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Conserved Interaction of the Papillomavirus E2 Transcriptional Activator Proteins with Human and Yeast TFIIB Proteins

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Papillomavirus early gene expression is regulated by the virus gene-encoded E2 proteins. The best-characterized E2 protein, encoded by bovine papillomavirus type 1 (BPV-1), has been shown to interact with basal transcription factor IIB (TFIIB) and the TATA binding protein basal transcription factor (N. M. Rank and P. F. Lambert, J. Virol. 69:6323–6334, 1995). We demonstrate that the potent E2 transcriptional activator protein encoded by a gene of human PV type 16 also interacts with TFIIB in vitro. Moreover, a direct comparison of domains within human TFIIB (hTFIIB) required for VP16 and BPV-1 E2 indicates that these acidic activators interact with hTFIIB in a qualitatively similar manner. Our mapping experiments identify hTFIIB interaction domains within the amino-terminal activation domain of BPV-1 E2. Finally, we demonstrate in vitro interaction between *Saccharomyces cerevisiae* TFIIB and BPV-1 E2, an observation that is consistent with the importance of the E2-TFIIB interaction for BPV-1 E2 transactivation in both systems.

The papillomavirus E2 proteins are the major regulatory factors involved in viral gene expression and viral DNA replication (reviewed in reference 36). E2 proteins bind specifically to the 12-bp palindromic sequence $ACCN_6GGT$. These sites are located throughout papillomavirus genomes but are particularly concentrated within the viral long control region, where they act to regulate transcription of the early viral genes (2, 19, 20, 29, 34, 40). The E2 protein encoded by bovine papillomavirus (BPV) has served as the prototype in many analyses, and has been most extensively characterized. BPV type 1 (BPV-1) E2, functioning through E2 binding sites, can activate viral promoters over relatively large distances within the viral genome (52, 56) and has been shown to cooperate with a number of cellular transcription factors (18, 30, 55). E2 binding sites within the papillomavirus long control region also participate in viral DNA replication by recruiting the viral E1 replication protein to the adjacent viral origin of replication (37). Furthermore, BPV-1 E2 can transactivate promoters containing E2 binding sites in *Saccharomyces cerevisiae* (28, 40, 53), suggesting that at least some aspects the E2 transactivation mechanism must be conserved between higher and lower eukaryotes.

Transcriptional activator proteins are thought to act, at least in part, by influencing the assembly and/or activity of preinitiation complexes (9, 50; for reviews see references 44 and 54). Moreover, members of the class of transcriptional activator proteins known as acidic activators share a number of features, including interaction with transcription factor IIB (TFIIB) (3, 10, 23, 31, 33, 47, 49, 51, 58). Moreover, the mechanism of transactivation by acidic activator proteins appears to be conserved, since in contrast to members of other classes of mammalian transcriptional activator proteins (e.g., SP1 [42]), many acidic activators can function in both yeast and mammalian cells (3, 5, 11, 27, 38, 43, 49) and bind $TAF_{II}31$ (25, 32, 57).

The BPV-1 E2 protein is known to share a number of characteristics with other acidic transcriptional activator proteins, including the presence of a predicted α-helical region with amphipathic character within its amino-terminal activation domain (14), the ability to interact with the TFIIB and TATA binding protein (TBP) basal transcription factors (45), and the capacity to function in yeast (28, 39, 53). In this study, we demonstrate that, like BPV-1 E2, human PV type 16 (HPV-16) E2 can interact with TFIIB in vitro. Mapping experiments were performed to determine the regions within BPV-1 E2 and human TFIIB (hTFIIB) required for this interaction. Finally, we demonstrate that BPV-1 and HPV-16 E2 proteins can interact with yeast TFIIB (yTFIIB), suggesting that this conserved interaction may be important in the ability of PV E2 to act as a potent transcriptional activator in yeast.

In vitro TFIIB interaction is a conserved function of BPV-1 E2 and HPV-16 E2. Interaction between TFIIB and glutathione *S*-transferase (GST)–BPV-1 E2 fusion proteins has been demonstrated previously (45). To further examine this interaction, we tested the ability of native E2 proteins to interact with recombinant hTFIIB (Santa Cruz Biotechnology) by coimmunoprecipitation in vitro. ^{35}S-labeled BPV-1 E2 was generated by coupled in vitro transcription and translation and incubated with increasing amounts of bacterially expressed recombinant hTFIIB. Following incubation for 1 h at room temperature in 0.7 ml of binding buffer (10 mM Tris [pH 7.5], 5 mM $MgCl_2$, 1 mM EDTA, 2 mM dithiothreitol, 100 mM NaCl, 0.5% Nonidet P-40), 7.5 μl (100 μg/100 μl) of anti-TFIIB antiserum (Santa Cruz Biotechnology) was added and incubation was continued for 30 min. A 50% protein A-agarose slurry (30 μl) preequilibrated in binding buffer was then added. After 30 min, beads were washed four times in 1 ml of binding buffer and samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Precipitated radiolabeled E2 was detected by autoradiography.

The results of such an experiment are shown in Fig. 1A. Addition of increasing amounts of recombinant hTFIIB resulted in coimmunoprecipitation of proportionally increasing amounts of BPV-1 E2. The small amount of BPV-1 E2 that was precipitated by hTFIIB antiserum in the absence of exogenously added recombinant TFIIB was probably not due to trace amounts of TFIIB present in reticulocyte extracts (see below) and is attributable to background levels of BPV-1 E2 precipitation by the anti-TFIIB antiserum. These experiments

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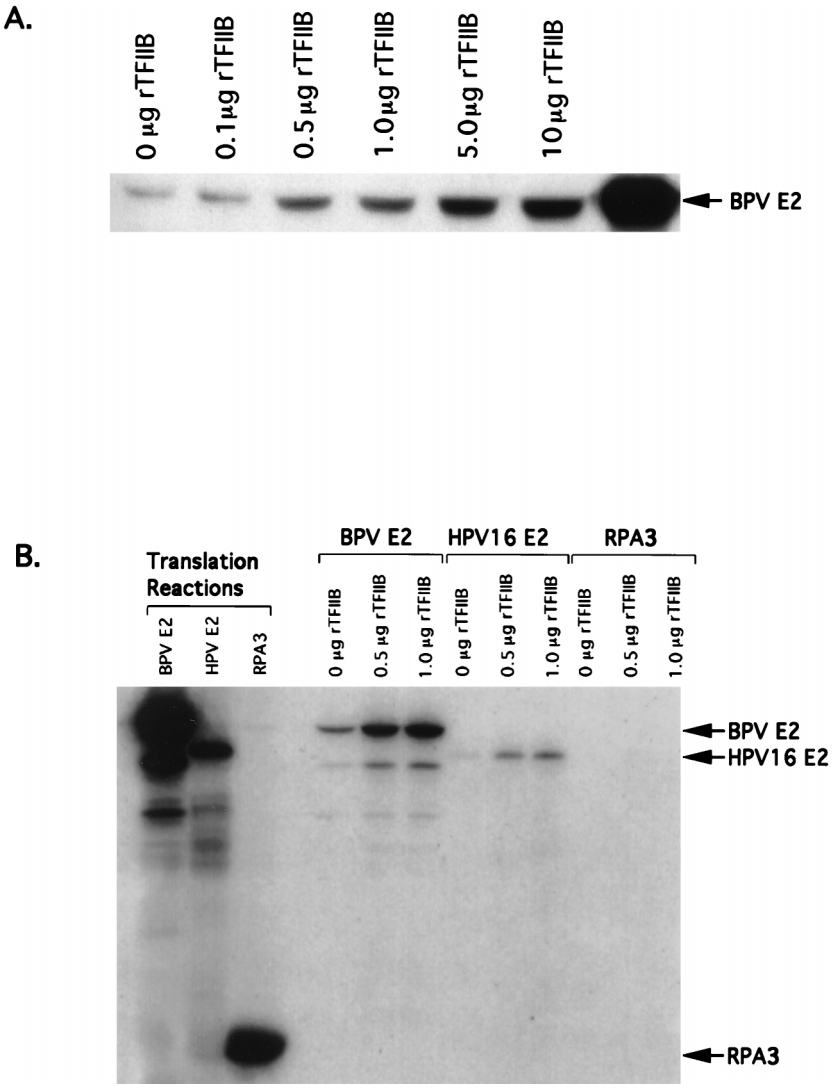

FIG. 1. In vitro coimmunoprecipitation of papillomavirus E2 proteins and recombinant hTFIIB. (A) [^{35}S]methionine-labeled E2 proteins were generated by the T7-TNT coupled reticulocyte lysate system (Promega) with pCMV-E2 as the full-length BPV-1 E2 template (4). Indicated amounts of recombinant hTFIIB (rTFIIB) were incubated with radiolabeled in vitro-translated E2 and then with 7.5 μg of hTFIIB antiserum and protein A-Sepharose. The far right lane represents 50% of the amount of BPV-1 E2 used in each binding reaction. (B) Binding and immunoprecipitation reactions were carried out as described for panel A. Bands representing full-length BPV-1 E2, HPV-16 E2, and RPA3 are indicated.

confirm the previous study that documented an in vitro interaction between BPV-1 E2 and hTFIIB (45).

Considering the fact that BPV-1 E2 and HPV16 E2 are both potent transcriptional activators (26), we next tested whether hTFIIB interaction was a shared characteristic of these two proteins. A T7 template for HPV-16 E2 was generated by PCR with a 5′ oligonucleotide that contained a T7 promoter and an initiation methionine codon in an optimal Kozak context. ^{35}S-labeled BPV-1 and HPV-16 E2 were tested for interaction with hTFIIB in vitro. Bacterially expressed recombinant hTFIIB (0, 0.5, or 5.0 μg) was incubated with radiolabeled BPV-1 or HPV-16 E2. An E2-hTFIIB interaction was detected by immunoprecipitation with anti-hTFIIB antibody (Fig. 1B). In vitro-translated RPA3 (5) was used as a negative control to ensure the specificities of the observed hTFIIB interactions. In these experiments, hTFIIB bound approximately 10% of the input radiolabeled BPV-1 and HPV-16 E2, indicating that TFIIB binding is a shared characteristic of both E2 proteins. No interaction was observed between hTFIIB and RPA3.

The in vitro BPV-1 E2-hTFIIB interaction is direct and not mediated by TBP. The observed immunoprecipitation of E2 with TFIIB antiserum might be due, at least in part, to the presence of endogenous TFIIB or TBP in the reticulocyte lysate. This was a potentially important consideration, because TBP and TFIIB are known to form a complex (8, 21). In order to address this issue, aliquots of radiolabeled E2 translated in rabbit reticulocyte lysate were precleared with 10 μg of antiserum to TBP (Santa Cruz Biotechnology) (Fig. 2, lanes 1 through 3) or 10 μg of anti-hTFIIB antiserum (Santa Cruz Biotechnology) (lanes 4 through 6) in 0.7 ml of binding buffer, and then 30 μl of a 50% (vol/vol) protein A slurry was added. After a 30-min incubation at room temperature, samples were briefly microcentrifuged and the supernatants were transferred to fresh tubes. This process was followed by immunoprecipi-

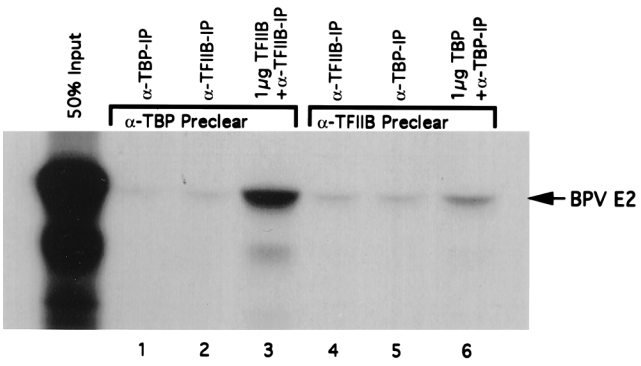

FIG. 2. Interaction of BPV-1 E2 with hTFIIB is direct and not mediated by TBP. Aliquots of radiolabeled BPV-1 E2 translated in rabbit reticulocyte lysate were precleared with anti-TBP (lanes 1 to 3) or anti-hTFIIB (lanes 4 to 6) antiserum. Recombinant hTFIIB (lane 3) or TBP (lane 6) was then added. Coimmunoprecipitations using anti-hTFIIB are shown in lanes 2 to 4; coimmunoprecipitations using anti-TBP are shown in lanes 1, 5, and 6. Bound E2 was analyzed by SDS-PAGE and autoradiography. The far left lane represents 50% of the amount of BPV-1 E2 used in each binding assay. IP, immunoprecipitate.

tation with anti-TBP (Fig. 2, lane 1) or anti-TFIIB (lane 2) or by addition of recombinant hTFIIB and subsequent precipitation with anti-TFIIB antiserum (lane 3) as shown in Fig. 1. Thus, after eliminating any endogenous TBP, radiolabeled E2 could be precipitated by TFIIB antiserum only after addition of exogenous recombinant TFIIB. These results indicate that the observed TFIIB-E2 interaction was not due to the presence of TBP in the reticulocyte lysate. Lanes 4 through 6 of Fig. 2 represent a parallel experiment that examined the possibility that the TBP-E2 interaction occurs through endogenous TFIIB. In these lanes, radiolabeled E2 in binding buffer was precleared with TFIIB antiserum. Immunoprecipitation was performed with anti-TFIIB (lane 4) or anti-TBP (lane 5). In the lane 6 sample, recombinant TBP was added to the anti-TFIIB precleared sample, followed by immunoprecipitation with TBP antiserum. Although the amount of E2 precipitated in lane 6 indicates some binding of BPV-1 E2 to TBP (see lanes 1 and 4), this binding appears to be much less efficient than that observed between recombinant TFIIB and BPV-1 E2. Separate experiments using radiolabeled TBP indicated that the TBP antiserum used in this experiment was competent and not limiting for TBP immunoprecipitation under these conditions (data not shown). In summary, these results indicate that the observed TFIIB-E2 interaction was not mediated by endogenous TBP in the reticulocyte lysate. Furthermore, only a small level of TBP-E2 interaction was observed, suggesting that like VP16 (31), BPV-1 E2 interacts much more strongly with TFIIB than with TBP. These experiments, however, do not address or rule out the possibility that a three-way complex of TFIIB, TBP, and E2 plays an important part in E2-mediated transactivation.

Interaction of hTFIIB with BPV-1 E2 involves determinants within the amino-terminal transactivation domain. We next performed mapping of the BPV-1 E2 domain necessary for interaction with hTFIIB. Template plasmids for T7 in vitro transcription that carry genes encoding full-length and deletion mutants of BPV-1 E2 (p2434 encodes BPV E2 amino acids [aa] 53 to 410, p2423 encodes aa 162 to 410, p2439 encodes BPV E2 deleted of aa 158 to 282, and p2430 encodes aa 207 to 410) were obtained from Alison McBride (34). Figure 3A shows the result of an assay in which in vitro-translated, radiolabeled full-length BPV-1 E2, as well as various BPV-1 E2 deletion mutants, was tested for hTFIIB binding. These assays were performed as described for Fig. 1 with 0.5 μg of recombinant hTFIIB and 7.5 μg of TFIIB antiserum. BPV-1 E2 proteins deleted of aa 1 to 52, 1 to 161, or 162 to 282 could bind hTFIIB in vitro, whereas no binding to a domain of BPV-1 E2 encompassing aa 207 to 410 was observed. In separate experiments (data not shown), no binding was observed between recombinant hTFIIB and the DNA binding domain (aa 310 to 410) of BPV-1 E2. These results (Fig. 3A) suggested that the BPV-1 E2 amino terminus might be sufficient for interaction with TFIIB in vitro. A T7 template encoding aa 1 to 262 of BPV-1 E2 was generated by PCR, and binding of this in vitro-translated [^{35}S]methionine-labeled protein to TFIIB was tested as described for Fig. 3A. Figure 3B shows that this BPV-1 E2 amino-terminal domain (aa 1 to 262) could interact with recombinant TFIIB in vitro.

The observation that regions of BPV-1 E2 capable of binding hTFIIB did not all overlap (Fig. 3C) suggests that more than one hTFIIB interaction domain may exist in BPV-1 E2. One hTFIIB interaction domain appears to reside amino terminal to residue 161, and a separate domain may lie between aa 161 and 207. Each of these domains lies within a region of BPV-1 E2 that is essential for transactivation (35). The aa 162 to 410 mutant corresponds to E2-TR, an E2 species present in BPV-1-transformed cells. This form of BPV-1 E2 includes a portion of the transactivation domain, as well as the hinge and DNA binding domain. E2-TR is capable of binding E2 DNA sites and represses transcriptional activation by full-length BPV-1 E2 (reviewed in reference 36). E2-TR alone can, however, transactivate in certain contexts (12), suggesting that interaction of E2-TR with TFIIB may sponsor transactivation under certain circumstances. The functional redundancy we observe in BPV-1 E2 has precedent: the gene of herpes simplex virus that encodes the VP16 acidic activator protein contains two distinct separable activation domains (46).

Our results indicate that all domains of BPV-1 E2 capable of in vitro hTFIIB interaction reside upstream of the BPV-1 E2 DNA binding domain and are at variance with the previous description of in vitro interaction between TFIIB and BPV-1 E2, where this interaction was mapped exclusively to a carboxy-terminal domain of E2 encompassing aa 311 to 410 (45). Furthermore, the specificities of BPV-1 E2 interaction are different in these respective assays. Our studies detected only low levels of interaction between native BPV-1 E2 and TBP in vitro, whereas GST-BPV E2 chimeric proteins used by Rank and Lambert showed comparable TFIIB and TBP binding activities within the BPV-1 E2 carboxy terminus. It is doubtful that different binding conditions account for these discrepancies, since both assays were performed under conditions of almost identical stringency. One obvious dissimilarity between the two studies is that Rank and Lambert (45) used GST-E2 fusion proteins in their assays, whereas we used native E2 proteins. It is possible that the context or amount of BPV-1 E2 protein used in such interaction assays has a dramatic effect upon its binding characteristics.

In BPV-1 E2-hTFIIB and VP16-hTFIIB interactions, analogous regions within the hTFIIB protein are used. VP16 is perhaps the most extensively studied mammalian acidic transcriptional activator protein. Interaction between an activator and basal transcription factors was first demonstrated with this activation domain (31, 47). Mapping studies by Roberts et al. (47), using a series of hTFIIB deletion and missense mutants, have established domains and specific amino acids within hTFIIB that are involved in TFIIB-VP16 interaction. However, the question of whether these same regions of TFIIB are involved in interactions with other activators has not yet been examined experimentally.

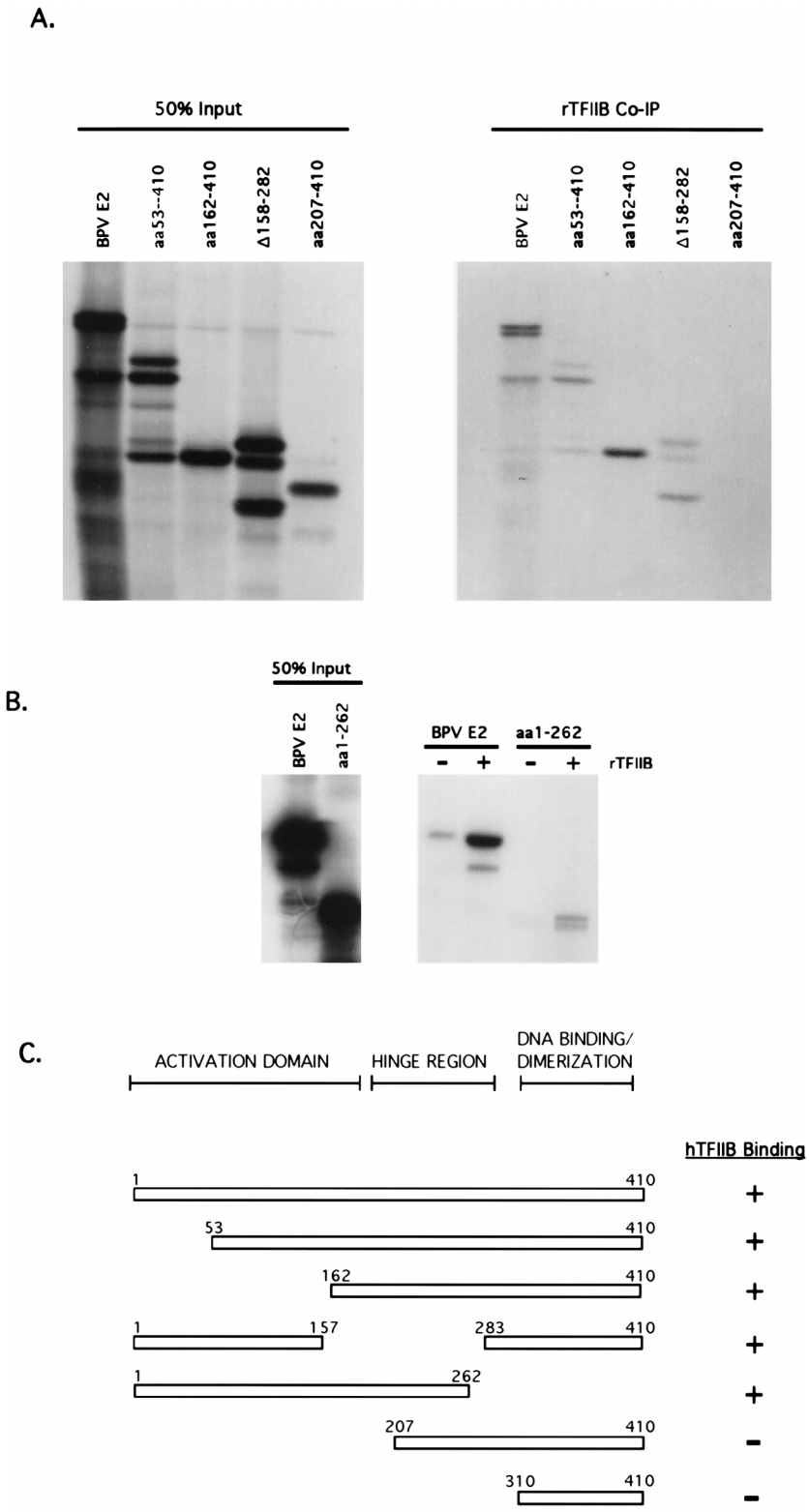

FIG. 3. Mapping the hTFIIB binding domain of BPV-1 E2. (A) hTFIIB interaction with various forms of BPV-1 E2 was tested by coimmunoprecipitation with 0.5 μg of recombinant hTFIIB (rTFIIB), as described in the legend to Fig. 1. Co-IP, coimmunoprecipitate. (B) Binding of hTFIIB to full-length BPV-1 E2 or an amino-terminal domain of BPV-1 E2 (aa 1 to 262). One-half microgram of recombinant hTFIIB was added (+) or not added (−) to radiolabeled E2 in 0.7 ml of binding buffer, followed by immunoprecipitation with 7.5 μg of anti-TFIIB. (C) Schematic summary of the binding data presented in panels A and B. Forms of BPV-1 E2 for which interaction with hTFIIB was observed are indicated with a plus sign. No interaction was observed between recombinant hTFIIB and aa 207 to 410 or aa 310 to 410 of BPV-1 E2 (see the text).

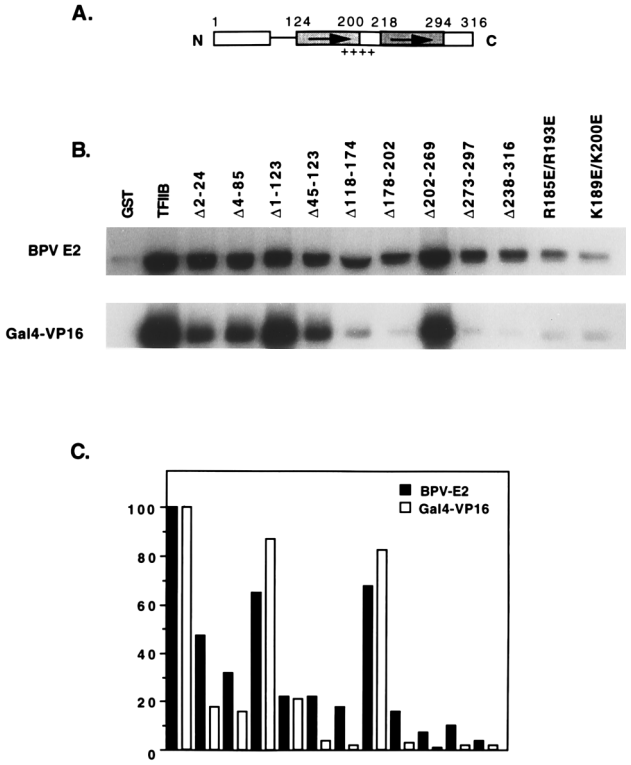

FIG. 4. Comparison of hTFIIB domains required for BPV-1 E2 and Gal4-VP16 interactions in vitro. (A) Schematic diagram of hTFIIB denoting the imperfect repeats and the basic amino acid residues involved in acidic activator interaction. (B) Radiolabeled BPV-1 E2 and Gal4-VP16 were tested for binding to GST alone, wild-type GST-hTFIIB, or GST-hTFIIB deletion mutants. The positions of the deleted amino acids are indicated by the numbers above each lane. R185E/R193E and K185E/K200E are GST-hTFIIB mutant proteins in which the basic amino acids at the indicated positions within hTFIIB were replaced by acidic residues. Bound BPV-1 E2 or Gal4-VP16 was analyzed by SDS-PAGE followed by autoradiography. (C) Graph representing GST-TFIIB binding by BPV-E2 (filled bars) or Gal4-VP16 (open bars) in panel A. Binding to each GST-TFIIB protein was normalized to the value observed with wild-type GST-TFIIB, which was assigned a value of 100%.

Using the same panel of GST-hTFIIB mutants that had been used previously to discern the details of VP16-TFIIB interaction, we tested interactions with BPV-1 E2 in vitro (Fig. 4A). Plasmid constructs carrying genes expressing wild-type or mutant forms of hTFIIB as GST fusion proteins were gifts of S. Roberts and M. R. Green. GST fusion proteins were prepared by standard procedures (24). pGEM-Gal4 VP16 (47) was translated with SP6-TNT wheat germ lysate (Promega). Radiolabeled BPV-1 E2 and Gal4-VP16 were tested for binding in 0.7 ml of binding buffer (room temperature for 1 h) to 0.5 μg of GST alone, wild-type GST-hTFIIB, or GST-hTFIIB deletion mutants. Samples were washed four times with 1 ml of binding buffer. R185E/R193E and K185E/K200E are GST-hTFIIB mutants proteins in which the basic amino acids at the indicated positions within hTFIIB were replaced by acidic residues.

The results of this experiment are shown in Fig. 4B. Quantitation of these results, by using the NIH Image program, is shown in Fig. 4C. While absolute binding of BPV-1 E2 to various forms of TFIIB differed from that of Gal4-VP16, the relative abilities of these various hTFIIB mutants to bind BPV-1 E2 closely paralleled their Gal4-VP16 binding activities. In addition, substitution of negatively charged amino acids for positively charged residues that are known to be important

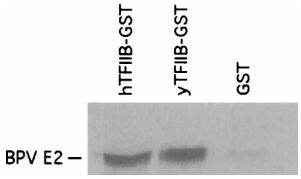

FIG. 5. BPV-1 E2 interaction is a conserved function of the hTFIIB and yTFIIB proteins. Radiolabeled in vitro-translated BPV-1 E2 was split into three equal aliquots and tested for binding to 0.5 μg of GST-hTFIIB or GST-yTFIIB fusion proteins or to GST protein alone under the conditions described in the legend to Fig. 4. Full-length BPV-1 E2 and E2-TR are indicated.

for VP16 interaction (R185E/R193E and K189E/K200E) also significantly reduced the in vitro interaction between BPV-1 E2 and hTFIIB. BPV-1 E2 appeared to bind all forms of hTFIIB more strongly than Gal4-VP16. This may have been due to the potential presence of two TFIIB interaction domains within E2, whereas only one VP16 activation domain is present in the form of Gal4VP16 used in these experiments. Thus, it appears that the character of interactions between BPV-1 E2 and hTFIIB (i.e., the domains of hTFIIB involved) is generally the same as that of interactions between VP16 and TFIIB.

As shown in Fig. 4A, TFIIB contains an amino-terminal tail, followed by two imperfect direct repeats (16, 17). The carboxy-terminal portion of the first repeat contains a positively charged amphipathic α-helix. The results presented here suggest that like VP16, domains within TFIIB at the carboxy terminus of each imperfect repeat are involved in the interaction of TFIIB with PV E2. Moreover, substitution of acidic residues for the basic residues within the amphipathic helix significantly decreases TFIIB binding by both BPV-1 E2 and the VP16 activation domain, suggesting that ionic interactions may be involved in formation of both complexes.

Interaction between BPV-1 E2 and yTFIIB. As mentioned above, BPV-1 E2, like other acidic activators, is a potent transcriptional activator in yeast (28, 39, 53). This finding has led to the conclusion that the mechanism of transactivation by E2 is likely conserved between higher and lower eukaryotes. The high level of conservation between h- and yTFIIB (52% similarity and 35% identity) (41) also suggests that the ability of E2 to interact with TFIIB is a conserved feature of the h- and yTFIIB proteins. This possibility was tested by cloning sequences encoding wild-type yTFIIB and expressing yTFIIB as a GST-fusion protein. A PCR fragment representing the yTFIIB (*SUA7*) open reading frame (41) was generated by PCR with purified DNA from yeast strain INVSC1 (Invitrogen) as a template. These oligonucleotides introduced an in-frame *Eco*RI site followed by the second codon of *SUA7* and a *Sal*I site immediately following the *SUA7* termination codon. This fragment was cloned into the *Eco*RI-*Xho*I sites of pGEX 4T-1 (Pharmacia).

Figure 5 shows the result of an experiment in which levels of binding of radiolabeled BPV-1 E2 to both GST-hTFIIB and GST-yTFIIB were compared. In these experiments, the GST-yTFIIB interaction with BPV-1 E2 was comparable to that observed with GST-hTFIIB. These results are consistent with the importance of E2 interaction with TFIIB in transactivation in both mammalian and yeast cells, although we certainly do not rule out the possible existence of other conserved and relevant E2 activities.

Many recent studies have utilized various mutagenesis strategies to identify residues within the papillomavirus E2 transactivation domain that are critical for transactivation (1, 6, 7,

13, 48). However, the specific function(s) of E2 affected by these mutations is not known. Our assessment of the in vitro hTFIIB binding activities of HPV-16 E2 missense mutants that were previously shown to be defective for transactivation (48) has revealed no quantitative change in hTFIIB binding activity (28a). It is possible that these mutants are defective for another essential E2 function that is unrelated to TFIIB interaction. Alternatively, VP16 has been shown to induce a conformational change in TFIIB (22, 47). Thus, the transactivation-defective HPV-16 E2 mutants we tested may be capable of binding TFIIB with affinities indistinguishable from that of wild-type E2 but bear qualitative defects rendering them unable to induce a TFIIB conformational change. Whatever the case, we do believe that the E2 function or functions disrupted by these HPV-16 E2 mutations are conserved between mammalian and yeast cells, since we have observed that HPV-16 E2 mutants which are unable to transactivate in mammalian cells are also defective for transactivation in yeast cells (4a). Testing of an independently derived set of transactivation-defective mutant proteins for activity in mammalian and yeast cells has shown similar correlations of BPV-1 E2 transactivation activity (6).

The results presented here suggest that PV E2 transactivation is mediated in a manner similar if not identical to that of other acidic activator proteins. Our assays indicate that E2 proteins participate in protein-protein interactions analogous to those of VP16, the prototypic mammalian acidic transcriptional activator. We have also observed interaction between both BPV-1 and HPV-16 E2 and human $TAF_{II}31$ in vitro (28a); $TAF_{II}31$ interaction is a known characteristic of both VP16 (15, 25) and p53 (32, 57). Moreover, our results affirm previous hypotheses that these functions which mediate E2 transactivation are conserved, both among the PV E2 proteins and between the TFIIB molecules of higher and lower eukaryotes. Further analysis of wild-type and mutant forms of the E2 proteins encoded by various PVs, along with comparison of their activities in various experimental systems, may allow clearer identification of the cellular transcription machinery through which the papillomavirus E2 proteins bring about transcriptional activation.

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REFERENCES

1. **Abroi, A., R. Kurg, and M. Ustav.** 1996. Transcriptional and replicational activation functions in the bovine papillomavirus type 1 E2 protein are encoded by different structural determinants. J. Virol. **70:**6169–6179.
2. **Androphy, E. J., D. R. Lowy, and J. T. Schiller.** 1987. Bovine papillomavirus E2 trans-activating gene product binds to specific sites in papillomavirus DNA. Nature **325:**70–73.
3. **Baniahmad, A., I. Ha, D. Reinberg, S. Tsai, M.-J. Tsai, and B. W. O'Malley.** 1993. Interaction of human thyroid receptor beta with transcription factor TFIIB may mediate target gene derepression and activation by thyroid hormone. Proc. Natl. Acad. Sci. USA **90:**8832–8836.
4. **Benson, J. D., and P. M. Howley.** 1995. Amino-terminal domains of the bovine papillomavirus type 1 E1 and E2 proteins participate in complex formation. J. Virol. **69:**4364–4372.

4a. **Benson, J. D., and H. Sakai.** Unpublished data.

5. **Blair, W. S., H. Bogerd, and B. H. Cullen.** 1994. Genetic analysis indicates that the human foamy virus Bel-1 protein contains a transcription activation domain of the acidic class. J. Virol. **68:**3803–3808.
6. **Breiding, D. E., M. J. Grossel, and E. J. Androphy.** 1996. Genetic analysis of the bovine papillomavirus E2 transcriptional activation domain. Virology **221:**34–43.
7. **Brokaw, J. L., M. Blanco, and A. A. McBride.** 1996. Amino acids critical for the functions of the bovine papillomavirus type 1 E2 transactivator. J. Virol. **70:**23–29.
8. **Buratowski, S., and H. Zhou.** 1993. Functional domains of transcription factor TFIIB. Proc. Natl. Acad. Sci. USA **90:**5633–5637.
9. **Choy, B., and M. R. Green.** 1993. Eukaryotic activators function during multiple steps of preinitiation complex assembly. Nature **366:**531–536.
10. **Colgan, J., S. Wampler, and J. L. Manley.** 1993. Interaction between a transcriptional activator and transcription factor IIB in vivo. Nature **362:** 549–553.
11. **Countryman, J. K., L. Heston, J. Gradoville, H. Himmelfarb, S. Serdy, and G. Miller.** 1994. Activation of the Epstein-Barr virus BMRF1 and BZLF1 promoters by ZEBRA in *Saccharomyces cerevisiae*. J. Virol. **68:**7628–7633.
12. **Demeret, C., M. Yaniv, and F. Thierry.** 1994. The E2 transcriptional repressor can compensate for SP1 activation of the human papillomavirus type 18 early promoter. J. Virol. **68:**7075–7082.
13. **Ferguson, M. K., and M. R. Botchan.** 1996. Genetic analysis of the activation domain of bovine papillomavirus protein E2: its role in transcription and replication. J. Virol. **70:**4193–4199.
14. **Giri, I., and M. Yaniv.** 1988. Structural and mutational analysis of E2 transactivating proteins of papillomaviruses reveals three distinct functional domains. EMBO J. **7:**2823–2829.
15. **Goodrich, J. A., T. Hoey, C. J. Thut, A. Admon, and R. Tijan.** 1993. Drosophila $TAF_{II}40$ interacts with both a VP16 activation domain and the basal transcription factor TFIIB. Cell **75:**519–530.
16. **Ha, I., W. S. Lane, and D. Reinberg.** 1991. Cloning of a human gene encoding the general transcription initiation factor IIB. Nature **352:**689–695.
17. **Ha, I., S. Roberts, E. Maldonado, X. Sun, L. U. Kim, M. Green, and D. Reinberg.** 1993. Multiple functional domains of human transcription factor IIB: distinct interactions with two general transcription factors and RNA polymerase II. Genes Dev. **7:**1021–1032.
18. **Ham, J., N. Dostatni, F. Amos, and M. Yaniv.** 1991. Several different upstream promoter elements can potentiate transactivation by the BPV-1 E2 protein. EMBO J. **10:**2931–2940.
19. **Hawley-Nelson, P., E. J. Androphy, D. R. Lowy, and J. T. Schiller.** 1988. The specific DNA recognition sequence of the bovine papillomavirus E2 protein is an E2-dependent enhancer. EMBO J. **7:**525–531.
20. **Hirochika, H., R. Hirochika, T. R. Broker, and L. T. Chow.** 1988. Functional mapping of the human papillomavirus type 11 transcriptional enhancer and its interaction with the trans-acting E2 proteins. Genes Dev. **2:**54–67.
21. **Hisataike, K., R. G. Roeder, and M. Horikoshi.** 1993. Functional dissection of TFIIB domains required for TFIIB-TFIID-promoter complex formation and basal transcription activity. Nature **363:**744–747.
22. **Horil, R., S. Pyo, and M. Carey.** 1995. Protease footprinting reveals a surface on transcription factor TFIIB that serves as an interface for activators and coactivators. Proc. Natl. Acad. Sci. USA **92:**6047–6051.
23. **Ing, N. H., J. M. Beckman, S. Y. Tsai, M.-J. Tsai, and B. W. O'Malley.** 1992. Members of the steroid hormone receptor superfamily interact with TFIIB (S300-II). J. Biol. Chem. **267:**17617–17623.
24. **Kaelin, W. G., W. Krek, W. R. Sellers, J. A. DeCaprio, F. Ajchenbaum, C. S. Fuchs, T. Chittenden, Y. Li, P. J. Farnham, and M. A. Blanar.** 1992. Expression cloning of a cDNA encoding a retinoblastoma-binding protein with E2F-like properties. Cell **70:**351–364.
25. **Klemm, R. D., J. A. Goodrich, S. Zhou, and R. Tijan.** 1995. Molecular cloning of the 32-kDa subunit of human TFIID reveals interactions with VP16 and TFIIB that mediate transcriptional activation. Proc. Natl. Acad. Sci. USA **92:**5788–5792.
26. **Kovelman, R., G. K. Bilter, E. Glezer, A. Y. Tsou, and M. S. Barbosa.** 1996. Enhanced transcriptional activation by E2 proteins from the oncogenic human papillomaviruses. J. Virol. **70:**7549–7560.
27. **Kunzler, M., G. H. Braus, O. Georgiev, K. Seipel, and W. Schaffer.** 1994. Functional differences between mammalian transcription activation domains at the yeast GAL1 promoter. EMBO J. **13:**641–645.
28. **Lambert, P. F., N. Dostatni, A. A. McBride, M. Yaniv, P. M. Howley, and B. Arcangioli.** 1989. Functional analysis of the papillomavirus E2 trans-activator in Saccharomyces cerevisiae. Genes Dev. **3:**38–48.

28a. **Lawande, R., and J. Benson.** Unpublished data.

29. **Li, R., J. Knight, G. Bream, A. Stenlund, and M. Botchan.** 1989. Specific recognition nucleotides and their DNA context determine the affinity of E2 protein for 17 binding sites in the BPV-1 genome. Genes Dev. **3:**510–526.
30. **Li, R., J. D. Knight, S. P. Jackson, R. Tijan, and M. R. Botchan.** 1991. Direct interaction between Sp1 and the BPV enhancer E2 protein mediates synergistic activation of transcription. Cell **65:**493–505.
31. **Lin, Y.-S., I. Ha, E. Maldonado, D. Reinberg, and M. R. Green.** 1991. Binding of general transcription factor TFIIB to an acidic activating region. Nature **353:**569–571.
32. **Lu, H., and A. J. Levine.** 1995. Human $TAF_{II}31$ protein is a transcriptional coactivator of the p53 protein. Proc. Natl. Acad. Sci. USA **92:**5154–5158.
33. **Manet, E., C. Allera, H. Gruffat, I. Mikaelian, A. Rigolet, and A. Sergant.** 1993. The acidic activation domain of the Epstein-Barr virus transcription factor R interacts in vitro with both TBP and TFIIB and is cell-specifically potentiated by a proline-rich region. Gene Expr. **3:**49–59.
34. **McBride, A. A., R. Schlegel, and P. M. Howley.** 1988. The carboxy-terminal domain shared by the bovine papillomavirus E2 transactivator and repressor proteins contains a specific DNA binding activity. EMBO J. **7:**533–539.

35. **McBride, A. A., J. C. Byrne, and P. M. Howley.** 1989. E2 polypeptides encoded by bovine papillomavirus I form dimers through the carboxyl-terminal DNA binding domain: transactivation is mediated through the conserved amino-terminal domain. Proc. Natl. Acad. Sci. USA **84:**510–514.
36. **McBride, A. A., H. Romanczuk, and P. M. Howley.** 1991. The papillomavirus E2 regulatory proteins. J. Biol. Chem. **28:**18411–18414.
37. **Mohr, I., R. Clark, R. Sun, E. J. Androphy, P. MacPherson, and M. R. Botchan.** 1990. Targeting the E1 replication protein to the papillomavirus origin of replication by complex formation with the E2 transactivator. Science **250:**1694–1699.
38. **Moore, P. A., S. M. Ruben, and C. A. Rosen.** 1993. Conservation of transcriptional activation functions of the NF-κB p50 and p65 subunits in mammalian cells and *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:**1666–1674.
39. **Morrissey, L. C., J. Barsoum, and E. J. Androphy.** 1989. *trans* activation by the bovine papillomavirus E2 protein in *Saccharomyces cerevisiae*. J. Virol. **63:**4422–4425.
40. **Moskaluk, C., and D. Bastia.** 1987. The E2 "gene" of bovine papillomavirus encodes an enhancer binding protein. Proc. Natl. Acad. Sci. USA **84:**1215–1218.
41. **Pinto, I., D. E. Ware, and M. Hampsey.** 1992. The yeast SUA7 gene encodes a homolog of human transcription factor TFIIB and is required for normal start site selection in vivo. Cell **68:**977–988.
42. **Ponticelli, A. S., T. S. Pardee, and K. Struhl.** 1995. The glutamine-rich activation domains of human SP1 do not stimulate transcription in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **15:**983–988.
43. **Privalsky, M. L., M. Sharif, and K. R. Yamamoto.** 1990. The viral erbA oncogene protein, a constitutive repressor in animal cells, is a hormone-regulated activator in yeast. Cell **63:**1277–1286.
44. **Ptashne, M., and A. Gann.** 1997. Transcriptional activation by recruitment. Nature **386:**569–577.
45. **Rank, N. M., and P. F. Lambert.** 1995. Bovine papillomavirus type 1 E2 transcriptional regulators directly bind two cellular transcription factors, TFIID and TFIIB. J. Virol. **69:**6323–6334.
46. **Regier, J. L., F. Shen, and S. J. Treizenberg.** 1993. Pattern of aromatic and hydrophobic amino acids critical for one of two subdomains of the VP16 transcriptional activator. Proc. Natl. Acad. Sci. USA **90:**883–887.
47. **Roberts, S. G. E., I. Ha, E. Maldonado, D. Reinberg, and M. R. Green.** 1993. Interaction between an acidic activator and transcription factor TFIIB is required for transcriptional activation. Nature **363:**741–744.
48. **Sakai, H., T. Yasugi, J. D. Benson, J. J. Dowhanick, and P. M. Howley.** 1996. Targeted mutagenesis of the human papillomavirus type 16 E2 transactivation domain reveals separable transcriptional activation and DNA replication functions. J. Virol. **70:**1602–1611.
49. **Scharer, E., and R. Iggo.** 1992. Mammalian p53 can function as a transcription factor in yeast. Nucleic Acids Res. **20:**1539–1545.
50. **Sharp, P. A.** 1991. Gene transcription. TFIIB or not TFIIB? Nature **351:**16–18.
51. **Smith, C. A., P. Bates, R. Rivera-Gonzales, B. Gu, and N. A. DeLuca.** 1993. ICP4, the major transcriptional regulator protein of herpes simplex virus type 1, forms a tripartite complex with TATA-binding protein and TFIIB. J. Virol. **67:**4676–4687.
52. **Spalholz, B. A., P. F. Lambert, C. L. Yee, and P. M. Howley.** 1987. Bovine papillomavirus transcriptional regulation: localization of the E2-responsive elements of the long control region. J. Virol. **61:**2128–2137.
53. **Stanway, C. A., M. P. Sowden, L. E. Wilson, A. J. Kingsman, and S. M. Kingsman.** 1989. Efficient activation of transcription in yeast by the BPV1 E2 protein. Nucleic Acids Res. **17:**2187–2196.
54. **Stargell, L. A., and K. Struhl.** 1996. Mechanisms of transcriptional activation in vivo: two steps forward. Trends Genet. **12:**311–315.
55. **Steger, G., J. Ham, O. Lefebvre, and M. Yaniv.** 1995. The bovine papillomavirus 1 E2 protein contains two activation domains: one that interacts with TBP and another that functions after TBP binding. EMBO J. **14:**329–340.
56. **Thierry, F., N. Dostatni, F. Arnos, and M. Yaniv.** 1990. Cooperative activation of transcription by bovine papillomavirus type 1 E2 can occur over a large distance. Mol. Cell. Biol. **10:**4431–4437.
57. **Thut, C. J., J. L. Chan, R. Klemm, and R. Tjian.** 1995. p53 transcriptional activation mediated by coactivators TAFII40 and TAFII60. Science **267:**100–104.
58. **Xu, X., C. Prorock, H. Ishikawa, E. Maldonado, Y. Ito, and C. Gélinas.** 1993. Functional interaction of the v-Rel and c-Rel oncoproteins with the TATA-binding protein and association with transcription factor IIB. Mol. Cell. Biol. **13:**6733–6741.