Cellular transcription factors regulate human papillomavirus type 16 gene expression by binding to a subset of the DNA sequences recognized by the viral E2 protein

Hannah Lewis, Kenneth Webster, Ana-Maria Sanchez-Perez† and Kevin Gaston

Department of Biochemistry, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD, UK

Human papillomavirus type 16 (HPV-16) is a DNA tumour virus that has been implicated in the development of cervical cancer. The HPV-16 E2 protein binds to four sites that are present upstream of the viral P97 promoter and regulates transcription of the E6 and E7 oncogenes. Here, it is shown that cellular transcription factors bind to two of these E2 sites. One cellular E2 site-binding factor, which is here named CEF-1, binds tightly to E2 site 1. CEF-2, an unrelated cellular E2 site-binding factor, binds tightly to E2 site 3. Transient transfection studies performed in the absence of the E2 protein showed that mutations that blocked the binding of CEF-1 to E2 site 1 or CEF-2 to E2 site 3 significantly reduced P97 promoter activity. Further characterization of CEF-1 indicated that this factor has not previously been identified and that CEF-1 and E2 competed for binding at E2 site 1.

Introduction

To date, more than 70 different types of human papillomavirus (HPV) have been identified (reviewed by van Ranst et al., 1996). Many of these papillomaviruses cause benign neoplasias (or warts). However, a group of so-called ‘high-risk’ papillomaviruses, including HPV-16 and HPV-18, are associated with malignant tumours of the cervix (Dürst et al., 1983; Peto & zur Hausen, 1986). Malignant transformation is brought about by the products of the viral E6 and E7 oncogenes, which inactivate the tumour suppressor proteins p53 and Rb, respectively (Dyson et al., 1989; Hawley-Nelson et al., 1989; Münger et al., 1989; Watanabe et al., 1989; Werness et al., 1990). In HPV-16, transcription of the E6 and E7 oncogenes is under the control of a single promoter (P97) located at the 3′ end of an approximately 1 kb long control region, or LCR (Smotkin & Wettstein, 1986; Smotkin et al., 1989).

The HPV-16 LCR consists of a complex array of transcription factor-binding sites that includes four binding sites for the viral E2 protein, a single binding site for the viral E1 protein and multiple binding sites for at least twelve different cellular transcription factors: AP-1 (Chan et al., 1990), cEBP (Bauknecht et al., 1996), glucocorticoid receptor (Gloss et al., 1987), progesterone receptor (Chan et al., 1989), NF1 (Chong et al., 1990), NF-IL6 (Kyo et al., 1993), Oct-1 (O’Connor & Bernard, 1995), PEF-1 (Cuthill et al., 1993), TEF-1 (Ishiji et al., 1992), TEF-2 (Chong et al., 1991), Sp1 (Gloss & Bernard, 1990) and YY1 (Dong et al., 1994; May et al., 1994b). In HPV-transformed cervical carcinoma cell lines and malignant cervical lesions, HPV sequences are often found integrated into the host genome (Dürst et al., 1985). Integration frequently occurs within the E2 open reading frame, resulting in the loss of the E2 protein (Baker et al., 1987). These observations led to the proposal that disruption of the E2 gene results in deregulated P97 promoter activity, uncontrolled expression of the E6 and E7 oncogenes and, ultimately, tumorigenesis. Consistent with this hypothesis, we have shown that re-introduction of the HPV-16 E2 protein into HPV-16-transformed cervical carcinoma cells up-regulates P97 promoter activity and triggers cell death via apoptosis (Sanchez-Perez et al., 1997).

Although the experiment described above suggests that the HPV-16 E2 protein activates transcription from the P97 promoter, the exact role of the E2 protein in the regulation of HPV-16 transcription has been the subject of some controversy. Depending on the E2 expression system used and the type of reporter construct studied, the HPV-16 E2 protein has been shown both to activate (Bouvard et al., 1994; Cripe et al., 1994) and to have no effect on P97 promoter activity (Cripe et al., 1995).
studies on related viruses have indicated that such factors might exist. In bovine papillomavirus type 4 (BPV-4), for example, the cellular transcription factor PEBP-2 binds to an E2 site located around 100 bp upstream of the BPV-4 TATA box and mutations that prevent the binding of PEBP-2 significantly reduce promoter activity (Jackson & Campo, 1995). Similarly, cellular factors bind to sequences that overlap an E2 site immediately downstream of the BPV-1 P_{185} promoter and stimulate promoter activity (Stenlund & Botchan, 1990; Vande Pol & Howley, 1990). Finally, in HPV-8, cellular factors have been shown to bind to an E2 site located immediately upstream of the late promoter TATA box (May et al., 1994a; Stubenauch et al., 1996).

In this study, we show that cellular transcription factors bind tightly to E2 sites 1 and 3 within the HPV-16 LCR. Mutations that prevent the binding of these cellular factors dramatically reduce P97 promoter activity, suggesting that these factors play an important role in the regulation of HPV-16 gene expression.

**Methods**

- **Nuclear extract preparation and gel retardation assays.** Nuclear extracts were prepared from HeLa, HaCat and SiHa cells as described by Dignam et al. (1983) with modifications described by Dorn et al. (1989). Single-stranded oligonucleotides carrying either wild-type or mutated E2-binding sites (shown in Figs 1 and 5) were labelled at the 5' end with [γ-32P]ATP (ICN) by using T4 polynucleotide kinase (Gibco-BRL). After annealing to complementary oligonucleotides, unincorporated label was removed by using a NucTrap column (Stratagene) according to the manufacturer's instructions. Labelled oligonucleotides (20000 c.p.m.) were incubated with 5 µg nuclear extract (unless indicated otherwise in the figure legends) and 3 µg poly(dI.dC):poly(dC.dI) in a buffer containing 20 mM HEPES, pH 7.9, 25 mM KCl, 1 mM DTT, 0.1% NP-40 and 10% glycerol. After 20 min at 20°C, free and bound DNA were separated on 5% non-denaturing polyacrylamide gels run in 0.5× TBE and visualized by autoradiography. Competitor oligonucleotides (400 or 800 ng) were added at the beginning of the incubation.

- **Plasmid constructs.** Fig. 1 shows the organization of the HPV-16 LCR and the positions of the four E2-binding sites (Chong et al., 1990). The reporter plasmids used in this study are derivatives of the pGL2-basic and pGL2-Enhancer vectors (Promega) and contain the HPV-16 LCR cloned upstream of the luciferase gene. HPV-16 sequences from bp 7837 to bp 77 were amplified by viral DNA by PCR (30 cycles of 94°C for 1 min, 53°C for 3 min and 72°C for 3 min) with the KpnI primer 5' CGGGGTTACCCTGCACATGGGTGTGTGC 3' and the HindIII primer 5' TCAGAAAGCTTGCTCGTTATTACATAC 3' and cloned between the KpnI and HindIII sites of pGL2-Enhancer to create the plasmid pGL2-A7837. The primers 5' CCAAGGTACCTATGGAATTAGTG 3' and 5' GCAAGGTACCAAATGAGATGA CTAACC 3' were then used to amplify HPV-16 LCR sequences from bp 7165 to bp 7830 under the conditions described above. The fragment containing bp 7165–7830 was then cloned into the KpnI site of pGL2-A7837 to create pGL2-LCR, except for the four base changes needed to create the KpnI site between bp 7831 and 7836, this plasmid contains the entire HPV-16 LCR/P97 region (bp 7165 to bp 77) directing expression of the luciferase gene.
The E2-binding sites within pGL2-LCR were mutated by PCR-directed mutagenesis. E2-binding site 4 was mutated from 5′ ACCN₆CGT 3′ to 5′ ACAN₆TGT 3′, where N₆ represents the six base pairs that differ between the four sites. In gel retardation assays, this mutation completely abolished binding of HPV-16 E2 protein (data not shown). E2 site 4 was mutated by using the primers Site 4 forward, 5′ GCTCTAACGCCATTCGCAGATGC 3′, and Site 4 reverse, 5′ GCATGCAACAGAATTTGCAGTGAAC 3′. The underlined bases do not match the template and introduce the desired mutations. The forward primer was used in combination with the pGL2-specific primer GL1 (Promega) to amplify one half of the LCR sequence. The reverse primer was used in combination with the pGL2-specific primer GL2 (Promega) to amplify the opposite half of the LCR sequence. The LCR fragments were then mixed and a full-length LCR was amplified by using the pGL2-specific primers GL1 and GL2 (Promega). Sequences were verified by using the PCR primers and the pGL2-specific primer dIII primer 5′-TCGGGTCGACGGTTTCTGCAACG-3′ and the wild-type HindIII primer. The amplified product was cloned between the KpnI and HindIII sites of pGL2-Enhancer, creating pGL2-7837CEF-1m. HPV-16 LCR sequences from bp 7165 to bp 7830 were then cloned into the KpnI site of pGL2-7837CEF-1m to create pGL2-LCR-CEF-1m.

E2 sites 1 and 2 were mutated by using the HindIII primer 5′ TCGAAAGCTGTCTGTTTACTACTACGGTTTCTGAAACACAGTTTCTGTTACGGCC-3′ and the KpnI primer described above. Combinations of mutations were obtained by cloning and by further rounds of PCR-directed mutagenesis with mutated constructs as template. The full-length wild-type LCR and the full-length construct containing mutations in all four E2-binding sites were transferred from pGL2-Enhancer into pGL2-basic as Smal–HindIII fragments to create plasmids pGLb-LCR and pGLb-LCR-1234, respectively.

CEF-1 (Fig. 2 B). E2 site 1 was mutated by using the A7837-KpnI primer and the HindIII primer 5′ TCGGGTCGACGGTTTCTGCAACG-3′, creating pGL2-7837CEF-1m. HPV-16 LCR sequences from bp 7165 to bp 7830 were then cloned into the KpnI site of pGL2-7837CEF-1m to create pGL2-LCR-CEF-1m.

All of the constructs used in this study were sequenced by using a Sequenase kit according to the supplier’s instructions (USB). The LCR sequence was verified by using the PCR primers and the pGL2-specific primers GL1 and GL2 (Promega).

**Cell culture and transfections.** All the cell lines used in this study were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% foetal calf serum, 10 μg/ml penicillin and 10 μg/ml streptomycin (Gibco-BRL). Cells were transiently transfected with 2.5 μg of each reporter plasmid by calcium phosphate precipitation. Luciferase activity was determined 24 h after transfection by using the Luciferase assay.
system (Promega) according to the manufacturer’s instructions. The β-galactosidase-expressing plasmid pRSV-βgal (5 μg) was included in each transfection to determine the transfection efficiency.

Results

Cellular transcription factors bind to E2 sites 1 and 3

In several papillomavirus types, cellular transcription factors have been shown to bind at, or near, E2-binding sites. To determine whether cellular factors bind to any of the E2-binding sites in HPV-16, we used labelled oligonucleotides carrying E2 site 1, 2, 3 or 4 in gel retardation experiments (band shifts) with HeLa cell nuclear extracts. Although HeLa cells are an HPV-18-transformed cervical carcinoma cell line, these cells do not produce E2 protein. The addition of HeLa nuclear extract to a labelled E2 site 1 oligonucleotide resulted in the formation of a single protein–DNA complex (indicated by the arrowhead in Fig. 2 A, lanes 2 and 3). We shall refer to this factor as the cellular E2 site 1-binding factor or CEF-1. Under the conditions used in this experiment, CEF-1 failed to bind E2 site 2, 3 or 4. A cellular factor also bound E2 site 3 (Fig. 2 A, lanes 8 and 9), but the resulting complex had much greater mobility than the CEF-1–E2 site 1 complex, indicating that these factors are probably unrelated. This E2 site 3-binding factor, or CEF-2, bound tightly to E2 site 3, bind very weakly to E2 sites 1 and 2 and failed to bind E2 site 4. Both CEF-1 and CEF-2 were also present in nuclear extracts made from SiHa cells, an HPV-16-transformed cervical carcinoma cell line, and HaCaT cells, a spontaneously immortalized human keratinocyte cell line that does not contain HPV sequences (data not shown).

To investigate the DNA-binding specificity of CEF-1 and CEF-2, we tested the ability of these factors to bind mutated copies of E2 sites 1 and 3, respectively. E2 site 1 was mutated from 5’ ACCGAAACCAGT 3’ to 5’ ACCGAATCCGTT 3’, where the mutated base pairs are underlined. In gel retardation assays, this mutation completely abolished the ability of E2 site 1 to compete away the CEF-1–E2 site 1 complex (Fig. 2 B, lanes 5 and 6). E2 site 3 was mutated from 5’ ACCGTITTTGGGT 3’ to 5’ ACAGTTTTTGTGT 3’ (mutated base pairs underlined). This mutation completely abolished the ability of E2 site 3 to compete away the CEF-2–E2 site 3 complex (Fig. 2 C, lanes 5 and 6). Thus, CEF-1 and CEF-2 bound wild-type but not mutated E2-binding sites 1 and 3, respectively. These data were confirmed by assaying the binding of CEF-1 and CEF-2 to the mutated sites (data not shown).

Mutations that block the binding of CEF-1 or CEF-2 reduce P97 promoter activity

To determine whether CEF-1 and/or CEF-2 play an important role in the control of HPV-16 gene expression in intact cells, we cloned the wild-type HPV-16 regulatory region and regulatory regions containing mutations in the E2-binding sites upstream of the luciferase gene in the promoterless reporter plasmid pGL2-Enhancer (Promega). The mutation in E2 site 1 described in the previous section blocked the binding of CEF-1 in vitro and was introduced into the HPV-16 LCR by PCR-directed mutagenesis. The other E2-binding sites were mutated individually from 5’ ACCNNGGT 3’ to 5’ ACA N_6 TGT 3’ (N_6 represents the six base pairs that vary between the sites and the mutated bases are underlined). This mutation blocked the binding of CEF-2 to E2 site 3 in vitro (Fig. 2 C) and also blocked the binding of E2 to these sites (data not shown). Combinations of mutated sites were obtained by cloning and by further rounds of PCR with mutated constructs as template.

The series of constructs shown schematically in Fig. 3(A) were transiently transfected into HeLa cells and assayed for promoter activity. Since HeLa cells do not produce E2 protein, mutations in the E2-binding sites might be expected to have little or no effect on P97 promoter activity in these cells. However, as can be seen from the data shown in Fig. 3(B), a mutation in either E2 site 1 or 3 severely reduced promoter activity (Fig. 3 B, columns 3 and 4, respectively). In contrast, a mutation in E2 site 4 had little or no effect on promoter activity (Fig. 3 B, column 5) and this site failed to bind any cellular factors in vitro (Fig. 2 A). Constructs carrying mutations in two, three or all four E2-binding sites also showed significant reductions in promoter activity. To examine the generality of these results, we repeated the transfections in SiHa cells; these cells contain a disrupted copy of the HPV-16 E2 gene and, like HeLa cells, do not produce E2 protein. Although the overall level of promoter activity varied between the two cell lines, the mutations had similar effects in SiHa cells to those seen in HeLa cells (data not shown). Taken together, these data confirm that mutations that block the binding of either CEF-1 to E2 site 1 or CEF-2 to E2 site 3 bring about severe reductions in P97 promoter activity.

The reporter plasmid used in these experiments carries the SV40 enhancer downstream of the luciferase reporter gene. To determine whether the presence of this enhancer had an effect on the outcome of our experiments, we placed the wild-type HPV-16 regulatory region and the construct containing mutations in all four E2-binding sites upstream of the luciferase gene in the enhancer-less reporter plasmid pGL2-basic (Promega) and compared the activity of these reporters to the equivalent pGL-Enhancer constructs. The results of this comparison are shown in Fig. 3(C). As can be seen from the figure, mutations in the E2-binding sites decreased promoter activity in both reporter backgrounds and in both HeLa cells and C33A cells. However, whilst in the presence of the SV40 enhancer the mutation of all four E2-binding sites reduced promoter activity by around 90%, in the absence of the SV40 enhancer, promoter activity was reduced by around 50 and 80%, respectively, in HeLa cells and C33A cells. Thus, although the extent of the effect varied, mutations in the E2-binding sites significantly reduced P97 promoter activity in both the presence and the absence of the SV40 enhancer.
Regulation of HPV-16 gene expression

Fig. 3. Mutations that block the binding of CEF-1 or CEF-2 reduce P97 promoter activity. (A) The reporter plasmids used in this study contained the HPV-16 LCR and P97 promoter region cloned upstream of the luciferase gene (Luc). Functional E2-binding sites are indicated by open boxes, whereas mutated E2-binding sites are marked with a cross. (B) Levels of luciferase activity found in HeLa cell extracts 24 h after transient transfection with (1) pGL2-Enhancer, (2) pGL2-LCR, (3) pGL2-LCR-CEF-1m, which contains a mutation in E2 site 1, (4) pGL2-LCR-CEF-2m, which contains a mutation in site 3, (5) pGL2-LCR-4, which contains mutations in site 4, (6) pGL2-LCR-12, which contains mutations in sites 1 and 2, (7) pGL2-LCR-34, which contains mutations in sites 3 and 4, (8) pGL2-LCR-124, which contains mutations in sites 1, 2, and 4, (9) pGL2-LCR-123, which contains mutations in sites 1, 2, and 3, and (10) pGL2-LCR-1234, which contains mutations in all four E2-binding sites. Transfection efficiency was determined by co-transfection with the β-galactosidase-expressing plasmid pRSV-βgal. Values represent the means of at least three independent experiments and are shown as promoter activity relative to the wild-type construct. (C) The pGL2-Enhancer-based reporter plasmids pGL2-LCR and pGL2-LCR-1234 and their pGL2-basic-based counterparts pGL2-LCR and pGL2-LCR-1234 were transiently transfected into HeLa cells (1–6) and C33A cells (7–9) and promoter activity was determined as described in (B).

Characterization of CEF-1

Since E2 site 1 has been intensively investigated and shown to play a key role in the regulation of HPV-16 gene expression, we decided to focus on CEF-1 for the remainder of this work. The experiments described above show that CEF-1 is present in a HeLa cell nuclear extract. To characterize the DNA-binding activity of this factor further, we performed gel retardation assays using a labelled oligonucleotide carrying E2 site 1 and nuclear extracts prepared from HeLa and HaCat cells (Fig. 4A). The addition of HeLa cell nuclear extract resulted in the formation of the CEF-1–E2 site 1 complex (Fig. 4A, lane 2). This complex was competed away by the addition of an excess of unlabelled E2 site 1 oligonucleotide (Fig. 4A, lanes 3 and 4) but was not competed away by an unrelated oligonucleotide that carries a YY1-binding site (lanes 5 and 6). Similar experiments using nuclear extract from HaCat cells revealed a band of the same mobility that was also competed away by the E2 site 1 oligonucleotide but not by the YY1 oligonucleotide (Fig. 4A, lanes 7–11). The cellular transcription factor PEBP-2 binds to an E2 site present within the BPV-4 regulatory region (Jackson & Campo, 1995). We wondered whether CEF-1 might be the human homologue of PEBP-2, a transcription factor known as AML-1 (Ogawa et al., 1993). However, an oligonucleotide carrying an AML-1/PEBP-2-binding site failed to compete away the CEF-1–E2 site 1 complex (data not shown).

A mutation from C:G to A:T at position −4 of HPV-16 E2 site 1 (shown in Fig. 1) has previously been shown to have no effect on P97 promoter activity (Romanczuk et al., 1990). Fig. 4(B) shows the results of a gel retardation assay in which an unlabelled E2 site 1 oligonucleotide carrying this mutation (−4A:T) was added to binding reactions containing E2 site 1 and nuclear extract. As can been seen from the figure, the
CEF-1 recognizes the central region of the E2-binding site

The experiments described above show that CEF-1 bound to DNA fragments carrying E2 site 1 but not to DNA fragments carrying E2 site 4. E2 sites 1 and 4 contain perfect copies of the consensus E2-binding site (5' ACCGNNCGGT 3', where N represents any nucleotide), base pairs outside the consensus E2 site must be critically important for the binding of CEF-1. To identify these base pairs, we mutated E2 site 1 at the positions that differ between sites 1 and 4 and tested the ability of these mutated sites to compete away the CEF-1–E2 site 1 complex. The sequences of the mutated E2 sites and the results of the competition assay are shown in Fig. 5(A) and (B), respectively. The exchange of two base pairs in the central region of the E2-binding site abolished the ability of E2 site 1 to compete away the CEF-1–E2 site 1 complex (Fig. 5B, lanes 7 and 8). In contrast, the exchange of four base pairs in the regions that flank the E2-binding site had little effect on competition (Fig. 5B, lanes 9 and 10). These results were confirmed by assaying the binding of CEF-1 to labelled oligonucleotides carrying each of the competitor sequences. CEF-1 bound the E2(e) site but did not bind E2 site 4 or E2(m) (data not shown). Taken together, these data show that mutation of the A:T and C:G base pairs at positions +1 and +2 of E2 site 1 blocked the binding of CEF-1. Mutations at these positions had little or no effect on the binding of E2 (not shown).

CEF-1 and E2 compete for binding at E2 site 1

Given the overlap of the CEF-1- and E2-binding sites, we reasoned that these factors might compete for binding to E2
Fig. 5. The central base pairs of the E2 site are important for the binding of CEF-1. (A) HPV-16 E2 sites 1 and 4 are shown aligned to the consensus E2-binding site (top-strand sequences only). E2(m) corresponds to E2 site 1 except at positions +1 and +2, where the A:T and C:G base pairs were replaced by the equivalent base pairs from E2 site 4 (underlined and in bold). E2(e) corresponds to E2 site 1 except for the C:G base pair at +8 and the G:C, C:G and A:T base pairs from +8 to +10, which were replaced by the equivalent base pairs from site 4 (underlined and in bold). (B) A labelled oligonucleotide carrying E2 site 1 was incubated with HeLa cell nuclear extract and the resulting CEF-1–E2 site 1 complex was visualized exactly as described in Fig. 2. Formation of the CEF-1–E2 site 1 complex (CEF,) was challenged with 400 or 800 ng of either E2 site 1, E2 site 4, E2(m) or E2(e).

Fig. 6. CEF-1 and E2 compete for binding at E2 site 1. A labelled oligonucleotide carrying E2 site 1 [E2(1)] was incubated with 5 µg HeLa cell nuclear extract (lanes 3–12) and/or a truncated E2 protein (E2Ct) consisting of the 86 C-terminal amino acids of HPV-16 E2 (lanes 2 and 4–12). The protein–DNA complexes were separated on a 5% non-denaturing polyacrylamide gel and visualized exactly as described in Fig. 2. The CEF-1–E2 site 1 complex (CEF,) and the E2–E2 site 1 complex (E2c) are indicated by arrowheads.

E2 DNA-binding domain alone (E2Ct, consisting of the 86 C-terminal amino acids of the HPV-16 E2 protein) or the full-length E2 protein. E2Ct bound E2 site 1 and formed a specific complex (Fig. 6, lane 2). In the absence of E2, CEF-1 bound E2 site 1 and formed a slower-migrating complex (Fig. 6, lane 3). The addition of increasing amounts of the E2Ct protein resulted in the formation of increasing amounts of the E2–DNA complex (Fig. 6, lanes 4–12). However, no additional bands that might represent ternary complexes were observed. Increasing the amount of the E2Ct protein in the binding reaction brought about a decrease in the amount of CEF-1–DNA complex, suggesting that E2Ct and CEF-1 compete for binding to the E2 site. Because CEF-1 is present in limiting amounts in nuclear extract, a large amount of free probe was used in these experiments and a large amount of E2 was therefore needed to compete out CEF-1 binding. Similar results were obtained with the full-length HPV-16 E2 protein. In this case, however, the E2–DNA complex and the CEF-1–DNA complex had similar (but not identical) electrophoretic mobilities, making it difficult to see the decrease in the amount of the CEF-1–DNA complex (data not shown). Although our data suggest that the binding of CEF-1 and E2 are mutually exclusive, it is important to point out that this method might not be sufficiently sensitive to detect weak interactions.
Discussion

The HPV-16 E2 protein binds to four sites that are present upstream of the viral P97 promoter and regulates viral gene expression. In conjunction with the viral E1 protein, the E2 protein also plays a role in the regulation of viral DNA replication (Mohr et al., 1990). Here we have shown that cellular transcription factors, which we have named CEF-1 and CEF-2, bind tightly to two of the sites recognized by E2. Mutations that blocked the binding of CEF-1 to E2 site 1 or CEF-2 to E2 site 3 significantly reduced P97 promoter activity, suggesting that both of these factors are important in the regulation of HPV-16 gene expression.

Since E2 site 1 has been shown to play a key role in the regulation of P97 promoter activity, the existence of a cellular transcription factor that competes with E2 for binding to this site has important consequences for the regulation of viral gene expression and the origin of cervical cancer. In vitro experiments have shown that the HPV-16 E2 protein binds most tightly to E2 site 4, the promoter-distal site, and less tightly to E2 sites 1, 2 and 3, the promoter-proximal sites (Sanders & Maitland, 1994; Thain et al., 1997). The E2 protein activates transcription from the full-length HPV-16 LCR (Bouvard et al., 1994; Kovelman et al., 1996; Phelps & Howley, 1987). However, E2 can repress transcription from P97 promoter derivatives that contain only the promoter-proximal E2-binding sites. The binding of E2 to sites 1 and 2 has been shown to block the binding of TBP and Sp1 to their respective sites within the P97 promoter (Dostatni et al., 1991; Tan et al., 1992, 1994). These experiments suggest a plausible model wherein high concentrations of E2 might repress the P97 promoter by preventing the binding of Sp1 and TBP. However, we have shown that mutations that blocked the binding of CEF-1 to E2 site 1 dramatically reduced P97 promoter activity. These data suggest that the binding of E2 to E2 site 1 might repress P97 promoter activity by blocking the binding of CEF-1 rather than, or as well as, TBP.

Several studies on other papillomavirus systems have revealed cellular factors that can bind at, or near, E2-binding sites. The cellular transcription factor PEBP-2 binds to an E2 site present within the BPV-4 LCR and mutations that block the binding of PEBP-2 significantly reduce BPV-4 LCR promoter activity (Jackson & Campo, 1995). The human homologue of PEBP-2 is a transcription factor known as AML-1 (Ogawa et al., 1993). We have used competition experiments to show that CEF-1 and AML-1/PEBP-2 are unrelated. Cellular transcription factors also bind to sequences that overlap an E2 site immediately downstream of the BPV-1 P1 promoter and stimulate promoter activity (Stenlund & Botchan, 1990). Unlike CEF-1, the cellular factors that bind to this site do not require A:T and C:G base pairs at positions +1 and +2 of the E2-binding site. Furthermore, the BPV-1 factors require DNA sequences that are well outside the consensus E2-binding site, whereas CEF-1 binds tightly to short oligonucleotides that span the E2-binding site and include only four base pairs of the flanking DNA sequences. Finally, a cellular factor has been shown to bind to a negative regulatory element (NRE) present upstream of the HPV-8 late promoter P7355 (May et al., 1994a). Although this NRE contains an E2-binding site, the cellular NRE-binding factor, unlike CEF-1, binds to sequences well outside the E2 site (May et al., 1994a; Stubbenrauch et al., 1996). Whilst these observations suggest that CEF-1 is unrelated to any of these previously identified transcription factors, they do not exclude the possibility that CEF-1 might be involved in the regulation of other papillomaviruses.

In malignant cervical lesions, HPV DNA sequences are often found integrated into the host genome (Baker et al., 1987). Virus integration frequently disrupts the E2 gene, resulting in loss of the E2 protein, deregulated expression of the E6 and E7 oncogenes and, finally, the transformed phenotype. The data presented in this paper suggest that loss of the E2 protein would allow CEF-1 and CEF-2 free access to E2-binding sites 1 and 3. Increased binding of these factors might result in increased P97 promoter activity, leading in turn to increased transcription of the E6 and E7 genes and higher levels of the E6 and E7 oncoproteins.

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References


Regulation of HPV-16 gene expression


H. Lewis and others


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