JOURNAL OF VIROLOGY, June 1997, p. 4866–4870
0022-538X/97/$04.00+0
Copyright © 1997, American Society for Microbiology
Vol. 71, No. 6

Transformation by Bovine Papillomavirus Type 1 E6 Is Independent of Transcriptional Activation by E6

RENEE NED, STACEY ALLEN, AND SCOTT VANDE POL*

Institute of Pathology, Case Western Reserve University School of Medicine, Cleveland, Ohio

Received 13 January 1997/Accepted 6 March 1997

We have generated mutants of bovine papillomavirus type 1 E6 (BE6) that are defective for transcriptional activation and have analyzed these mutants for transformation of contact-inhibited cells and association with the mammalian protein E6-AP. These BE6 mutants demonstrate that transformation by BE6 does not require transcriptional activation and that association of BE6 with E6-AP is a function separable from transcriptional activation by BE6. Association of BE6 with E6-AP appears to be necessary but not sufficient for transformation by BE6. In addition, the mutational analysis of BE6 demonstrates that transactivation, transformation, and association with E6-AP all require sequences throughout the BE6 gene, indicating that the papillomavirus E6 proteins do not have a simple domain structure.

The bovine papillomavirus type 1 (BPV-1) E6 oncoprotein (BE6) is a zinc-binding protein that shares conservation of numbers and spacing of zinc-binding CXXC motifs with other papillomavirus E6 proteins. Despite this similarity in structure, studies have indicated surprising biochemical diversity among E6 genes of different papillomavirus types. E6 genes from the cancer-associated human papillomaviruses (HPVs) complex with a cellular protein termed E6-AP and together with E6-AP bind to the p53 tumor suppressor protein thereby degrading p53 through ubiquitin-mediated proteolysis (5). E6 genes from the non-cancer-associated HPV types do not bind E6-AP or degrade p53. BE6 binds E6-AP but fails either to complex with p53 or to degrade associated proteins, implying that BE6 might transform cells through a mechanism different from that of the HPVs (11). In addition to targeting p53, the E6 genes of both cancer-associated HPVs and BPV-1 have been shown to associate with a cellular-calcium-binding protein localized to the endoplasmic reticulum (1).

Previous studies demonstrated that BE6 is a strong transcriptional activator when fused to the BPV-1 E2 DNA binding domain and targeted to promoters containing E2 binding sites (7). Retroviral oncogenes such as v-*myc*, v-*jun*, v-*myb*, v-*fos*, v-*ets*, v-*ski*, and others can be transcription factors. While small DNA tumor virus oncogenes have not been shown to transform cells by acting directly as transcription factors, oncoproteins from many DNA tumor viruses activate transcription indirectly, and this activation of transcription is essential for their transforming function. The adenovirus E1a protein and the similarly acting simian virus 40 TAg, HPV E7, and polyomavirus TAg proteins activate E2F-responsive promoters through their interaction with retinoblastoma family members and the release of E2F transcription factors from Rb, and this function is essential for the transformation or immortalization function of these oncoproteins (references 3 and 17 and references therein). Given that the transformation of a cell requires the transcriptional induction of cellular genes, it would not be surprising if a viral oncogene from a small DNA tumor virus was found to transform cells through acting directly as a transcription factor. It is in this context that BE6, which was found to have transcriptional activating properties, was proposed to transform cells by acting as a transcription factor. This model was supported by a limited analysis of mutations in the zinc-binding motifs of BE6, which eliminated both transformation and transactivation (7). However, mutations in the zinc fingers of BE6 might have global effects upon protein structure, so zinc finger mutants could be devoid of all BE6 functions.

We decided to select BE6 mutants defective for transcriptional activation in the yeast *Saccharomyces cerevisiae* in order to obtain a greater insight into the domains of BE6 required for transcriptional activation and the role transcriptional activation might play in transformation by BE6. To test the feasibility of such an approach, wild-type BE6, HPV-16 E6, and BE6 deletion mutants from this study as well as mutants carrying BE6 point mutations from an earlier mutational analysis of BE6 (16) were cloned as fusion proteins to the DNA binding domain of *lexA* in plasmid pEG202 and tested for activation of a *lexA*-responsive *lacZ* reporter. Figure 1 demonstrates that wild-type *lexA*-BE6 is a transactivator in yeast when fused to the *lexA* DNA binding domain and that transactivation for BE6 deletion mutants as well as for zinc finger point mutation mutants was decreased. HPV-16 E6 was also devoid of transcriptional activation in this assay, indicating that strong transcriptional activation in this assay is not a conserved feature of all papillomavirus E6 proteins. The same yeast strains used for transactivation analysis in Fig. 1A were analyzed for levels of the *lexA*-BE6 fusion protein by Western blotting (Fig. 1B); this showed that the lack of transactivation by BE6 mutants is not a consequence of low levels of expression by BE6 mutants. The previous results with C-terminal fusions of BE2 to the mammalian E2 DNA binding domain (7) and the results here with N-terminal fusions of the bacterial *lexA* domain to BE6 both demonstrate that BE6 can function as a transcriptional activation domain.

Transactivation and association with E6-AP can be dissociated. In order to gain greater insights into the structure of the BE6 protein and the role of transcriptional activation in BE6 transformation, random mutagenesis of BE6 was performed by gap repair transfection in yeast (Fig. 2) (10). BE6 mutants altered in transcriptional activation were identified as white yeast colonies on X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) plates. In order to identify BE6 mutants not

* Corresponding author. Mailing address: Department of Pathology, Case Western Reserve University, 10900 Euclid Ave., Cleveland, OH 44106. Phone: (216) 368-1679. Fax: (216) 368-1300. E-mail: sbv@pop.cwru.edu.

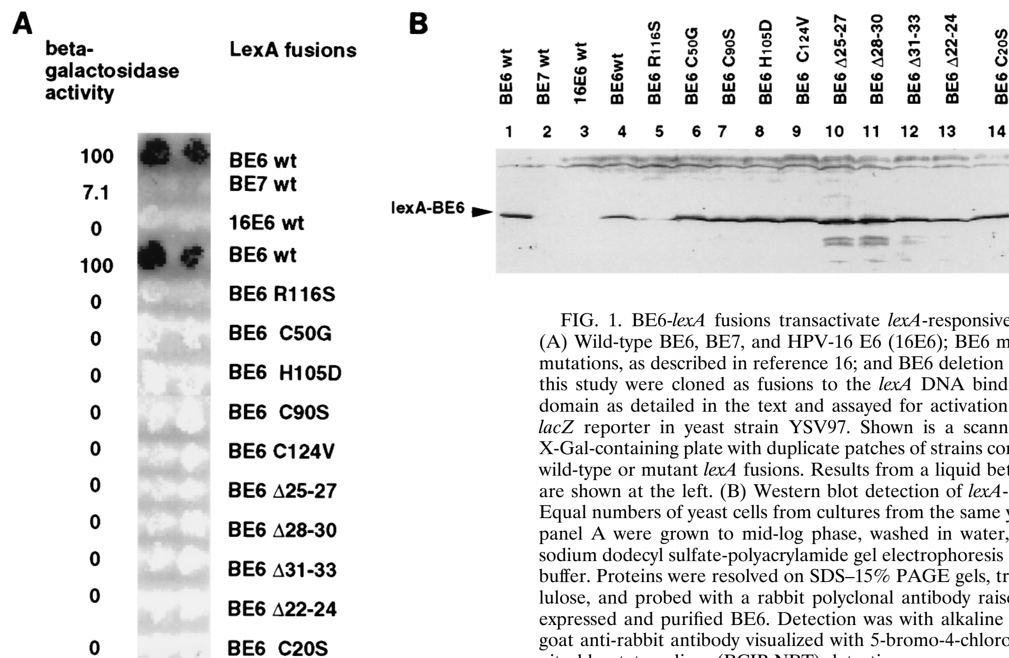

FIG. 1. BE6-*lexA* fusions transactivate *lexA*-responsive promoters in yeast. (A) Wild-type BE6, BE7, and HPV-16 E6 (16E6); BE6 mutants carrying point mutations, as described in reference 16; and BE6 deletion mutants generated in this study were cloned as fusions to the *lexA* DNA binding and dimerization domain as detailed in the text and assayed for activation of a *lexA*-responsive *lacZ* reporter in yeast strain YSV97. Shown is a scanned image of a yeast X-Gal-containing plate with duplicate patches of strains containing the indicated wild-type or mutant *lexA* fusions. Results from a liquid beta-galactosidase assay are shown at the left. (B) Western blot detection of *lexA*-BE6 fusions in yeast. Equal numbers of yeast cells from cultures from the same yeast strains shown in panel A were grown to mid-log phase, washed in water, and resuspended in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer. Proteins were resolved on SDS–15% PAGE gels, transferred to nitrocellulose, and probed with a rabbit polyclonal antibody raised against bacterially expressed and purified BE6. Detection was with alkaline phosphatase-coupled goat anti-rabbit antibody visualized with 5-bromo-4-chloro-3-indolylphosphate–nitroblue tetrazolium (BCIP-NBT) detection.

globally defective for all E6 functions, these colonies were then screened for the ability of the BE6-*lexA* fusions to interact in yeast with the E6-AP protein in a two-hybrid analysis. Of 135 transactivation-defective colonies observed to express full-length *lexA*-BE6 fusion proteins by Western blotting (data not shown), 28 were positive for association with E6-AP, demonstrating that transcriptional activation and association with E6-AP can be separated. However, the great majority (107/135 or 79%) of random amino acid substitutions that are selected on the basis of defects in one BE6 property (transcriptional activation) also result in defects in a second property (association with E6-AP), indicating the importance of screening for at least two properties when evaluating mutations in E6 genes. The mutant pLexA-BE6 plasmids were recovered from selected colonies, and the BE6 genes were sequenced, with the results shown in Table 1; the locations of amino acid substitutions are shown in Fig. 3.

Figure 3 demonstrates that mutations that disrupt transcriptional activation while retaining association with E6-AP are distributed throughout the BE6 primary amino acid sequence and do not cluster into a single region of the BE6 primary sequence. Most of the yeast-selected mutations involved amino acids that are highly conserved among either all papillomaviruses or among a subgroup of the papillomaviruses. A mutant with a single amino acid change at the last amino acid of BE6, BE6-P137R, was defective for transcriptional activation, despite the fact that there is no conservation of proline at this position (Fig. 3). All of the site-directed deletion mutants except for BE6-Δ131-133/S134A were defective for transcriptional activation and association with E6-AP.

In some BE6 mutants there was a difference between relative binding of E6-AP in vitro and association as measured by two-hybrid analysis in yeast. Some of the mutants with a temperature-sensitive (ts) designation in Table 1 revealed two-hybrid interactions at room temperature not seen in the standard assay conditions of 30°C (BE6-E101V, BE6Δ131-133/S134A, and BE6-P137R). Since the in vitro binding assay is performed at 4°C, these mutants might be expected to show greater interaction at 4°C in vitro than at 30°C in vivo. Two mutants, BE6-R123H and BE6-R116G, had greater interaction with E6-AP in yeast than in vitro for unknown reasons.

Transformation by BE6 does not require strong transcriptional activation. A subset of the BE6 mutants shown in Table 1 were cloned into the retroviral expression plasmid pLXSN and transfected into C127 cells to test for the ability of BE6 and BE6 mutants to induce anchorage-independent cell growth. Table 1 shows that only two mutants that are greatly decreased (>80% decreased) for transcriptional activation in yeast consistently induced anchorage independence: BE6-R42W and BE6-R116G. BE6-R42W was very efficient at the induction of anchorage independence, forming colonies at approximately the same efficiency as that of wild-type BE6 and demonstrating that anchorage independence in mammalian cells is not strongly associated with transcriptional activation by BE6 in yeast. Two separate cloned and sequenced DNA preparations of pLXSN-BE6-R42W were tested to confirm this result. BE6 mutants BE6-K114E, BE6-R129S, and BE6-Δ131-133/S134A, although efficient at the induction of anchorage independence, still retained significant transcriptional activation. Although all of the mutants that induced anchorage independence retained association with E6-AP (Table 1), the ability to associate with E6-AP was not sufficient to induce anchorage independence, as BE6-K35E and BE6-H105R/I88T retained strong E6-AP associations yet were defective for the induction of anchorage independence. In an earlier mutational analysis of BE6, a mutant with a mutation of codon 50 (BE6-C50G; also known as mutant 238 in reference 16) retained the ability to induce anchorage independence despite the mutation of a conserved zinc finger. A mutant with a different mutation at the same codon in this study (BE6-C50R) was defective for association with E6-AP and the induction of anchorage independence (Table 1).

Mutants that were defective for transcriptional activation but positive for anchorage independence, as shown by data in Table 1, were cloned as fusion proteins to the DNA binding and dimerization domain of BPV-1 E2, first in order to prove

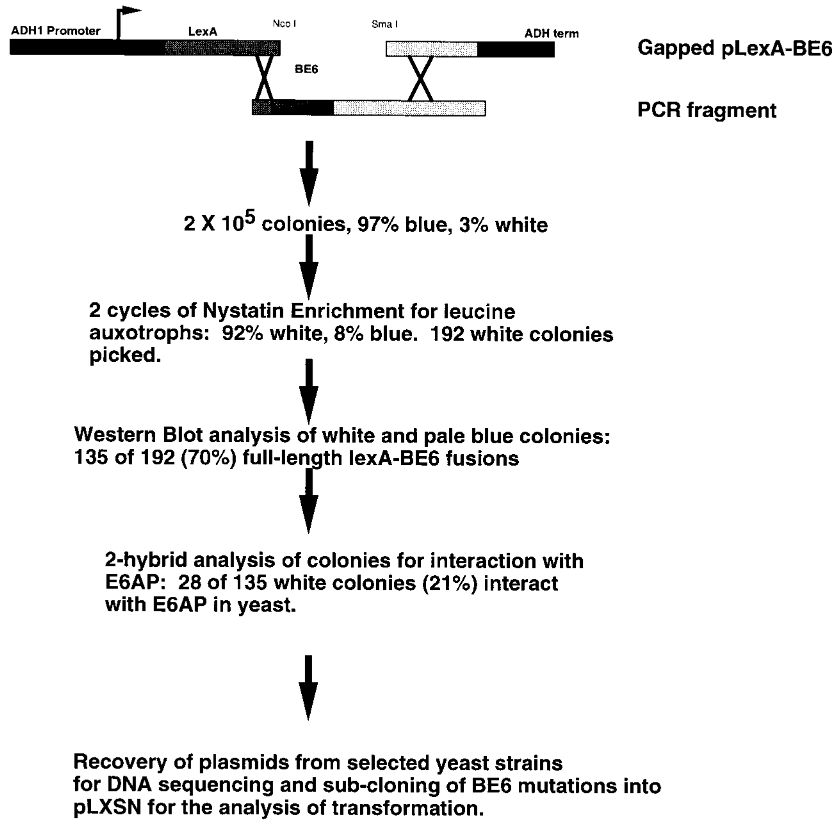

FIG. 2. Gap repair mutagenesis of BE6. The wild-type BPV-1 E6 and E7 regions were modified by oligonucleotide-mediated site-directed mutagenesis to eliminate a nonconserved stop codon at nucleotide (nt) 48 (TAG-to-TAC mutation), to introduce an *Nco*I site at amino acid 6 of BE6, to introduce an *Eco*RI site just upstream of the *Hpa*I site at the origin of replication of BPV-1, and to insert an *Xho*I site into the E1 open reading frame at nt 1470. The resulting 1,478-nt *Eco*RI-to-*Xho*I fragment was cloned into pEG202 such that the sequences from the *Eco*RI site at the origin of replication to the end of BE6 are a continuous open reading frame fused to *lexA*. pEG202, *lexA*-responsive *lacZ* reporter plasmid pSH18-34, and yeast reporter strain EGY-48 were a generous gift from Roger Brent (Harvard University) (2). Gap repair mutagenesis (10) was performed by cotransfection of a 1.7-kb PCR-amplified fragment of BPV-1 sequences from pLexA-BE6 together with 1 μg of pLexA-BE6 from which the BE6 sequences had been excised by restriction digestion. The diagram at the top illustrates homologous recombination in yeast between a gapped plasmid and a PCR product used to repair the plasmid. The results for the transfection and subsequent screening for BE6 mutants are shown below. BE6 mutant association with E6-AP was determined by two-hybrid analysis following mating to strains of YPH499 yeast containing either E6-AP preys or the empty prey plasmid pJG4-5.

that the phenotypes of the mutants were not a consequence of fusion to *lexA* and second to determine if the mutant phenotypes selected in yeast were also observed in mammalian cells. Wild-type and mutant BE6-E2 fusions were assayed in monkey CV-1 cells for transactivation of an E2-responsive chloramphenicol acetyltransferase (CAT) reporter. Table 2 reveals that wild-type BE6 fused to E2 strongly transactivated the E2-responsive CAT reporter, confirming earlier findings that BE6 is a transcriptional activation domain in mammalian cells (7). BE6 mutants BE6-R42W, BE6-R116G, and BE6-Δ13-15 all failed to activate the E2-responsive reporter in monkey CV-1 cells, demonstrating that transcriptional activation in

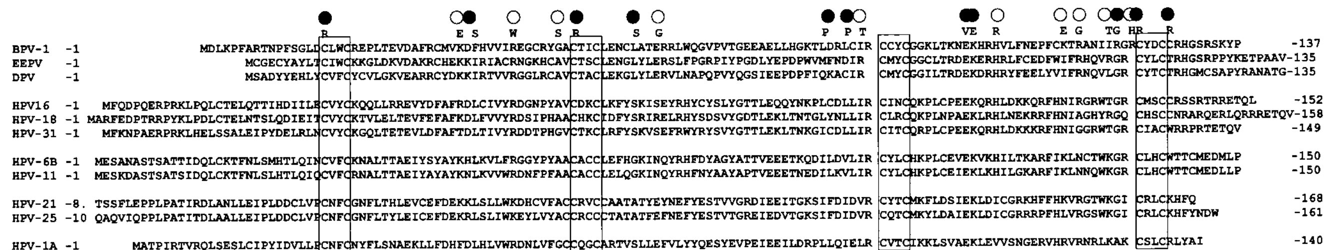

FIG. 3. Location of BE6 mutants selected for defects in transcriptional activation. The locations and phenotypes of yeast-selected mutations in BE6 from Table 1 are illustrated graphically. Conceptual translations of the indicated E6 genes were aligned at conserved zinc-binding motifs (CXXC; boxed). The locations and amino acid substitutions from Table 1 are shown above the BE6 sequence. BPV-1, deer papillomavirus (DPV), and European elk papillomavirus (EEPV) are all fibropapillomaviruses. HPV-16, -18, and -31 are human mucosal cancer-associated papillomaviruses, HPV-6B and -11 are human mucosal non-cancer-associated viruses, HPV-21 and -25 are epidermodysplasia verruciformis-associated viruses, and HPV-1A is a cutaneous-wart-associated virus. Open circles above the indicated BE6 mutations indicate mutants that have decreased transcriptional activation yet retain association with E6-AP, and the solid circles illustrate the locations of mutations that result in both decreased transcriptional activation and decreased association with E6-AP.

TABLE 1. Transcriptional activation and interaction with E6-AP by BE6 mutants

BE6 wild type or mutant[a]	Mutation	Transactivation[b]	E6-AP interaction (% wild type)		Transformation[g]
			Association[c]	Binding[e]	
BE6-WT	NA[d]	100	100	100	100, 100, 100
BE6-Δ13-15[h]	NA	1.1	1.0	0	0, 0, 0
BE6-C20R	TGC to CGC	0	0	0	0, 0, 0
BE6-Δ22-24[h]	NA	0	0	0	0, 0, 0
BE6-Δ31-33[h]	NA	0	1.1	0	0, 0, 0
BE6-K35E	AAA to GAA	51	65	76	0, 0, 0
BE6-F37S	TTT to TCT	0	0	0	0, 0, 0
BE6-R42W	CGG to TGG	4.0	77	58	55, 47, 69
BE6-G48S	GGT to AGT	31	49	43	ND[f]
BE6-C50R	TGT to CGT	5.8	0	0	0, 0, 0
BE6-L58S	TTA to TCA	8.7	1.3	0	0, 0, 0
BE6-E61G	GAA to GGA	15	48	15	ND
BE6-L83P	CTT to CCT	0	0	3	0, 0, 0
BE6-L86P	CTT to CCT	2.0	2.4	0	0, 0, 0
BE6-E101V	GAA to GTA	4.6	0 (ts)[j]	21	0, 0, 0
BE6-K102E	AAA to GAA	0	0	0	0, 0, 0
BE6-I88T/H105R	ATA to ACA/CAT to CGT	11	62	0	0, 0, 0
BE6-K114E	AAA to GAA	70	79	63	25, 16, 33
BE6-R116G	AGA to GGA	6.4	93	14	1.5, 0.5, 0.7
BE6-I120T	ATT to ACT	2.9	27	11	ND
BE6-R121G	AGA to GGA	0	4.5	0	0, 0, 0
BE6-R123H	CGC to CAC	18	58	12	0, 0, 0
BE6-C124R	TGC to CGC	0	0	0	0, 0, 0
BE6-C128R	TGC to CGC	1.0	15	13	ND
BE6-R129S[h]	AGA to AGC	197	71	97	96, 87, 93
BE6-Δ131-133/S134A[h]	NA	23 (ts)[i]	63	88	16, 21, 3.3
BE6-Δ135-137[h]	NA	2.0	2.0	2.2	0, 0, 0
BE6-P137R[h]	CCA to CGA	2.0	1.0 (ts)[j]	74	0, 0, 0

[a] Mutants (except for those to which footnote *h* applies) are designated by the wild-type amino acid followed by the position followed by the substituted amino acid. For example, BE6-C20R refers to the mutant with the substitution of arginine for cysteine at amino acid 20. WT, wild type.

[b] Transactivation results are the results of liquid beta-galactosidase assays performed in triplicate on log-phase cultures with beta-galactosidase assay buffer (Z buffer) containing *o*-nitrophenyl-β-D-galactopyranoside and 0.2% sarkosyl to permeabilize the yeast (6). The results are expressed as percentages of wild-type BE6 beta-galactosidase units.

[c] Human E6-AP (provided by John Huibregtse, Rutgers University) was cloned as a fusion to the B42 transactivation domain in the yeast two-hybrid prey plasmid pJG4-5 (2). The E6-AP gene contains a mutation in the conserved cysteine necessary for ubiquitin ligation. This mutant E6-AP binds to E6 genes but does not initiate ubiquitin-mediated degradation (4). The interaction with E6-AP was observed in yeast by a two-hybrid analysis in parallel with transactivation assays after mating the *lexA*-BE6-expressing yeast strains to a strain containing an E6-AP prey plasmid. Optical density at 410 nm (OD_{410}) values from the transactivation assay (see footnote *b*) were subtracted from the E6-AP OD_{410} association results in order to correct for the inherent transactivation of BE6 or the BE6 mutants. The results are expressed as the percentages of wild-type BE6 beta-galactosidase units.

[d] NA, not applicable.

[e] For E6-AP in vitro association assays, in vitro transcription templates for wild-type BE6 and mutant BE6 genes were generated by PCR of the yeast *lexA* fusion plasmids isolated from yeast with a 5′ primer containing a synthetic T7 RNA polymerase promoter and annealing to the beginning of the E6 gene and a 3′ PCR primer located at nucleotide 710 within the E7 gene. The PCR product was then translated in a coupled transcription and translation reaction with [^{35}S]cysteine. One microgram of GST–E6-AP fusion protein (a gift from John Huibregtse, Rutgers University) immobilized on glutathione agarose beads was resuspended in 150 μl of binding buffer (0.15 M NaCl, 50 mM Tris [pH 7.4], 1% Nonidet P-40, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl floride) together with 10 μl of in vitro-translated and [^{35}S]cysteine-labeled rabbit reticulocyte lysate programmed to translate wild-type BE6 or BE6 mutants. The binding reaction was incubated for 16 h at 4°C prior to washing the beads three times with 1.0 ml of binding buffer. E6 proteins were then resolved on SDS–15% PAGE protein gels and visualized by autoradiography. E6 was quantitated by scanning the dried gels on a Packard Instant Imager. The percentages of E6 bound by the mutants are relative to the percentage of wild-type E6 bound by immobilized GST–E6-AP. In this experiment, 26% of the wild-type BE6 input counts bound to GST–E6-AP beads.

[f] ND, not done.

[g] Wild-type BE6 or BE6 mutants were cloned directly as 1.4-kB *Eco*RI-to-*Xho*I fragments from plexA-BE6 (for wild-type BE6) or mutant plexA-BE6 plasmids recovered from yeast into pLXSN, a retroviral expression plasmid with a Moloney leukemia virus promoter and pSV2neo selection (9). Equal numbers of pooled G418-resistant mouse C127 cells were seeded into agar 14 days after transfection with either wild-type BE6 or BE6 mutants as previously described (14). Cultures were scored for anchorage independence 10 to 14 days later. Shown are the results of three separate transfections for each mutant; results are expressed as percentages of anchorage independence relative to that of wild-type BE6. Pooled G418-resistant colonies transfected with wild-type BE6 induced anchorage-independent colonies at 55 to 70% efficiency in these assays.

[h] Mutant was not selected in yeast but rather was the result of oligonucleotide-directed site-specific mutagenesis.

[i] Mutant was found in separate assays performed at room temperature to have transactivation significantly greater than that seen in the 30°C assay result shown in the table.

[j] Mutant was found in separate assays performed at room temperature to have a slight interaction with E6-AP not seen in the 30°C assay result shown in the table.

mammalian cells and the induction of anchorage independence can be dissociated. BE6-Δ13-15 was used as a negative control plasmid since it is defective for transactivation, transformation, and association with E6-AP. In experiments not shown here, we have found that wild-type and mutant BE6-E2 fusions were expressed at equivalent levels in *cos-1* transfections, indicating that the lack of transactivation by mutant BE6-E2 fusions is not a consequence of protein instability. In this study we are comparing the properties of wild-type BE6 determined from transformation assays to those of *lexA*-BE6 and BE6-E2 fusions determined from transcriptional activation assays. While the transformation phenotype of the *lexA*-BE6 fusion is unknown, other fusions to the N terminus of BE6 (such as glutathione *S*-transferase [GST] [12] or the monoclo-

TABLE 2. Transcriptional activation by BE6 mutants in mammalian cells

Plasmid[a]	Transcriptional activation (fold)[b]
pSVe vector	1.0
pSVeBE6-E2	64
pSVeBE6-R116G-E2	1.66
pSVeBE6-R42W-E2	1.67
pSVeBE6-Δ13-15-E2	0.88

[a] The 121 C-terminal amino acids of BPV-1 E2 were amplified with PCR primers that added a 5′ *Not*I site and a 3′ *Stu*I site; this fragment was cloned into p1135B. A PCR-amplified BE6 gene with a 5′ *Nco*I site at the translation initiation site for BE6 and a 3′ *Xho*I site fused in frame to the 3′ end of BE6 then was cloned in frame to the E2 DNA binding and dimerization domain in p1135B, generating pSVeBE6-E2. BE6 mutants were PCR amplified with 5′ *Nco*I and 3′ *Xho*I sites and cloned as E2 fusions into pSVeBE6-E2. All of the resultant plasmids were sequenced through the E6 and E2 genes and were found not to contain any unexpected PCR-generated mutations. pSVe vector is p1135, the parent plasmid for pSVeBE6-E2. Five micrograms of the E2-responsive reporter plasmid pTKM17.9 was transfected together with 1 μg of activating cDNA expression plasmid for a total of 10 μg per 6-cm-diameter plate as described in reference 15.

[b] Fold activation relative to that of pSVe plasmid; results are the averages of triplicate plates for each sample.

nal antibody epitope EE [18] are still compatible with transformation by the resulting GST-BE6 or EE-BE6 fusion protein (13).

The BE6-R42W mutant demonstrates that the induction of wild-type levels of anchorage independence by BE6 does not require transcriptional activation. While none of the mutants that were defective for association with E6-AP retained the ability to induce anchorage independence, strong association with E6-AP was not sufficient for anchorage independence because mutant BE6-K35E was defective for anchorage independence while retaining strong association with E6-AP. These results make the role of BE6 transcriptional activation unknown. BPV-1 transcription appears to be under the control of virally encoded E2 products as well as *cis* constitutive transcription elements (15; reviewed in reference 8). It is possible that in the context of a natural infection BE6 might influence viral transcription or BE6 might play some role in BPV-1 late gene transcription. It is also possible that transcriptional activation may have a role in the transformation or immortalization of primary cells or cell lines other than C127 cells.

One surprising feature of the yeast-selected mutants was their distribution throughout the primary BE6 amino acid sequence. Even mutants that are selectively defective for transcriptional activation while retaining some association with E6-AP (i.e., mutants that are not globally defective for all BE6 functions), such as BE6-R42W, -G48S, -E61G, -H105R/I88T, -R116G, -I120T, -R123H, and -Δ131-133/S134A are found in both zinc finger regions and the region between the two zinc fingers and the C terminus. There was no obvious clustering of mutations to a short linear stretch of amino acids. Thus, BE6 transcriptional interaction and association with E6-AP both require sequences throughout E6. The interpretation of this result is not yet clear. It is possible that E6-AP or the transcription apparatus recognizes only a short linear domain of BE6 and that sequences elsewhere in BE6 do not directly contact E6-AP but rather contribute to the proper conformation of a small domain. The same may be true for transcriptional activation. However, interactions between BE6 and the transcriptional apparatus or E6-AP may be more complex, requiring dispersed contacts throughout the BE6 primary sequence. The mutants derived in this study will be useful for the interpretation of structural models of the E6 proteins that may be developed in the future.

We thank Francoise Thierry for the reporter plasmid pTKM17.9, John Huibregtse for the E6-AP gene, and Roger Brent for yeast strain EGY-48 and plasmids pSH18-34 and pEG202. We also thank Dennis Templeton and Adam Goldfarb for critical reading of the manuscript.

This work was supported by NIH grant CA-69292-01A1, ACS grant IRG-186A, and institutional support from UPATH Associates.

REFERENCES

1. **Chen, J. J., C. E. Ried, V. Band, and E. J. Androphy.** 1995. Interaction of papillomavirus E6 oncogenes with a putative calcium binding protein. Science **269:**529–531.
2. **Gyuris, J., E. Golemis, H. Chertkov, and R. Brent.** 1993. Cdi1, a human G1 and S phase protein phosphatase that associates with Cdk2. Cell **75:**791–803.
3. **Howley, P. M., K. Munger, H. Romanczuk, M. Scheffner, and J. M. Huibregtse.** 1991. Cellular targets of the oncoproteins encoded by the cancer associated human papillomaviruses. Princess Takamatsu Symp. **22:**239–248.
4. **Huigbregtse, J. M., M. Scheffner, S. Beaudenon, and P. M. Howley.** 1995. A family of proteins structurally and functionally related to the E6-AP ubiquitin-protein ligase. Proc. Natl. Acad. Sci. USA **92:**2563–2567.
5. **Huibregtse, J. M., M. Scheffner, and P. M. Howley.** 1993. Cloning and expression of the cDNA for E6-AP, a protein that mediates the interaction of the human papillomavirus E6 oncoprotein with p53. Mol. Cell. Biol. **13:**775–784.
6. **Kippert, F.** 1995. A rapid permeabilization procedure for accurate quantitative determination of beta-galactosidase activity in yeast cells. FEMS Microbiol. Lett. **128:**201–206.
7. **Lamberti, C., L. C. Morrissey, S. R. Grossman, and E. J. Androphy.** 1990. Transcriptional activation by the papillomavirus E6 zinc finger oncoprotein. EMBO J. **9:**1907–1913.
8. **McBride, A. A., H. Romanczuk, and P. M. Howley.** 1991. The papillomavirus E2 regulatory proteins. J. Biol. Chem. **266:**18411–18414.
9. **Miller, A. D., and G. J. Rosman.** 1989. Improved retroviral vectors for gene transfer and expression. BioTechniques **7:**980–990.
10. **Muhlrad, D., R. Hunter, and R. Parker.** 1992. A rapid method for localized mutagenesis of yeast genes. Yeast **8:**79–82.
11. **Scheffner, M., K. Munger, J. Huibregtse, and P. Howley.** 1992. Targeted degradation of the retinoblastoma protein by human papillomavirus E7-E6 fusion proteins. EMBO J. **11:**2425–2431.
12. **Smith, D. B., and K. S. Johnson.** 1988. Single-step purification of polypeptides expressed in Escherichia coli as fusions with glutathione S-transferase. Gene **67:**31–40.
13. **Vande Pol, S.** Unpublished observations.
14. **Vande Pol, S., and P. Howley.** 1995. Negative regulation of the bovine papillomavirus E5, E6, and E7 oncogenes by the viral E1 and E2 genes. J. Virol. **69:**395–402.
15. **Vande Pol, S. B., and P. M. Howley.** 1992. The bovine papillomavirus constitutive enhancer is essential for viral transformation, DNA replication, and the maintenance of latency. J. Virol. **66:**2346–2358.
16. **Vousden, K., E. Androphy, J. Schiller, and D. Lowy.** 1989. Mutational analysis of bovine papillomavirus E6 gene. J. Virol. **63:**2340–2342.
17. **Vousden, K. H.** 1994. Interactions between papillomavirus proteins and tumor suppressor gene products. Adv. Cancer Res. **64:**1–24.
18. **Walter, G., R. Ruediger, C. Slaughter, and M. Mumby.** 1990. Association of protein phosphatase 2A with polyoma virus medium tumor antigen. Proc. Natl. Acad. Sci. USA **87:**2521–2525.