Journal of General Virology (2002), **83**, 503–510. Printed in Great Britain SHORT COMMUNICATION

The hinge region of the human papillomavirus type 8 E2 protein activates the human p21$^{WAF1/CIP1}$ promoter via interaction with Sp1

Gertrud Steger, Carsta Schnabel and Hanns-Martin Schmidt†

Institute of Virology, University of Cologne, Fürst-Pückler-Strasse 56, 50935 Cologne, Germany

The E2 proteins regulate papillomavirus (PV) gene expression by sequence-specific DNA binding. However, E2 is also able to activate in the absence of E2 binding sites. We show here that the E2 protein of human PV type 8 (HPV8) can activate the expression of p21$^{WAF1/CIP1}$ via promoter-proximal 200 nucleotides, which contain several Sp1 binding sites and no E2 binding sites. HPV8 E2 lacking the activation domain, which is rather conserved among E2 proteins, cooperated with co-expressed Sp1 in stimulation of the p21$^{WAF1/CIP1}$ promoter, in contrast to HPV18 E2 lacking the activation domain. We can demonstrate that the internal non-conserved hinge region of HPV8 E2 is sufficient for this functional cooperativity with Sp1. In correlation, the hinge of HPV8 E2 directly binds to Sp1. These results suggest that HPV8 E2 might be able to 'super'-activate Sp1-mediated transcription by a direct interaction via the non-conserved hinge region.

The E2 protein of papillomaviruses (PV) regulates virus transcription and is required for replication of the viral DNA. E2 is a sequence-specific DNA binding factor, which recognizes the palindromic recognition sequence ACCN$_6$GGT, present in multiple copies within the virus genome (Spalholz *et al.*, 1985; Li *et al.*, 1989). The various E2 proteins average 30% amino acid sequence identity (Giri & Yaniv, 1988). The conserved amino acids localize within the N-terminal activation domain (AD) and the C-terminal DNA binding and dimerization domain (DBD). These two conserved domains are separated by a hinge region of variable length and amino acid composition (McBride *et al.*, 1989b). Activation of transcription by E2 involves the direct interaction of the AD with components of the preinitiation complex (Miller Rank & Lambert, 1995; Benson *et al.*, 1997; Yao *et al.*, 1998) and with co-factors like CBP and its homologue p300 or AMF-1/Gps2 (Breiding *et al.*, 1997; Peng *et al.*, 2000; Lee *et al.*, 2000). In addition to these contacts, E2 has to cooperate with other sequence-specific DNA binding factors for efficient activation. Activation of gene expression in cooperation with Sp1 seems to be characteristic of all E2 proteins (Ham *et al.*, 1991, 1994; Spalholz *et al.*, 1991; Li *et al.*, 1991; Ushikai *et al.*, 1994; Steger *et al.*, 1995). It has been suggested that a direct interaction between bovine PV type 1 (BPV1) E2 and Sp1 will be involved (Li *et al.*, 1991). In addition, E2 proteins are able to stimulate transcription also in the absence of E2 binding sites (Haugen *et al.*, 1988; Heike *et al.*, 1989).

We have observed that the E2 protein of human PV type 8 (HPV8), which is associated with the rare disease epidermodysplasia verruciformis (ev), is able to enhance the protein level of the cyclin-dependent kinase inhibitor p21$^{WAF1/CIP1}$ (p21) in the HPV-negative and p53-negative skin keratinocyte cell line RTS3b (Purdie *et al.*, 1993) (data not shown). P21 plays important roles in regulation of cell growth, arrest or progression, DNA methylation, cell senescence, apoptosis and differentiation (reviewed in Dotto, 2000). Its expression is regulated by many inducers, among them p53 and factors that control differentiation of diverse cell types including skin cells (Prowse *et al.*, 1997). Some of these factors mediate their effects on p21 gene expression via the promoter-proximal 210 nucleotides (Kardassis *et al.*, 1999 and references therein). This region is GC-rich and contains five sequences that resemble Sp1 binding sites and there is no E2 consensus sequence present within this promoter fragment (Fig. 1A). In order to test whether this promoter-proximal region might be sufficient for HPV8 E2 to stimulate p21 activity, we transiently transfected a CMV promoter-driven HPV8 E2 expression vector (Stubenrauch & Pfister, 1994) together with a luciferase reporter construct comprising the promoter-proximal region from +15 to −210 of p21 (Prowse *et al.*, 1997) (Fig. 1A) in RTS3b cells. HPV8 E2 led to a low but distinct 2-fold activation. Surprisingly, when we expressed the HPV8 E2ΔN, lacking the AD, the promoter was stimulated 10-fold, suggesting that the AD of HPV8 E2 is not necessary for activation of

Author for correspondence: Gertrud Steger.
Fax +49 221 478 3902. e-mail Gertrud.Steger@uni-koeln.de

† **Present address:** Amaxa Biosystems GmbH, Gottfried-Hagen-Strasse 60–62, 51105 Cologne, Germany.

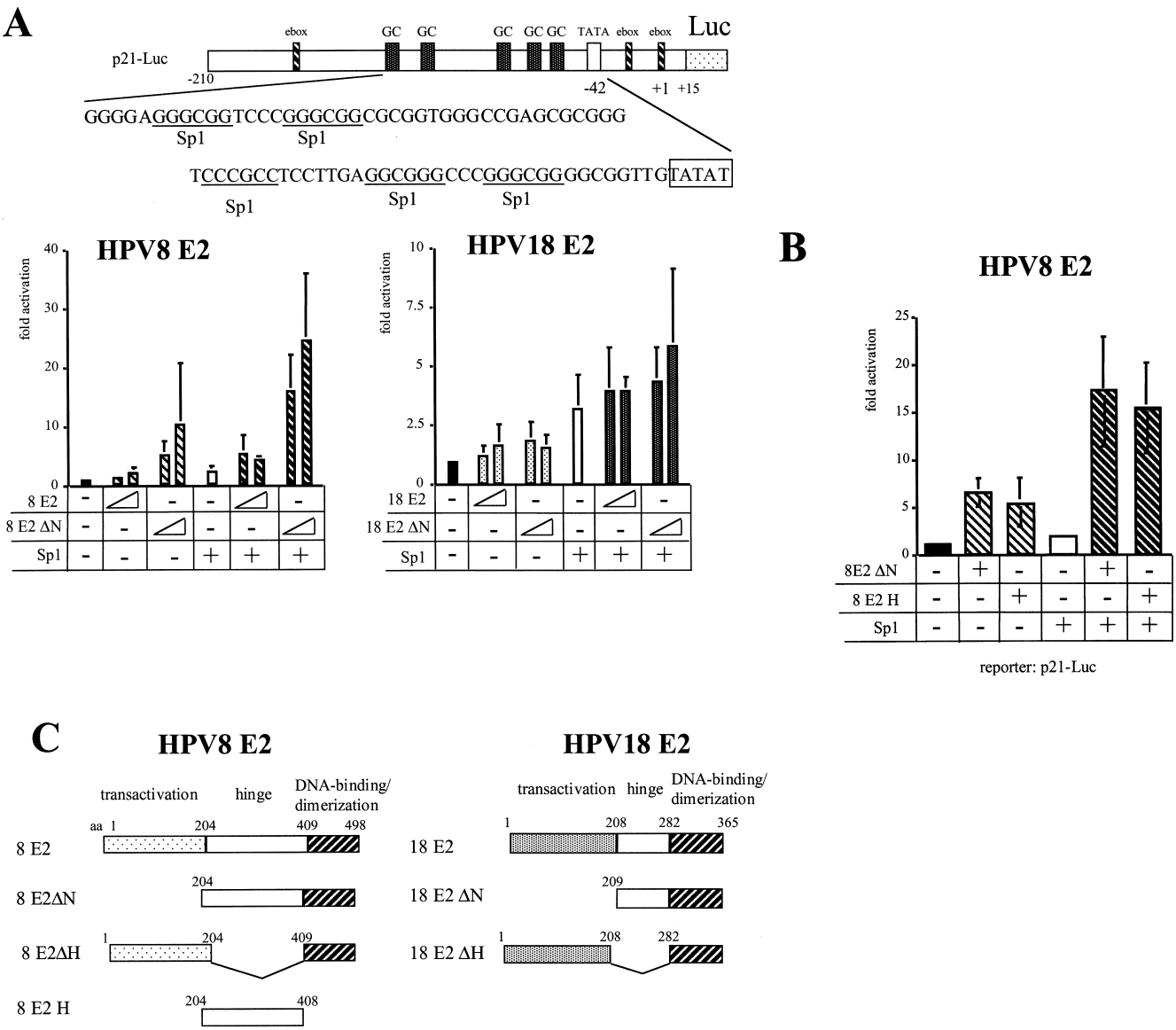
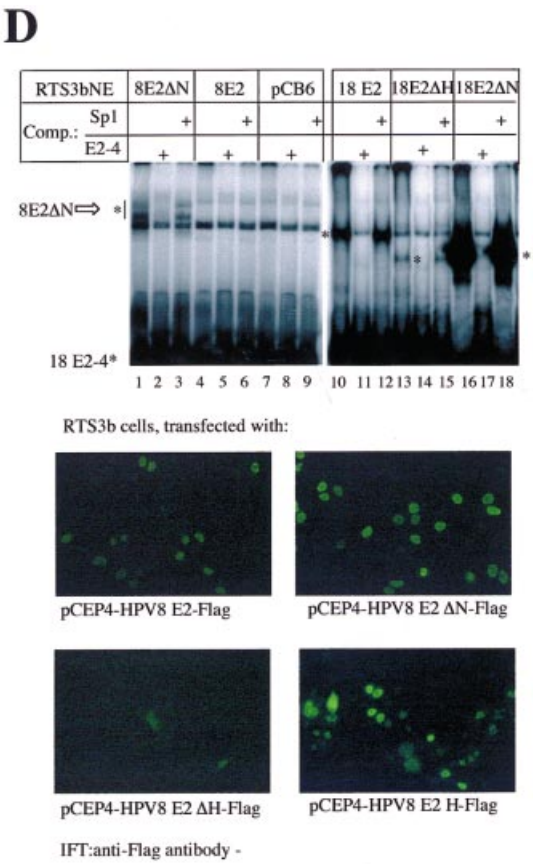


Fig. 1. The hinge region of HPV8 E2 is sufficient for cooperation with Sp1 in activation of the p21 promoter. (A) RTS3b cells were transfected using the FuGene reagent (Roche Diagnostics) with a luciferase reporter construct containing the human p21 promoter from position −210 to +15. A schematic representation of the promoter is shown above the graphs, including the sequence from position −120 to −42 containing five Sp1 binding sites and the TATA box, as indicated. The graph on the left shows the results of transiently co-transfecting this p21-Luc reporter construct with increasing amounts (200 ng and 400 ng, indicated by triangles) of pCB6-based expression vectors encoding HPV8 E2 (8 E2) (Stubenrauch & Pfister, 1994) or a deletion mutant lacking the N-terminal AD (8 E2ΔN) either alone or in combination with 150 ng of an expression vector for human Sp1, indicated by a plus sign. The graph on the right shows the results of co-transfecting 200 or 400 ng of CMV promoter-driven expression vectors for HPV18 E2 (18 E2) or for a deletion mutant lacking the N-terminal AD (18 E2ΔN) either alone or in combination with 150 ng of the expression vector for Sp1. Note the different scales of the two graphs. (B) The p21-Luc reporter construct has been co-transfected with 400 ng of pCB6-based expression vectors either encoding HPV8 E2 lacking the N-terminal AD (8 E2ΔN) or encoding the hinge region (8 E2H) in the absence or presence of 150 ng expression vector for Sp1. In each case, the activity of the p21-Luc construct on its own was set as 1 and the fold activation has been calculated. The values represent the means of three to five independent experiments and the standard deviations are given. (C) Schematic representation of E2 proteins of HPV8 and -18. The positions of the N-terminal AD (transactivation), the internal hinge and the C-terminal DBD (DNA binding dimerization) are indicated. The numbers above the entire ORF refer to the amino acids (aa) encoding the different domains in the two proteins. The structures of the deletion mutants of HPV8 E2 and HPV18 E2 used in Figs 1 and 2 are also shown. (D) Gel shift assays with nuclear extracts and immunofluorescence tests of transiently transfected RTS3b cells to monitor the expression levels of the various E2 proteins. Gel shift assays using 15 μg of nuclear extracts from RTS3b cells which have been transiently transfected with the pCB6-based expression vectors for HPV8 E2, HPV8 E2ΔN, the vector alone (pCB6) or with the pCMV2-HPV18 E2, pCMV2-HPV18 E2ΔN and pCMV2-HPV18 E2ΔH (used in Fig. 2) as indicated above were performed as described in Boeckle *et al.* (2002). As probe we used the ^{32}P-labelled



the p21 promoter. Since the promoter fragment contains a cluster of Sp1 binding sites we wondered whether HPV8 E2 functionally interacts with Sp1 in activation and co-transfected an expression vector for Sp1. Sp1 on its own stimulated promoter activity 2·2-fold. HPV8 E2 and co-expressed Sp1 led to a 5·3-fold activation, indicating weak or no cooperativity. In contrast, co-expression of HPV8 E2ΔN and Sp1 resulted in a 24-fold activation. The enhancement of the activation mediated by HPV8 E2ΔN due to overexpression of Sp1 is significant ($P = 0{\cdot}012$), suggesting that activation by HPV8 E2ΔN involves functional interaction with Sp1. Since E2 proteins are rather conserved within their structure and function, we used the E2 protein of HPV18 to test whether this is true for another E2 protein. HPV18 E2 on its own also weakly stimulated the promoter (2-fold) as well as the mutant lacking the AD (HPV18 E2ΔN). These activations increased up to 4- and 5·5-fold, respectively, when Sp1 was co-expressed (Fig. 1A). Thus, HPV18 E2ΔN does not cooperate with Sp1 in activation of the p21 promoter, in contrast to HPV8 E2ΔN. This difference is not related to differential expression of both proteins. Gel-shift assays using nuclear extracts from RTS3b cells transiently transfected with the corresponding expression vectors revealed that HPV18 E2ΔN was even present in higher amounts than HPV8 E2ΔN (Fig. 1D). The lack of cooperativity between HPV18 E2ΔN and Sp1 does not result from squelching due to unphysiologically high amounts, since we tested a broad range of concentrations of expression vectors (data not shown). The E2 proteins are conserved within their DBDs and not within their internal hinge region (Giri & Yaniv, 1988). Therefore, we wondered whether the hinge region of HPV8 E2 might be responsible for this cooperation with Sp1. Transfecting an expression vector encoding solely the hinge region of HPV8 E2 (HPV8 E2H) revealed that it was able to activate and to cooperate with co-expressed Sp1 to the same extend as HPV8 E2ΔN (Fig. 1B). As shown by immunofluorescence tests of RTS3b cells transiently transfected with the plasmids expressing Flag-tagged HPV8 E2 proteins, HPV8 E2ΔN and HPV8 E2H were localized in the nucleus and expressed to comparable levels (Fig. 1D).

As in the case of other E2 proteins, the AD of HPV8 E2 is absolutely required for activation of a classical E2 responsive promoter, whereas the hinge region is dispensable (Fig. 2). A reporter construct with a synthetic promoter composed of the adenovirus major late minimal promoter downstream of two high affinity Sp1 binding sites and four classical E2 binding sites (described in Ham *et al.*, 1994) has been co-transfected with expression vectors for HPV8 E2 or deletion mutants, which are indicated in Fig. 1(C), into the cervical carcinoma cell line C33A, as described previously (Steger & Corbach, 1997). The E2 protein of HPV8, expressed from the vector CEP4 (Invitrogen), which is able to replicate to high copy number, was able to activate the promoter 16-fold. HPV8 E2ΔH, lacking the hinge region, reached the same maximal activation when transfecting high amounts of expression vector. This was necessary since HPV8 E2ΔH was expressed in lower levels than wild-type E2 (Fig. 1D). HPV8 E2ΔN did not activate. HPV18 E2 stimulated the promoter on average 12-fold. A similar level of activation was achieved by co-transfection of

high affinity promoter-distal E2 binding site −4 of HPV18 (Steger & Corbach, 1997). The specificity of the protein–DNA complexes has been demonstrated by adding a 400-fold excess of unlabelled unrelated oligonucleotide, which codes for an Sp1 binding site, or by the addition of a 400-fold excess of the homologous oligonucleotide as competitor (Comp.), as indicated. The positions of the various E2 proteins are labelled by an asterisk. In order to detect the HPV8 E2H, the full-length HPV8 E2 and the HPV8 E2ΔH, the latter two were expressed in too low concentrations to be detected in gel shift assays, the ORFs for the different HPV8 E2 versions were fused with a Flag epitope and cloned into the expression vector pCEP4 (Invitrogen). Immunofluoresence tests of RTS3b cells, which have been transfected with these constructs, and the anti-Flag M5 antibody (Kodak) have been performed as described in Boeckle *et al.* (2002) and are shown at the bottom.

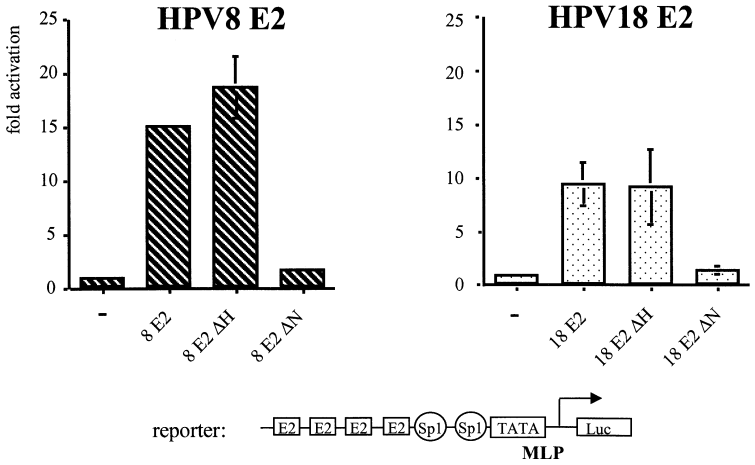


Fig. 2. The N-terminal AD is necessary for activation of a classical E2 responsive promoter. C33A cells have been transfected by the $CaCl_2$-method with a luciferase reporter construct containing a synthetic promoter, composed of the minimal adenovirus major late promoter (MLP) in front of four E2 and two Sp1 binding sites, as indicated beneath. In the case of HPV8 E2, 750 ng of empty pCEP4 vector (lane −) or pCEP4-based expression vectors encoding either the E2 protein of HPV8 and truncated versions thereof, as indicated in the graph, were transiently co-transfected with the reporter construct. In the case of HPV18 E2, the results of transfecting 200 ng of pCMV2 (lane −), pCMV2-HPV18 E2, pCMV2-HPV18 E2ΔH and pCMV2-HPV18 E2ΔN are shown. The values represent the fold activation of the promoter activity in the presence of any E2 expression vector and are the means of three independent experiments. The error bars represent the standard deviations.

an expression vector for HPV18 E2 lacking the hinge region (HPV18 E2ΔH). The AD was absolutely required since HPV18 E2ΔN failed to activate (Fig. 2).

In order to test whether a direct interaction between HPV8 E2 and Sp1 might be involved in the cooperative activation of the p21 promoter we performed GST pull down assays, as described in Enzenauer *et al*. (1998). Radioactively labelled E2 proteins of HPV8 and HPV18, produced by *in vitro*-translation via a rabbit reticulocyte lysate, were passed over a GST–Sp1-loaded column. The Sp1 protein lacks the 90 N-terminal amino acids. HPV8 E2 as well as HPV18 E2 was specifically retained by GST–Sp1 (Fig. 3A). In order to map the regions of E2 binding to Sp1, the various domains of the E2 proteins have been fused to GST to incubate them with purified Sp1 (Promega). Bound Sp1 was analysed in a Western blot with a polyclonal antiserum directed against Sp1 (Santa Cruz). Fig. 3(B) shows that purified Sp1 interacts with the AD and the DBD of HPV18 E2. Neither the GST–HPV18 E2 hinge fusion protein nor GST alone was able to precipitate Sp1. In a vice versa experiment, we could detect binding of *in vitro*-translated HPV18 E2 DBD as well as of HPV18 E2 AD to GST–Sp1 (data not shown). BPV1 E2 seems to interact in a similar way with Sp1, since purified Sp1 was retained by the AD and the DBD of BPV1 E2 (Fig. 3B).

In the case of HPV8 E2, purified Sp1 specifically bound to the hinge region and to the DBD, expressed as GST fusion

Fig. 3. (A) The E2 proteins of HPV8 and HPV18 bind Sp1. An Sp1 protein, lacking the N-terminal 90 amino acids fused to GST (lane 2) and GST alone (lane 1) were incubated with *in vitro*-translated (IVT), radioactively labelled full-length E2 proteins of HPV8 (lanes 5, 6) and HPV18 (lanes 3, 4). 10% of the input was loaded in each case. GST (lane 1) and the GST–Sp1 fusion protein (lane 2) used in this assay are shown on the protein gel stained with Coomassie blue on the left. The positions of marker proteins as well as those of the E2 proteins are indicated. (B) Sp1 directly binds to the N-terminal ADs of HPV18 and BPV1 E2, as well as to their C-terminal domains. The upper part shows Western blots developed with an antibody against Sp1 to analyse the binding of purified Sp1 to the GST fusion proteins encoding the N-terminal activation domain (AD, lane 1), the DNA binding/dimerization domain (DBD, lane 2), the hinge region (H, lane 3) and a protein encoding the AD and the hinge (lane 4) of HPV18 E2. In the case of BPV1, the interaction analysis of Sp1 with the DBD (lane 5), the hinge (H, lane 6) and the activation domain (AD, lane 7) all fused to GST as well as with GST alone is shown. The lane '10% inputs' represents 10% of the amount of Sp1 used in the interaction assay. The position of Sp1 is indicated. SDS gels stained by Coomassie blue reveal the various GST fusion proteins used for the interaction assays. The domains within the E2 ORFs including the amino acids (aa) which have been fused to GST are indicated at the bottom, respectively. (C) Sp1 directly binds to the hinge region and to the DNA binding dimerization/domain of HPV8 E2. HPV8 E2 (lane 1), its N-terminal activation domain (AD, lane 2), its hinge region (H, lane 3) and its C-terminal DNA binding/dimerization domain (DBD, lane 4) have been fused to GST. The purified fusion proteins have been either incubated with purified Sp1 or with HeLa nuclear extracts, as indicated, and bound Sp1 was analysed in a Western blot. 10% of purified Sp1 or HeLa nuclear extracts used in the experiment was included in the Western blot (indicated by 10% input). A protein gel with the different GST HPV8 E2 fusion proteins is shown above a schematic representation of the E2 protein and the corresponding domains, which have been fused to GST. The positions of the full-length GST–HPV8 E2 proteins in the SDS gel are indicated by an asterisk. The amino acids encoding the different domains fused to GST are indicated.

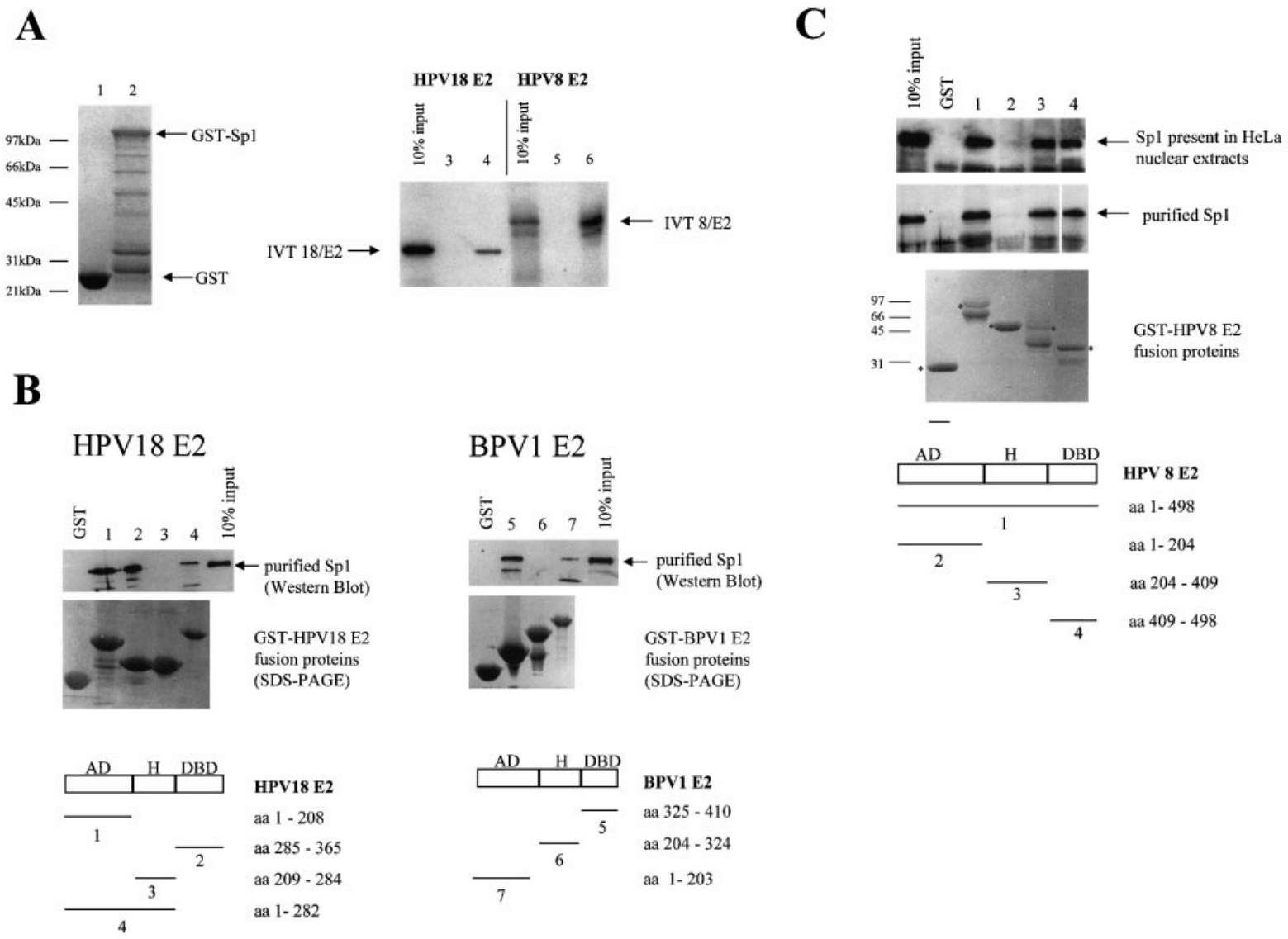


Fig. 3. For legend see facing page.

proteins (Fig. 3C). The results were identical when the HPV8 E2 GST fusion proteins were incubated with nuclear extracts from HeLa cells to analyse binding of endogenous Sp1 (Fig. 3C). Furthermore, the binding of Sp1 to the hinge and to the DBD could be confirmed in a vice versa experiment using *in vitro*-translated HPV8 E2 derivatives and Sp1 fused to GST (data not shown). Surprisingly, we could not detect any interaction of the AD of HPV8 E2 with Sp1 (Fig. 3C), although it is related to the ADs of the E2 proteins of HPV18 and BPV1 (Giri & Yaniv, 1988), which bind to Sp1 (Fig. 3B). For GST pull down assays we used E2 proteins either fused to GST or produced via a rabbit reticulocyte lysate to reduce the risk of misfolded proteins. However, we cannot exclude that this was the reason for the lack of interaction of the AD of HPV8 E2 with Sp1. Sp1 was applied either purified or fused to GST. Since interactions could also be confirmed with endogenous Sp1 present in HeLa cells (Fig. 3C) we exclude artificial binding of Sp1 due to misfolding.

As in the case of HPV18 E2 (Fig. 2) and BPV1 E2 (Steger *et al*., 1995), the AD of HPV8 E2 is required for cooperation with Sp1 in activation of the promoter containing high affinity Sp1 and E2 binding sites (Fig. 2). This activation may involve the binding of the N terminus to components of the preinitiation complex or to co-factors, as identified for other E2 proteins (Miller Rank & Lambert, 1995; Benson *et al*., 1997; Yao *et al*., 1998). These contacts seem to be absolutely required also for HPV8 E2, since HPV8 E2ΔN does not activate the synthetic promoter. Thus, the role of the direct interaction of the AD of BPV1 E2 and that of HPV18 E2 with Sp1 remains unclear for this kind of activation. In addition to Sp1, E2 shows cooperative activation with a variety of sequence-specific DNA binding factors such as AP1, USF, TEF-1, NF1/CTF when the corresponding binding sites as well as the E2 binding sites have been cloned upstream of the promoter (Ham *et al*., 1991; Ushikai *et al*., 1994). A direct interaction between E2 and these cooperation partners has not yet been shown, implying that this kind of cooperativity (also) may occur without direct binding. The role of the binding of the DBD to Sp1 in activation of transcription remains unclear as well. It is unlikely that this interaction is mediated by contaminating DNA since it occurs also in the presence of ethidium bromide (data not shown). Furthermore, we used purified proteins for our protein–protein interaction studies. Interactions between transcription factors without any relevance for transactivation have been observed previously. For example, the binding of the DNA binding domain of Oct1 to TBP does not play any role in activation (Arnosti *et al*., 1993). However, it might still be possible that these interactions are involved in other not yet characterized processes.

The mechanism of activation of the p21 promoter by the hinge region of HPV8 remains unclear. Our data presented here suggest that the direct interaction of this hinge region with Sp1 may be involved. It might be possible that the hinge mediates oligomerization of Sp1, which increases its affinity to the p21 promoter. Furthermore, the HPV8 E2H bound on the promoter via Sp1 may promote the recruitment of other cooperating factors such as p300. P300 has been shown to be required for p21 induction in differentiating keratinocytes (Xiao *et al*., 2000). In line with this model, we could show that p300 also interacts with the hinge of HPV8 E2 (A. Müller & G. Steger, unpublished results). A similar mechanism in stimulation of p21 gene expression has been described for c-Jun and the Smads protein (Kardassis *et al*., 1999; Pardali *et al*., 2000). Also in the case of c-Jun, which binds via its leucine zipper to Sp1, the AD is not necessary for activation of the p21 promoter.

The stronger activation of the p21 promoter by HPV8 E2ΔN and E2H compared to the wild-type HPV8 E2 protein (Fig. 1A, B) might be due to the fact that E2ΔN and E2H are expressed to higher levels than the full-length protein (Fig. 1D). Thus, small amounts of HPV8 E2 may not be sufficient for efficient cooperation with Sp1. Furthermore, the N-terminal AD may somehow interfere with Sp1 function, thereby inhibiting cooperativity mediated by the hinge. Moreover, regions required for functional interaction might only be accessible in the N-terminally truncated version. It is not known whether these forms of E2 are expressed in the case of HPV8, as has been shown for other E2 proteins (Lambert *et al*., 1987; Liu *et al*., 1995; Stubenrauch *et al*., 2000). However, in an ev lesion induced by HPV5, which is closely related to HPV8, a series of spliced transcripts, with the potential to encode an E2 protein starting at amino acid 202, have been identified (Haller *et al*., 1995).

Initially, the hinge region was suggested to function as a flexible linker to connect the AD and the DBD. Meanwhile, discrete functions for the hinge region of various E2 proteins have been described. For example, signals for phosphorylation and nuclear localization have been mapped to the hinge of BPV1 E2 and HPV11 E2, respectively (McBride *et al*., 1989a; Lehman *et al*., 1997; Pernose & McBride, 2000; Zou *et al*., 2000). Previously, we could demonstrate an involvement of the hinge of BPV1 E2 in regulation of gene expression (Steger *et al*., 1995). In contrast to other E2 proteins, the E2 proteins of PV associated with ev display a very long hinge region, rich in arginine, serine and glycine residues, indicating specialized function. In correlation with this, the hinge of HPV5 E2 was shown to interact with splicing factors and to enhance splicing (Lai *et al*., 2000). Our data presented here suggest a novel mechanism of transactivation by the hinge region of HPV8 E2, probably mediated through protein–protein interaction with the important cellular regulator Sp1. By inducing the expression of p21 in infected keratinocytes, the hinge of HPV8 E2 might affect keratinocyte differentiation and thus contribute to HPV8-induced pathogenesis.

We thank G. P. Dotto for providing plasmids and Andrew Barker for critical reading of the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft (STE 604/3-1; SFB 274/A8).

References

Arnosti, D. N., Merino, A., Reinberg, D. & Schaffner, W. (1993). Oct-2 facilitates functional preinitiation complex assembly and is continuously required at the promoter for multiple rounds of transcription. *EMBO Journal* **12**, 157–166.

Benson, J. D., Lawande, R. & Howley, P. M. (1997). Conserved interaction of the papillomavirus E2 transcriptional activator proteins with human and yeast TFIIB proteins. *Journal of Virology* **71**, 8041–8047.

Boeckle, S., Pfister, H. & Steger, G. (2002). A new cellular factor recognizes E2 binding sites of papillomaviruses which mediate transcriptional repression by E2. *Virology* (in press).

Breiding, D. E., Sverdrup, F., Grossel, M., Moscufo, N., Boonchai, W. & Androphy, E. J. (1997). Functional interaction of a novel cellular protein with the papillomavirus E2 transactivation domain. *Molecular and Cellular Biology* **17**, 7208–7219.

Dotto, G. P. (2000). $p21^{WAF1/Cip1}$: more than a break to the cell cycle? *Biochimica et Biophysica Acta* **1471**, M43–M56.

Enzenauer, C., Mengus, G., Lavigne, A.-C., Davidson, I., Pfister, H. & May, M. (1998). Interaction of human papillomavirus 8 regulatory proteins E2, E6 and E7 with components of the TFIID complex. *Intervirology* **41**, 80–90.

Giri, I. & Yaniv, M. (1988). Structural and mutational analysis of E2 transactivating proteins of papillomaviruses reveal three distinct functional domains. *EMBO Journal* **7**, 2823–2829.

Haller, K., Stubenrauch, F. & Pfister, H. (1995). Differentiation-dependent transcription of the epidermodysplasia verruciformis-associated human papillomavirus type 5 in benign lesions. *Virology* **214**, 245–255.

Ham, J., Dostatni, N., Arnos, F. & Yaniv, M. (1991). Several different upstream promoter elements can potentiate transactivation by the BPV-1 E2 protein. *EMBO Journal* **10**, 2931–2940.

Ham, J., Steger, G. & Yaniv, M. (1994). Cooperativity in vivo between the E2 transactivator and the TATA box binding protein depends on core promoter structure. *EMBO Journal* **13**, 147–157.

Haugen, T. H., Turek, L., Mercurio, F. M., Cripe, T. P., Olson, B. J., Anderson, R. D., Seidl, D., Karin, M. & Schiller, J. (1988). Sequence-specific and general transcriptional activation by the bovine papillomavirus-1 E2 trans-activator require an N-terminal amphipathic helix-containing E2 domain. *EMBO Journal* **7**, 4245–4253.

Heike, T., Miyatake, S., Yoshida, M., Arai, K. I. & Naoka, A. (1989). Bovine papilloma virus encoded E2 protein activates lymphokine genes through DNA elements distinct from the consensus motif, in the long control region of its own genome. *EMBO Journal* **8**, 1411–1417.

Kardassis, D., Papakosta, P., Pardali, K. & Moustakas, A. (1999). c-Jun transactivates the promoter of the human $p21^{WAF1/Cip1}$ gene by acting as a super activator of the ubiquitous transcription factor Sp1. *Journal of Biological Chemistry* **274**, 29572–29581.

Lai, M.-C., Teh, B. & Tarn, W.-Y. (2000). A human papillomavirus E2 transcriptional activator. *Journal of Biological Chemistry* **274**, 11832–11841.

Lambert, P. F., Spalholz, B. A. & Howley, P. M. (1987). A transcriptional repressor encoded by BPV1 shares a common carboxy-terminal domain with the E2 transactivator. *Cell* **50**, 69–78.

Lee, D., Lee, B., Kim, J., Kim, D. W. & Choe, J. (2000). cAMP response element binding protein-binding protein binds to human papillomavirus E2 protein and activates E2 dependent transcription. *Journal of Biological Chemistry* **275**, 7045–7051.

Lehman, C. W., King, D. S. & Botchan, M. R. (1997). A papillomavirus E2 phosphorylation mutant exhibits normal transient replication and transformation but is defective in transformation and plasmid retention. *Journal of Virology* **71**, 3652–3665.

Li, R., Knight, J., Bream, G., Stenlund, A. & Botchan, M. (1989). Specific recognition nucleotides and their DNA context determine the affinity of E2 protein for 17 binding sites in the BPV-1 genome. *Genes and Development* **3**, 510–526.

Li, R., Knight, J. D., Jackson, S. P., Tjian, R. & Botchan, M. R. (1991). Direct interaction between Sp1 and the BPV enhancer E2 protein mediates synergistic activation of transcription. *Cell* **65**, 493–505.

Liu, J. S., Kuo, S. R., Broker, T. & Chow, L. T. (1995). The function of the human papillomavirus type 11 E1, E2 and E2C proteins in cell-free DNA replication. *Journal of Biological Chemistry* **270**, 27283–27291.

McBride, A. A., Bolen, J. B. & Howley, P. M. (1989a). Phosphorylation sites of the E2 transcriptional regulatory proteins of bovine papillomavirus type 1. *Journal of Virology* **63**, 5076–5085.

McBride, A. A., Byrne, J. C. & Howley, P. M. (1989b). E2 polypeptides encoded by the bovine papillomavirus type 1 form dimers through the common carboxyl-terminal domain: transactivation is mediated by the conserved amino-terminal domain. *Proceedings of the National Academy of Sciences, USA* **86**, 510–514.

Miller Rank, N. & Lambert, P. F. (1995). Bovine papillomavirus type 1 E2 transcriptional regulators directly bind two cellular transcription factors, TFIID and TFIIB. *Journal of Virology* **69**, 6323–6334.

Pardali, K., Kurisaki, A., Moren, A., ten Dijke, P., Kardassis, D. & Moustakas, A. (2000). Role of Smad proteins and transcription factor Sp1 in $p21^{Waf1/Cip1}$ regulation by transforming growth factor-beta. *Journal of Biological Chemistry* **275**, 29244–29256.

Peng, Y.-C., Breiding, D. E., Sverdrup, F., Richard, J. & Androphy, E. J. (2000). AMF-1/Gps2 binds p300 and enhances its interaction with papillomavirus E2 proteins. *Journal of Virology* **74**, 5872–5879.

Pernose, K. J. & McBride, A. A. (2000). Proteasome-mediated degradation of the papillomavirus E2-TA protein is regulated by phosphorylation and can modulate viral genome copy number. *Journal of Virology* **74**, 6031–6038.

Prowse, D. M., Bolgan, L., Molnar, A. & Dotto, G. P. (1997). Involvement of the Sp3 transcription factor in induction of $p21^{Cip1/WAF1}$ in keratinocyte differentiation. *Journal of Biological Chemistry* **272**, 1308–1314.

Purdie, K. J., Sexton, C. J., Proby, C. M., Glover, M. T., Williams, A. T., Stables, J. N. & Leigh, I. M. (1993). Malignant transformation of cutaneous lesions in renal allograft patients: a role of human papillomaviruses. *Cancer Research* **53**, 5328–5333.

Spalholz, B. A., Yang, Y. C. & Howley, P. M. (1985). Transactivation of a bovine papillomavirus transcriptional regulatory element by the E2 gene product. *Cell* **42**, 183–191.

Spalholz, B. A., Van de Pol, S. B. & Howley, P. M. (1991). Characterization of the cis elements involved in basal and E2 transactivated expression of the bovine papillomavirus P_{2443} promoter. *Journal of Virology* **65**, 743–753.

Steger, G. & Corbach, S. (1997). Dose-dependent regulation of the early promoter of human papillomavirus type 18 by the viral E2 protein. *Journal of Virology* **71**, 50–58.

Steger, G., Ham, J., Lefebvre, O. & Yaniv, M. (1995). The bovine papillomavirus 1 E2 protein contains two activation domains: one that interacts with TBP and another that functions after TBP binding. *EMBO Journal* **14**, 329–340.

Stubenrauch, F. & Pfister, H. (1994). Low-affinity E2 binding site mediates downmodulation of E2 transactivation of the human papillomavirus type 8 late promoter. *Journal of Virology* **68**, 6959–6966.

Stubenrauch, F., Hummel, M., Iftner, T. & Laimins, L. A. (2000). The E8E2C protein, a negative regulator of viral transcription and replication, is required for chromosomal maintenance of human papillomavirus type 31 in keratinocytes. *Journal of Virology* **74**, 1178–1186.

Ushikai, M., Lace, M. J., Yamakawa, Y., Kono, M., Anson, J. and others (1994). trans activation by the full-length E2 protein of human papillomavirus type 16 and bovine papillomavirus type 1 in vitro and in vivo: cooperation with activation domains of cellular transcription factors. *Journal of Virology* **68**, 6655–6666.

Xiao, H., Hasegawa, T. & Isobe, K. I. (2000). p300 collaborates with Sp1 and Sp3 in p21waf1/cip1 promoter activation induced by histone deacetylase inhibitor. *Journal of Biological Chemistry* **275**, 1371–1376.

Yao, J.-M., Breiding, D. E. & Androphy, E. J. (1998). Functional interaction of the bovine papillomavirus E2 transactivation domain with TFIIB. *Journal of Virology* **72**, 1013–1019.

Zou, N., Lin, B. Y., Duan, F., Lee, K.-Y., Jin, G., Guan, R., Yao, G., Lefkowitz, E. J., Broker, T. & Chow, L. T. (2000). The hinge of human papillomavirus type 11 E2 protein contains major determinants for nuclear localization and nuclear matrix association. *Journal of Virology* **74**, 3761–3770.

Received 29 July 2001; Accepted 25 October 2001