Sp1 is critical for basal and E2-transactivated transcription from the bovine papillomavirus type 1 P_{89} promoter

Abby B. Sandler,* Carl C. Baker and Barbara A. Spalholz†

Laboratory of Tumor Virus Biology, Bldg 41, Room C111, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA

The bovine papillomavirus type 1 (BPV-1) long control region (LCR) contains at least three consensus binding sites for the transcription factor Sp1 at nucleotides (nt) 7800, 7833 and 7854. A high basal-level P_{89} expression vector consisting of an origin-deleted LCR fused to the chloramphenicol acetyltransferase (CAT) gene was utilized to determine the role of these Sp1 sites in the regulation of transcription from the BPV-1 P_{89} promoter. The three Sp1 sites were capable of binding Sp1 in vitro. Mutation of these sites in the background of the origin-deleted LCR–CAT or a wild-type LCR–CAT construct resulted in decreased basal expression from P_{89}. In addition, mutation of the Sp1 sites in the wild-type background caused a reduction in E2-transactivation potential. These data illustrate the importance of these Sp1 sites in regulating both basal and E2-transactivated P_{89} expression.

Introduction

Bovine papillomavirus type 1 (BPV-1) transcription is a tightly regulated process (Baker, 1990). Seven different viral promoters have been identified to date, based on examination of transcripts present in BPV-1-transformed rodent cells and in bovine wart tissue (Ahola et al., 1987; Stenlund et al., 1985; Yang et al., 1985). The BPV-1 P_{89} promoter is located within the long control region (LCR) of the viral genome and adjacent to the viral origin of DNA replication (see Fig. 1a). This promoter is believed to be responsible for regulating the expression of the BPV-1 transforming genes E6 and E7 (Neary & DiMaio, 1989; Schiller et al., 1984). Transcription from P_{89} has previously been shown to be regulated by gene products encoded by the BPV-1 E2 open reading frame (ORF) (Spalholz et al., 1987). The full-length E2 gene product is a potent transcriptional activator (Spalholz et al., 1985) which binds as a dimer to its cognate binding site ACCN_{6}GGT (Androphy et al., 1987; Dostatni et al., 1988; McBride et al., 1988). Twelve E2-binding sites are located within the LCR (Li et al., 1989), including the four that constitute the E2-dependent enhancer E2RE_{1}, which is involved in the E2 transactivation of P_{89} (Li et al., 1991; Spalholz et al., 1987, 1991; Szymanski & Stenlund, 1991) and two sites which flank the replication origin just upstream of P_{89}. The viral E1 replication protein (Ustav et al., 1991; Wilson & Ludes-Meyers, 1991) also binds near the P_{89} promoter at the replication origin. Binding is greatly enhanced when E1 forms a complex with the E2 transactivator protein (Blitz & Laimins, 1991; Mohr et al., 1990; Spalholz et al., 1993; Yang et al., 1991) and the two E2-binding sites adjacent to the origin have been shown to be involved in this enhanced binding (Seo et al., 1993; Spalholz et al., 1993; Ustav et al., 1993).

Previous studies in our laboratory have shown that in primary bovine embryo dermal fibroblasts (BEFs), E2-transactivated expression from the BPV-1 P_{89} promoter could be repressed by the BPV-1 E1 replication protein (Sandler et al., 1993), in a manner consistent with binding of the E1/E2 complex to the origin. We postulated that the bound complex interfered with assembly or function of the transcriptional machinery at the P_{89} promoter. Evidence that E6 and E7 expression are normally repressed in BPV-1 transformed cells was published by Lambert & Howley (1988) and Schiller et al. (1989). These studies showed that E1 mutants exhibited a higher transformation potential and increased levels of P_{89} transcription. This type of regulation may be functional in other papillomaviruses as well, as evidenced by the fact that disruption of the E1 and E2 ORFs in HPV-16 leads to deregulation of E6 and E7 expression (Romanczuk & Howley, 1992). It will therefore be important to fully characterize the mech-
anism of E1 repression of the BPV-1 P<sub>ss</sub> promoter; however, a better understanding of the control of basal transcription must first be achieved.

Basal transcription from the P<sub>ss</sub> promoter has not been extensively studied. The 5' end of P<sub>ss</sub> mRNA is at nt 89 and sequence analysis shows that a consensus TATA box for P<sub>ss</sub> is located at nt 58. In addition, an upstream constitutive enhancer (see Fig. 1 a) is important for basal transcription from P<sub>ss</sub> in bovine cells (Vande Pol & Howley, 1990, 1992). The precise mechanisms and cellular factors involved in controlling P<sub>ss</sub> basal transcription, however, have yet to be elucidated. The purpose of this study was to begin to investigate these features to gain a clearer understanding of how P<sub>ss</sub> transcription is regulated in an infected cell.

Methods

Cells. Primary bovine embryo fibroblasts (BEFs) were harvested from fetal calf dermis as previously described (Sandler et al., 1993). BEF and monkey CV-1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml) and 10% fetal calf serum (GIBCO).

Plasmids. The wild-type BPV-1 P<sub>ss</sub> chloramphenicol acetyltransferase (CAT) reporter plasmid, p1066, has been previously described (Spalholz et al., 1987). Plasmid p2026, which contains an 85 bp deletion from nucleotide (nt) 7906 to 45 in the p1066 background has also been described previously (Spalholz et al., 1993). Sp1-binding site mutations were cloned into the p2026 background using the method of Tomic et al. (1991). The single base mismatches introduced into the BPV-1 P<sub>ss</sub> Sp1 sites at nt 7800, 7833 and 7854 changed the sequences of the sites from AGGCGG to AGGGGG. These substitutions into p2026 generated the plasmids p2541, p2035 and p2542, containing mutations in one, two or three of the Sp1 sites, respectively. In order to generate these mutations in the p1066 background, the Transformer site-directed mutagenesis system from Clontech was utilized. The single base mismatches for the sites at nt 7833 and 7854, and nt 7800, 7833 and 7854 as described above were used to generate p2702 and p2703, respectively.

Transient expression analysis. Transfections for CAT assays were performed by the calcium phosphate co-precipitation method (Graham & van der Eb, 1973) as described previously (Sandler et al., 1993), using 5 µg of CAT-containing plasmid per 60 mm dish, and 1 µg of the E2 expression plasmid pC59 (Yang et al., 1985) where indicated. Each dish also received enough salmon sperm DNA to keep the total amount of DNA transfected constant at 10 µg per dish.

 Primer extension analysis. Primer extension analysis of polyadenylated BEF mRNA was performed as described previously (Sandler et al., 1993). The oligonucleotide used is complementary to CAT mRNA, 25 nt downstream of the CAT AUG and is expected to yield a primer extension product of 88 nt for transcripts initiating at the P<sub>ss</sub> promoter.

 Gel retardation assays. Probes for gel shifts were made by PCR amplification of BPV-1 DNA from nt 7781 to 7879 using either wild-type or Sp1-mutated DNA as the template. One oligonucleotide was kinased with [γ-32P]ATP prior to performing PCR to generate radiolabelled probe.

 Binding reactions were performed under published conditions (Dignam et al., 1983) using approximately 50000 c.p.m. probe per sample (approximately 2 ng DNA). HeLa cell nuclear extract and purified Sp1 were obtained from Promega. Approximately 4 µg of HeLa nuclear cell extract or 15 ng of purified Sp1 was added per sample, where indicated. Competition with primers containing known transcription factor binding sites was as follows: AP-2, 5' GATCG AACTG ACCGC CGCG GCCT 3'; Sp1, 5' ATTCG ATCCG GCAGG GCAGA GC 3'. Electrophoresis was performed on 5% polyacrylamide gels in 0·25 × TBE at 17 mA at 4 °C.

Results

In order to analyse the transcriptional regulatory elements of the BPV-1 P<sub>ss</sub> promoter we made use of a plasmid, p2026, which exhibits elevated P<sub>ss</sub> transcription. p2026 is similar to p1066 (Fig. 1) in that it has the BPV-1 P<sub>ss</sub> promoter driving CAT expression; however, where p1066 contains the entire BPV-1 LCR upstream of P<sub>ss</sub>, p2026 has a deletion of 85 nt from nt 7906 to 45 which removes the origin of DNA replication. The elevated CAT expression and P<sub>ss</sub> transcription resulting from this plasmid after transfection into primary BEF cells is presented in Fig. 1. BEF cells transfected with p2026 DNA consistently exhibited a 35- to 100-fold elevation of basal CAT activity compared to cells transfected with p1066 DNA (Fig. 1 b). Primer extension analysis specific to CAT mRNA performed on the poly(A)+ RNA prepared from the p1066- and p2026-transfected primary BEF cells confirmed that the CAT transcripts from both p1066- and p2026-transfected cells originate from the P<sub>ss</sub> promoter (Fig. 1 c, lane ‘p2026’) and that the increase in the level of P<sub>ss</sub> transcripts between p2026- and p1066-transfected cells was consistent with the corresponding CAT expression levels. As the elevated CAT expression level of p2026 facilitated promoter analysis, it was used for the initial characterization of P<sub>ss</sub> transcriptional regulatory elements.

Examination of the BPV-1 DNA sequence between the 3' end of the E2-dependent enhancer E2RE<sub>1</sub> and the replication origin revealed the presence of three putative binding sites for the transcription factor Sp1. These sites are located at nt 7800 (AGGCGG, lower strand), 7833 (AGGCGG, upper strand) and 7854 (AGGCGG, upper strand). To confirm that the transcription factor Sp1 could bind to these sites in vitro, a gel shift assay was performed. A double-stranded DNA probe was generated by PCR amplification of the region between nt 7781 and 7879 (see Fig. 2 a) and this fragment was tested for its ability to bind purified Sp1 or Sp1 present in HeLa cell nuclear extract. The results of gel shift analysis are shown in Fig. 2(b). The first three lanes illustrate the position of a DNA complex containing Sp1 protein (complex B), obtained with either purified Sp1 (lane S) or HeLa nuclear extract (lane H), using a radiolabelled Sp1 consensus oligonucleotide as a probe. This DNA fragment binds one molecule of Sp1. When the gel shift
Fig. 1. (a) Structure of the wild-type reporter plasmid p1066. The 12 E2-binding sites present in the LCR are represented by filled circles. The position of the four E2-binding sites which constitute the E2-dependent enhancer E2RE\(_1\) are indicated, as are the positions of the viral replication origin (ORI) and the constitutive enhancer (CE) (Vande Pol & Howley, 1990). The three putative Sp1-binding sites, located between E2-binding sites 10 and 11, are indicated by the solid rectangles. The positions of the P\(_{89}\) promoter and the bacterial CAT gene are also indicated. The thick black bar indicates the location of the deleted sequences in plasmid p2026. (b) Basal CAT activity of p1066 and p2026 in primary BEF cells. CAT assays were performed at 37 °C for 90 min using 40 μg of cell extract. Percentage acetylation for this assay was 0.66 % for p1066 and 24.6 % for p2026. (c) Primer extension analysis of poly(A)\(^+\) mRNA from BEF cells transfected with p1066 or p2026. Poly(A)\(^+\) mRNA was prepared from 60 μg of total RNA per sample. The arrow indicates the position of the expected 88 nt primer extension product corresponding to transcripts initiating at the P\(_{89}\) promoter. M, molecular mass markers.
probe was a radiolabelled PCR fragment from p1066 containing three wild-type Sp1 sites, the same complex (B) could be observed in the presence of purified Sp1 (lane S), indicating that only one molecule of Sp1 bound to this BPV-1 DNA fragment. When the gel shift was performed with HeLa nuclear extract (lane H), two additional DNA–protein complexes (A and C) could be observed with the BPV-1 probe, suggesting that at least one other protein present in HeLa nuclear extract was capable of binding this DNA fragment. When a radiolabelled BPV-1 DNA fragment containing point mutations in all three putative Sp1 sites was used as a gel shift probe, we still observed binding in the presence of purified Sp1, but the gel shift pattern changed dramatically in the presence of HeLa nuclear extract (probe 2542, lanes S and H). Complex A was no longer present, complex B was greatly diminished, but complex C was still present and increased. This suggests that the slowest-migrating complex A may result from the binding of both Sp1 and another nuclear protein to the DNA fragment, while complex C may represent binding of only that nuclear protein or a different nuclear protein to the BPV-1 DNA fragment.

In order to determine which of the three Sp1 sites was binding Sp1 in the gel shift assay, the gel shift pattern of wild-type BPV-1 DNA observed with HeLa nuclear extract was compared to that seen when the radiolabelled probe contained a point mutation in the putative Sp1 site at nt 7800 (see Fig. 2a, probe 2541). The results of this comparison are illustrated in Fig. 2(c). No difference in the gel shift pattern could be detected with the two probes used, indicating that Sp1 binds predominantly to one of the two downstream sites. Gel shifts performed using a radiolabelled probe containing mutations in both
Spl and BPV-1 \( P_{\text{ss}} \) transcription

Fig. 2. Gel shift analysis of Spl binding to sites in the BPV-1 LCR. (a) DNA probes used for testing Spl binding. The arrows, with corresponding nucleotide numbers, indicate the endpoints of the probes. Filled ovals represent E2-binding sites, open rectangles represent Spl sites, and filled rectangles represent Spl sites containing point mutations as described in the text. (b) Binding of purified Spl and HeLa nuclear extract to BPV-1 probes containing wild-type or mutated Spl sites. Binding reactions were performed as described in Methods using consensus Spl oligonucleotide (Spl), wild-type (1066) or mutant (2542; three Spl sites mutated) radiolabelled probe as indicated below the gel. The presence of HeLa nuclear extract (H lanes) or purified Spl (S lanes) in the binding reactions is indicated at the top of the gel. The positions of the unbound probes are indicated, as are the positions of the three major retarded complexes A, B and C. (c) Binding of Spl to a wild-type or single Spl-mutated probe. Radiolabelled probes were wild-type (1066) or a fragment containing a point mutation in the Spl site at nt 7800 (2541). The presence (+) or absence (−) of HeLa nuclear extract is indicated above each lane. The position of the unbound probes is indicated, as are the positions of the shifted complexes A, B and C. (d) Competition of Spl binding. Probes are indicated below the gel; 1066 contains three wild-type Spl sites. The presence (+) or absence (−) of HeLa nuclear extract is indicated below each lane. Competitors were as follows: lane 3, sixfold molar excess of unlabelled Spl consensus oligonucleotide; lane 4, sixfold molar excess of unlabelled AP2 consensus oligonucleotide; lane 6, twofold molar excess of unlabelled wild-type BPV fragment; lane 7, eightfold molar excess of unlabelled wild-type BPV fragment; lane 8, twofold molar excess of unlabelled p2542 fragment (three mutated Spl sites); lane 9, eightfold molar excess of p2542 fragment; lane 10, tenfold molar excess of unlabelled Spl consensus oligonucleotide; lane 11, tenfold molar excess of unlabelled AP2 consensus oligonucleotide.

downstream Spl sites yielded a pattern of gel shift complexes identical to that seen using the probe containing mutations in all three Spl sites (Fig. 2b, probe 2542 lanes; data not shown).

To clarify the identity of the three shifted complexes observed with HeLa nuclear extract, competition assays were carried out (Fig. 2d). Lane 2 illustrates the position of the DNA–protein complex containing Spl using the radiolabelled Spl consensus oligonucleotide as a probe (complex B). This complex could be efficiently competed by the addition of a sixfold molar excess of unlabelled Spl oligonucleotide to the binding reaction (lane 3) but not by the addition of a sixfold molar excess of unlabelled AP2 consensus oligonucleotide (lane 4). The three DNA–protein complexes obtained after binding of HeLa nuclear extract to the wild-type BPV-1 probe (lane 5) could all be efficiently competed by the addition of an eightfold molar excess of unlabelled BPV-1 DNA (lane 7). When an unlabelled BPV-1 DNA fragment containing mutations in all three Spl sites was used as a competitor, a much lower level of competition was observed (Fig. 2d, compare lane 8 to lane 6 and lane 9 to lane 7). An
eightfold molar excess of mutant DNA competed only as well as a twofold molar excess of wild-type DNA. This suggests that the Sp1 mutations result in a greatly reduced affinity for Sp1 protein. When a tenfold molar excess of unlabelled Sp1 oligonucleotide was used as a competitor, complexes A and B were efficiently competed, but complex C could not be competed (lane 10). This result confirms that complex C represents binding of another nuclear protein and that complexes A and B result from Sp1 binding. As expected, the addition of a tenfold molar excess of the unlabelled non-specific competitor oligonucleotide AP2 did not affect binding (lane 11).

Having established that the region containing the three Sp1 sites in the BPV-1 LCR could specifically bind Sp1 \textit{in vitro}, it was necessary to determine whether these sites were functional \textit{in vivo}. Since disruption of functional Sp1 binding sites would be expected to reduce basal transcription, the high basal expression of p2026 was critical to facilitate detection and quantification of any reduced transcriptional activity from \( P_{89} \). Plasmids containing point mutations in one (p2541), two (p2035) or three (p2542) of the Sp1 sites (Fig. 3) were each transfected into primary BEF cells. Basal CAT expression was measured and compared to that from the parent plasmid, p2026. The results of CAT analyses are shown in Fig. 3. The results are presented as the average fold-reduction of CAT expression from p2541, p2035 or p2542 compared to p2026 in each experiment. Mutation of the putative Sp1 site at nt 7800 resulted in only a modest (1.9-fold) decrease in basal CAT activity. Mutation of the two putative Sp1 sites at nt 7833 and 7854 (p2035) resulted in a fivefold reduction in basal CAT activity compared to p2026. Mutation of all three Sp1 sites resulted in the greatest reduction in basal CAT activity (18.3-fold). These results indicate that the Sp1 sites are functional \textit{in vivo} in the background of p2026.

The results of the gel shift assays taken together with the CAT analyses indicate that Sp1 binding sites in the BPV-1 LCR function to activate \( P_{89} \) transcription in the context of an origin-deleted LCR–CAT construct. Since it was possible that the increase in \( P_{89} \) promoter activity from p2026 might be due to the repositioning of these sites closer to the \( P_{89} \) TATA box as a consequence of the deletion, it was necessary to determine whether the Sp1 sites were functional in the context of p1066, the wild-type LCR–CAT construct. The Sp1 binding site mutations present in plasmids p2035 and p2542 were cloned into the p1066 background to create the plasmids p2702 (two mutated Sp1 sites) and p2703 (three mutated Sp1 sites), respectively (Fig. 4). BEF cells were transfected with p1066, p2702 or p2703 and the results of CAT analyses are shown in Fig. 4. The basal CAT activity of p2702 was consistently twofold lower than
than primary BEF cells (A. Sandler, unpublished results).

The results of the CAT analysis in CV-1 cells are shown in Fig. 4 and demonstrate that these promoters are functional, the transfections were repeated in monkey CV-1 cells, which can be transfected at a higher efficiency than primary BEF cells (A. Sandler, unpublished results). The results of the CAT analysis in CV-1 cells are shown in Fig. 4 and demonstrate that these promoters are active, as their basal CAT expression is greater than that seen in a mock transfection. These data clearly confirm that the Sp1 binding sites in the BPV-1 LCR are involved in basal transcription from the P89 promoter.

Having established a role for the LCR Sp1 sites in P89 basal transcription, we next sought to determine their importance in E2 transactivation of P89. Previous studies have suggested that Sp1 is involved in E2-transactivation from the BPV-1 P2443 and P3050 promoters (Li et al., 1991; Spalholz et al., 1991). These downstream promoters can also be E2-transactivated through E2RE1, but require Sp1 sites located near the promoters for their activation. In order to determine whether the LCR Sp1 binding sites are critical for the strong E2 transactivation of P89, Sp1 binding site mutations were tested in E2-transactivation assays. The mutations tested were those cloned into the full-length LCR background of p1066 (p2702 and p2703). The plasmids were co-transfected into primary BEF cells or CV-1 cells alone or with either the E2-expressing plasmid pC59 (+ E2) or the plasmid pC59.Kpn.TTL (− E2), which contains a translation termination linker inserted into the E2 coding region and results in the production of a non-functional E2 protein (Winokur & McBride, 1992). As before, the transfection was also performed in CV-1 cells to ensure that any reduced level of E2 transactivation observed with plasmids p2702 and p2703 was not simply due to a complete lack of basal activity. The results of CAT analyses are shown in Table 1. Clearly, mutation of two (p2702) or three (p2703) of the Sp1 sites in the wild-type p1066 background greatly reduced the level of E2 transactivation in both cell types tested. The fact that in the wild-type context mutation of the Sp1 sites resulted in a drastic reduction in E2 transactivation indicates that these Sp1 sites are in fact involved in E2-transactivated P89 expression as well as basal expression.

## Discussion

The data presented in this study define a role for Sp1 in regulating both basal and E2-transactivated transcription from the BPV-1 P89 promoter. The observation that mutation of the BPV-1 Sp1 binding sites in the wild-type LCR causes a reduction in E2-transactivation potential supports the previous work published by Li et al. (1991). That study showed that Sp1 and E2 interact directly in vitro and suggested that an interaction between these transcription factors mediates transactivation of E2-responsive promoters in vivo. While the data presented here support the idea of an in vivo interaction between E2 and Sp1, the possible role of other cellular transcription factors in this activation cannot be dismissed. Interestingly, mutation of the Sp1 binding sites in the background of the deletion plasmid p2026 (plasmids p2035, p2541 and p2542) did not impair the ability of E2 to transactivate transcription from the P89 promoter (data not shown). The simplest explanation for this phenomenon is that when E2RE1 is close to the P89 TATA box, an E2/Sp1 interaction is no longer needed for E2 transactivation. This explanation, however, is unlikely in the light of a previous study by Ham et al. (1991). That study utilized synthetic test promoters to ascertain the role of Sp1 sites in E2 transactivation. They found that a minimal thymidine kinase or adenovirus major late promoter containing two E2-binding sites upstream of a TATA box could not be efficiently transactivated by E2, whether the sites were 50 nt or 109 nt upstream of the TATA box. However, the addition of Sp1 sites between the E2-binding sites and the TATA box greatly increased the E2-transactivation potential, showing that Sp1 is important for E2 transactivation. A more plausible explanation for the E2 transactivation seen with the Sp1 mutations in the deletion background may be that the deletion in p2026
has removed a binding site for another cellular factor which now allows transactivation to occur in the absence of Spl. In the context of the wild-type LCR, it is likely that other transcription factors binding in the vicinity of the Spl and E2-binding sites may interfere with E2 transactivation unless Spl is also present.

There is evidence that other cellular transcription factors in addition to Spl contribute to control of basal P99 transcription. Plasmid p2542, containing three Spl mutations in the p2026 background exhibits basal CAT activity that is still fivefold higher than the wild-type plasmid p1066 (data not shown). This observation supports the possibility that other transcription factor binding sites may play a role in normal basal P99 transcription. Results of the gel shift assays performed with DNA spanning the region containing the Spl sites and HeLa cell nuclear extract also indicate that some other factor present in the HeLa cell nuclear extract binds to this region (Fig. 2). The gel shift data indicate that Spl binds to only one of the three Spl sites, yet the in vivo functional data suggest a synergistic effect when multiple Spl sites are mutated (Fig. 3). This indicates that the synergistic effect observed in vivo probably results from the interaction of Spl with another nuclear protein. It is also possible that some transcriptional repressor binding sites are present between nt 7906 and 45 which account for the 35- to 100-fold increase in basal transcription of p2026 as compared to p1066. Therefore, it is likely that P99 basal transcription is subject to a combination of regulatory mechanisms.

Previous studies have investigated the role of Spl in regulating the BPV-1 promoters P2443 and P3880. These studies, however, could not definitively show a requirement for Spl in E2 transactivation of the BPV-1 promoters. It is possible that Spl was absolutely required for a functional basal promoter (Li et al., 1991; Spalholz et al., 1991). The study by Li et al. (1991) determined an in vivo role for Spl in basal transcription from examination of promoter activity in an Spl- cell line and showed that transcription was stimulated by the addition of exogenous Spl. These current experiments measured reduction of transcription when Spl sites were mutated in the presence of the normal, physiological amounts of Spl. The study by Spalholz et al. (1991) identified a critical Spl site upstream of P2443, but could not assess its function in E2 transactivation because mutation of that site reduced basal transcription to background levels and the subsequent loss of E2 transactivation could have been due to complete inactivation of the promoter. In contrast, the phenotype of p2702 and p2703 indicates that Spl is not required for a functional P99 promoter, since these plasmids retain some basal activity (Fig. 4), but that Spl can significantly enhance P99 activity. Furthermore, the impairment of E2 transactivation in p2702 and p2703 strengthens the data suggesting an important role for Spl in E2 transactivation.

The Spl involvement in BPV-1 P99 basal transcription is similar to that seen with transcription from the E6 and E7 promoters of the human papillomaviruses HPV-16 and HPV-18. Previously, Spl had been implicated in regulating expression from both the HPV-16 P99 promoter and the HPV-18 P106 promoter (Gloss & Bernard, 1990; Hoppe-Seyler & Butz, 1992). The HPV-16 and HPV-18 LCRs each contain one sequence-aberrant Spl site located just upstream of the P99 and P106 promoters, respectively. In HPV-18, mutation of this Spl site leads to a decrease in transcription from the P106 promoter in some cell types (Hoppe-Seyler & Butz, 1992), suggesting that regulation by Spl probably involves other cellular factors. In fact, characterization of the LCRs of HPV-16 and HPV-18 has revealed the presence of binding sites for the cellular transcription factors AP-1, NF-1 and Oct-1 in addition to the Spl site (Chan et al., 1990; Chong et al., 1990; Gloss et al., 1989). Control of E6 and E7 expression in the HPVs is probably achieved through interaction of several cellular transcription factors with their cognate sites in the LCR. Why BPV-1 uses three Spl sites to regulate the analogous P99 promoter is not clear, but is probably related to the differences in the BPV and HPV promoter regions. While transcription from the BPV-1 P99 promoter is strongly stimulated in the presence of E2, transcription from the analogous HPV-16 and HPV-18 P99 and P106 promoters is usually repressed because E2 binds to a site immediately adjacent to the promoter and interferes with binding of the transcriptional machinery (Dostatni et al., 1991). The HPV E6/E7 promoters can be E2-transactivated if the proximal E2-binding site is inactivated but this does not appear to be the predominant regulatory mechanism. Examination of the BPV-1 LCR reveals the presence of putative binding sites for the transcription factors AP-1 and NF-1 between E2RE1 and the replication origin. While the possible role of these transcription factors in regulating P99 transcription is currently being investigated, it seems reasonable that interaction between Spl and NF-1 or AP-1, or both, may also be important in the BPV-1 system. In fact, preliminary data suggest that the NF-1 site in the BPV LCR is important for P99 basal transcription (A. Sandler, unpublished results) and studies are in progress to identify other cellular factors which may be involved in BPV-1 P99 transcription.

We thank Alison McBride for critical reading of the manuscript and the members of LTVB for helpful discussions.

References


(Received 6 June 1995; Accepted 5 October 1995)