The EMBO Journal vol.6 no.1 pp.139-144, 1987

Identification of early proteins of the human papilloma viruses type 16 (HPV 16) and type 18 (HPV 18) in cervical carcinoma cells

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Communicated by H.zur Hausen

We have sequenced 1730 bp of human papilloma virus type 18 (HPV 18) DNA containing the open reading frames (ORF) E6, E7, the N-terminal part of E1 and, additionally, 120 bp of the N-terminal part of L1. Based on these sequencing data, together with the human papilloma virus type 16 (HPV 16) DNA sequence published recently, we identified and cloned the ORF E6, E7, E1 and L1 of HPV 18 and the ORF E6, E7, E1, E4, E5, L2 and L1 of HPV 16 into prokaryotic expression vectors. The expression system used provides fusions to the N-terminal part of the MS2 polymerase gene controlled by the heat-inducible lambda P_L promoter. Using the purified fusion proteins as immunogens we raised antisera against the proteins encoded by the ORF E6, E7 and E1 of HPV 18 as well as those encoded by the ORF E6, E7, E4 and L1 of HPV 16. By Western blot analysis we could show that the E7 gene product is the most abundant protein in cell lines containing HPV 16 or HPV 18 DNA. It is a cytoplasmic protein of 15 kd in the SiHa and the CaSki cell lines which contain HPV 16 DNA, and 12 kd in the HeLa, the C4-1 and the SW756 cell lines which contain HPV 18 DNA. These results were confirmed by *in vitro* translation of hybrid-selected HPV 16 and HPV 18 specific poly(A)$^+$ RNA from SiHa, CaSki and HeLa cells. Additionally, these experiments led to the identification of an 11-kd E6 and a 10-kd E4 protein in the CaSki cell line as well as a 70-kd E1 protein in HeLa cells.

Key words: HPV 16 and HPV 18 early proteins/human cervical carcinoma cell lines/expression vectors/antibodies

Introduction

DNA of human papilloma virus (HPV) type 16 and type 18 has been found in the majority of human genital carcinomas supporting the concept that papilloma viruses are a key factor in the etiology of these tumors (for review see zur Hausen and Schneider, 1987). HPV DNA has also been detected in cell lines derived from cervical cancers: HPV 16 DNA in the cell lines CaSki and SiHa, HPV 18 DNA in HeLa, C4-1 and SW756 (Boshart *et al.*, 1984; Schwarz *et al.*, 1985; Yee *et al.*, 1985; Pater and Pater, 1985). Since tissue culture systems susceptible to transformation by human papilloma viruses are not presently available, these cell lines provide unique systems to study the expression of HPV 16 and HPV 18 genes in cells derived from human tumors.

The complete DNA sequences of several papilloma virus genomes [e.g. HPV 1a, HPV 6b, HPV 11, HPV 16 and bovine papilloma virus type 1 (BPV 1)] have been reported recently (Chen *et al.*, 1982; Danos *et al.*, 1982; Schwarz *et al.*, 1983; Seedorf *et al.*, 1985; Dartmann *et al.*, 1986). These viral genomes have been divided into an early and late region by analogy to the polyoma and Simian virus 40 genome. The late region contains two open reading frames (ORF) L1 and L2 which code for viral structural proteins (reviewed by Danos *et al.*, 1984).

Information about the function of early genes of papilloma viruses originates almost exclusively from *in vitro* transfection experiments with BPV 1: the ORF E6 and E5 have been shown to encode transforming proteins (Schiller *et al.*, 1984; Yang *et al.*, 1985; Schiller *et al.*, 1986). Transforming activity of BPV 1 DNA is also influenced by ORF E2 which encodes a *trans*-activating factor (Spalholz *et al.*, 1985). Genetic studies using a mutated ORF of BPV 1 indicate that the E1 gene product appears to maintain BPV 1 DNA in the episomal state, while the E7 gene product controls replication of the episomes to a high copy number (Lusky and Botchan, 1984; Sarver *et al.*, 1984; Lusky and Botchan, 1985). Recently two gene products of the ORF E4 have been demonstrated as a 16/17-kd doublet in warts induced by HPV 1a (Doorbar *et al.*, 1986). The E6 protein of BPV 1 has been identified in the nuclear and membrane fractions of mouse cells transfected with cloned BPV 1 DNA, while an E5 protein could be demonstrated in the cellular membrane fraction (Androphy *et al.*, 1985; Schlegel *et al.*, 1986).

However, no early human papilloma virus protein could be demonstrated in papillomas, carcinomas, cell lines derived from carcinomas or in premalignant lesions, with the exceptions of the E4 protein from HPV 1a (Doorbar *et al.*, 1986) and the E7 protein from HPV 16, which was recently identified in the CaSki cell line as a 20-kd protein (Smotkin and Wettstein, 1986). The failure to detect additional viral proteins has been hampered by the lack of viral propagation systems and appropriate antisera.

Here we report the identification of an E6, E7 and E4 protein of HPV 16 and an E7 and E1 protein of HPV 18. To identify these gene products from the early and late regions of HPV 16 and the HPV 18 genomes we subcloned the ORF E6, E7, E1, E4, E5, L2, L1 of HPV 16 and E6, E7, E1 and L1 of HPV 18 into prokaryotic expression vectors and raised antisera against selected proteins expressed in *Escherichia coli*. We used these antisera to characterize viral gene products in cell lines derived from cervical carcinomas.

Results

Nucleotide sequence of the early region of HPV 18 and identification of open reading frames

Since the general organization of the HPV 18 genome was known (for details see Boshart *et al.*, 1984), we cloned four fragments [the 1.5-kb and 2.7-kb *Xba*I, the 2.5-kb *Bam*HI−*Hin*dIII and the 1.3-kb *Bam*HI fragments (see Figure 1b)] into the plasmid pUC19. Progressive deletions of the 1.5-kb fragment were obtained by *Bal*31 digestion, after opening the plasmid at the 5′ end of the inserted *Xba*I fragment. Subsequently these deleted fragments were recloned, after cleavage with *Hin*dIII, into plasmid pUC8. Following this strategy overlapping sequence data were generated starting at the CAT-box defined as position 1

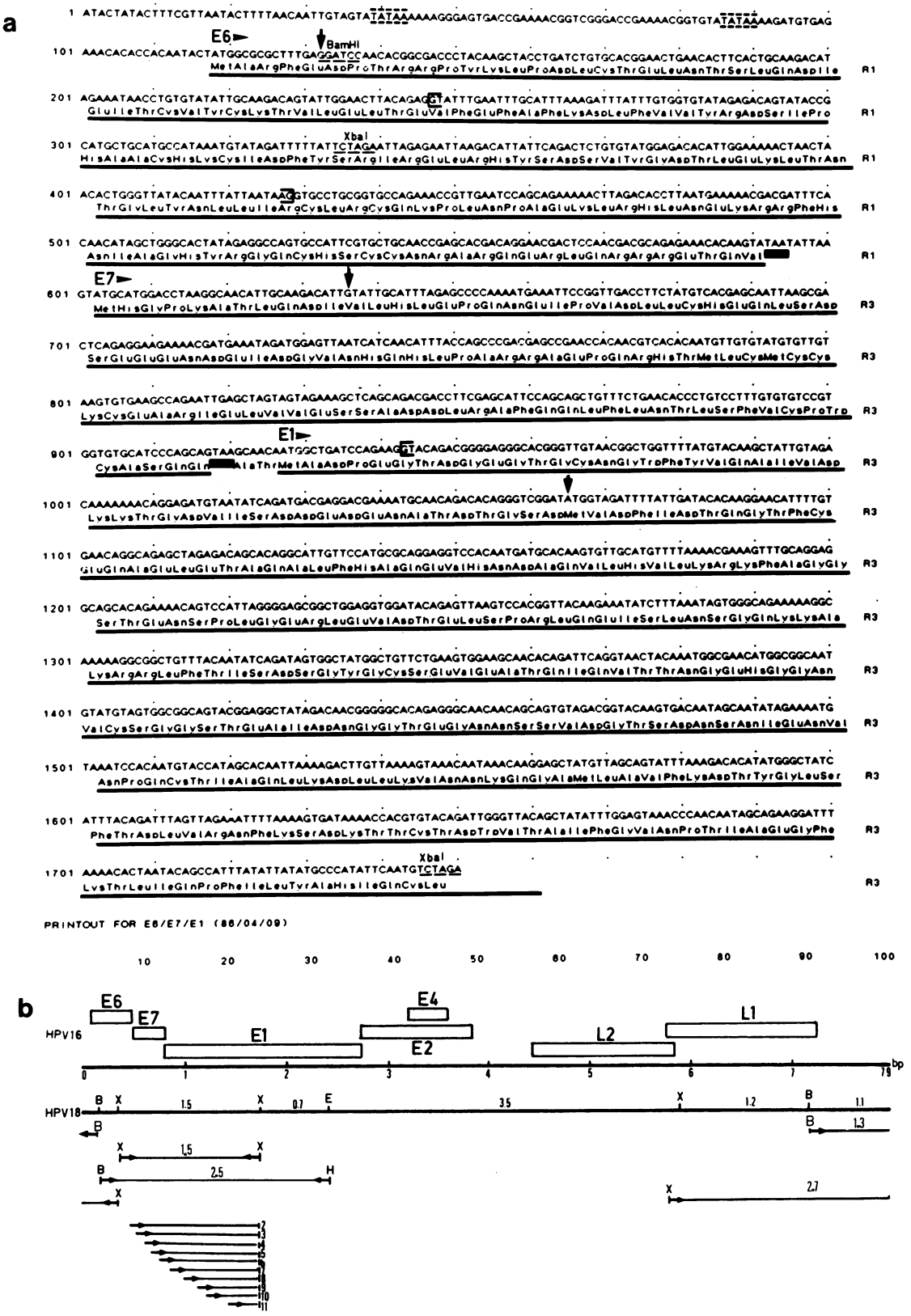

Fig. 1. (**a**) Nucleotide sequence and derived amino acid sequence of the early region of HPV 18 (1730 bp). The numbering system was chosen by analogy to other HPV DNA sequences based on alignment via the CAT-box region. The ORF E6, E7 and E1 are underlined, the corresponding translation initiation codons are marked by the name of the particular ORF (E6, E7 and E1). Stop codons are marked by black bars, reading frames are indicated by R1 and R3. Vertical arrows correspond to the 5′ boundary of the ORF introduced into the expression vector. Brackets demonstrate splice donor and acceptor sites deduced from cDNA sequences (Schneider-Gädicke and Schwarz, 1986). Two TATA boxes (position 19 and 66) upstream from the E6 translation initiation ATG are boxed by dashed lines. (**b**) Alignment of HPV 18 DNA with HPV 16 DNA according to nucleotide sequence homologies. On top the distribution of the ORF is shown. For partial sequence analysis restriction fragments of HPV 18 DNA generated by cleavage with *Bam*HI (B), *Hin*dIII (H) and *Xba*I (X) were used. The 1.5 kb *Xba*I fragment was digested from the 5′ end with exonuclease *Bal*31 resulting in progressive 5′ deletions (2−11). The complete DNA sequence information for the first 1730 bp was generated by overlapping sequencing as indicated by arrows.

Table I. Open reading frames expressed in *E. coli*

ORF	Position of the first nucleotide	Position of the last nucleotide	Antibodies obtained
HPV 16			
E6	110	556	+
E7	585	855	+
E1	1240	2811	–
E4	3399	3617	+
E5	3869	4097	–
L2	4139	5654	–
L1	5692	7152	–
L112	5692	6819	+
L1232	6819	7152	+
HPV 18			
E6	112	571	+
E7	616	897	+
E1	1045	1730	+
L1[a]	5754	7152	–

ORFs of HPV 16 and HPV 18 expressed as fusion proteins in *E. coli* C600/537. The numbering system for the HPV 16 ORFs was taken from the DNA sequence published recently (Seedorf *et al.*, 1985), for E6, E7 and E1 of HPV 18 as indicated in Figure 1.
[a]The numbering system was chosen analogous to HPV 16 after homology comparisons.

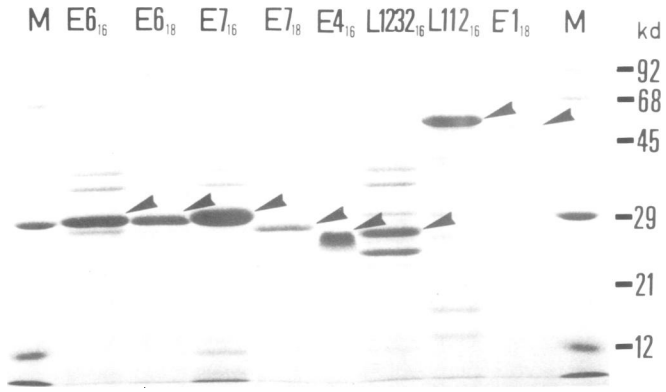

Fig. 2. Identification of HPV 16 and HPV 18 specific fusion proteins in cell extracts of transformed *E. coli* C600/537. The fusion proteins were enriched by differential extraction from *E. coli* cells (see Materials and methods) and separated on a 12.5% polyacrylamide gel (10 μg protein/lane). The position of HPV specific fusion proteins is indicated by arrows. **M** = mol. wt standard, **L1232/16** = C-terminal fragment of the late protein L1 of HPV 16; **L112/16** = N-terminal fragment of L1 of HPV 16; **E1/18** = early protein E1 of HPV 18, etc.

by analogy to other papilloma viruses, up to position 1730 (see Figure 1). Additionally, 120 bp of the N-terminal part of the L1 ORF of HPV 18 were sequenced (data not shown). The sequencing reaction was performed according to the dideoxy chain termination method (Sanger *et al.*, 1977) with modifications for double-stranded DNA as template (Seedorf *et al.*, 1985). This sequence information together with homology comparisons to the HPV 16 DNA sequence recently published (Seedorf *et al.*, 1985) allowed us to identify the ORF E6, E7, E1 and L1 of HPV 18.

Expression of open reading frames of HPV 16 and HPV 18 in E. coli and preparation of antisera

Based on this sequence information we cloned the ORF E6, E7, E1, L1 of HPV 18 and E6, E7, E1, E4, E5, L2 and L1 of HPV 16 into pUC8 or pUC19. In most cases we used blunt-ended fragments which were cloned into the *Hin*cII site of the polylinker. After confirming the sequence of the 5′ ends of the inserts, appropriate sticky-end fragments were recloned into expression vectors (see Table I). The expression vectors used were derivatives of plasmid pPLc 24 (Remaut *et al.*, 1981, 1983a,b) modified by introducing a polylinker fragment with the restriction sites for *Eco*RI, *Bam*HI, *Sal*I, *Pst*I, *Bgl*II, *Xba*I and *Hin*dIII in three reading frames with respect to the amino terminus of the MS2 polymerase. Details of construction will be published elsewhere (H.Krafft, G.Krämmer, K.Seedorf, U.M.Schätzle and W.G.Röwekamp, in preparation). *E. coli* C600/537 was transformed with the recombinant plasmids, synthesis of the fusion proteins was induced and the fusion proteins were purified as described in Materials and methods.

Following this procedure we were able to express and purify large amounts of protein (the yield from a 1 litre culture was in the range of 5–20 mg protein) for E6, E7, E4 and L1 of HPV 16 and E6 and E7 of HPV 18 (see Figure 2).

Subequently high-titre antisera were raised in rabbits against the fusion proteins from these recombinants (see Figure 2 and Table I). All antisera were purified by ammonium sulphate precipitation and immunoaffinity chromatography on columns with proteins bound which had been extracted from induced *E. coli* cells containing the parental expression vector.

Detection of HPV proteins in carcinoma cell lines by Western blot analysis

From studies on the integration of HPV 18 and HPV 16 DNA into the cellular genome it is known that the 3′ part of the early region is either deleted (HeLa, C4-1 and SiHa) or interrupted (SW756 and CaSki) by the integration event (Schwarz *et al.*, 1985; M.Dürst, personal communication). HPV 18 cDNA sequence information from HeLa, SW756 and C4-1 cells (Schneider-Gädicke and Schwarz, 1986) and HVP 16 transcripts mapped in the CaSki cell line (Smotkin and Wettstein, 1986) supports these findings and suggests that proteins from the early region can only be expected from the ORF E6, E7, E1 and possibly E4. Therefore, in our first Western blot experiments specific antisera were used to identify these proteins in total cell extracts from cell lines derived from cervical carcinomas. In the lines containing HPV 18 DNA, i.e. HeLa, C4-1 and SW756, a very strong 12-kd signal became visible with antibodies raised against the E7 fusion protein of HPV 18. This 12-kd protein band is specific for these three cell lines and was not detected in other lines (see Figure 3a). A similar result was obtained with antisera against the E7 fusion protein of HPV 16. In this case a specific 15-kd protein band can be identified in total protein extracts from the SiHa and CaSki cell lines (see Figure 3b). This 15-kd protein is more abundant in the CaSki line than in the SiHa line correlating with the viral RNA levels in these cell lines (Schwarz *et al.*, 1985). Cell fractionation into membrane, cytoplasmic and nuclear proteins indicated that the E7 protein is a cytoplasmic protein (data not shown).

In contrast, with antisera directed against E6, E4, E1 and L1 we were not able to identify specific proteins in any of these cell lines.

In vitro translation of hybrid selected viral poly(A)$^+$ RNA prepared from cervical carcinoma-derived cell lines

The failure to detect E6, E4 and E1 proteins might be due to the limited sensitivity of the Western blot analysis. To exclude the possibility and to confirm that the E7 specific 12-kd and 15-kd proteins are indeed encoded by HPV 18 and HPV 16, respectively, we isolated viral-specific poly(A)$^+$ RNA from HeLa SiHa and CaSki cells by hybrid selection. These RNAs were translated

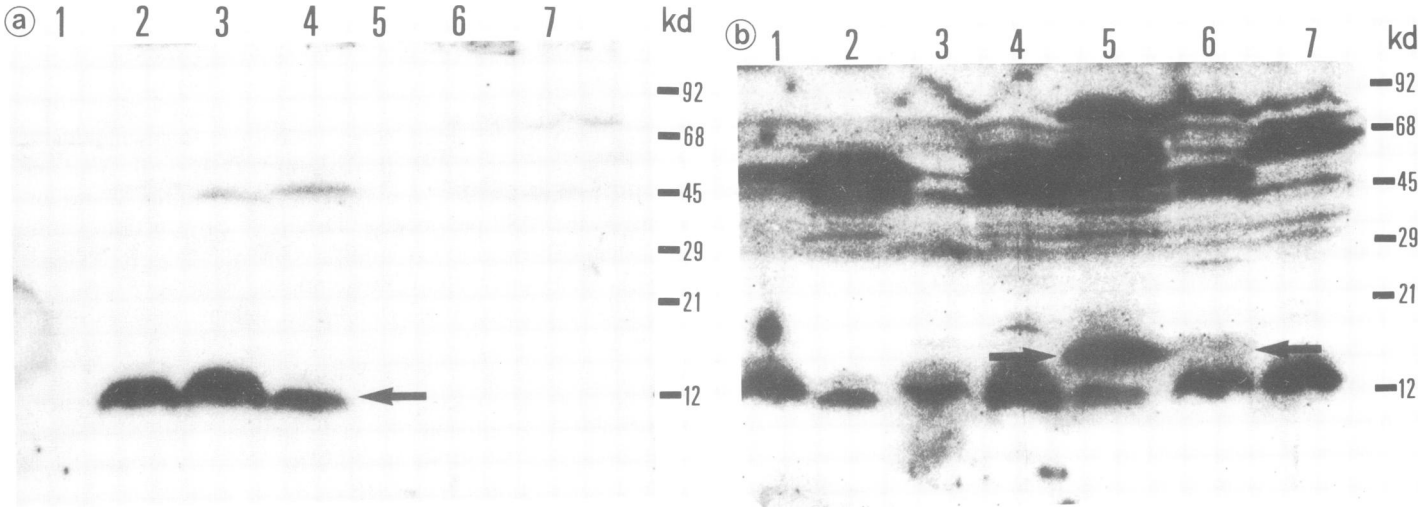

Fig. 3. Identification of E7 specific proteins of HPV 16 and HPV 18 by Western blot analysis in different cell lines. 50 μg of total cell proteins were separated on a 12.5% polyacrylamide gel, transferred by Western blotting to a nitrocellulose filter, 'immunolabeled' with HPV 18 E7 specific antibodies (**a**) and HPV 16 E7 specific antibodies (**b**) followed by incubation with 1 μCi ^{125}I-labeled protein A in both cases. Cell lines: **1** = HT-3 (control), **2** = C4-1, **3** = SW756, **4** = HeLa, **5** = CaSki, **6** = SiHa and **7** = C-33A (control). Filter a (probed with E7 HPV 18 antibodies) was exposed for 24 h, filter b (probed with E7 HPV 16 antibodies) for 9 days. The E7 specific signals (12 kd and 15 kd) are indicated by arrows.

in a rabbit reticulocyte system. After immunoprecipitation with the corresponding antisera and isolation of the immunocomplexes with protein A−Sepharose we detected specific protein bands for E7 (12 kd) and E1 (70 kd) of HPV 18 and for E6 (11 kd), E7 (15 kd) and E4 (10 kd) of HPV 16 in 12.5% polyacrylamide gels according to Laemmli (1970) (see Figure 4). No proteins were detected with antisera raised against E6 of HPV 18 and L1 of HPV 16.

Discussion

Northern blotting data from HeLa, SW756 and C4-1 as well as DNA sequence information obtained from HPV 18 cDNA clones suggest that the early region of HVP 18 is transcribed into three differentially spliced mRNA species containing cellular sequences at the 3′ end (Schneider-Gädicke and Schwarz, 1986). In HeLa cells these three early mRNAs can give rise to complete E6, E7 and possibly a complete E1, as well as a shortened version of an E6 protein. An analogous situation can also be considered in the case of SW756 and C4-1 cells, with the exception of E1-specific mRNA which is mainly spliced at the major splice donor site (Schneider-Gädicke and Schwarz, 1986).

The transcription data of Schneider-Gädicke and Schwarz (1986) and our Western blotting and *in vitro* translation data indicate that the HPV 18 early mRNAs must be transcribed as polycistronic RNA. The sizes of the E7 protein (12 kd) and the E1 protein (70 kd) estimated from polyacrylamide gels after hybrid selection or from Western blot experiments are consistent with the sizes predicted from the DNA sequence.

Also, in the CaSki cell line only parts of the HPV 16 early region are transcribed. Detailed mapping data of these transcripts suggest that they all code for a complete E7 protein. A complete as well as two shortened versions of an E6 protein can be generated by differential splicing. The ORF E2 is interrupted by splicing preventing synthesis of a complete E2 protein but allowing the expression of a complete E4 polypeptide (Smotkin and Wettstein, 1986). These results are confirmed by the identification of an E7 protein, as well as translatable mRNAs for E6 and E4 proteins in the CaSki cell line. The predicted sizes for E7 (12 kd) and E4 (10 kd) proteins are nearly identical to the values found on polyacrylamide gels. Since a complete E6 ORF would code for a 19-kd protein we assume that the E6 protein found in CaSki cells (11 kd) is generated by a splicing event.

The most abundant protein in cell lines containing HPV 16 or HPV 18 DNA is the E7 protein. This is in agreement with the fact that in the cell lines HeLa, C4-1, SW756 and CaSki all early transcripts contain a complete E7 ORF (Schneider-Gädicke, 1986; Smotkin and Wettstein, 1986). Genetic studies on the function of an E7 protein in BPV have shown that this gene product is involved in the maintenance of plasmids at a high copy number (Lusky and Botchan, 1985). Since neither HPV 16 nor HPV 18 DNA have been identified as episomal plasmids in these cell lines an additional function for this protein has to be postulated. A similar argument can be applied to E1 where published data suggest an involvement of the BPV E1 protein in plasmid maintenance in transformed mouse cells (Lusky and Botchan, 1984). Amino acid comparisons between the putative E1 gene products of papilloma viruses (BPV 1, HPV 1a and HPV 6b) and the large T protein of polyoma and Simian virus 40 show significant homologies in two blocks in their carboxy-terminal halves. These homologous regions correspond to sites involved in the ATPase and nucleotide binding activities suggesting an involvement in DNA replication (Clertant and Seif, 1984).

Since an E4 protein has been recently suggested to be involved in viral particle maturation, its function in the CaSki cell line remains unclear.

The identification of an E6 gene product in transformed cell lines containing HPV 16 or HPV 18 DNA could be important, because the E6 protein of BPV has been shown to function as a transforming protein in DNA transfection experiments (Androphy *et al.*, 1985). In fact, we find a E6 protein in the CaSki cells just above the detection limit of the hybrid selection method, thus not excluding the possibility that in HeLa and SiHa cells an E6 protein is also present but at even lower levels.

Based on the results reported here, HPV 16 and HPV 18 specific proteins (E7 E6, E4 of HPV 16, and E7 and E1 of HPV 18, respectively) can possibly now be isolated by immunoabsorption and thus assayed for their biological functions. It has to be shown whether we can identify any of these proteins

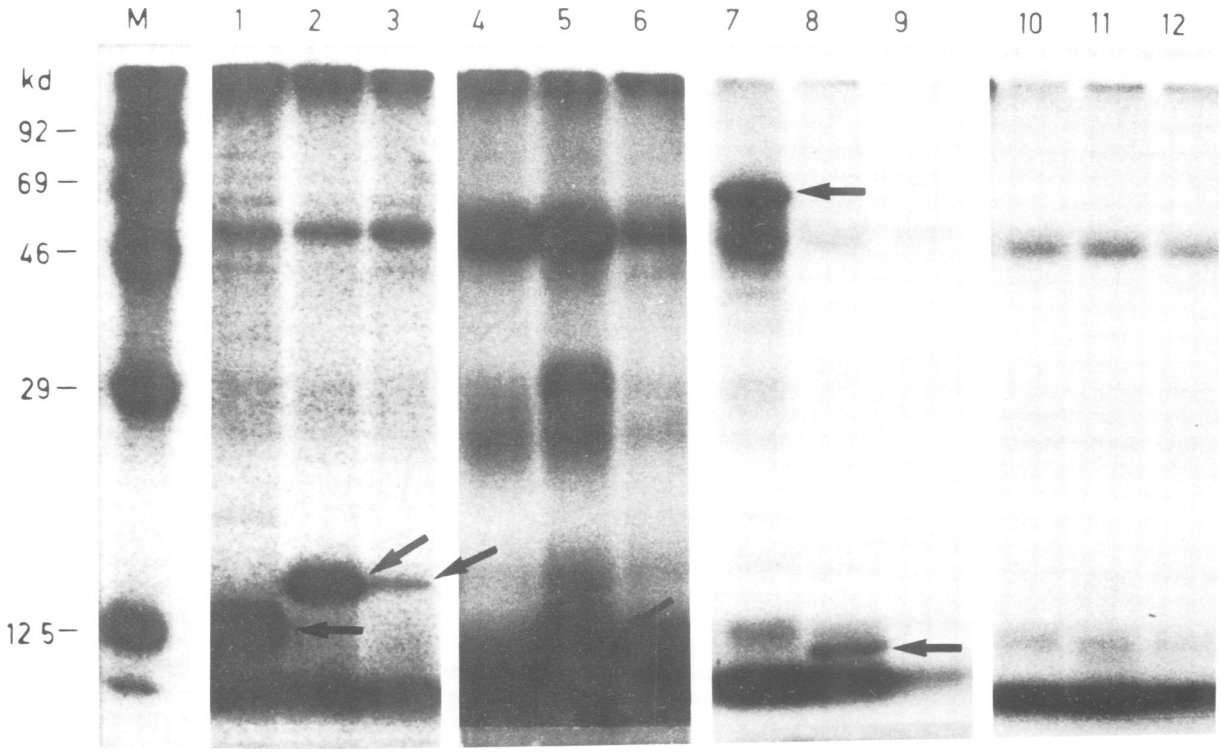

Fig. 4. Identification of the early proteins E6, E7, E4 of HPV 16 and E7 and E1 of HPV 18 after *in vitro* translation. Hybrid-selected poly(A)$^+$ RNA isolated from SiHa, CaSki (containing HPV 16 DNA) and HeLa cells (containing HPV 18 DNA) was translated *in vitro* in a rabbit reticulocyte lysate containing [^{35}S]methionine and [^{35}S]cysteine. Viral polypeptides were obtained by successive rounds of immunoprecipitation with specific antisera and protein A–Sepharose. The order of immunoprecipitation was E7, E6, E1, E4 and L1 (L1232 and L112). The immunoprecipitated proteins were separated on a 12.5% polyacrylamide gel. Specific proteins are indicated by arrows. **Lane 1–3** and **10–12** were exposed for 3 days; **lanes 4–9** for 3 weeks. (**M** = marker.) The schematic evaluation of this experiment is shown on the right.

Track	Antibody used for immuno-precipitation	Identification of specific proteins	Mol. wt
1 HeLa	E7 HPV 18	+	12 kd
2 CaSki	E7 HPV 16	+	15 kd
3 SiHa	E7 HPV 16	+	15 kd
4 HeLa	E6 HPV 18	–	
5 CaSki	E6 HPV 16	+	11 kd
6 SiHa	E6 HPV 16	–	
7 HeLa	E1 HPV 18	+	70 kd
8 CaSki	E4 HPV 16	+	10 kd
9 SiHa	E4 HPV 16	–	
10 HeLa	L1 HPV 16[a]	–	
11 CaSki	L1 HPV 16[a]	–	
12 SiHa	L1 HPV 16[a]	–	

[a]A mixture of antibodies directed against L112 and L1232 was used.

in HPV 16 or HPV 18 infected tissues (e.g. cervical carcinomas, vulvar and penile cancers, premalignant lesions). This should give us further insight into the different steps of the development of human cancer related to HPV infections. Expressed fusion proteins may also be useful in determining antibody titers in patients. Our results may thus permit a wide spectrum of basic and clinical applications.

Materials and methods

Bacterial strains, plasmid vectors and cell lines

Plasmid preparations and transfections of bacteria were performed as described by Maniatis *et al.* (1982). The ORFs of HPV 16 and HPV 18 used for sequencing were subcloned into the plasmids pUC8 or pUC19 and transformed into *E. coli* strain HB 101. The original expression vector pPLc24 (kindly supplied by E.Remaut, Gent) allows the expression of inserts fused to the first 98 amino acids of the MS2 polymerase under the control of the lambda P_L promoter (Remaut *et al.*, 1981, 1983a,b). The vector pPLc24 was modified by us as described elsewhere (Krafft *et al.*, in preparation). The appropriate host *E. coli* C600/537 was a gift from H.Schaller, Heidelberg. This strain harbours a temperature-sensitive CI repressor gene of phage lambda on a multicopy plasmid conferring kanamycin resistance.

Cells of the cervical carcinoma lines HeLa, C4-1, SW756, SiHa, CaSki, HT-3 and C-33A (originally obtained from the American Type Culture Collection and kindly supplied by L.Gissmann, Heidelberg) were grown in monolayer cultures in DMEM minimum essential medium containing 10% fetal calf serum.

DNA sequencing

The DNA sequencing reaction was performed according to Sanger *et al.* (1977) with modifications for double-stranded DNA as template (Seedorf *et al.*, 1985).

Expression and purification of fusion proteins

ORFs of HPV 16 and HPV 18 (see Table I) were subcloned into pUC8 or pUC19, in most cases by blunt-end cloning into the *Hin*cII site of the polylinker sequence. Sticky-end fragments were then recloned into the correct expression vectors after sequencing the 5′ ends of the inserts. The *E. coli* strain C600/537 was transfected with the expression vectors carrying the indicated HPV sequences. Growth of the cells and induction was essentially as described by Remaut *et al.* (1981). After 3 h of growth at the restricted temperature, the cells of a 1 litre culture were collected and suspended in 40 ml of 8% sucrose, 50 mM EDTA, 50 mM Tris–HCl pH 8.0, treated with lysozyme (200 μg/ml, final concentration) for 30 min and lysed by addition of 0.1% Triton X-100. After sonication and stirring for 15 min at 37°C the lysate was cleared by centrifugation at 40 000 *g* for 15 min. The resulting pellet was successively extracted with 25 ml of PBS containing 0.1% Triton X-100, then with 25 ml 1 M urea with sonication, further stirring at 37°C and centrifugation as described before. The fusion proteins could be purified from the 1 M urea residue by 7 M urea extraction yielding preparations which normally contained between 70 and 90% fusion protein. The overall yield from a 1 litre culture was in the range of 5–20 mg protein for most of the expressed fusion peptides.

Antisera and affinity chromatography

Antisera were raised by immunizing rabbits (Herbert, 1973). Purification of the antibodies was performed as described by Goding (1983). The sera were further

purified by affinity chromatography on a Sepharose column containing MS2 and *E. coli* proteins covalently linked. Activation of CNBr−Sepharose and protein coupling were performed according to the manufacturer's instructions (Pharmacia, Uppsala).

Total cell extracts and Western blot analysis

About 10^8 cells were collected (after trypsination), washed twice with PBS, resuspended in 200 μl PBS and lysed by adding 200 μl of a two-fold concentrated Laemmli sample buffer. About 50 μg of total cell protein prepared from the cell lines were separated on a 12.5% polyacrylamide gel (Laemmli, 1970) and transferred by Western blotting to nitrocellulose (Schleicher and Schüll, BA 85) as described by Towbin *et al.* (1979). The filters were coated by incubation in 1 × PBS, 5% milk powder, 0.02% Tween 20 for 1 h. The antibody reaction (dilution of the antisera was 1:200) was carried out overnight followed by three washes with the following buffers: PBS + 0.1% Triton X-100, PBS + 0.5% Triton X-100, PBS + 0.4 M NaCl. Staining with *Staphylococcus aureus* protein A (1 μCi ^{125}I-labeled protein A from Amersham, Braunschweig) was carried out for 3 h in PBS + 5% milk powder, followed by the washing procedure mentioned before. Filters were exposed to X-ray films with an intensifier screen.

RNA isolation, hybrid selection and in vitro translation of virus specific poly(A)$^+$ RNA

Total cellular RNA from ~ 10^7 cells was isolated using the guanidine thiocyanate method according to Chirgwin *et al.* (1979). Poly(A)$^+$ RNA purification and hybrid selection of viral RNA starting with 30 μg of poly(A)$^+$ RNA, was performed according to Maniatis *et al.* (1982). The viral enriched poly(A)$^+$ RNA (5 μl) was translated in 40 μl of a rabbit reticulocyte lysate (Amersham, Braunschweig) with 40 μCi [^{35}S]methionine and [^{35}S]cysteine each for 45 min at 30°C. The reaction was stopped by adding RNase (10 μg/ml) and EDTA (20 mM final concentration). Incorporation of labeled amino acids was monitored by TCA precipitation. Immunoprecipitation of viral specific proteins was performed overnight at 4°C after addition of 200 μl of PBS + 5% milk powder, 30 μl protein A−Sepharose (Pharmacia) and 30 μl antiserum diluted 1:20 in PBS. Immunocomplexes bound to protein A−Sepharose were collected by centrifugation (5 s, 5000 *g*). The pellets (protein A−Sepharose-antigen−antibody complexes) were washed three times with the buffers already mentioned (see Western blot analysis). The last pellet was resuspended in 30 μl Laemmli sample buffer and after incubation at 100°C for 5 min the proteins were separated in a 12.5% polyacrylamide gel. After electrophoresis the proteins were fixed wth 10% acetic acid (30 min), incubated for 30 min in Amplify (Amersham), dried and exposed at −70°C.

Acknowledgements

We thank Dr L.Gissman, Dr E.Schwarz and Dr H.zur Hausen for helpful discussions, U.Schätzle for technical assistance and M.Cole for typing the manuscript. Parts of this work were supported by a grant from the Deutsche Forschungsgemeinschaft to W.G.R. (Ro 444/6-4).

References

Androphy,E.J., Schiller,J.T. and Lowy,D.R. (1985) *Science*, **230**, 442−445.
Boshart,M., Gissmann,L., Ikenberg,H., Kleinheinz,A., Scheurlen,W. and zur Hausen,H. (1984) *EMBO J.*, **3**, 1151−1157.
Chen,E.Y., Howley,P.M., Levinson,A.D. and Seeburg,P.H. (1982) *Nature*, **299**, 529−534.
Chirgwin,J.M., Przybyla,A.E., MacDonald,R.J. and Rutter,W.J. (1979) *Biochemistry*, **18**, 5294−5299.
Clertant,P. and Seif,I. (1984) *Nature*, **311**, 276−279.
Danos,O., Katinka,M. and Yaniv,M. (1982) *EMBO J.*, **1**, 231−236.
Danos,O., Giri,I., Thierry,F. and Yaniv,M. (1984) *J. Invest. Dermatol.*, **83**, 7−11.
Dartmann,K., Schwarz,E., Gissmann,L. and zur Hausen,H. (1986) *Virology*, **151**, 124−130.
Doorbar,J., Campbell,D., Grand,R.J.H. and Gallimore,P.H. (1986) *EMBO J.*, **5**, 355−362.
Dürst,M., Gissmann,L., Ikenberg,H. and zur Hausen,H. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 3812−3815.
Goding,J.W. (1983) In *Monoclonal Antibodies: Principles and Practice*. Academic Press, Orlando.
Herbert,W.J. (1973) Laboratory animal techniques for immunology. In Weir,D.H. (ed.), *Handbook of Experimental Immunology*. Vol. 3, Blackwell, Oxford.
Laemmli,U.K. (1970) *Nature*, **227**, 680−685.
Lusky,M. and Botchan,M.R. (1984) *Cell*, **36**, 391−401.
Lusky,M. and Botchan,M.R. (1985) *J. Virol.*, **53**, 955−965.
Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) In *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Pater,M.M. and Pater,A. (1985) *Virology*, **145**, 313−318.
Remaut,E., Stanssens,P. and Fiers,W. (1981) *Gene*, **15**, 81−93.
Remaut,E., Stanssens,P. and Fiers,W. (1983a) *Nucleic Acids Res.*, **11**, 4677−4688.
Remaut,E., Tsao,H. and Fiers,W. (1983b) *Gene*, **22**, 103−113.
Sanger,F. Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463−5467.
Sarver,N., Rabson,M.S., Yang,Y.C., Byrne,J.C. and Howley,P.M. (1984) *J. Virol.*, **52**, 377−388.
Schlegel,R., Wade-Glass,M., Rabson,M.S. and Yang,Y.-C. (1986) *Science*, **233**, 464−466.
Schiller,J.T., Vass,W.C. and Lowy,D.R. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 7880−7884.
Schiller,J.T., Vass,W.C., Vousden,K.H. and Lowy,D.R. (1986) *J. Virol.*, **57**, 1−6.
Schneider-Gädicke,A. and Schwarz,E. (1986) *EMBO J.*, **5**, 2285−2292.
Schwarz,E., Dürst,M., Demankowski,C., Lattermann,O., Zeck,R., Wolfsperger,E., Suhai,S. and zur Hausen,H. (1983) *EMBO J.*, **2**, 2341−2348.
Schwarz,E., Freese,U.K., Gissmann,L., Mayer,W., Roggenbruck,B., Stremlau,H. and zur Hausen,H. (1985) *Nature*, **314**, 111−114.
Seedorf,K., Krämmer,G., Dürst,M., Suhai,S. and Röwekamp,W.G. (1985) *Virology*, **145**, 181−185.
Smotkin,D. and Wettstein,F.O. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 4680−4684.
Spalholz,B.A., Yang,Y.-C. and Howley,P.M. (1985) *Cell*, **42**, 183−191.
Towbin,H., Stähelin,T. and Gordon,J. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 4350−4354.
Yang,Y.C., Okayama,H. and Howley,P.M. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 1030−1034.
Yee,C., Krishnan-Hewlett,H.I., Buker,C.C., Schlegel,R. and Howley,P.M. (1985) *Am. J. Pathol.*, **119**, 361−366.
zur Hausen,H. and Schneider,A. (1987) In Howley,P.M. and Salzman,N. (eds), *The Papovaviridae: The Papilloma Viruses*. Plenum Press, NY, in press.

Received on 8 September 1986; revised on 10 October 1986