic segment position 7806 to 7827). Col-TRE/TkCAT has been published (15) and was supplied by P. Angel.

### DNaseI protection and band shift experiments

Footprint reactions (26) were carried out following modifications as previously published by us (11).

For band shift assays DNA fragments were cut out of a plasmid using SalI and BamHI restriction sites, labeled with $^{32}$P by filling in the protruding ends and subsequently purified via gel electrophoresis. Incubation conditions with HeLa nuclear extracts were as for DNase I footprinting assays, with the following modifications: Preincubation of 20 μg nuclear extract with 1.5 μg poly(dI-dC) and varying amounts of competitor oligonucleotides on ice for 10 min was followed by addition of approximately 10,000 cpm labeled DNA fragment and 10 min incubation at 25°C. The complexes were separated on a 5% polyacrylamide gel (60:1 Bis-acrylamide) containing 2.5% glycerol in 50 mM Tris-base, 380 mM glycin, and 2 mM EDTA. The following oligonucleotides were used as competitors: the two complementary 31 mers, 5'-CTAGAAGAGGTGTCTGACT-CATGC TTTATAT-3' and 5'-CTAGATATAAAGCATGAG-TCAGACACCTCTT-3', were hybridized, resulting in a 27 bp double stranded DNA resembling the AP1 site from the collagenase gene (15); the AP1 motif from fp4e (named fp4el) which was used to construct the plasmid pHPV16-fp4e-15, resulted from hybridizing the two 15 mers 5'-CTAGTCTGAATCACA-3' and 5'-CTAGTGTGATTC AGA-3' leading to a 11 base pairs double strand. The fp9e motif was represented by hybridization of the two oligonucleotides 5'-CTAGTAAGGT TAGTCATACATTGTTCAA-3' and 5'-CTAGTTGAACAATGTATGACTAACCTTA-3'. For bandshifting, oligonucleotides were cloned into the XbaI site of pBLCAT2, cleaved out, labelled by fill-in with Klenow-polymerase, and used in 10,000 cpm aliquots per reaction mix. The competitor oligonucleotide resembling the adenovirus NF1 binding site was described previously (12).

## RESULTS

### The HPV-16 promoter P97 is stimulated by phorbol esters

The CaSki cell line is derived from a cervical carcinoma and contains approximately 600 HPV-16 genomes (19). To test whether the viral P97 promoter is regulated by phorbol esters, we incubated serum starved CaSki cells for 3 h with or without 100 ng/ml TPA, isolated total RNA and analyzed P97 activity qualitatively and quantitatively by extension of a primer complementary to the E6/E7 mRNA at the E7 ATG start codon between the HPV-16 genomic positions 589 to 550. The 3 mRNAs coding for E6, E6* and E7 proteins lead to three extension products E6, E6*I and E6*II (22). Fig. 1 documents a strong stimulation of all three P97 derived transcripts by TPA.

### Location of phorbol ester responsive elements in the LCR of HPV-16

A 400 bp fragment between the genomic positions 7455 and 7855 contains a cluster of 9 footprints, which induce cell-type-specific transcription and regulation by glucocorticoids and progesterone of P97 (9,11,13). We reasoned that this enhancer may also be a good candidate for the location of a TPA responsive element (TRE) responsible for the P97 induction and tested this using the vector pHPV16-LCR1. In several independent experiments, the TPA induction of pHPV16-LCR1 was an average of 3 fold, while pBLCAT2 was not inducible (Fig.2A).

To localize TREs in the 400 bp fragment, we tested several subfragments of this region in a similar way. Three out of four

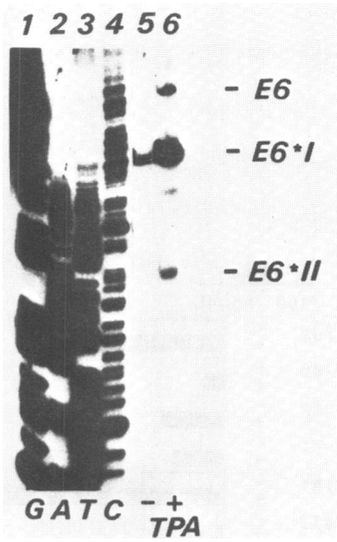


**Figure 1.** Phorbol ester induction of the HPV-16 P97 promoter in CaSki cells. Lanes 1–4, sequencing lanes; lanes 5 and 6, primer extension products, using RNA isolated from CaSki cells after 4 hours treatment with (lane 6), or without (lane 5) 100 ng/ml TPA. The three extension products correspond to unspliced E6 mRNA, and to the two alternatively spliced E6*I and E6*II mRNAs, as indicated. Induction of E6* mRNAs, which are believed to facilitate efficient translation of the E7 gene (22), should result in a increased level of the protein product of this transforming gene (7).

constructs containing smaller fragments with full or partial enhancer activity exhibited TPA response. We conclude, that at least one TRE is located 5′ of the HhaI site at position 7673. This element appears to be contained in the fragment in pHPV-16 fp4e-71, which covers fp4e.

Transcriptional response to phorbol esters is most frequently mediated through the factor AP1, which recognizes the palindromic sequences 5′- TGACTCA-3′ or 5′-TGAGTCA-3′ (18). Analysis of the published (25) HPV-16 sequence reveals 3 segments on the 400bp enhancer fragment which are related to this motif, namely between position 7631 and 7637, the sequence 5′-TGAATCA-3′, between position 7648 and 7754 5′-TGTGTCA-3′, and between position 7810 and 7816 the sequence 5′-TTAGTCA-3′ (Fig.3). All three motifs overlap with footprints which we identified in a previous study, the first two positioned within fp4e and the third within fp9e (11).

To test these elements functionally in transfection experiments in HeLa cells, we inserted short oligonucleotides representing these motifs into the XbaI site of pBLCAT2 or ptkCATdH/N. Fig.2B and 2C show that 3 constructs with the AP1 consensus element between position 7630 and 7638 (named fp4el) in various contexts are stimulated by phorbol esters, namely pHPV16-fp4e-32, pHPV16-fp4el-15 and pHPV16-fp4e1-15-5x. Similarly, one construct representing the fp9e AP1 motif is strongly stimulated by phorbol esters (Fig. 2C). In contrast, the AP1 motif between position 7648 and 7754 overlapping with the GRE/PRE (named fp4er) did not significantly induce phorbol ester dependent CAT expression. The 2 to 5 fold induction observed in our experiments is similar to that of the published control vector Col-TRE/TKCAT and corresponds quantitatively to published induction ratios (15,42).

To further confirm the identity of these AP1 sites with the TREs of HPV-16, we analyzed the properties of a 91 bp oligonucleotide (position 7629 to 7719) representing fp4e, fp5e and fp6e. Cloned into pktCATdH/N, this segment stimulated CAT expression 82 fold. Double-mutation of the 2 AP1 sites in fp4e, or alternatively, of the 2 NF1 sites fp5e and fp6e, reduced this enhancer activity by more than 90% (Chong et al., Nucl. Acids Res., in press). TPA responsiveness was 2.0 fold for the wild type segment, and undiminished for the NF1 mutation, while no TPA response was observed with the AP1 mutation (data not shown). The accumulated data from these experiments make it likely that the AP1 motifs within the enhancer are responsible for the TPA induction observed in Fig. 2A just as the enhancer function and the GRE/PRE influence P97 from this remote position (9,10,13). Our data do not rule out the possibility of additional phorbolester responsive elements outside the core enhancer, which might be suggested by the very strong transcriptional induction of P97 in Fig. 1.

### The TREs of HPV-16 are recognized by nuclear proteins with binding properties indistinguishable from AP1

fp4e of HPV-16 protects a complex and overlapping array of sequences suggestive of the binding of several different nuclear proteins (11). To test whether the motif 5′-CTGAATCAC-3′ (fp4el) isolated from surrounding sequences would recognize a nuclear protein we performed a footprint experiment with this cloned oligonucleotide. Fig.4 shows this DNaseI protection analysis with HeLa nuclear extracts on the relevant sequence (HPV-16 position 7630–7638). The lower footprint protects the motif 5′-CTGAATCAC-3′ from HPV-16, indicative of binding of AP1, while the upper footprints identify fortuitous transcription factor binding sites in plasmid sequences (M. Karin, R. Renkawitz, pers. comm.) which include the sequence 5′-TGGCA-3′, a likely artificial NF1 binding site. These sequences have been deleted in the vector ptkCATdH/N. This footprint experiment identifies the 9 bp AP1 motif as one out of several nuclear factor binding sites which form fp4e.

In order to clarify the identity of the factor binding to the motif within fp4e, we performed bandshift competition experiments. Two protein-DNA complexes formed when HeLa nuclear extracts were incubated with the fp4el motif. The dominant complex termed A2 was about 20 fold more abundant than the complex A1 (Fig.5A). Both bands were specifically competed by increasing amounts of the 'bona fide' collagenase gene AP1 motif (15). In contrast, both bands were completely resistant to competition with increasing amounts of an oligonucleotide representing a NF1 binding site (Fig.5B). We conclude, that the same sequence motif in the HPV-16 enhancer that mediates phorbol ester response exhibits binding to a nuclear factor indistinguishable from the binding of the factor AP1.

The second AP1 like motif in fp4e which overlaps with the GRE (fp4er) gives a different result in a bandshift competition. Instead of one dominant complex, a cluster of complexes with similar mobility forms, termed G1 to G5 (Fig.5C). Whereas the formation of all five complexes is competed by the homologous oligonucleotide, complexes G2, -3, -4 and -5 are resistant to competition by the collagenase and fp4el AP1 motifs, G1 is efficiently competed by the collagenase motif. This suggests that while AP1 may show weak affinity to the GRE sequence, other

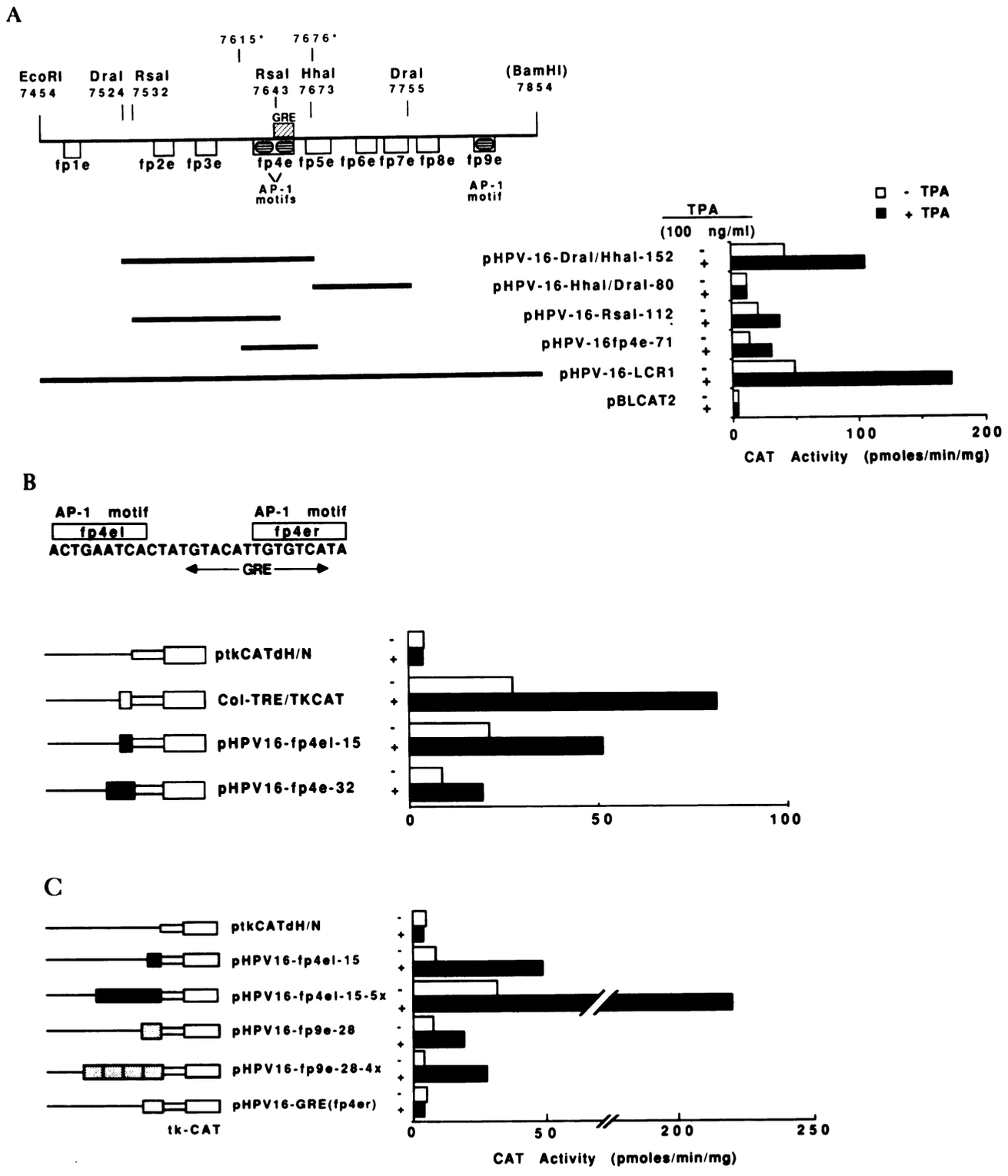


**Figure 2.** Phorbol ester stimulated CAT expression from test vectors with fragments (A) or oligonucleotides representing footprints (B,C) from HPV-16. A: Location of the footprints fp1e to fp9e (11) on the HPV-16 400bp enhancer fragment, that activates the promoter P97, 150 bp 3′ to this segment. Restriction fragments tested are described in detail in the Method section and are represented here by bars. Three AP1 like sequence motifs in the fp4e and fp9e regions are marked by dashes. B: Phorbol ester stimulation of the vector ptkCATdH/N (negative control) with inserts representing both AP1 sites (pHPV16-fp4e-32) or only the left AP1 site within fp4e (pHPV16-fp4el-15). The published vector Col-TRE/tkCAT is included as a positive control. C: the constructs tested in this experiment document the increased stimulation through oligomerization of one HPV-16 AP1 site in fp4e, as published for established AP1 sites (16), and the phorbolester-response through constructs containing fp9e. The lack of inducibility observed with pHPV16-GRE, is probably determined by proteins competing for binding to the same sequence (Fig. 5C).

proteins different from the glucocorticoid receptor which is at undetectable levels in the nuclear extracts used (11), bind more efficiently, thereby leading to an inefficient TPA response of this element. Fig. 5D shows the result of a similar bandshift competition using the AP1 motif from fp9e. This sequence shows a perfect homology to the PEA1 motif from the polyoma enhancer

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collagenase      5'- CTGACTCAT -3'
   AP-1          3'- GACTGAGTA -5'

fp4e-1 AP-1          CTGAATCAC
(7630-7638)          GACTTAGTG

fp4e-r GRE           TTGTGTCAT
(7647-7655)          AACACAGTA

fp9e AP-1            GTTAGTCAT
(7809-7817)          CAATCAGTA

polyoma PEA1         GTTAGTCAC
                     CAATCAGTG
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**Figure 3**. Sequence comparison of the AP1 motifs from HPV-16. Three sequences of the HPV-16 LCR with good homology to known AP1 sites (see Fig. 2A) were compared to the AP1 binding site from the collagenase promoter (15); identical bases are marked with open bars. The fp9e sequence shows good homology (black bar) with the PEA1 element from the polyoma enhancer, a sequence binding the murine analogue of AP1 (22).

which is a likely mouse homologue of the human AP1 factor (27). Two complexes (D1 and D2) formed with this DNA fragment are efficiently competed by the collagenase AP1 site, suggesting that AP1 also binds to the fp9e region. Three other complexes are competed by the homologues fp9e oligo but not by the collogenase AP1 motif suggestive of a complex protein binding behaviour of this site.

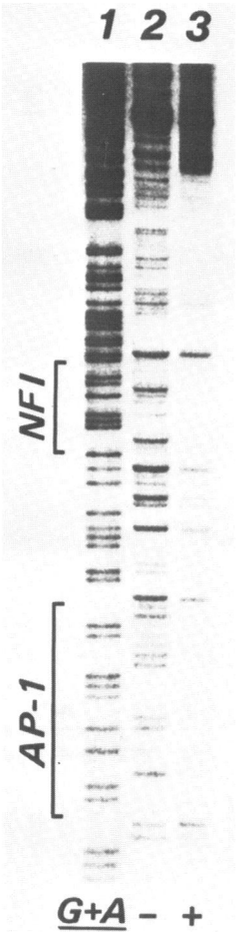


**Figure 4**. DNaseI footprint on the HPV-16 fp4el AP1 motif. Lane 1, G+A sequence marker; DNaseI digestion without (lane 2), or with protein extract added (lane 3). The fp4el sequence 5'-CTGAATCAC-3' plus five bp (5'), and six bp (3') were protected from cleavage (marked with 'AP1'). The additional footprint is in the polylinker/vector part on a fortuitous NF1 sequence motif (5'-TGCCAA-3').

## DISCUSSION

The long control region (LCR) of HPV-16 contains a transcriptional enhancer, which can function independently of the papillomavirus E2 proteins but is stimulated by cellular transcription factors (9,10). Enhancers with similar properties exist in the LCR of HPV-6, 11 and 18 (28–30). In this paper we have localized two phorbol ester responsive elements (TREs) to two footprints, fp4e and fp9e within the cell-type-specific enhancer of the HPV-16 LCR. These TREs appear to be embedded in a network of other transcriptional enhancer elements which consist of a GRE/PRE (mediating glucocorticoid and progesterone regulation), several NF1 binding sites, and some other binding sites for yet unspecified transcription factors (Chong et al., Nucl. Acids Res., in press).

Both TREs coincide with AP1 motifs. When isolated from flanking sequences, both gave rise to protein-DNA complexes as visualized by a bandshift assay. One of them, the fp4el motif could be DNaseI footprinted when isolated from the tight cluster of transcription factor binding sites of fp4e. We have demonstrated by bandshift competition experiments that these complexes can be competed by the presence of the collagenase AP1 site. A second AP1 like motif within fp4e, termed here fp4er, which was previously shown to overlap with a glucocorticoid/ progesterone receptor binding site, shows affinity to several nuclear proteins. While AP1 seems to have a weak affinity to this sequence, it does not function as an efficient TRE, possibly due to competition of various factors for this short DNA segment. We have recently published footprints on sequences reminiscent of the collagenase promoter AP1 binding site 5'-TGACTCA-3' in the LCR of HPV-11, 16 and 18 (12) which were suspected to stimulate HPV transcription by phorbol esters (31,32). Similar motifs suggestive of AP1 binding sites occur in the LCR of HPV-6 (33), (position 7436–7442), HPV-8 (34), (position 7461–7467), HPV-33 (35), (position 7351–7357, 7676–7682 and 7831–7837), BPV-1 (36), (position 7183–7189) and of the cottontail rabbit papillomavirus (37), (position 7819–7825).

AP1, originally defined as the transcription factor binding to the motif 5'-TGACTCA-3' in the collagenase gene promoter (15) and to a related sequence in the SV40 enhancer (16), consists of a heterodimer of the Fos and Jun oncoproteins. The molecular mechanisms that lead to activation of these AP1 protein complexes, via PKC stimulation are not known. PKC is induced by diacylglycerol, a second messenger arising after the binding of certain signal ligands to their corresponding receptors, and through synthetic analogues of diacylglycerol such as phorbol esters. The latter group of substances has been classified as tumor promoters in certain animal models of carcinogenesis and it is

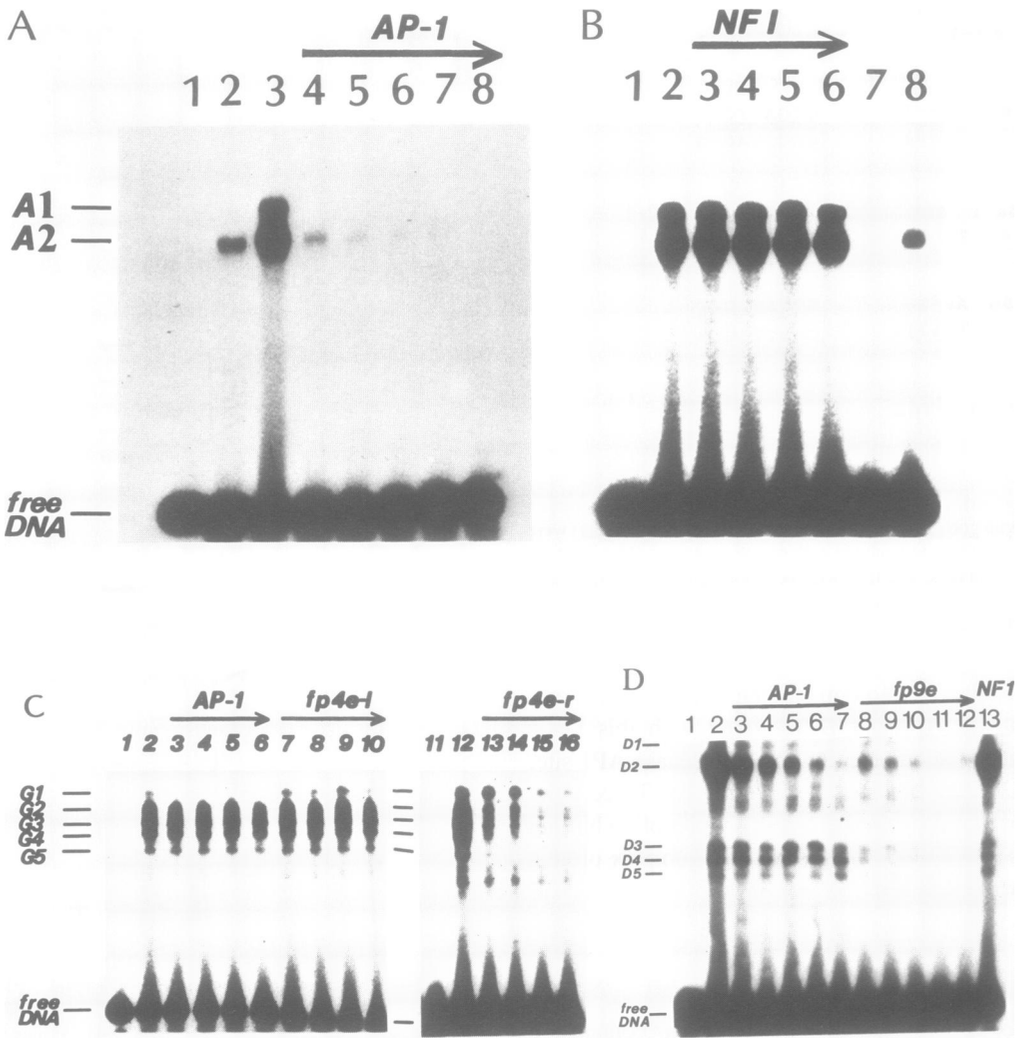


**Figure 5.** Bandshift competition of protein complexes formed on AP1 motifs. (A) Competition of complexes of the fp4el motif by an AP1 site. Two complexes A1 and A2 were formed by incubation of the AP1 motif fp4el, with 1.5 μg polyd(I-C) and 10 (lane 2) or 20 μg HeLa nuclear extract (lane 3); lane 1, no extract. Lanes 2 and 3, extract only; lanes 4–8 competition by collagenase AP1 oligo (10, 20, 40, 60 and 100 ng respectively). (B) Competition with a NF1 site. Lane 1, no extract; lane 2, 20μg extract only; Competitors: lanes 3–6, NF1 oligo (10, 20, 50 and 100 ng); lane 7, 40 ng collagenase AP1 oligo, lane 8, 50 ng fp4el oligo. (C) Bandshift assay of the GRE oligonucleotide overlapping with a potential AP1 site (fp4er). The complexes were named G1 to G5. Lane 1 and 11, no extract; lanes 2 and 12, with extract only; competitors lanes 3–6, collagenase AP1 oligo, (10, 20, 40 and 100 ng); lanes 7–10, fp4el oligo (5, 10, 20 and 50ng); lanes 13–16, homologous fp4er oligo (10, 20, 50 and 100 ng). (D) Bandshift assay of the fp9e oligo: lane 1, no extract, lane 2, with 20 mg extract only. Competitors: lanes 3 to 7 homologous fp9e oligo (2.5, 5, 10, 20, 40 and 80 ng). Lane 13 competition with 40 ng of adenovirus NF1 oligo.

likely that this property arises from the mimicking of diacylglycerol, i.e. the superinduction of certain intracellular regulatory pathways (for reviews see 17,18).

HPV-16, and possibly other papillomaviruses, may use AP1 binding sites to couple their gene regulation to downstream events of intracellular signalling. The property of AP1 to couple promoter function to the cell cycle has been described (38). As non-lytic viruses, papillomaviruses have the need to maintain a constant genomic copy number and transcriptional level. Beyond this possibility, AP1 sites may mediate papillomavirus gene regulation in response to extracellular signals such as the epidermal growth factor (39), a possible explanation of the efficient amplification of HPV-11 in human epithelial cells transplanted into the kidney capsule of the nude mouse (40).

Considering the tumorigenicity of genital human papillomaviruses, attention must be given to the fact that these AP1 sites are bringing the activation of the viral tumor genes E6 and E7 under the influence of cellular tumor genes, namely the genes of the jun, fos and possibly the ras family as found for the polyomavirus (27). The viral genes may in turn bring about changes in transcription of cellular genes, like the activation of the transcription factor E2F through the HPV-16 E7 protein (7), which may in turn induce the transcription of the myc gene (41). It will be interesting to explore whether transcriptional induction of these viral genes through peptide hormones and growth factors, steroid hormones, tumor promoting substances, in cooperation with mutations in cellular tumor genes, contribute significantly to the multifactorial etiology of genital carcinoma. This possibility is suggested by the cooperativity of ras and HPV-16 (5) and glucocorticoids and HPV-16 (4) in transformation essays, the latter one probably mediated by the GRE in the HPV-16 enhancer (9).

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