JOURNAL OF VIROLOGY, Oct. 1990, p. 4792–4798
0022-538X/90/104792-07$02.00/0

Vol. 64, No. 10

A Member of the Activator Protein 1 Family Found in Keratinocytes but Not in Fibroblasts Required for Transcription from a Human Papillomavirus Type 18 Promoter

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Received 26 March 1990/Accepted 30 June 1990

Papillomaviruses are tissue specific and replicate in differentiating keratinocytes. We are interested in the question of tissue specificity at the level of transcription. We used extracts from human keratinocytes and human fibroblasts at low passage number and from HeLa cells to look for factors binding to the E6 promoter of human papillomavirus type 18 (HPV-18) DNA by footprint and gel mobility shift experiments. We found a factor present in HeLa and keratinocyte extracts but not in fibroblast extracts which bound about 160 base pairs upstream from the start of E6. The binding site included the sequence TGACTAAG, which resembles the consensus binding site for the AP-1 family of proteins. Synthetic oligonucleotides containing this binding site specifically competed with factor binding to HPV-18 DNA, as did the AP-1 sequence of simian virus 40. They also inhibited transcription from the E6 promoter in vitro in extracts from HeLa cells. Thus, the presence of this keratinocyte-specific factor seems to be important for HPV-18 transcription.

Human papillomavirus type 18 (HPV-18), along with certain other viruses in the human papillomavirus group, has been associated with genital lesions, including cervical carcinoma (7, 13). HPV-18 DNA from a cervical lesion was cloned (7), and its nucleotide sequence was determined (11). Part of the HPV-18 genome is present in some cell lines derived from cervical tumors (for example, HeLa cells), and in these cells the viral early genes E6 and E7 are expressed (26). HPV-18 DNA transforms rodent cells and immortalizes human keratinocytes in culture (19, 27, 36). The E6 and E7 genes are sufficient for transformation by HPV-18 (3, 4) and by the related HPV-16 (22, 27, 29, 35).

The major start sites for synthesis of HPV-18 mRNA in HeLa cells are near the junction of the noncoding long control region (LCR) and the E6 open reading frame (ORF) (26, 30). The LCR contains a transcriptional promoter activity (31) and at least one enhancer. The enhancer is between 200 and 400 base pairs (bp) upstream from the E6 RNA start site and needs no viral gene product for activity (17, 30). An E2 binding site is close to the E6 RNA start site and is responsive to the E2 gene product of bovine and human papillomaviruses (6, 17, 32). This E2-responsive sequence is activated by E2 when positioned in an enhancer configuration upstream of a heterologous promoter. However, it is repressed by E2 when functioning as part of the normal E6 promoter (6).

Several cellular proteins interact with HPV-18 LCR DNA (16). Most of these proteins have not yet been identified. Two of the protein-binding sites on the DNA have homology to the consensus binding site for the activator protein 1 (AP-1) family (1, 16).

The papillomaviruses are apparently very specific as to the type of cell that they infect. The host cells of HPV-16 and HPV-18 are believed to be genital mucosal keratinocytes (18, 22). Human papillomaviruses do not grow in culture. The constitutive enhancer in the HPV-18 LCR can function in keratinocytes, epithelial cell lines, and also fibroblasts (6, 17). The transcriptional promoter activity detected in the LCR, on the other hand, works in keratinocytes but not fibroblasts (6). The promoter therefore seems to be cell type specific.

In the work reported here, we used DNase I footprinting and gel mobility shift assays to look at the interaction of proteins from different types of cells with the HPV-18 E6 promoter. We found a protein(s) present in human keratinocyte but not human fibroblast cells which binds to an AP-1-like sequence in the HPV-18 E6 promoter and which is necessary for transcription from the E6 promoter in vitro.

MATERIALS AND METHODS

Cells. The cells used were HeLa (strain S3), HaCaT (human keratinocytes [8]), SV61 (human fetal epidermal keratinocytes immortalized with a mutant of simian virus 40 [SV40] lacking the origin of DNA replication, originally named SV61 Bam HFK [23]), FEK4 (human foreskin fibroblasts [34]), and 3229 (37). All cells were grown in Dulbecco minimal essential medium containing either 5% (HeLa, SV61, HaCaT) or 10% (FEK4, 3229) fetal calf serum. Hydrocortisone (4 μg/ml) was added to the medium for SV61 cells. The SV61 and FEK4 cells were kindly provided by R. Tyrell; 3229 and HaCaT cells were provided by N. Fusenig and P. Cerutti.

Cell extracts. Nuclear and whole-cell extracts of HeLa cells were prepared as described by Dignam et al. (14) and Manley et al. (21), respectively. Extracts from SV61 and FEK4 cells were prepared by the same methods slightly modified for use with cells grown as monolayers. Some FEK4 whole-cell extracts were also made as described by Paterson and Everett (24).

Synthesis and labeling of oligonucleotides. The oligonucleotides used were as follows: the HPV-18 AP-1 (41-mer) was the sequence from positions 7775 to 7815 (11, 17), including the AP-1-like core, 5′-**ATGACTAAGC**, and the SV40 AP-1 (30-mer) was the sequence from positions 102 to 131 of SV40 (33), including the AP-1 core sequence, 5′-**CTGACTAATT** (nucleotide sequence of the strand in the sense of late RNA). Complementary, single-stranded oligonucleotides were syn-

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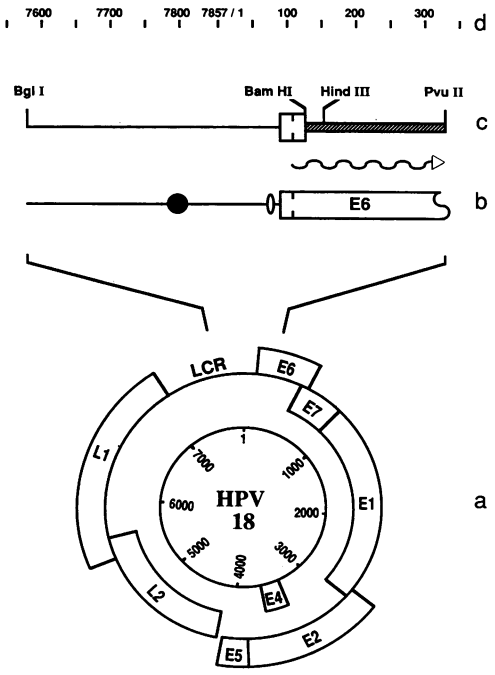

FIG. 1. HPV-18 genome organization and plasmid construct. (a) Circular HPV-18 genome is shown with early and late ORFs. The LCR upstream of E6 was cloned into the vector pSP65 at the *Bam*HI site. The *Bgl*I-*Pvu*II fragment (c) was isolated and used for in vitro transcription studies. Symbols: hatched box, vector sequences; wavy arrow, run-off transcript. The *Bgl*I-*Hin*dIII fragment (c) was used for footprinting studies. (b) E6-proximal promoter region with (●) AP-1 Jun/Fos recognition site and (○) TATA box. (d) Scale of HPV-18 nucleotide positions for the LCR and E6 sequences.

thesized and purified on 18% polyacrylamide–urea gels. One strand was labeled at the 5′ end with [γ-^{32}P]dATP by using polynucleotide kinase and annealed to the complementary strand by incubation in 10 mM Tris (pH 8.3)–10 mM $MgCl_2$ at 80°C for 8 min, followed by slow cooling to room temperature. For unlabeled competing oligonucleotides, the complementary strands were annealed without prior labeling.

Transcription in vitro. Transcription reaction mixes (containing about 100 μg of protein extract in a reaction volume of 50 μl) and conditions, analysis of ^{32}P-labeled RNA products by denaturation with glyoxal, agarose gel electrophoresis, and autoradiography were as described previously (2) except that the agarose gels contained 3% NuSieve agarose (FMC BioProducts). The DNA templates are described in the legends to Fig. 2 and 4. For competition studies, the in vitro transcription mix was incubated on ice without DNA template for 10 min with a 25-fold molar excess of competing oligonucleotide. The in vitro transcription was started by addition of the DNA template and transfer to 30°C.

DNase I footprinting. The plasmid containing the 1-kilobase (kb) *Bam*HI fragment of HPV-18 cloned in pSP65 (Fig. 1) was digested with *Bgl*I and *Hin*dIII. The *Bgl*I-*Hin*dIII 450-bp fragment (*Bgl*I in the LCR of HPV-18 to *Hin*dIII in the polylinker of the vector) was isolated by preparative gel electrophoresis and labeled by using the Klenow fragment of DNA polymerase with [α-^{32}P]dATP at the *Hin*dIII site. Protein-DNA binding reaction mixes (50 μl) contained 0.2 ng of labeled DNA fragment, 25 to 100 μg of protein extract, and Dignam buffer D (14) to a combined volume of 25 μl, 0.5 mM dithiothreitol, 6 mM $MgCl_2$, and 1 μg of poly(dI-dC). The reaction mix was incubated on ice for 15 to 20 min and then transferred to a water bath at 22°C for 60 s. DNase I (Boehringer Mannheim Biochemicals; the amount was determined empirically but was on the order of 0.1 to 0.3 U) was added, and incubation was continued for 60 s at 22°C. The digestion was stopped by the addition of 50 μl of a solution containing 0.2 M NaCl, 0.03 M EDTA, 1% sodium dodecyl sulfate, and 0.4 μg of pronase per μl. After 15 min at 37°C, the reaction mixes were extracted with phenol-chloroform (1:1) and adjusted to 0.3 M sodium acetate, and 10 μg of tRNA was added. The nucleic acids were precipitated at −70°C with 2.5 volumes of ethanol and centrifuged for 10 min in an Eppendorf microfuge at 4°C. The pellets were washed in 70% ethanol, dried, and dissolved in gel loading buffer containing 80% (vol/vol) formamide, 1× Tris-borate-EDTA buffer–0.01% bromophenol blue (28), heated for 5 min at 95°C, and then analyzed by electrophoresis through a 6% polyacrylamide sequencing gel.

Gel mobility shift assay. Standard binding reaction mixes (20 μl) contained 8 μg of nuclear protein (dialyzed against buffer D of Dignam et al. [14]) plus buffer D to a combined volume of 12 μl, 1 mM dithiothreitol, 8 mM $MgCl_2$, 1 μg of poly(dI-dC), and 20 fmol of end-labeled oligonucleotide probe. In the case of FEK4 cells, whole-cell extracts were also made as described by Paterson and Everett (24). This extract was not dialyzed, but the salt concentration in the protein-DNA binding reaction mix was adjusted to 60 mM as when the nuclear extract was used. Protein-DNA binding reaction mixes were incubated on ice for 20 min, analyzed by electrophoresis through a 5% polyacrylamide gel in 0.5× Tris-borate-EDTA buffer, and electrophoresed at 10 V/cm at 4°C. The gel was then dried and autoradiographed. For competitive binding studies, the reaction mix was incubated with unlabeled competing oligonucleotide for 10 min on ice before the addition of labeled probe.

RESULTS

Transcription in vitro from the HPV-18 E6 promoter. The LCR of HPV-18, contained within a 1-kb *Bam*HI restriction fragment (nucleotides [nt] 6929 to 119 [11]), was cloned into the *Bam*HI site of vector pSP65 (Fig. 1). (ORF L1 terminates at nt 7133, and the AUG of the E6 ORF is at nt 105 [11].) The plasmid DNA was then cut with *Eco*RI in the polylinker of pSP65 and *Pvu*II in the vector sequences to release a fragment containing the whole of the LCR with about 200 bp of vector sequences downstream. A second fragment containing only the E6-proximal part of the LCR was isolated by cutting with *Bgl*I at position 7582 in the middle of the LCR and *Pvu*II in the downstream vector sequences. These linear templates were used for in vitro runoff transcription experiments with whole-cell (21) and nuclear (14) extracts of HeLa cells. The resulting transcripts were compared with those from SV40 DNA cut with *Pst*I. The results are shown in Fig. 2.

The specific HPV-18 transcript of about 230 nt was seen with both nuclear and whole-cell extracts and with both the *Eco*RI-*Pvu*II and *Bgl*I-*Pvu*II templates (Fig. 2A). The length of this in vitro transcript corresponded to a start site at about position 104 on the HPV-18 genome. This result is in agreement with previous analysis of transcripts from transfected cloned HPV-18 DNA (30) and from endogenous HPV-18 in HeLa cells (26) showing multiple RNA start sites in this region. The top bands in lanes 1 and 2 of Fig. 2A corresponded to the end-to-end transcript of about 600 nt

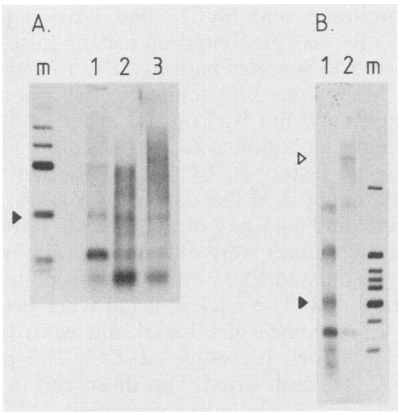

FIG. 2. Transcription in vitro from the HPV-18 and SV40 early promoter. Whole-cell (A, lanes 2 and 3) or nuclear (A, lane 1; B, lanes 1 and 2) extracts from HeLa cells were used for in vitro transcription reactions. The template DNA (0.5 μg) (see Fig. 1 for restriction sites) was *Bgl*I-*Pvu*II (A, lanes 1 and 2; B, lane 1), *Eco*RI-*Pvu*II (A, lane 3) (*Eco*RI cuts upstream of the insert in the polylinker of pSP65; thus, the *Eco*RI-*Pvu*II fragment contains all of the long control region), and SV40 cut with *Pst*I (B, lane 2). The runoff RNA transcripts were glyoxylated and analyzed by agarose (3% NuSieve) gel electrophoresis. The size markers (lanes m) were radiolabeled (A) SV40 *Hin*fI (1,845, 1,085, 766, 543/525, 237, 109, and 83 bp) and (B) pBR322 *Hin*fI (1,631, 516/506, 396, 344, 298, 221/220, 154, and 75 bp) fragments. The solid arrowhead shows the transcript of about 230 nt from the HPV-18 early promoter. The open arrowhead shows the 2-kb transcript from the SV40 early promoter.

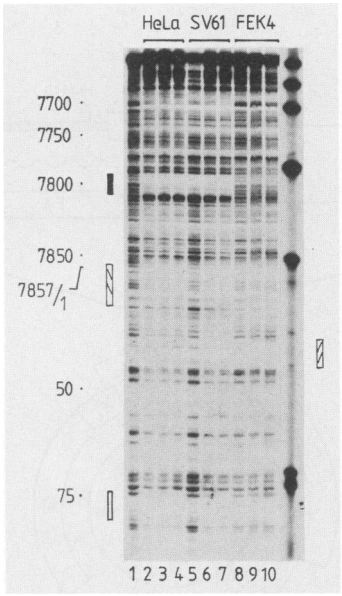

FIG. 3. DNase I protection by an epithelial cell-specific factor bound to the HPV-18 early promoter. DNase I protection studies were done with nuclear extracts from HeLa, SV61 keratinocyte, and FEK4 fibroblast cells. The probe was *Bgl*I-*Hin*dIII DNA (Fig. 1) labeled with [α-^{32}P]dATP at the *Hin*dIII site. The DNase I digestion pattern is shown for the probe alone (lane 1); with 25, 50, and 100 μg of HeLa extract (lanes 2 to 4); with 25, 50, and 100 μg of SV61 extract (lanes 5 to 7); and with 25, 50, and 100 μg of FEK4 extract (lanes 8 to 10). The size markers were radiolabeled pBR322 *Hin*fI restriction fragments. Solid box, Epithelial cell-specific factor; open box, TATA box factor; hatched box on left, CCAAT box factor; hatched box on right, fibroblast factor. The scale on the left-hand side corresponds to nucleotide positions on the HPV-18 genome.

from the *Bgl*I-*Pvu*II template. When all of the LCR was included in the template (the *Eco*RI-*Pvu*II DNA fragment), several additional transcripts starting further upstream than the 230-nt transcript were seen (Fig. 2A, lane 3). As we have focused on the main start site near the E6 ORF, we have not, as yet, further analyzed these putative upstream start sites. The two lowest bands in Fig. 2A were nonspecific and probably came from the extract itself, as they were also seen when DNA unrelated to HPV-18 (e.g., SV40, Fig. 2B) was transcribed.

Figure 2B shows a comparison of transcription in vitro of the HPV-18 *Bgl*I-*Pvu*II template and SV40 DNA cut with *Pst*I. Lane 2 shows the main 2-kb early transcript of SV40 described previously (28) as well as the 1.2-kb end-to-end transcript of the shorter SV40 *Pst*I restriction fragment. The 230-nt transcript seen in lane 1 with HPV-18 DNA was not seen when SV40 DNA was transcribed. Thus, the HeLa extracts transcribed specifically the HPV-18 and SV40 viral DNA templates.

Proteins binding to the E6 promoter. We were interested to know what factors were important for transcription from the HPV-18 E6 promoter and whether we could identify any cell type-specific factors which allow transcription to occur in epithelial but not fibroblast cells. DNase I footprinting experiments were done with extracts from HeLa cells and human foreskin keratinocyte (SV61) and fibroblast (FEK4) cells. The DNA used in the protein-DNA binding reactions was the *Bgl*I-*Hin*dIII fragment (Fig. 1) labeled at the *Hin*dIII site. Using this fragment, we could look at the proteins binding to the region proximal to the E6 promoter.

Figure 3 shows the results of DNase I protection analysis. A region of strong protection was seen around position 7800 (the outside limits of the footprint being nt 7785 to 7810 by sequence analysis) with HeLa and SV61 keratinocyte extracts. This protected region was not seen with FEK4 fibroblast extracts or with extracts from 3229 human fibroblast (34) cells (data not shown). A footprint at this position has been observed previously with HeLa cell extracts (16). It covers a region containing the sequence 5′-TGACTAA, which resembles the AP-1 Jun/Fos recognition site (1). The results in Fig. 3 show that the factor giving this footprint was also present in a human keratinocyte cell line but not in human fibroblast cells. Footprints corresponding to the TATA box and CCAAT box binding factors were seen with both the epithelial (HeLa and SV61) and fibroblast extracts. In contrast, there appeared to be another factor in the FEK4 extracts which was not present in the HeLa and SV61 extracts. This factor mapped at about position 40.

To confirm the specificity of factor binding to the HPV-18 AP-1-like sequence, gel mobility shift assays were done with extracts from HeLa, SV61, and FEK4 cells and a 41-bp oligonucleotide corresponding to HPV-18 nt 7775 to 7815. Extracts of HeLa and SV61 cells gave one strong retarded band (band b, Fig. 4, lanes 2 and 4) and two weaker bands (bands a and c). Formation of the strong band b was specific, as it was prevented by a 25-fold molar excess of unlabeled oligonucleotides (lanes 3 and 5). The protein-DNA complexes observed with extracts from HeLa and SV61 cells were also seen with extracts of HaCaT keratinocytes (Fig. 4, lanes 8 to 11), which contain no SV40 or HPV sequences (8). Addition of an unrelated oligonucleotide had no effect on the pattern of retarded bands (see Fig. 6). Extracts of FEK4 cells did not yield retarded band b or a but did give several

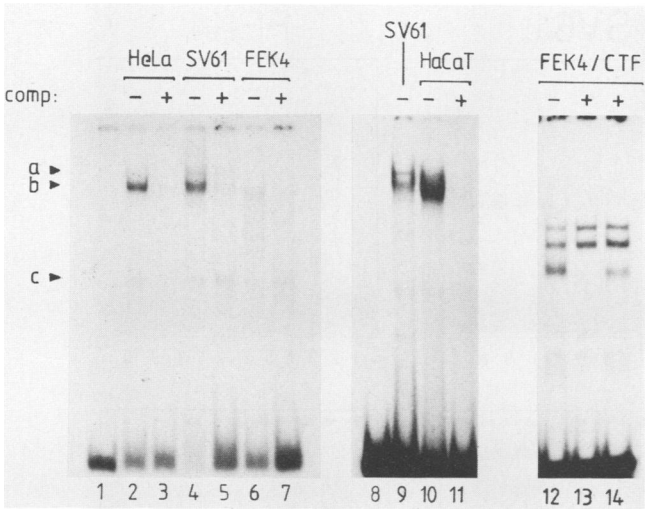

FIG. 4. Factor binding to the AP-1-like sequence of HPV-18. Gel mobility shift assays were done with nuclear extracts of HeLa (lanes 2 and 3), SV61 (lanes 4, 5, and 9), FEK4 (lanes 6 and 7), and HaCaT (lanes 10 and 11) cells and an end-labeled 41-mer oligonucleotide containing the HPV-18 AP-1 sequence. Lanes 1 and 8 show the labeled oligonucleotide alone. Lanes 2, 4, 6, 9, and 10 show the complexes formed in the absence of competing, unlabeled oligonucleotide. Lanes 3, 5, 7, and 11 show the results of adding a 25-fold molar excess of unlabeled HPV-18 oligonucleotide. Lanes 12 to 14 show the results of a control experiment with a whole-cell extract of FEK4 cells and labeled CTF oligonucleotide (5). Lane 12 shows the complexes formed in the absence of competing, unlabeled oligonucleotide. Lane 13 shows competition of the lower complex by addition of a 25-fold molar excess of unlabeled CTF oligonucleotide. Lane 14 shows that the addition of a 25-fold excess of the HPV-18 AP-1 sequence did not compete with the binding of CTF.

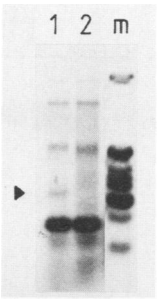

FIG. 5. Competition of HPV-18 early transcription by an oligonucleotide containing the HPV-18 AP-1-like sequence. *BglI-PvuII* DNA (0.5 μg) was transcribed in nuclear extracts from HeLa cells without (lane 1) or with (lane 2) a 25-fold molar excess of a 41-mer oligonucleotide representing nt 7775 to 7815 of the HPV-18 sequence. The arrowhead shows the early runoff transcript of about 230 nt. The size markers were radiolabeled pBR322 *HinfI* restriction fragments.

faint bands, including band c (Fig. 4, lanes 6 and 7). The FEK4 extracts contained the CCAAT-binding factor CTF (Fig. 4, lanes 12 to 14) and Sp1 factor (data not shown).

Factor binding to the AP-1-like sequence is needed for transcription. To investigate whether the proteins which interact with the AP-1-like sequence are important for transcription in vitro, the HPV-18 41-bp oligonucleotide was used as a competitor in in vitro transcription experiments. The *BglI-PvuII* template was transcribed with nuclear extracts of HeLa cells which had been incubated or not with a 25-fold molar excess of competing oligonucleotide. All transcription reaction mixes contained 1 μg of poly(dI-dC) nonspecific competitor DNA. Transcription in vitro from the E6 promoter was specifically blocked by the competing AP-1-like sequence, whereas synthesis of the end-to-end transcript was not reduced but rather increased (Fig. 5). Transcription was not reduced by a nonspecific 45-mer, the polylinker of the plasmid pSP65 (data not shown). Thus, binding of a protein factor to the AP-1-like sequence is important for transcription from the E6 promoter in vitro.

Epithelial cell and fibroblast factors recognizing the HPV-18 and SV40 AP-1 sequences—comparison by gel mobility shift assay. DNA sequences related to the AP-1 consensus are recognized by an extended family of proteins, members of which are regulated in different ways and are made in different types of cells (12). The enhancer of SV40 contains AP-1 sequences with the same core as but different flanking sequences than the HPV-18 AP-1 sequence. We wanted to test whether similar protein-DNA complexes were formed with the SV40 AP-1 sequence as with that of HPV-18 or whether certain complexes were specific for HPV-18.

Competition gel mobility shift assays were done with extracts from HeLa, SV61, and FEK4 cells. Two oligonucleotides containing AP-1-like sequences were used. One was the HPV-18 41-mer described above. The other was a 30-mer containing the original AP-1 site (5′-CTGACTAATT) from the SV40 72-bp enhancer region. Figure 6A shows the binding pattern with labeled HPV-18 oligonucleotide either alone (lane 2) or in competition with increasing amounts of unlabeled HPV-18 (lanes 3 to 6) or SV40 (lanes 7 to 10) oligonucleotide.

Figure 6B shows the results of a similar experiment with labeled SV40 AP-1 oligonucleotide either alone (lane 2) or in competition with increasing amounts of unlabeled SV40 (lanes 3 to 6) or HPV-18 (lanes 7 to 10) oligonucleotide. Lane 1, in all cases, shows the labeled DNA alone. The specific protein-DNA complexes a and b appeared as a doublet with HeLa and SV61 cell extracts in both cases (the complexes formed with the HPV-18 or SV40 sequences migrated similarly when run on the same gel; data not shown). Both bands were reduced by competition with similar excesses of the HPV-18 and SV40 AP-1-related sequences but not by an unrelated 30-mer oligonucleotide containing the transactivation-responsive element (15) from the parvovirus minute virus of mice (Fig. 6A, SV61 extract, lane 11) or by a mutated AP-1 sequence (data not shown). The doublet was absent when FEK4 cell extracts were used, although other, fainter bands were seen. This binding was specific, as it was reduced by an excess of unlabeled HPV-18 and SV40 AP-1 sequences (Fig. 6, FEK4) but not by CTF, Sp1, or mutated AP-1 sequences (data not shown). We know that the fibroblast extracts were active, as they showed binding to CTF- and Sp1-specific oligonucleotide sequences (see above) and gave a fibroblast-specific footprint and a footprint over the CCAAT box (Fig. 3).

Subtle differences in the formation of complexes a and b were seen, depending on the cell type and the oligonucleotide sequence used. For example, with the HPV-18 sequence and HeLa cell proteins, complex b was predominant, while with the SV40 sequence and SV61 cell proteins, complex a was formed almost exclusively. Thus, the AP-1-related sequences of SV40 and HPV-18 form similar protein-DNA complexes, but the proteins which form these complexes differ in their relative affinities for the two viral DNAs.

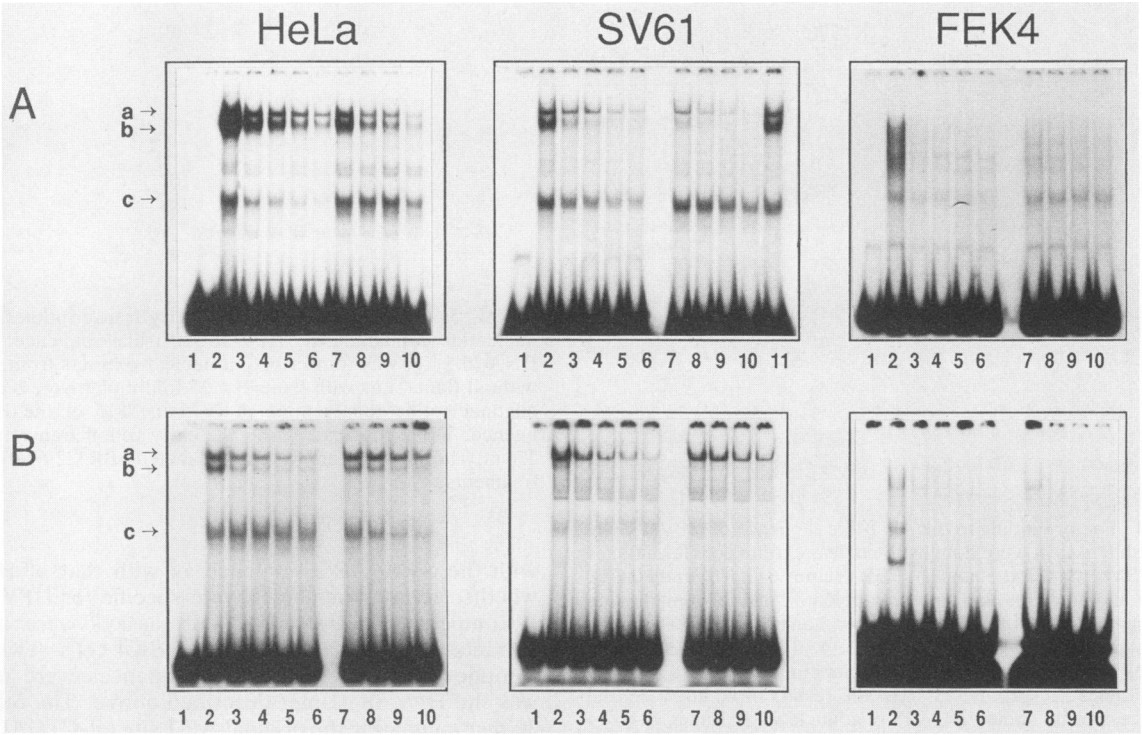

FIG. 6. Competition of binding between AP-1-related sequences from HPV-18 and SV40. Gel mobility shift assays were done with nuclear extracts of HeLa and SV61 cells and a whole-cell extract of FEK4 cells. The [γ-^{32}P]dATP-labeled oligonucleotides used in the binding reactions were: (A) 41-mer containing the HPV-18 AP-1-like sequence 5'-ATGACTAAGC; and (B) 30-mer containing an SV40 AP-1 site, 5'-CTGACTAATT. Lanes 1 show the labeled oligonucleotide without protein extract. Lanes 2 show the protein-DNA binding pattern in the absence of competing oligonucleotide. (A) Lanes 3 to 6 and 7 to 10 show competition by a 5-, 10-, 20-, and 30-fold molar excess of HPV-18 and SV40 oligonucleotides, respectively. In addition, lane 11 in the case of SV61 shows binding in the presence of a 30-mer oligonucleotide with a sequence unrelated to either the HPV or the SV40 sequence (see text). (B) Lanes 3 to 6 and 7 to 10 show competition by a 5-, 10-, 20-, and 30-fold molar excess of competing SV40 and HPV-18 oligonucleotides, respectively.

DISCUSSION

We detected in vitro the activity of a promoter which functions in the integrated HPV-18 DNA of HeLa cells. The activity of this promoter in vitro was about the same when the template DNA was cut upstream at the *Bgl*I site or contained the whole LCR. Therefore, under these conditions, the enhancer between positions −200 and −400 (30) is not needed. In the in vitro assay, additional RNA start sites upstream in the LCR were visible. Such upstream initiation of transcription was also observed for the endogenous HPV-18 DNA in HeLa cells (26) but it was relatively minor. Clearly, the major HPV-18 transcriptional start site seen in HeLa cells is not the only one; the activity of these different start sites could be modulated by proteins during different phases of the viral growth cycle and by the presence of nucleosomes on the intracellular viral DNA (28).

Within the region close to the E6 promoter, the major protein-binding sites we saw by DNase I footprinting corresponded to a CCAAT-related sequence, a TATA sequence, and, as noted before (16), a sequence related to the AP-1 Jun/Fos recognition site. Different cell types gave different patterns of binding. The CCAAT box-binding protein was present in each cell type tested. A footprint over the AP-1-related sequence was seen with extracts of HeLa cells and the immortalized keratinocytes SV61 but not with extracts of human fibroblast lines FEK4 and 3229. The fibroblast extracts were functional, giving a different, specific footprint. Protection over the TATA box, interestingly, was seen only when the AP-1-related sequence was also protected.

A protein which binds to the AP-1-related sequence is needed for the function of this promoter in vitro, because competition with an oligonucleotide containing the AP-1 sequence blocked transcription. In HPV-16, AP-1 sites upstream of the E6 mRNA start site have also been shown to be important for transcription from this promoter (9, 10). The central core AP-1 sequence in HPV-18 DNA is the same as that in the SV40 promoter, but the flanking sequences are different. The gel mobility assays suggest that the protein binding to HPV-18 DNA is a member of the AP-1 Jun/Fos family. First, an oligonucleotide containing the SV40 AP-1 sequence gave a similar pattern of retarded bands as the HPV-18 sequence, and second, the SV40 sequence competed effectively for complex formation on HPV-18 DNA. Three protein-DNA complexes were detected. Complex c was apparently not cell type specific, being formed by all cell extracts tested, while complexes a and b were formed by extracts of HeLa, SV61, and HaCaT keratinocytes but not extracts of FEK4 fibroblasts. Although the three complexes formed with both HPV-18 and SV40 sequences, clear differences in the apparent relative affinities were noticeable. Complexes a, b, and c could contain different proteins or differently modified versions of the same proteins. Further work should show which proteins or modifications are involved.

We conclude that a protein(s) present in HeLa, SV61, and HaCaT cells but not in FEK4 or 3229 human fibroblasts recognizes the AP-1-related sequence of HPV-18. Although FEK4 cell extracts do not form the major AP-1 protein-DNA

complex seen with HeLa and HaCaT extracts, they do contain lower amounts of other proteins which recognize the AP-1 sequence. Such proteins are therefore not ubiquitous, a conclusion supported by the observations of others. Member proteins of the AP-1 Jun/Fos family are believed to be expressed in a cell type-specific manner (12). Their activity can be induced by phorbol esters (1). In mouse embryonal carcinoma stem cells, the activity of PEA-1, an AP-1 homolog, is very low. When the cells differentiate, PEA-1 is induced, and this could explain the host specificity of polyomavirus for these cells (20). In the mouse NIH 3T3 cell line, AP-1 activity is increased by phorbol ester, serum, and viral transformation (25).

HeLa and SV61 cells contain viral sequences (of HPV-18 and SV40, respectively), but HaCaT cells do not (8). These three cell types have similar levels of factors binding to the AP-1 sequence. Therefore, the presence of these factors does not correlate with the presence of the viral sequences but rather with the epithelial origin of the cells.

Could the cell type-specific expression of proteins of the AP-1 family account, at least in part, for the host cell specificity of HPV-18? Recent transfection experiments (6) have shown that the HPV-18 E6 promoter is active in primary human keratinocytes but not in fibroblasts. Our finding that keratinocytes contain factors not detectable in the low-passage human fibroblasts used here that recognize the AP-1 sequence therefore provides a reasonable explanation for the cell type-specific transcription from this promoter.

ACKNOWLEDGMENTS

We thank B. Hirt for helpful comments and critical reading of the manuscript and B. Bentele and H. Bruggmann for excellent technical assistance. We are very grateful to H. zur Hausen for kindly donating cloned HPV-18 DNA, to R. Tyrell for providing the FEK4 and SV61 cells, to P. Cerutti for the 3229 cells, and to N. Fusenig and P. Cerutti for HaCaT cells.

This work was supported by grants from the Ligue Suisse Contre le Cancer and the Fonds National Suisse de la Recherche Scientifique.

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