VIROLOGY 214, 289–293 (1995)

SHORT COMMUNICATION

Dimerization of the Human Papillomavirus E7 Oncoprotein *in Vivo*

KAREN E. CLEMENS,* ROGER BRENT,† JENÖ GYURIS,†,[1] and KARL MÜNGER‡,[2]

*Laboratory of Molecular Virology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892; †Department of Molecular Biology, Massachusetts General Hospital, and Department of Genetics, Harvard Medical School, Boston, Massachusetts 02114; and ‡Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115

Received August 1, 1995; accepted October 3, 1995

We have used a yeast two-hybrid system to show that human papillomavirus E7 proteins can form oligomeric complexes *in vivo.* The carboxyl-terminal cysteine-rich metal-binding domain is critical for this activity although amino-terminal sequences also contribute to oligomerization. Our experiments also reveal that E7 possesses an intrinsic transcription activation activity in yeast, which resides in the amino terminus of the protein.

Papillomaviruses are small DNA viruses which infect epithelial cells and cause the formation of generally benign hyperplasias or warts. Of the more than 70 different human papillomaviruses (HPVs), approximately 20 are associated with lesions of the anogenital tract. These HPVs are classified into two groups: "low-risk" HPVs (such as HPV-6 and HPV-11), associated with genital warts (condyloma acuminata), which are at low risk for carcinogenic progression, and "high-risk" HPVs (such as HPV-16 and HPV-18), associated with squamous intraepithelial neoplasias, which are at risk for carcinogenic progression. Consistent with this classification, approximately 80% of all cervical carcinomas contain high-risk HPVs (reviewed in *1*).

The high-risk HPVs encode two oncoproteins, E6 and E7, which are consistently expressed in HPV-positive cervical cancers (reviewed in *2*). In tissue culture, expression of high-risk HPV E6 and E7 induces immortalization of primary human genital epithelial cells, the normal host cells of these viruses (*3–5*). Like the oncoproteins of other small DNA tumor viruses — including the adenovirus (Ad) E1A and E1B proteins and the large tumor antigens (TAgs) of polyomaviruses — HPV E6 and E7 exert their oncogenic functions at least in part by interacting with and functionally inactivating cellular regulatory proteins. The high-risk HPV E6 proteins bind to and inactivate the p53 tumor suppressor protein by inducing its rapid degradation through the ubiquitin-mediated proteolysis system (*6, 7*). The HPV E7 proteins interact with and functionally inactivate a family of structurally related proteins including the retinoblastoma tumor suppressor protein pRB, p107, and p130 (*8–13*). Complex formation with these cellular proteins is thought to account for many biological properties of high-risk HPV E7 proteins, including cellular transformation and transcriptional activation of the E2F-responsive viral and cellular promoters (reviewed in *14*). These biological properties are shared between high-risk HPV E7, the Ad E1A protein, and the TAgs of polyomaviruses. As predicted by their functional similarity, the three classes of viral oncoproteins also share structural similarity (*15*). The amino-terminal 38 amino acids of E7 are similar to two portions of the Ad E1A and the polyomavirus TAg sequences which include a short portion of conserved region (CR) 1 and the entire CR2. The carboxyl termini of the E7 proteins contain two copies of a Cys-X-X-Cys sequence motif which has been implicated in the binding of zinc ions (*16–21;* Fig. 1). Interestingly, the papillomavirus E6 proteins also contain similar motifs (*16*). The spacing between the Cys-X-X-Cys motifs is quite large (29 amino acid residues) in the conserved regions and does not contain the highly conserved amino acid residues found in typical zinc finger structures.

Mutations in the metal-binding domain of E7 either have no distinguishable biological phenotypes or interfere with protein stability (*22, 23*), suggesting that the carboxyl terminus is critical for the structural and functional integrity of the E7 protein (*20, 24*). Several studies have suggested that specific biochemical and biological functions are associated with the carboxyl terminus of E7. This sequence is necessary for the abrogation of pRB's nonspecific DNA-binding property (*25*), for the disruption of pRB/E2F-1 transcription factor complexes by HPV-16 E7 (*26, 27*), and may constitute a low-affinity pRB-binding site (*28*). A carboxyl-terminal peptide of E7 was

[1] Present address: Mitotix Inc., Cambridge MA 02142.

[2] To whom correspondence and reprint requests should be addressed at Pathology Department, Harvard Medical School, Goldenson Building, Room 113A, Boston, MA 02115. Fax: (617) 432-0426.

0042-6822/95 $12.00

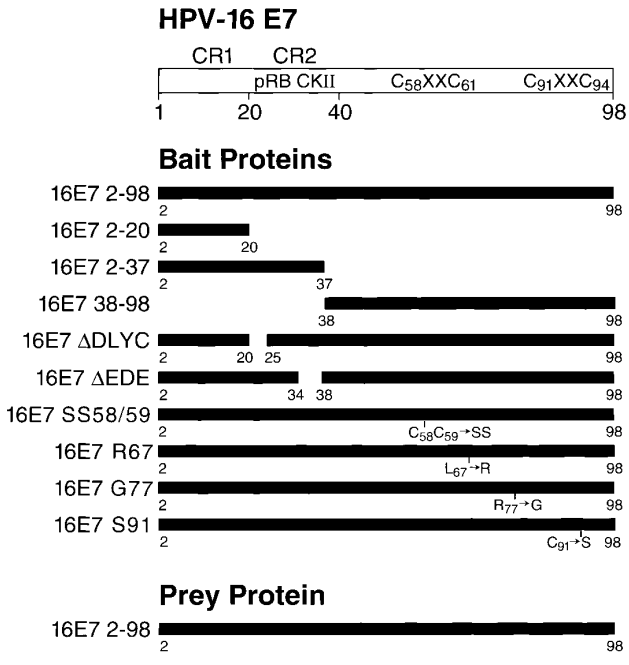

FIG. 1. Structure of the HPV-16 E7 "bait" and "prey" fusion proteins. The 2-μm *HIS3* plasmid pLex202 was used for construction of the baits. In pLex202 the LexA DNA-binding and dimerization domains (LexA amino acid residues 1–202) (*39*) are expressed from the constitutive alcohol dehydrogenase (ADH) promoter. The LexA hybrid genes ("baits") were prepared using PCR amplification according to the manufacturer's conditions (Perkin–Elmer). The amplified products were generated with oligonucleotide primers generating *Eco*RI and *Bam*HI restriction sites at the 5′ and 3′ termini, respectively, to allow cloning into the pLex202 plasmid at the *Eco*RI and *Bam*HI restriction sites. The sequences of the PCR-amplified baits were confirmed by dideoxynucleotide sequence analysis (Sequenase, USB). The prey plasmid, pJG4-5, is a 2-μm, *TRP1* plasmid that directs the synthesis of the SV40 nuclear localization signal, the B42 acidic activation domain, and the influenza virus HA1 epitope tag — totaling 107 amino acids (*31*). Fusion protein expression is directed from the glucose-repressed, galactose-inducible *GAL1* promoter. Amino acids 2–98 of HPV-16 E7 and 371–928 of the retinoblastoma protein pRB were PCR-generated and cloned into the *Eco*RI and *Xho*I sites of pJG4-5. The cloned products were analyzed by dideoxynucleotide sequence analysis (Sequenase, USB). The pRB prey contains a valine to alanine substitution at amino acid 520. Since this mutant protein was able to interact strongly with the wild-type E7 bait, it was used in all of the assays presented here. The 2-μm, *URA3* reporter plasmid, pJK103 (*40*), is a medium-sensitivity LexAop-GAL1-lacZ reporter; it contains a single high-affinity colE1-derived LexA operator and presumably binds four monomers of the LexA fusion bait (*41*). The *Saccharomyces cerevisiae* strain EGY188 (*MATa trp1 ura3 his3 LEU::pLexop2-LEU2*) was used as a host to analyze bait/prey interactions (*32*). Both the yeast *LEU2* gene and the *lacZ* gene on pJK103 are transcriptionally silent unless activated by a LexA-containing protein or protein complex. Yeast transformations were performed according to standard methods (*42*).

able to transactivate the Ad E2 promoter when microinjected into HeLa cells (*29*). One study also suggests that the carboxyl terminus of E7 contains important determinants for the immortalization of primary human genital keratinocytes in cooperation with E6 (*24*). Spectroscopic analyses and biochemical studies have indicated that the carboxyl-terminal, cysteine-rich, zinc-binding domain may be involved in mediating dimer/multimer formation (*18–20*). A detailed characterization of this activity was complicated by the fact that mutations in this region often led to decreased protein stability (*22, 23*). When expressed in yeast as fusions to the LexA moiety, the carboxyl-terminal E7 mutants encoded stable proteins and were expressed at levels comparable to those of the wild-type LexA–E7 protein (data not shown). We used these mutants in a two-hybrid system, the interaction trap (*30–32*), to analyze oligomerization of E7 *in vivo* and to examine the molecular determinants that are necessary for oligomerization.

Several LexA–E7 fusion proteins, which encode either the wild-type or the mutant forms of HPV-16 E7, were used as baits. Additional baits were constructed encoding previously identified structural and functional domains of HPV-16 E7. These include amino acids 2–20 (CR1 homology domain), 2–37 (CR1/CR2 homology domains), and 38–98 (carboxyl-terminal zinc-binding domain) (Fig. 1). We found that the E7 protein, when fused to LexA, activates transcription in the absence of an interacting prey (Table 1). This intrinsic transcriptional activation property of E7 mapped primarily to the amino-terminal 37 amino acids of E7, while the carboxyl terminus of E7 (amino acids 38–98) had a much lower activation potential. The level of activation was considerably lower for a bait containing the full-length HPV-6 E7 coding region fused to LexA (data not shown). The intrinsic transcriptional activity of the LexA HPV-16 E7 protein was sufficient to induce expression of the integrated LexA-responsive *LEU2* reporter gene to a level that induced growth of LexA–E7-expressing yeast reporter cells on leucine-deficient plates (data not shown), which has effectively precluded the use of these E7 baits for the screening of a cDNA library to identify novel potential interaction partners of the HPV E7 oncoprotein. Consistent with the generally high sensitivity of this bait and reporter system, this activity was detected only in yeast and the LexA–HPV-16 E7 fusion protein did not activate transcription of a LexA-responsive reporter plasmid in mammalian cells (J. L. Brokaw and K. Münger, unpublished results). Although the possible biological relevance of the intrinsic transcriptional activation potential of E7, therefore, is presently unknown, it is possible that, when tethered to DNA, the cryptic activation domain may also function in mammalian cells. Interestingly, it has been shown that HPV-16 E7 can interact with p107/E2F transcription factor complexes (*33*). Although E7 binding does not lead to a physical disruption of this complex (*27, 34, 35*), it can result in the activation of specific E2F-responsive promoters (*36*). It is therefore an intriguing possibility that, under certain conditions, the transcriptional activation function of E7 may indeed contribute to its biological activity.

To evaluate the overall structural integrity and to characterize the carboxyl-terminal mutant E7 baits further, we also generated a prey where a fragment of pRB (amino acid residues 371–928) was fused to the B42 activator

TABLE 1

E7 Oligomerization *in Vivo*[a]

Bait	No interacting prey (β-galactosidase units[b])	pRB prey (% β-galactosidase activity[c])	Full-length E7 prey (% β-galactosidase activity[c])
16 E7 2–98	14	100	100
16 E7 2–20	2	3 ± 3	0
16 E7 2–37	50	88 ± 11	11 ± 10
16 E7 38–98	2	0	59 ± 16
16 E7 ΔDLYC	7	25 ± 11	67 ± 22
16 E7 ΔEDE	13	125 ± 65	88 ± 9
16 E7 SS58/59	5	30 ± 3	0
16 E7 R67	26	92 ± 19	0
16 E7 G77	12	64	98 ± 61
16 E7 S91	19	75 ± 25	0

[a] The yeast strain EGY188 was initially transformed with the *URA3*$^+$ reporter, pJK103, selected on uracil-deficient glucose plates followed by transformation with bait and prey plasmids (*43*). One milliliter of overnight culture grown in selective glucose media was washed once in H_2O and resuspended in 10 ml of selective media with galactose to induce prey protein synthesis. At 12 hr postinduction, liquid β-galactosidase assays were conducted according to standard procedures (*44*).

[b] The β-galactosidase activity was calculated with the following formula:

$$\frac{1000 \times OD_{420\ nm}}{OD_{600\ nm} \times \text{vol (ml)} \times \text{time (min)}}.$$

[c] The values were corrected by subtracting the intrinsic transactivation activity of each individual bait in the absence of an interacting prey. The values are given in % of transactivation by wild-type E7, which was arbitrarily set to 100%, and represent averages of three experiments.

sequence. In agreement with a previous study (*37*), HPV-16 E7 was able to efficiently interact with the pRB prey in this system and as established by our previous biochemical experiments, pRB binding was mediated by sequences in CR2 (*23*). The full-length HPV-16 E7 and the CR1/CR2 bait (16 E7 2–37) but not the HPV-16 E7 CR1 bait (16 E7 2–20) efficiently interacted with the pRB prey. Similarly, the deletion of amino acid residues 21 to 24 (16 E7 ΔDLYC) but not 35 to 37 (16 E7 ΔEDE) in the context of the full-length E7 protein resulted in a marked decrease in interaction with pRB. The four mutants in the carboxyl terminus of E7 were all able to interact with the pRB prey, although at somewhat reduced levels. Most strikingly, the reduced pRB-binding efficiency as a consequence of the mutation of the two cysteine residues in the 16 E7 SS58/59 bait protein may indicate that the structural integrity of the E7 protein may be critically dependent on the metal-binding sites in the carboxyl terminus (Table 1). Interestingly, deletion of the core pRB-binding site in CR2 did not completely abrogate pRB prey binding, possibly supporting some role of the carboxyl terminus in pRB binding (*28*). Although it has been reported that the carboxyl terminus of HPV E7 contains an independent, low-affinity pRB-binding site (*28*), the HPV-16 E7 carboxyl terminus bait (16 E7 38–98) did not interact with the pRB prey in our experiments. Similar results were obtained by β-galactosidase (Table 1) and coimmunoprecipitation analyses (Fig. 2). These results clearly illustrate the high sensitivity and selectivity of this version of the two-hybrid system.

Since the results with the pRB interaction indicated that the carboxyl-terminal mutant LexA–E7 fusion proteins had no major defects in protein folding, they were used to analyze the dimerization domain of E7 in more detail. The HPV-16 E7 baits and the HPV-16 E7 prey shown in Fig. 1 were coexpressed, and protein/protein interactions were analyzed using the LexAop-lacZ reporter and by coimmunoprecipitation (Table 1 and Fig. 2). Expression of the E7 prey was slightly toxic in yeast, in that the cell growth rate was slightly reduced upon induction of prey expression with galactose. This effect was independent of bait protein coexpression (data not shown). As shown in Table 1 and Fig. 2, the full-length HPV-16 E7 bait protein can efficiently interact with the HPV-16 E7 prey. We found that the carboxyl terminus of E7 (amino acids 38–98) was also able to efficiently interact with the full-length E7 prey (transcription at 59% of wild-type E7; Table 1). The 16 E7 2–37 bait also interacted at a very low efficiency with full-length LexA E7 (transcription at 11% of wild-type E7; Table 1), suggesting that the carboxyl terminus may not be the sole determinant for oligomerization. In agreement with this idea, the two amino-terminal mutants 16 E7 ΔDLYC and 16 E7 ΔEDE interacted with the E7 prey at somewhat reduced levels (transcription at 67 and 88%, respectively; Table 1). However, the concept that the carboxyl terminus is the major determinant for dimerization is clearly supported by the observation that three of the four tested LexA E7 preys with mutations in the carboxyl terminus (16 E7 SS58/59, 16 E7 R67, and 16E7 S91 of E7) had lost their ability to bind to the E7 prey (no activation of transcription above background level; Table 1). Similar results were obtained by coimmunoprecipitation analyses (Fig. 2). Not every mutation in the carboxyl terminus

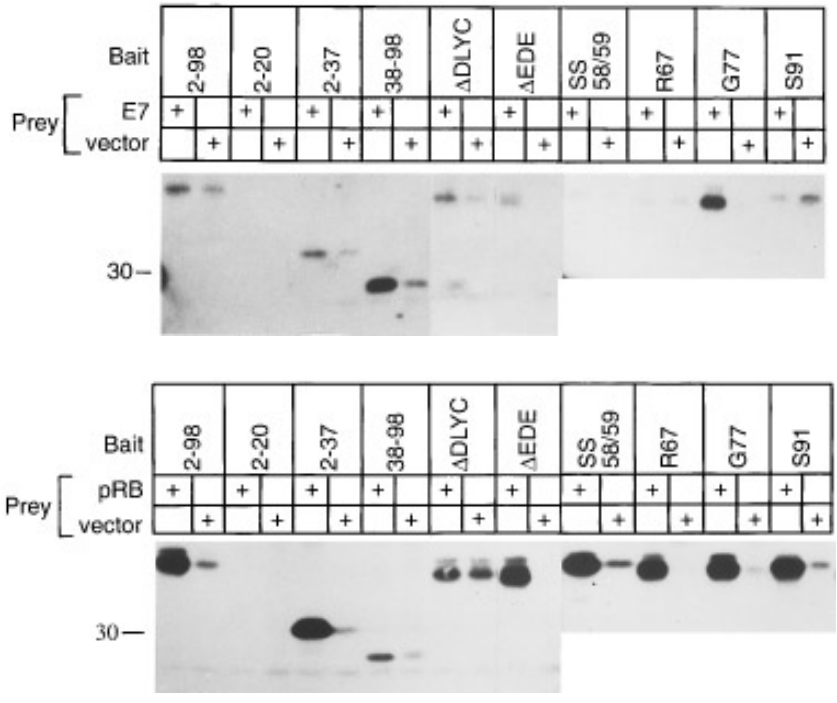

FIG. 2. Representative coimmunoprecipitation assays of the LexA–E7 fusion proteins with the E7 prey (top) and the pRB prey (bottom). A 10-ml culture of cells expressing the corresponding hybrid proteins was grown overnight in selective glucose media, washed in water, and grown in selective galactose media for 12 hr. Cells were lysed in breaking buffer (0.1 *M* NaCl, 50 m*M* Tris, 0.5% NP-40, 0.1 mg/ml phenymethylsulfonylfluoride, 2 μg/ml aprotinin, 2 μg/ml leupeptin, and 0.7 μg/ml pepstatin, pH 8.0) by vortexing in an equal volume of glass beads (Sigma; 425–600 μm). After centrifugation, the protein concentration of the supernatant was determined by the Bio-Rad assay. An aliquot containing 400 μg of total protein was immunoprecipitated using 5 μg of 12CA5 antibody (BAbCo) directed against the HA epitope in the prey fusion protein. Immunoprecipitated proteins were separated on a sodium dodecyl sulfate–10% polyacrylamide gel and electroblotted to Immobilon P membranes (Millipore). Coimmunoprecipitation of LexA–E7 bait proteins was accomplished using a 1:2000 dilution of a rabbit polyclonal antibody to the LexA portion of the bait (a kind gift of Russ Finley). After incubation with a horseradish peroxidase-linked secondary antibody (Amersham), the baits were detected by enhanced chemiluminescence (ECL kit, Amersham). Although bait and prey plasmids were expressed at approximately equal levels in all of these experiments (not shown), no further efforts were made to allow a quantitative interpretation of the data shown in this figure.

interfered with the dimerization/multimerization property of E7, and 16 E7 G77 strongly interacted with E7 by both β-galactosidase (transcription at 98% of wild-type E7; Table 1) and coimmunoprecipitation (Fig. 2) assays. Taken together, these results suggest that the HPV-16 E7 protein can form oligomeric complexes *in vivo.* The cysteine-rich carboxyl terminus of E7 is the major determinant but the amino-terminal CR1/CR2 homology domain of E7 may also contribute to oligomerization.

As summarized earlier, the carboxyl terminus of E7 exhibits a number of interesting biochemical properties and biological activities. Since alterations in the carboxyl terminus often compromise protein stability, it has not been possible to establish clearly the importance of oligomer formation for the functional integrity of E7. It is intriguing to note, however, that the one carboxyl-terminal E7 mutant that maintained the ability to form oligomeric complexes in this study (16 E7 G77) also retained transforming and transcriptional activities in a previous mutagenic analysis conducted in mammalian cells (*23*). In contrast, the dimerization-negative mutants 16 E7 SS58/59 and 16 E7 R67 were impaired in their biological activities although they were expressed at similar levels. The third dimerization-negative mutant, 16 E7 S91, did not express a stable protein in mammalian cells (*23*). Although alternative explanations are possible, these results are consistent with a model where oligomerization may serve as an indicator for the structural and functional integrity of the papillomavirus E7 proteins.

The carboxyl termini of the low- and high-risk E7 proteins are highly conserved and found to be functionally interchangeable (*38*). Thus, it was not surprising that a HPV-6 full-length E7 bait interacted with the HPV-16 E7 prey (data not shown). As with the high-risk HPV E7 protein, the interaction is dependent on carboxyl-terminal sequences since a HPV-6 amino-terminal E7 bait (amino acids 2–36) did not interact with the full-length HPV-16 E7 prey (data not shown).

The carboxyl-terminal Cys-X-X-Cys motifs and the 29-amino-acid spacing, which appear too large to form a typical zinc finger structure (*20*), are conserved in all E7 proteins so far examined and are also present in two copies in the papillomavirus E6 proteins (*16*). Therefore, the E6 protein may also form dimeric/multimeric complexes. Furthermore, the structurally similar Cys-X-X-Cys domains of E6 and E7 may allow the formation of heteromeric complexes which might possess interesting biological activities. Although, due to the low intracellular

levels of these proteins and inadequate immunological reagents, we have not yet been able to detect such complexes in mammalian cells, such complexes are readily detectable by coimmunoprecipitation from insect cells coinfected by E6- and E7-expressing recombinant baculoviruses (Münger, unpublished observations).

In conclusion, we have shown that the HPV E7 protein possesses an intrinsic transcriptional activation function in yeast. The amino-terminal CR2 homology domain is the major determinant for this activity. Furthermore, we show by functional and biochemical assays that the HPV E7 oncoprotein can form oligomeric complexes *in vivo* and that the carboxyl terminus of HPV E7 functions as a major determinant for this activity. Our results are consistent with the model that the ability to form homomeric complexes is an indicator of the structural and functional integrity of the carboxyl-terminal domain of the E7 protein.

ACKNOWLEDGMENTS

This work was supported by intramural research funds of the Division of Cancer Etiology of the NCI and grants from the Funds for Discovery, the Council for Tobacco Research (3859), and the American Cancer Society (VM-97) to K.M. We thank Russ Finley for anti-LexA antiserum and valuable suggestions, Jon Huibregtse, William C. Phelps, Jennifer Pietenpol, and Boris Schilling for helpful discussions and comments on the manuscript, and Ann C. Frenning for her expert editorial assistance.

REFERENCES

1. Zur Hausen, H., and De Villiers, E. M., *Annu. Rev. Microbiol.* 48, 427–447 (1994).
2. Münger, K., Scheffner, M., Huibregtse, J. M., and Howley, P. M., *Cancer Surv.* 12, 197–217 (1992).
3. Münger, K., Phelps, W. C., Bubb, V., Howley, P. M., and Schlegel, R., *J. Virol.* 63, 4417–4421 (1989).
4. Hawley-Nelson, P., Vousden, K. H., Hubbert, N. L., Lowy, D. R., and Schiller, J. T., *EMBO J.* 8, 3905–3910 (1989).
5. Kaur, P., and McDougall, J. K., *Virology* 173, 302–310 (1989).
6. Werness, B. A., Levine, A. J., and Howley, P. M., *Science* 248, 76–79 (1990).
7. Scheffner, M., Werness, B. A., Huibregtse, J. M., Levine, A. J., and Howley, P. M., *Cell* 63, 1129–1136 (1990).
8. Dyson, N., Howley, P. M., Münger, K., and Harlow, E., *Science* 243, 934–937 (1989).
9. Münger, K., Werness, B. A., Dyson, N., Phelps, W. C., Harlow, E., and Howley, P. M., *EMBO J.* 8, 4099–4105 (1989).
10. Barbosa, M. S., Edmonds, C., Fisher, C., Schiller, J. T., Lowy, D. R., and Vousden, K. H., *EMBO J.* 9, 153–160 (1990).
11. Gage, J. R., Meyers, C., and Wettstein, F. O., *J. Virol.* 46, 723–730 (1990).
12. Dyson, N., Guida, P., Münger, K., and Harlow, E., *J. Virol.* 66, 6893–6902 (1992).
13. Davies, R., Hicks, R., Crook, T., Morris, J., and Vousden, K., *J. Virol.* 67, 2521–2528 (1993).
14. Münger, K., and Phelps, W. C., *Biochem. Biophys. Acta* 1155, 111–123 (1993).
15. Phelps, W. C., Yee, C. L., Münger, K., and Howley, P. M., *Curr. Top. Microbiol. Immunol.* 144, 153–166 (1989).
16. Cole, S. T., and Danos, O., *J. Mol. Biol.* 193, 599–608 (1987).
17. Barbosa, M. S., Lowy, D. R., and Schiller, J. T., *J. Virol.* 63, 1404–1407 (1989).
18. Roth, E. J., Kurz, B., Liang, L., Hansen, C. L., Dameron, C. T., Winge, D. R., and Smotkin, D., *J. Biol. Chem.* 267, 16390–16395 (1992).
19. Patrick, D. R., Zhang, K., Defeo-Jones, D., Vuocolo, G. R., Maigetter, R. Z., Sardana, M. K., Oliff, A., and Heimbrook, D. C., *J. Biol. Chem.* 267, 6910–6915 (1992).
20. McIntyre, M. C., Frattini, M. G., Grossman, S. R., and Laimins, L. A., *J. Virol.* 67, 3142–3150 (1993).
21. Pahel, G., Aulabaugh, A., Short, S. A., Barnes, J. A., Painter, G. R., Ray, P., and Phelps, W. C., *J. Biol. Chem.* 268, 26018–26025 (1993).
22. Edmonds, C., and Vousden, K. H., *J. Virol.* 63, 2650–2656 (1989).
23. Phelps, W. C., Münger, K., Yee, C. L., Barnes, J. A., and Howley, P. M., *J. Virol.* 66, 2418–2427 (1992).
24. Jewers, R. J., Hildebrandt, P., Ludlow, J. W., Kell, B., and McCance, D. J., *J. Virol.* 66, 1329–1335 (1992).
25. Stirdivant, S. M., Huber, H. E., Patrick, D. R., Defeo-Jones, D., McAvoy, E. M., Garsky, V. M., Oliff, A., and Heimbrook, D. C., *Mol. Cell. Biol.* 12, 1905–1914 (1992).
26. Huang, P. S., Patrick, D. R., Edwards, G., Goodhart, P. J., Huber, H. E., Miles, L., Garsky, V. M., Oliff, A., and Heimbrook, D. C., *Mol. Cell. Biol.* 13, 953–960 (1993).
27. Wu, E. W., Clemens, K. E., Heck, D. V., and Münger, K., *J. Virol.* 67, 2402–2407 (1993).
28. Patrick, D. R., Oliff, A., and Heimbrook, D. C., *J. Biol. Chem.* 269, 6842–6850 (1994).
29. Rawls, J. A., Pusztai, R., and Green, M., *J. Virol.* 64, 6121–6129 (1990).
30. Fields, S., and Song, O., *Nature* 340, 245–246 (1989).
31. Gyuris, J., Golemis, E., Chertkov, H., and Brent, R., *Cell* 75, 791–803 (1993).
32. Golemis, E. A., Gyuris, J., and Brent, R., *In* "Current Protocols in Molecular Biology" (F. A. Ausubel *et al.,* Eds.), pp. 13.14.1–13.14.17. Greene Publishing and Wiley–Interscience, New York, 1994.
33. Arroyo, M., Bagchi, S., and Raychaudhuri, P., *Mol. Cell. Biol.* 13, 6537–6546 (1993).
34. Chellappan, S., Kraus, V. B., Kroger, B., Münger, K., Howley, P. M., Phelps, W. C., and Nevins, J. R., *Proc. Natl. Acad. Sci. USA* 89, 4549–4553 (1992).
35. Pagano, M., Dürst, M., Joswig, S., Draetta, G., and Jansen-Dürr, P. *Oncogene* 7, 1681–1686 (1992).
36. Lam, E. W. F., Morris, J. D. H., Davies, R., Crook, T., Watson, R. J., and Vousden, K. H., *EMBO J.* 13, 871–878 (1994).
37. Ciccolini, F., Dipasquale, G., Carlotti, F., Crawford, L., and Tommasino, M., *Oncogene* 9, 2633–2638 (1994).
38. Münger, K., Yee, C. L., Phelps, W. C., Pietenpol, J. A., Moses, H. L., and Howley, P. M., *J. Virol.* 65, 3943–3948 (1991).
39. Golemis, E. A., and Brent, R., *Mol. Cell. Biol.* 12, 3006–3014 (1992).
40. Kamens, J., Richardson, P., Mosialos, G., Brent, R., and Gilmore, T., *Mol. Cell. Biol.* 10, 2840–2847 (1990).
41. Ebina, Y., Takahara, Y., Kishi, F., Nakazawa, A., and Brent, R., *J. Biol. Chem.* 258, 13258–13261 (1983).
42. Schiestl, R. H., and Gietz, R. D., *Curr. Genet.* 16, 339–346 (1989).
43. Lundblad, V., and Treco, D. A., *In* "Current Protocols in Molecular Biology" (F. A. Ausubel *et al.,* Eds.), pp. 13.1.1–13.1.7. Greene Publishing and Wiley–Interscience, New York, 1994.
44. Reynolds, A., and Lundblad, V., *In* "Current Protocols in Molecular Biology" (F. A. Ausubel *et al.,* Eds.), pp. 13.6.1–13.6.4. Greene Publishing and Wiley–Interscience, New York, 1994.