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# Activation of human papillomavirus type 18 E6-E7 oncogene expression by transcription factor Sp1

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## ABSTRACT

**The human papillomavirus 18 (HPV18) E6 and E7 proteins are considered to be primarily responsive for the transforming activity of the virus. In order to analyse the molecular mechanisms resulting in viral oncoprotein expression, it is necessary to identify the factors involved in the transcriptional regulation of the E6/E7 genes. Here we define by gel retardation experiments a sequence aberrant Sp1 binding site present in the promoter proximal part of the viral transcriptional control region (Upstream Regulatory Region, URR). Functional analyses employing transient reporter assays reveal that this Sp1 element is required for an efficient stimulation of the HPV18 E6/E7-promoter. Mutation of the Sp1 element in the natural context of the HPV18 URR leads to a strong decrease in the activity of the E6/E7-promoter in several cell lines. The magnitude of reduction varies between different cell types and is higher in cell lines of epithelial origin when compared with nonepithelial cells. Cotransfection assays using Sp1 expression vector systems further define the promoter proximal HPV18 Sp1 binding motif as a functional Sp1 element in vivo and show that its integrity is essential for the stimulation of the E6/E7-promoter by augmented levels of Sp1. These results indicate, that the cellular transcription factor Sp1 plays an important role for the stimulation of the E6/E7-promoter by the viral URR and represents a major determinant for the expression of HPV18 transforming genes E6 and E7.**

## INTRODUCTION

Human papillomaviruses (HPVs) are small epitheliotropic DNA viruses responsible for a variety of benign proliferative lesions of the skin and mucosal epithelium, such as warts and condylomata acuminata. In addition, certain genotypes are closely associated with the development of human anogenital malignancies such as carcinoma of the uterine cervix (1).

Several lines of investigations indicate a causative role for these viruses in cervical carcinogenesis. Studies demonstrated, that more than 90% of cervical tumor biopsies contain the DNA of specific HPVs, particularly HPV types 16 (HPV16) and 18 (HPV18), usually with parts or all of the HPV genome found integrated into the host chromosomes (1). Transcripts mapping to the E6/E7 part of the early region within the viral genome are transcribed both in tumor tissue and in tumor-derived cell lines (2, 3). The products of the E6 and E7 genes possess transforming potential in vitro (4, 5, 6) and have been shown to be required for the maintenance of the transformed phenotype of cervical cancer cells in vitro (7) and in vivo (8). On the molecular level, the complex formation of both viral proteins with the products of cellular tumor-suppressor genes, specifically E6 with p53 (9) and E7 with the Rb protein (10), is likely to be relevant for the process of cell transformation by these viruses. Taken together, these findings strongly suggest that the expression of the viral E6 and E7 genes plays an important role in the etiology of HPV associated malignancies.

HPV18 E6/E7-expression is regulated on the transcriptional level by an intricate network of cis-regulatory elements contained within the viral upstream regulatory region (URR), which separates the viral early (E) and late (L) genes in the viral genome (Fig. 1A). A variety of regulatory proteins have been shown to recognize distinct binding sites within the HPV18 URR and have been implicated to participate in the transcriptional control of the E6/E7-promoter located at the 3'-terminus of the URR. These factors include the cellular transcription factors NF1, AP1, KRF, Oct-1 and the glucocorticoid-receptor (11, 12, 13, 14, 15, 16). The HPV16 URR has been proposed to possess a similar functional composition (17). However, it should be noted that the HPV18 URR is a much stronger activator of the E6/E7-promoter as the HPV16 URR (about 5 to 10 fold difference in cervical carcinoma cells; Hoppe-Seyler and Butz, unpublished observation) and has been shown to be responsible for the significantly higher immortalisation potential of HPV18 when compared to HPV16 (18). Different factor requirements might well account for these functional differences. A recent report indeed suggested the binding of an keratinocyte-specific activatory protein, KRF, to the HPV18 URR but not to the HPV16 URR (14). These findings indicate the necessity to examine the binding and functional significance of regulatory factors specifically in the context of the respective HPV URR and prohibit a simple extrapolation of data from one virus type to the other.

Recent studies indicated the presence of a sequence aberrant binding site for the transcription factor Sp1 within the promoter proximal region of the HPV16 URR, which exhibits a single base

mismatch when compared with the Sp1 consensus binding motif (19). A G-rich DNA sequence is also found in the corresponding region of the HPV18 URR, showing a two base deviation from the Sp1 consensus binding sequence (Fig. 1B).

In this paper we show by binding analysis, that these sequences within the HPV18 URR represent a bona fide Sp1 recognition element. As both the proper spacing between individual cis-acting elements and the overall composition of a transcriptional control region can influence the activity of a given regulatory element, we analysed the functional significance of the promoter proximal Sp1 element in the natural context of the complete HPV18 URR. Transient luciferase assays employing wildtype and site-specifically mutated HPV18 URR reporter constructs reveal that the Sp1 element plays an important role for the stimulation of the HPV18 E6/E7-promoter, indicating that it is required for an efficient activation of viral transforming gene expression in vivo. The amount of cis-activation conferred by the promoter proximal Sp1 element varies between cell types of either epithelial or non-epithelial origin. Cotransfection assays utilizing Sp1 expression vectors further define the element as a functional Sp1 element in vivo and show that its integrity is essential for the Sp1 induced stimulation of the HPV18 E6/E7-promoter.

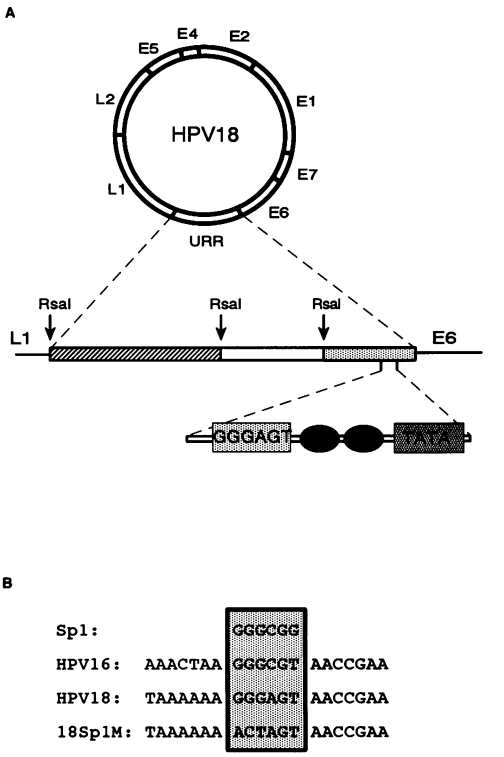


**Figure 1.** Organisation of the HPV18 genome. **A:** Early (E) and late (L) open reading frames separated by the Upstream Regulatory Region (URR) are indicated schematically. Below: Representation of the HPV18 URR divided into the 389 bp 5'-region (dashed area), the constitutive enhancer (open area) and the promoter proximal portion (stippled area) containing the G-rich sequence motif (HPV18 nucleotides 34 to 39) located directly upstream to the two palindromic E2 binding sites and the E6/E7-promoter TATA box. **B:** Sequence comparison of the HPV18 promoter proximal G-rich sequence (HPV18) with the sequence aberrant Sp1 recognition motif (19) of the HPV16 URR (HPV16) and the Sp1 consensus binding motif (Sp1). 18Sp1M: Mutated sequence analysed in gel retardation assays and introduced for functional analysis into the natural context of the HPV18 URR by site-directed mutagenesis.

## MATERIALS AND METHODS

### Cells

Cells of HeLa−, SW756−, Me180−, CaSki, SiHa and C33A− human cervical carcinoma cell lines, primary human fibroblasts from oral mucosa, human embryonic lung fibroblasts (HEL-cells), CV-1 monkey kidney fibroblasts, NIH3T3 mouse fibroblasts, Saos-2 osteosarcoma cells and cells of the spontaneously immortalized human keratinocyte cell line HaCaT (20) were maintained in Dulbeccos' minimal essential medium (D-MEM) pH 7.2 supplemented with 10% fetal calf serum.

### Plasmids and oligonucleotides

Both basic vector pBL and reporter plasmid p18URRL, which contains the HPV18 URR linked to the firefly *P.pyralis* luciferase gene, have been described in detail previously (13). Site specific mutagenesis of the Sp1 binding sites within the HPV18 URR was performed with a polymerase chain reaction (PCR) based protocol (21). All DNA manipulations followed standard protocols. The DNA sequences of wildtype and mutated constructs were verified by the dideoxynucleotide termination reaction. HPV18 nucleotide numbering throughout the text followed the paper of Cole and Danos (22) Sp1 expression vector pSVSp1-F and control vector pSV2A101 were generously provided by Dr. J.D.Saffer and have been described previously (23).

Oligonucleotides were synthesized with a Gene Assembler Plus (Pharmacia) and purified by polyacrylamide gel electrophoresis. The double-stranded oligonucleotides used for binding analysis were derived from the HPV18 URR and bear the following sequences (only sense strands are indicated): 18Sp1 (HPV18 nt 19 to 45): 5'-GTAGTATATAAAAAAGGGAGTGACCGA-3'; 18Sp1M (mutated bases are underlined): 5'-GTAGTATAT AAAAAAACTAGTGACCGA-3' and Ap1E (nt 7583 to 7624): 5'-TGGCGATACAAGGCGCACCTGGTATTAGTCATTTT-CCTGTCC-3'. The Sp1 consensus oligonucleotide Sp1K is derived from the HSV IE-3 gene promoter (24) and contains the sequence 5'-CCGGCCCCGCCCATCCCCGGCCCCG-CCCATCC-3'.

### Nuclear extracts and gel retardation assays

Nuclear extracts were prepared according to Dignam et al. (25). For gel retardation assays, 5 $\mu$g of nuclear extract were mixed with 3 $\mu$g poly (dI·dC) in a 25 $\mu$l reaction volume containing 10% glycerol, 10 mM Hepes pH 7.9, 4 mM $MgCl_2$, 30 mM KCl, 0.1 mM EDTA, 0.5 mM dithiotreitol (DTT) and 0.5 mM phenylmethylsulfonyl fluoride (PMSF). After 5 min incubation at 4°C, 12000 cpm of gamma-32-P-ATP end-labeled double stranded oligonucleotide (10−20 fmol) were added as a probe and incubation was continued for 15 min at 4°C. For competition assays, a 200 fold molar excess of unlabeled double-stranded oligonucleotides was included before addition of probe DNA. DNA-protein complexes were separated from unbound probe on a 4% (29:1 cross-linking ratio) nondenaturing polyacrylamide gel and were visualized by autoradiography. Purified Sp1 protein was purchased from Stratagene and used at a concentration of 0.2 fpu (Footprint units as specified by the supplier) for gel retardation analysis.

### Transfections and transient expression assays

Approximately $2\times10^5$ cells per 30 mm dish were transfected by the calcium phosphate coprecipitation technique (26). Transfections usually contained 3 $\mu$g reporter plasmid and were

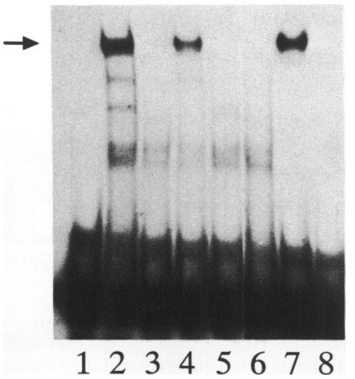


**Figure 2.** Sp1 binds to the promoter proximal portion of the HPV18 URR. Gel retardation analysis using HeLa-cell nuclear extract and purified human Sp1 protein. Lane 1: Wildtype oligonucleotide 18Sp1, free probe. Lanes 2–5: HeLa cell nuclear extract forms a specific protein–DNA complex (arrow) with 18Sp1 (lane 2), which is efficiently competed by a 200 fold molar excess of both unlabeled homologous oligonucleotide 18Sp1 (lane 3) and the Sp1 consensus oligonucleotide Sp1K (lane 5), but not by the heterologous oligonucleotide AP1E (lane 4). Lane 6: Loss of binding of HeLa cell nuclear extract to mutant oligonucleotide 18Sp1M. Lanes 7 and 8: Binding of purified human Sp1 protein to wildtype oligonucleotide 18Sp1 (lane 7), which is abolished by the mutation introduced into oligonucleotide 18Sp1M (lane 8).

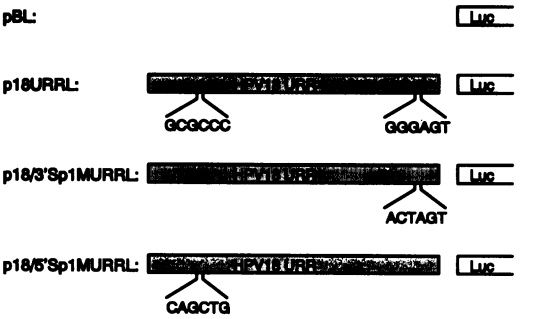


**Figure 3.** Wildtype and mutated HPV18 URR luciferase reporter plasmids used in the study. **A:** Reporter constructs employed in transient expression assays. pBL, basic luciferase vector (13). Vector p18URRL contains the HPV18 URR linked to the luciferase reporter gene, which is under transcriptional control of the E6/E7-promoter (13). The sequences of both the 5'- and the 3'-Sp1 elements are indicated. As shown, plasmid p18/3'Sp1MURRL and p18/5'Sp1MURRL contain a specific mutation in the 3'- or 5'- Sp1 recognition element, respectively, but otherwise are identical to p18URRL.

adjusted to 6.5 μg by addition of Bluescript DNA as carrier. For cotransfection assays, 0.5 μg of the reporter construct were added to 3 μg of the respective expression vector and 3 μg Bluescript DNA. For luciferase assays, cells were processed as described (27) and activities were quantitated using a lumat luminometer (Berthold).

## RESULTS

### The cellular transcription factor Sp1 binds to the 3'-portion of the HPV18 URR

A G-rich sequence GGGAGT (HPV18 nucleotides 34 to 39), bearing two mismatches to the Sp1 consensus recognition sequence GGGCGG (Fig. 1B), is located in the promoter proximal region of the HPV18 URR (Fig. 1A). To test whether this sequence, despite its two base deviation from the consensus binding motif, represents a bona fide recognition site for Sp1, gel retardation assays were performed.

Incubation of crude nuclear extract from HeLa cervical carcinoma cells with oligonucleotide 18Sp1 encompassing the HPV18 G-rich motif (for DNA sequence of 18Sp1 see Materials and Methods) led to the detection of a prominent protein-DNA complex (Fig. 2, lane 2). Formation of the complex was efficiently competed by the addition of a 200 fold molar excess of the unlabeled homologous oligonucleotide 18Sp1 (lane 3), but not by the addition of the same molar amount of the heterologous oligonuleotide AP1E (lane 4), which is derived from the HPV18 enhancer region and contains an AP1 binding site (12). These data indicate, that the complex is formed by a protein specifically binding to a motif within the DNA sequence of oligonucleotide 18Sp1.

Importantly, complex formation could be efficiently competed by an 200 fold molar excess of oligonucleotide Sp1K which is derived from the Herpes simplex virus immediate early 3 (HSV IE-3) gene promoter (lane 5) and contains a well defined Sp1 recognition site (24). This result indicates, that the complex formed on oligonucleotide 18Sp1 results from the binding of Sp1 to a recognition site within these sequences.

To investigate whether the G-rich motif within 18Sp1 is required for the binding of Sp1, gel shift assays were performed using oligonucleotide 18Sp1M, which contains a specific mutation in these sequences (Fig. 1B). As shown in Figure 2, this mutation abolishes complex formation (lane 6), mapping the Sp1 contacts on oligonucleotide 18Sp1 to the Sp1 like recognition sequences.

Further evidence that the promoter proximal G-rich motif represents a bona fide Sp1 recognition element is derived from the observation that purified human Sp1 protein specifically binds to oligonucleotide 18Sp1, resulting in a complex co-migrating with the prominent protein-DNA complex obtained with HeLa crude nuclear extract (lane 7). In contrast, mutant oligonucleotide 18Sp1 is not bound by purified Sp1 protein (lane 8).

In conclusion, these experiments demonstrate, that the HPV18 URR contains a sequence-aberrant Sp1 recognition site located close to the E6/E7-promoter.

### The HPV18 promoter proximal Sp1 element is important for the activation of the E6/E7-promoter

To investigate the functional significance of the sequence aberrant Sp1 element for the regulation of the HPV18 E6/E7-promoter, transient transfection experiments using reporter gene systems were performed in several cell lines. Plasmid p18URRL (Fig. 3) contains the HPV18 URR linked to the firefly *P.pyralis* luciferase gene and directs transcription of the reporter gene from the E6/E7-promoter p105 (13). To assess the relative contribution of the Sp1 recognition site to the activity of the E6/E7-promoter, the Sp1 binding motif was mutated in the natural context of the HPV18 URR by a PCR-based protocol for site-specific mutagenesis (21), resulting in reporter construct p18/3'Sp1MURRL (Fig. 3). As shown in Fig. 2, the introduced mutated sequence abolishes Sp1 binding to the promoter proximal part of the URR.

The activities of the mutated and wildtype URR were subsequently tested in transient expression assays. The comparative analysis of these constructs demonstrates, that the

**Table 1.** Relative luciferase activities (fold activation above basis vector pBL) of the wildtype HPV18 URR and mutated constructs p18/3'Sp1MURRL and p18/5'Sp1MURRL (see Figure 3) in cells of epithelial and non-epithelial origin. Percent repression indicates the reduction in luciferase activity of the respective mutated construct versus the wildtype URR. Results represent the average values of at least three independent transfections, each performed in triplicates. Results from individual transfections varied by less than 20%.

| Cells | pBL rel. activ. | p18URRL rel. activ. | p18/3'Sp1MURRL rel. activ. | %Repr. | p18/5'Sp1MURRL rel. activ. | %Repr. |
|---|---|---|---|---|---|---|
| HeLa | 1.0 | 33.2 | 8.9 | 73 | 32.4 | 2 |
| SW756 | 1.0 | 23.3 | 8.6 | 63 | 19.8 | 15 |
| SiHa | 1.0 | 63.8 | 22.3 | 65 | 57.9 | 9 |
| CaSki | 1.0 | 39.4 | 12.6 | 68 | 36.4 | 8 |
| Me180 | 1.0 | 88.5 | 20.4 | 77 | 80.8 | 9 |
| HaCaT | 1.0 | 53.1 | 17.3 | 67 | N.D. | N.D. |
| C33A | 1.0 | 27.9 | 25.4 | 9 | 27.8 | 0 |
| Fib | 1.0 | 6.8 | 5.0 | 26 | 6.8 | 0 |
| HEL | 1.0 | 7.0 | 4.8 | 31 | 5.8 | 17 |
| NIH3T3 | 1.0 | 9.0 | 6.3 | 30 | 8.4 | 7 |
| CV-1 | 1.0 | 5.3 | 3.7 | 30 | 4.7 | 11 |
| Saos-2 | 1.0 | 9.0 | 6.8 | 24 | 8.8 | 2 |

selective mutation of the promoter proximal Sp1 element in plasmid p18/3'Sp1MURRL results in an strong (73%) decrease of the activity of the E6/E7-promoter in HeLa cells (Table 1). Comparable repression (between 63–77%) was observed in other cervical carcinoma cells as SW756, SiHa, CaSki, Me180 and in the spontaneously immortalized human keratinocyte cell line HaCaT (Table 1). These results indicate, that the promoter proximal Sp1 element plays an important role for the activity of the HPV18 URR in cells of epithelial origin.

Interestingly, when tested in the cervical carcinoma cell line C33A, the mutation of the Sp1 element led to only a marginal reduction (9%) of the activity of the E6/E7-promoter, suggesting that in this particular cell line the activity of the HPV18 URR is regulated by alternative control mechanisms.

The HPV18 URR has been reported to be either inactive or weakly active in non-epithelial cells such as fibroblasts (12, 13, 28). While we did not detect significant activity of the HPV18 URR in SV80 fibroblasts or HepG2 hepatoma cells (not shown), we could measure a low stimulation of the E6/E7-promoter in primary human dermal fibroblasts (Fib), in human embryonic lung fibroblasts (HEL), in the mammalian fibroblast cell lines CV-1 and NIH 3T3 and in Saos-2 osteosarcoma cells (between 10% to 17% of the activity obtained with HaCaT keratinocytes, see Table 1). In these cell lines, the selective mutation of the Sp1 element in the context of the complete HPV18 URR led to only a weak decrease (between 26–31%) in the overall stimulatory activity of the E6/E7-promoter (Table 1). Thus, with the exception of C33A cells, mutation of the promoter proximal Sp1 element consistently resulted in a much higher decrease in the activity of HPV18 URR in epithelial cells when compared with cell lines of fibroblast origin.

Binding studies indicated the presence of an additional sequence-aberrant Sp1 recognition site (GCGCCC, HPV18 nucleotides 7316–7321) in the 5'-portion of the HPV18 URR (28). Sp1 elements have been shown to possess the potential to

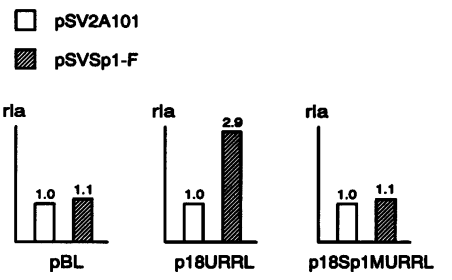


**Figure 4.** Stimulation of the HPV18 URR by overexpressed Sp1 protein. CV-1 cells were transfected with luciferase plasmids pBL (left panel), p18URRL (central panel) and p18/3'Sp1MURRL (right panel) together with either control vector pSV2A101 (open columns) or Sp1 expression vector pSVSp1-F (dashed columns). rla, relative luciferase activities above cotransfection with control vector pSV2A101. Results represent the average fold activation of six seperate transfections, each performed in triplicates. Results from individual transfections varied by less then 20%.

synergistically activate transcription even when separated by a large distance (29). We therefore adressed the question whether the Sp1 element located in the 5'-URR might contribute to the transcriptional activation of the HPV18 E6/E7-promoter by functionally cooperating with the promoter proximal Sp1 binding site. To maintain the physiological spacing between these two elements, the Sp1 element within the 5'-URR was mutated by site specific mutagenesis in the context of the complete HPV18 URR resulting in construct p18/5'Sp1MURRL (Fig. 3).

Introduction of this mutation, which abolishes Sp1 binding in vitro (28), did not significantly effect the activity of the E6/E7-promoter as assessed by transient luciferase assays in cells of epithelial and non-epithelial origin (Table 1). These results indicate that the activation of the HPV18 E6/E7-promoter by the promoter proximal Sp1 element does not require functional cooperativity with the Sp1 element contained within the 5'-portion of the URR.

## Overexpression of Sp1 stimulates the expression from the HPV18 E6/E7-promoter

To further analyse the influence of Sp1 on the activity of the HPV18 E6/E7-promoter, we performed cotransfection assays using HPV18 reporter plasmids and expression vectors for human Sp1-protein (kindly provided by Dr. J.D.Saffer). For cotransfection experiments we chose CV-1 cells, since these cells were found to be suitable for Sp1 overexpression studies as they contain only limiting amounts of endogenous Sp1 (23). Two vectors were used for cotransfection with HPV18 reporter plasmids: (i) plasmid pSVSp1-F, which encodes a fully functional wild-type Sp1 protein devoid of the extreme amino-terminus and (ii) control vector pSV2A101, which is identical to pSVSp1-F except that the Sp1-coding sequence is missing. Both constructs have been described in detail elsewhere (23).

As shown in Figure 4 (central panel), overexpression of Sp1 by cotransfected pSVSp1-F led to an approximately three fold activation of reporter plasmid p18URRL. In contrast, the activity of the basic luciferase vector pBL was not influenced by overexpressed Sp1 (left panel), indicating that the Sp1 mediated transactivation is not due to sequences within the vector backbone of pBL.

Furthermore, reporter plasmid p18/3'Sp1MURRL, which carries a mutation in the promoter proximal Sp1 recognition sequence but otherwise is identical to p18URRL, was not responsive to Sp1 (right panel). These findings indicate, that this Sp1 recognition motif is essential for the stimulation of E6/E7 transcription by overexpressed Sp1 protein.

In conclusion, these results demonstrate that the activity of the HPV18 URR can be increased in vivo by augmented Sp1 levels and show, that this response is mediated by the sequence aberrant Sp1 element located close to the E6/E7-promoter.

## DISCUSSION

The results of this study indicate that the cellular transcription factor Sp1 binds specifically to a sequence aberrant recognition motif within the promoter proximal part of the HPV18 URR and plays an important role for the activation of the E6/E7-promoter in cell lines of epithelial origin.

We and others (13, 30) previously reported that the promoter proximal region of the HPV18 URR by itself exhibits only weak cis-stimulatory activity, indicating that the strong contribution of the Sp1 element to the overall stimulation of the E6/E7-promoter requires the cooperative interaction with transcriptional elements contained within other parts of the URR. It is known that both the exact spacial arrangement between distinct cis-regulatory elements and the specific constitution of a particular transcriptional control region determines the functional significance of a individual regulatory element (31, 32). Based on these findings, it was important to analyse the regulatory role of the promoter proximal Sp1 element by site-specific mutagenesis within the natural context of the HPV18 URR.

Comparative analyses of wildtype and mutated reporter constructs indicate, that the promoter proximal Sp1 element plays a key role for the stimulation of the E6/E7-promoter in a variety of cell lines. Although the number of cell lines investigated in this study is limited, our results raise the possibility, that the contribution of this element to the overall activity of the HPV18 URR is somewhat cell-type dependent. After mutation of the promoter proximal Sp1 element we consistently observed a much stronger decrease in E6/E7-promoter activity in epithelial cell lines when compared with cells of non-epithelial origin. These findings could indicate, that Sp1, although being considered as a rather ubiquitous factor, contributes to the epithelial-cell preferent activity of the HPV18 URR, possibly by the functional cooperation with an epithelial cell specific regulatory protein. Indeed, it has been shown, that the transcriptional activation of TATA-box containing promoters by Sp1 requires the interaction with coactivatory proteins (33), some of which might be cell-type specific.

Interestingly, the HPV-negative cervical carcinoma cell line C33A behaved differently to all other cell lines investigated in this study. In these cells, the mutation of the promoter proximal Sp1 element had almost no effect on the overall activity of the HPV18 URR (Table 1), although we found that C33A cells contain endogenous Sp1 protein capable of binding to the promoter proximal Sp1 element (Hoppe-Seyler and Butz, unpublished data). These findings indicate, that in C33A cells the efficient stimulation of the HPV18 E6/E7-promoter results from alternative regulatory pathways which can substitute for the activity of the promoter proximal Sp1 element. Consistent with the notion of alternative regulatory pathways for HPV18 E6/E7-promoter stimulation in this particular cell line, recent experiments designed to analyse the functional role of AP1 for the activation of the HPV18 URR revealed that the mutation of the AP1 elements within the HPV18 URR led to a much weaker reduction in the activity of the HPV18 URR in C33A cells when compared to HaCaT or HeLa cells (34). Furthermore, footprint analyses in C33A cells showed marked differences in the protein-binding pattern to subregions of the HPV18 URR when compared with other cervical carcinoma cell lines (15).

We show by cotransfection assays, that an elevated level of Sp1 within a cell can lead to an increase in the activity of the HPV18 E6/E7-promoter. This stimulation is dependent on the integrity of the promoter proximal Sp1 binding motif. In this context it is noteworthy, that another small DNA virus, namely SV40, can enhance the level of Sp1-protein in infected cells, most likely by an T antigen mediated activity (23). Since the SV40 T antigen and the HPV E7 proteins share both structural homologies (35) and functional activities (36), one should be aware of the possibility that papillomaviruses could possess an SV40 analogous mechanism for the benefit of Sp1 stimulated viral regulatory elements in the sense of a positive autoregulatory loop.

Sp1 elements have been shown to play an important role for the biology of bovine papillomavirus type 1 (BPV1), being involved in transformation and viral replication (37) as well as in the activation of the BPV1 E2 and E5 genes (38). These results suggest, that Sp1 is involved in several important regulatory aspects in the life cycle of papillomaviruses. Consistent with this notion, in addition to the E6/E7-promoter proximal Sp1 binding site another Sp1 element is present in the 5'-part of the HPV18 URR (28). As the mutation of this element did not significantly influence the activity of the HPV18 E6/E7-promoter (Table 1) it probably does not contribute to the activation of the E6/E7-transcription but rather might be either involved in the stimulation of a yet unknown viral promoter or participate in HPV replication analogous to an Sp1 element within the BPV1 5'-URR (37).

Intriguingly, the activity of Sp1 elements can be modulated by papillomavirus gene products. It has been demonstrated, that the viral E2 transregulatory protein can functionally cooperate with Sp1 in transactivation (39, 40). In addition, in HPV16 the viral E2 protein has been proposed to repress Sp1 mediated activation of the E6/E7-promoter by sterically interfering with the binding of Sp1 to its recognition sequence (41). A similar regulatory mechanism might apply to the situation in HPV18, where the Sp1 binding sequence analysed in the present study is also located directly adjacent to an E2 binding site. Taken together, these findings imply that both the positive and negative interaction of viral gene products with Sp1 plays an important role in the transcriptional regulation of papillomaviruses.

The results obtained in this study indicate, that the promoter proximal Sp1 element plays a major role for the activation of the viral E6/E7-promoter, thus suggesting that Sp1 is a key factor for an efficient stimulation of viral transforming gene expression. The activity of the Sp1 element requires the cooperative interaction with yet uncharacterised regulatory elements within the HPV18 URR. Their identification will be required to gain more insight into the molecular mechanisms involved in the activation of the E6/E7-promoter. This hopefully will contribute to a better understanding of the regulatory mechanisms resulting in HPV18 transforming gene expression.

## ACKNOWLEDGEMENTS

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