PKN Binds and Phosphorylates Human Papillomavirus E6 Oncoprotein*

Qingshen Gao‡, Ajay Kumar‡, Seetha Srinivasan‡, Latika Singh‡, Hideyuki Mukaï§, Yoshitaka Ono§, David E. Wazer‡, and Vimla Band‡¶

Received for publication, December 22, 1999

The high risk human papillomaviruses (HPVs) are associated with carcinomas of cervix and other genital tumors. Previous studies have identified two viral oncoproteins E6 and E7, which are expressed in the majority of HPV-associated carcinomas. The ability of high risk HPV E6 protein to immortalize human mammary epithelial cells has provided a single gene model to study the mechanisms of E6-induced oncogenic transformation. In recent years, it has become clear that in addition to E6-induced degradation of p53 tumor suppressor protein, other targets of E6 are required for mammary epithelial cells immortalization. Using the yeast two-hybrid system, we have identified a novel interaction of HPV16 E6 with protein kinase PKN, a fatty acid- and Rho small G protein-activated serine/threonine kinase with a catalytic domain highly homologous to protein kinase C. We demonstrate direct binding of high risk HPV E6 proteins to PKN in wheat-germ lysate in vitro and in 293T cells in vivo. Importantly, E6 proteins of high risk HPVs but not low risk HPVs were able to bind PKN. Furthermore, all the immortalization-competent and many immortalization-non-competent E6 mutants bind PKN. These data suggest that binding to PKN may be required but not sufficient for immortalizing normal mammary epithelial cells. Finally, we show that PKN phosphorylates E6, demonstrating for the first time that HPV E6 is a phosphoprotein. Our finding suggests a novel link between HPV E6 mediated oncogenesis and regulation of a well known phosphorylation cascade.

The human papillomaviruses (HPVs)1 are associated with epithelial tumors or benign lesions, especially those of anogenital origin (1, 2). HPVs are categorized into low risk and high risk HPVs. Low risk HPVs, such as HPV6 and HPV11, are usually associated with benign warts, whereas high risk HPVs, such as HPV16 and HPV18, are associated with carcinomas (1, 2). Transfection of the high risk HPV DNA into primary human keratinocytes results in their immortalization, indicating that the HPV genome encodes oncoproteins that mediate cellular transformation (3–5). Further studies revealed that only the E6 and E7 genes were necessary for the immortalization activity (4, 5). HPV E6 and E7 oncoproteins bind to and inactivate critical tumor suppressor proteins enabling the virus to override checkpoints that regulate cell proliferation (6–9). HPV E6 binds to the tumor suppressor protein p53 via the E6AP protein, a ubiquitin ligase, and induces its degradation through ubiquitin-proteasomal pathway (10–13). The p53 protein is critical for protection against propagation of DNA damage, and mediates apoptotic and cell cycle arrest responses to DNA damage (14, 15). The HPV E7 oncogene binds to the retinoblas-toma (Rb) gene product (6, 7) resulting in its degradation through the ubiquitin-proteasomal pathway (16). The unphosphorylated Rb protein is a critical cell cycle regulatory protein that is in complex with transcriptional factors, E2Fs during G1 phase of the cell cycle. Upon phosphorylation or association with oncoproteins such as E7, E2F complex is released, leading to S phase or continuous proliferation.

Unlike keratinocytes, discrete subpopulations of mammary epithelial cells are uniquely susceptible to immortalizing effects of E6 or E7 oncoproteins alone (17–19). This mammary epithelial cell model provides an opportunity to dissect out cellular pathways that are targeted by individual HPV onco-proteins. Although p53 is an important target of E6, other potential targets have recently emerged. Recently, it has been shown that introduction of HPV16 E6 into epithelial cells results in an early increase in telomerase activity and essentially all HPV E6-immortalized cells have a dramatic increase in telomerase activity (20, 21). Telomerase enzyme is responsible for replicating telomeres, the DNA elements located at the ends of chromosomes (22–24). Telomerase is composed of an RNA subunit, which acts as a template for replication, and the catalytic subunit hTERT, which functions as a reverse transcriptase (22–24). Telomerase activity has been found to be low in most of the normal tissues in vivo but is known to be elevated during tumorigenesis (22–24). Recent findings have directly implicated telomerase in escape from cellular senescence. Indeed, transfection of hTERT component of telomerase into selected cell types can itself induce immortalization (25, 26). It is likely that the ability of E6 to activate telomerase is one mechanism by which E6 can immortalize normal mammary epithelial cells. However, there is no direct evidence that E6-induced telomerase activity is responsible for E6-induced immortalization. E6 has also been shown to stabilize the c-Myc protein levels in cells through a post-transcriptional mechanism (27). Interestingly, c-Myc-binding sites have been identified in the promoter of hTERT (28–31). Since the E6-induced telomerase activation may be c-Myc-dependent and c-Myc can activate hTERT transcription, E6 may immortalize cells via c-Myc-induced telomerase activation. However, c-Myc and hTERT do not directly interact with E6.

1 The abbreviations used are: HPV, human papillomavirus; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; aa, amino acid(s); PKC, protein kinase C.
In addition to hTERT and c-Myc, a number of other E6-interacting proteins have been reported recently. These include E6BP (also referred to as ERC55), a putative calcium binding protein; paxillin, a focal adhesion protein involved in transducing signals from the plasma membrane to the actin cytoskeleton; clathrin adaptor complex AP-1; the human homologue of the Drosophila discs large tumor suppressor protein; interferon regulatory factor-3, multicytoplasmic maintenance protein 7, a subunit of the replication licensing-factor-M; Bak, a Bcl2 antagonist that promotes apoptosis (E6 inhibits Bak-induced apoptosis); and E6TP1, a novel protein with homology to Rap1GAP (32–40). Recent studies have demonstrated that E6 binding to hDlg, E6BP, and IRF3 are not required for E6-induced immortalization of cells (21, 38, 41). Studies are under way in many laboratories to define the role of remaining E6-binding proteins in E6-induced immortalization.

Using the yeast two-hybrid system, we report here a novel interaction of E6 protein with protein kinase PKN, a fatty acid- and Rho-small G protein-activated serine/threonine kinase (42–44). PKN was originally cloned by screening a human and Rho-small G protein-activated serine/threonine kinase (42–44). PKN was originally cloned by screening a human and Rho-small G protein-activated serine/threonine kinase (42–44). PKN was originally cloned by screening a human and Rho-small G protein-activated serine/threonine kinase (42–44). PKN was originally cloned by screening a human and Rho-small G protein-activated serine/threonine kinase (42–44). PKN was originally cloned by screening a human and Rho-small G protein-activated serine/threonine kinase (42–44). PKN was originally cloned by screening a human and Rho-small G protein-activated serine/threonine kinase (42–44). PKN was originally cloned by screening a human and Rho-small G protein-activated serine/threonine kinase (42–44). PKN was originally cloned by screening a human and Rho-small G protein-activated serine/threonine kinase (42–44). PKN was originally cloned by screening a human and Rho-small G protein-activated serine/threonine kinase (42–44). PKN was originally cloned by screening a human and Rho-small G protein-activated serine/threonine kinase (42–44). PKN was originally cloned by screening a human and Rho-small G protein-activated serine/threonine kinase (42–44).

In Vivo Binding of E6 and PKN—Twenty-eight clones were transduced in vitro in the presence of [35S]cysteine using a rabbit reticulocyte lysate-based coupled transcription/translation system (TNT rabbit reticulocyte lysate system; Promega). Five-μl aliquots of p53 or PKN translation reactions were incubated together with 5 μL of HPV E6 or water-primed (control) translation reaction. After a 12-h incubation at 30 °C, the degradation reaction was stopped by adding 100 μl of sample buffer, and proteins were resolved by 7.5% SDS-PAGE and visualized by fluorography.

In Vivo E6-dependent Degradation—293T cells were transfected with 10-μg DNA of pSG5 vector, pSG5–16E6, pSG5–E6TP1, pHmPKN7, or pHmPKN PK-2 individually or in the indicated combination. The total amount of DNA was held constant at 20 μg/dish by adding vector DNA. Cells were harvested in sample buffer after 48 h, and 10 μg of total protein was resolved on 6% SDS-PAGE, and transferred to a polyvinylidene difluoride membrane. Membranes were blotted with a rabbit anti-PKN (42) or anti-E6TP1 antisera (33), and detected using the enhanced chemiluminescence (ECL) detection system (Amersham Pharmacia Biotech).

RESULTS

Identification of PKN as a HPV16 E6 Interacting Protein

Using the Yeast Two-hybrid System—Screening of 1.5 × 10⁶ individual recombinants of normal mammary epithelial cell yeast two-hybrid cDNA library with HPV16 E6 (amino acids 2–158) identified clones capable of growth on Trp+, Leu+, and His+ selection buffer. Further screening of these clones using LacZ complementation yielded 88 colonies. These clones were re-tested for their interaction with E6 relative to control baits pLam 5′ and pVA3 murine p53. Twenty-eight clones were

Experimental Procedures

Yeast Two-hybrid Constructs and Screening—The detailed yeast two hybrid screening procedures have been described previously (33). Briefly, the HPV16 E6 bait in pGBT9 (E6 residues 2–158) was used to screen a normal mammary epithelial cell strain 76N cDNA library in pGAD10 vector (custom-made through CLONTECH) with 1.5 × 10⁶ primary recombinants and an average insert size of 1.5 kilobase pairs. The HPV16 E6 interacting proteins were identified by screening for growth on Trp+, Leu+, and His+ selection buffer (0.15M NaCl, 50 mM Tris, pH 7.4, 1 mM EDTA, 1% Triton X-100, 100 mM NaCl, 0.5% Nonidet P-40) for 2 h at 4 °C, and bound [35S]-labeled proteins were resolved by a 17% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and visualized by fluorography.

In Vitro Binding of E6 and PKN—The HPV16, -18, -11, -6 E6, and -16 E6 mutant proteins were generated by in vitro translation in the presence of [35S]cysteine (NEN Life Science Products) using a wheat germ lysate-based coupled transcription/translation system (TNT wheat germ lysate system; Promega) according to supplier’s recommendations.

Cloning of HPV16, -18, -11, and -6 E6 into pSP65 vector for screening of 1.5 × 10⁶ cells/100-mm dish, two 100-mm dishes per transfection) were transfected with 5 μg DNA of pSG5 vector, pSG5–16E6, pSG5–E6TP1, pHmPKN7, or pHmPKN PK-2 (K644E), or their indicated combinations using the Fugene reagent. The total amount of DNA was held constant at 20 μg/dish by adding vector DNA. One set of transfected cells were labeled in 3 ml of phosphorus-free Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) containing 30 μCi/ml [35S]phosphophoric acid (NEN Life Science Products) for 3 h. The other set of cells was labeled in 15 μCi/ml [35S]cysteine for 3 h. Each dish of cells was harvested in 5 ml of radioimmunoprecipitation buffer (0.15 mM NaCl, 50 mM Tris, pH 7.4, 1 mM EDTA, 1% Triton X-100, 0.1% deoxycholate, 0.1% SDS). HPV16 E6 protein was immunoprecipitated with anti-HPV16 E6 antisera (33) and resolved on a 17% SDS-PAGE, and immunoprecipitated proteins were visualized by autoradiography.
found to specifically interact with HPV16 E6 and were sequenced. One relatively weak positive clone encoded the 476 carboxy-terminal amino acids of E6-AP, a known E6-binding protein, including its 18-aa E6-binding motif (13). This result indicated that the cDNA library and the method of screening were suitable for isolating E6-binding proteins. A set of 11 identical and 1 distinct strongly positive clone identified overlapping regions of a novel E6-binding putative Rap-specific GAP, E6TP1, which we have reported recently (33). One of the remaining positive clones encoded the carboxy-terminal 169-aa protein kinase PKN, a previously identified serine/threonine kinase (Fig. 1). The region of PKN present in the isolated clone lacked basic and leucine zipper-like regions, which are known to be involved in intramolecular regulation via interaction with and binding to Rho, which regulates the activity of PKN. This region of PKN includes part of the catalytic domain and the extreme COOH-terminal region of PKN, which corresponds to the substrate binding region in PKC.

PKN Binds More Efficiently to E6 Proteins of High Risk HPV s in in Vitro System—To further confirm the interaction between E6 and PKN, we prepared a GST fusion protein encoding the full-length PKN and used it for in vitro binding experiments with the in vitro wheat germ lysate-translated \(^{35}\)S-labeled E6 protein of two high risk HPV (HPV16 and HPV18) and two low risk (HPV6 and HPV11) HPVs. As shown in Fig. 2, GST-E6AP (aa 37–865), used as a positive control, showed substantial binding to HPV16 but no binding to HPV6 E6 or HPV11 E6 proteins, as expected. As has been previously published, E6AP binds significantly less to HPV16 E6 as compared with HPV16 E6 (12). GST-E6AP mutant (\(\Delta 391–408\)), that lacks the 18-aa E6 binding motif, did not bind to the HPV E6 proteins above background level demonstrating the specificity of binding; GST-PKN protein showed substantial binding to the high risk HPV E6 proteins (HPV16 E6 and HPV18 E6), but a relatively low level of binding to the low risk HPV E6 proteins (HPV6 E6 and HPV11 E6). Thus, PKN appears to preferentially bind to E6 proteins of high risk HPV s. As binding assays were carried out in wheat germ lysates, which lack E6AP, the interaction between PKN and HPV E6 proteins is likely to be independent of E6AP similar to E6 interaction with E6AP but unlike E6 interaction with p53 (8, 11).

Binding of PKN and HPV16 E6 Protein in Vivo—To assess whether E6 can interact with PKN in vivo, 293T cells were transfected with either vector or a plasmid encoding myc-tagged E6 protein with or without plasmid encoding the FLAG-tagged wild-type PKN-AF3 or the kinase-defective PKN-AF3 (K644E). Forty-eight hours after transfection, the exogenously expressed PKN was immunoprecipitated using anti-FLAG monoclonal antibody (M2), followed by Western blotting for E6 using the anti-myc antibody (9E10). As expected, no E6 protein was detected in anti-FLAG immunoprecipitates of cells transfected with either vector alone or when E6 and PKN constructs were transfected individually (Fig. 3, lanes 1–3). In contrast, anti-myc-reactive E6 protein was clearly detected in anti-FLAG immunoprecipitates of cells co-transfected with E6 and PKN constructs (lane 4). Western blotting of whole cell lysates with anti-myc antibody indicated that E6 was expressed in all of the transfectants where E6 construct was introduced. These results demonstrate that E6 can associate with PKN in vivo.

Further, the kinase-defective PKN mutant (K644E) also associated with HPV16 E6 at levels similar to wild-type PKN, demonstrating that the kinase activity of PKN is not required for binding to HPV E6.

HPV16 E6 Protein Does Not Target PKN for Degradation—Previous analyses have revealed that the high risk HPV E6 proteins target a number of interacting proteins for degradation, whereas other binding proteins are not targeted for degradation (8, 32–40). In particular, HPV E6 proteins target p53 for degradation via the E6AP-mediated ubiquitination pathway (8, 10–13), which is thought to be critical for the transforming ability of E6. In addition, interaction with HPV16 E6 also targets other binding proteins such as E6TP1, Myc, Bak, and Mdm7 for degradation (33, 36, 37, 49). Given the preferential interaction of PKN with high risk HPV E6 proteins, we wished to examine if PKN was targeted for degradation upon interaction with E6. For this purpose, we incubated the in vitro translated PKN or p53 (as a positive control) with in vitro translated HPV16 E6 or HPV6 E6 proteins, and assessed the E6-induced degradation as described under “Experimental Procedures.” As expected, the level of p53 protein decreased upon incubation with HPV16 but not HPV6 E6 (Fig. 4A). In contrast, no decrease in the level of PKN protein was observed upon incubation with either HPV16 or HPV6 E6, indicating that E6 does not target PKN for degradation in this in vitro system.

To further assess the possibility that E6 may target PKN for degradation in vivo, we co-transfected PKN and HPV16 E6 into...
293T cells and lysates of these cells were subjected to immunoblotting with anti-PKN antibody. The endogenous levels of PKN in 293T cells were quite low (Fig. 4B, lanes 5 and 6), allowing the assessment of the effect of E6 expression on exogenously introduced PKN. As a positive control, HPV16 E6 was co-expressed with E6TP1, which we have shown to be targeted by E6 for in vivo degradation (33). After 48 h of transfection, the PKN or E6TP1 protein levels were determined using Western blotting. As expected, co-expression of E6 with E6TP1 led to a marked E6-induced loss of E6TP1 (left panel, compare lanes 3 and 4). However, co-expression with HPV16 E6 did not influence the protein levels of either the wild-type PKN or the kinase-defective mutant of PKN (Fig. 4B). Thus, both in vitro and in vivo analyses demonstrate that HPV16 E6 does not target PKN for degradation.

Analysis of E6 Mutants Binding to PKN in Vitro—Previous analyses of HPV16 E6 have identified immortalizing and non-immortalizing mutants of HPV16 E6 (21, 41, 48); using these mutants, we examined whether the ability of E6 protein to bind to PKN correlates with its immortalizing abilities. As shown in Fig. 5 and Table I, we examined 23 mutants for their ability to bind to PKN. Binding experiment were performed with in vitro wheat germ translated wild-type or mutant E6 proteins and GST fusion protein of PKN as described under “Experimental Procedures.” Notably, out of 23 mutants tested, all the six immortalizing substitution mutants and the two immortalizing small deletion mutants were capable of binding to PKN. However, a number of non-immortalizing substitution mutants (four out of six) and all of the six non-immortalizing small deletion mutants retained the ability to bind to PKN. Thus, it would appear that E6 binding to PKN is required for immortalization, although PKN binding may not be sufficient for immortalization.

PKN Phosphorylates HPV16 E6 Protein in Vivo—While HPV E6 proteins have been demonstrated to interact with a number of cellular proteins, none of the previously identified binding protein is protein kinase. Given the in vitro and in vivo association of E6 with PKN (Figs. 2 and 3), and the role of phos-
phorylation as a potential switch in protein function, we wished to address the possibility that E6 may become phosphorylated in vivo. In this regard, it is noteworthy that the short form of cottontail rabbit papillomavirus E6 and HPV E7 oncoprotein have been shown to be phosphorylated (51–55). To assess potential phosphorylation of E6, we co-transfected PKN or kinase-defective PKN (K644E) with HPV16 E6 into 293T cells. Transfected cells were either metabolically labeled with $[^{32}P]$orthophosphate for examining phosphorylation or with $[^{35}S]$cysteine for detecting E6 protein. These analyses revealed that when E6 was co-expressed with wild-type PKN, it was detected as a phosphoprotein (Fig. 6, lane 4, upper panel). However, when E6 was co-expressed with kinase-defective PKN, it was not detectably phosphorylated. Immunoprecipitation of $[^{35}S]$cysteine-labeled E6 proteins showed the expression of E6 proteins (Fig. 6, lower panel). This analysis demonstrates that in vivo interaction with PKN leads to phosphorylation of E6 and provides the first evidence that HPV E6 proteins are phosphorylated.

**DISCUSSION**

The ability of viral oncoproteins to induce single-step cellular transformation has provided an excellent approach to delineate the various biochemical pathways that control normal cell growth and differentiation. Indeed, recent studies have provided ample evidence that viral oncoproteins target cellular pathways whose aberrations are also critical in the development of human cancer (6–9). The ability of E6 oncoproteins of high risk HPVs to efficiently immortalize human epithelial cells has led to considerable interest in identifying their cellu-
PKN Binds and Phosphorylates HPV16 E6

14829

A role in cellular transformation. Notably, however, PKN binding was also retained in certain E6 mutants that do not immortalize epithelial cells, suggesting that binding to PKN by itself is not sufficient for E6-induced cellular transformation.

What role may PKN play in cellular transforming activity of E6? At present, the precise physiological role of PKN remains to be defined. PKN and a related protein, PKR2, are serine threonine kinases with a kinase domain significantly homologous to PKC (42, 56). However, these kinases appear to perform distinct function (57, 58). Recently, another isoform of PKN, PKNδ, was identified. The expression pattern and arachidonic acid dependence is different from those of PKN (59). Recent studies have focused attention on the ability of PKN to interact with GTP-bound form of Rho family of small GTPases, an interaction mediated via the NH2-terminal region of PKN (45, 46, 60–62). Importantly, Rho-GTP binding activates PKN (45, 46). Interestingly, PKN is also possibly regulated by autophosphorylation and binding to lipids, such as arachidonic acid (43, 63, 64), and is negatively regulated by intramolecular binding of a putative pseudosubstrate in the NH2-terminal region of catalytic domain. Given the ability of multiple modalities to regulate PKN, it is conceivable that E6 binding may achieve a positive or negative regulation of its activity. In this regard, it is notable that E6 binds to COOH-terminal region of PKN. This would suggest a possibility that Rho and E6 may concurrently interact with PKN, potentially allowing E6 to influence Rho-mediated signaling.

In recent years, Rho family GTPases have emerged as critical regulators of actin cytoskeleton remodeling that accompanies cellular activation by extracellular stimulation (65–68). E6 also has been shown to directly interact with paxillin, a tyrosine phosphoprotein localized in focal adhesions, which play an important role in co-ordinating actin cytoskeletal rearrangements in response to integrin-mediated cellular stimulation (40, 69, 70). It would appear that multiple interactions of E6 might be needed to alter biochemical pathways that regulate cell spreading, migration, and shape, processes that are markedly affected during oncogenesis.

At present, the role of E6-PKN interaction must remain speculative. One clue for a functional interaction between these two proteins is provided by our observation that E6 serves as a substrate for PKN when both were co-expressed in vivo. Phosphorylation of E6 appeared to be directly mediated by PKN, as the kinase-defective mutant of PKN did not lead to phosphorylation. Importantly, this is the first evidence that HPV E6 is a phosphoprotein. Phosphorylation of other DNA tumor virus oncoproteins has dramatic effects on their function (50, 71–78). For example, SV40 and polyoma large T phosphorylation affect their DNA replication functions (71, 72). Polyoma middle T phosphorylated on tyrosine residues mediates its association with SHC, phosphatidylinositol 3-kinase, and phospholipase Cyl (73–78), whereas its serine phosphorylation mediates binding to 14-3-3 protein (50). Notably, HPV E7 phosphorylation by casein kinase II on serines 31 and 32 appeared to be important for its ability to transform primary cells when co-introduced with Ras (53, 54). These studies suggest that phosphorylation of E6 may also play a role either in E6-induced immortalization or in the regulation of viral life cycle.

In conclusion, we have identified a novel interaction of high risk HPV E6 proteins with a lipid- and Rho small G protein-regulated serine/threonine kinase PKN. Our results implicate this protein phosphorylation cascade, thought to participate in the regulation of actin cytoskeleton and other cellular functions, in E6-induced cellular transformation or in the regulation of HPV viral cycle.

Acknowledgments—We thank Dr. Elliot Androphy for E6 mutants, Dr. Ishiihashi for pE6-10E6-5-myc constructs, Dr. Howley for GST-E6AP and GST-ERAP mutant, and Hamid Band for critical reading of the manuscript.

REFERENCES

1. Zur Hausen, H. (1987) Appl. Pathol. 5, 19–24
2. Zur Hausen, H., and Salzman, N. P. (eds) (1987) The Papillomaviruses: The Papovaviridae, Vol. 2, Plenum, New York
3. Woodworth, C. D., Doniger, J., and DiPaolo, J. A. (1989) J. Virol. 63, 159–164
4. Hawley-Nelson, P., Vosden, K. L., and Hartzell, N. L., Loey, D. R., and Schiller, J. T. (1989) EMBO J. 8, 3905–3910
5. Munger, K., Phelps, W. C., Bubb, V., Howley, P. M., and Schlegel, R. (1989) J. Virol. 63, 4417–4421
6. Dyson, N., Howley, P. M., Munger, K., and Harlow, E. (1989) Science 243, 934–940
7. Munger, K., Werners, B. A., Dyson, N., Phelps, W. C., Harlow, E., and Howley, P. M. (1989) EMBO J. 8, 4099–4105
8. Scheffner, M., Werners, B. A., Huibregtse, J. M., Levine, A. J., and Howley, P. M. (1990) Cell 63, 1129–1136
9. Werners, B. A., Levine, A. J., and Howley, P. M. (1990) Science 248, 76–79
10. Scheffner, M., Huibregtse, J. M., Vierstra, R. D., and Howley, P. M. (1993) Cell 78, 495–505
11. Huibregtse, J. M., Scheffner, M., and Howley, P. M. (1991) EMBO J. 10, 4129–4135
12. Huibregtse, J. M., Scheffner, M., and Howley, P. M. (1993) Mol. Cell. Biol. 13, 775–784
13. Huibregtse, J. M., Scheffner, M., and Howley, P. M. (1993) Mol. Cell. Biol. 13, 4918–4927
14. Levine, A. J. (1997) Cell 88, 323–331
15. Hansen, R., and Oren, M. (1997) Curr. Opin. Genet. Dev. 7, 46–51
16. Wazer, D. E., Liu, X. L., Chu, Q., Gao, G., and Band, V. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3867–3871
17. Band, V., Dalal, S., Delmolino, L., and Androphy, E. J. (1993) EMBO J. 12, 1847–1852
18. Band, V., De Caprio, J. A., Delmolino, L., Kulesa, V., and Sager, R. (1991) J. Virol. 65, 6671–6676
19. Klingelhoft, A. J., Foster, S. A., and McDougal, J. K. (1996) Nature 380, 79–82
20. Kiyono, T., Foster, S., Koop, J., McDougal, J., Galloway, D., and Klingelhoft, A. J. (1996) Nature 380, 84–88
21. Creider, G. W. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 90–92
22. Creider, G. W. (1998) Curr. Biol. 8, R178–R181
23. Takakura, M., Kyo, S., Kanaya, T., Hirano, K., Honda, M., Kurosawa, A., and Weidner, N. (1999) J. Natl. Cancer Inst. 91, 159–164
24. Morales, C., Holt, S. E., Ouellette, M., Kaur, K. J., Yan, Y., Wilson, K. S., White, M. A., Wright, W. E., and Shay, J. W. (1999) Nat. Biotechnol. 17, 836–839
25. Jiang, X. R., Jimenez, C., Chang, E., Frolkis, M., Kessler, B., Magee, S., Beeche, M., Bodnar, A., Wahl, G. M., Tisty, T. D., and Chua, C. P. (1999) Nat. Genet. 21, 111–114
26. Greenberg, R., O'Hagan, R. C., Deng, H., Xiao, Q., Hannon, S., Adams, R., Lichtsteiner, S., Chin, L., Morn, G., and DePinho, R. (1999) Oncogene 18, 1219–1226
27. Takakura, M., Kyo, S., Kanaata, T., Hirano, K., Hakeda, J., Yatsudo, M., and Inoue, M. (1999) Cancer Res. 59, 551–557
28. Wu, K. J., Grandori, C., Amacker, M., Simon-Vermot, N., Polack, A., and Linger, J. (1999) Nat. Genet. 21, 120–224
29. Tong, X., Boll, W., Kirchhausen, T., and Linger, J. (1999) Science 286, 529–531
30. Gao, Q., Sinnivasan, S., Boyer, S., Warber, D., and Band, V. (1999) Mol. Cell. Biol. 19, 793–744
31. Kiyono, T., Hiraiwa, A., Fujita, M., Hayashi, Y., Akiyama, T., and Ishibashi, M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 11612–11616
32. Lee, S. S., Weiss, R. S., and Javier, R. T. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 6670–6675
33. Thomas, M., and Banks, L. (1998) Oncogene 17, 2943–2954
34. Kuhn, C., and Banks, L. (1998) J. Biol. Chem. 273, 34302–34309
35. Band, V., Karpova, A., Vidal, M., and Howley, P. M. (1998) Genes Dev. 12, 2061–2072
36. Tong, X., Boll, W., Kirchhausen, T., and Howley, P. (1998) J. Virol. 72, 476–482
40. Tong, X., and Howley, P. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4412–4417
41. Liu, Y., Chen, J. J., Gao, Q., Dalal, S., Hong, Y., Mansur, C. P., Band, V., and
Proc. Natl. Acad. Sci. U. S. A. 40. Tong, X., and Howley, P. M. (1997)
43. Mukai, H., Kitagawa, M., Shibata, H., Takanaga, H., Mori, K., Shimakawa,
Biochem. Biophys. Res. Commun. 204, 348–356
44. Palmer, R. H., Ridden, J., and Parker, P. J. (1995) Eur. J. Biochem.
46. Watanabe, G., Saito, Y., Madaule, P., Ishizaki, T., Fujisawa, K., Morii, N.,
44. Palmer, R. H., Ridden, J., and Parker, P. J. (1995)
47. Takahashi, M., Mukai, H., Toshimori, M., Miyamoto, M., and Ono, Y. (1998)
48. Dalal, S., Gao, Q., Androphy, E., and Band, V. (1996)
49. Gross-Mesilaty, S., Reinstein, E., Bercovich, B., Tobias, K., Schwartz, A.,
50. Cullere, X., Rose, P., Thathamangalam, U., Chatterjee, A., Mullane, K. P.,
51. Selvey, L. A., Dunn, L. A., Tindle, R. W., Park, D. S., and Frazer, I. H. (1994)
52. Barbosa, M. S., and Wettstein, F. O. (1988)
54. Firzlaff, J. M., Luscher, B., and Eisenman, R. N. (1991)
55. Storey, A., Almond, N., Osborn, K., and Crawford, L. (1990) J. Gen. Virol. 71, 965–970
56. Palmer, R. H., and Parker, P. J. (1995) Biochem. J. 309, 315–320
57. Quilliam, L. A., Lambert, Q. T., Mickelson-Young, L. A., Westwick, J. K.,
Sparks, A. B., Kay, B. R., Jenkins, N. A., Gilbert, D. J., Copeland, N. G., and
Der, C. J. (1996) J. Biol. Chem. 271, 28772–28776
58. Vincent, S., and Settlement, J. (1997) Mol. Cell. Biol. 17, 2247–2256
59. Oishi, K., Mukai, H., Shibata, H., Takahashi, M., and Ono, Y. (1999) Biochem.
Biophys. Res. Commun. 261, 808–814
60. Maesaki, R., Shimizu, T., Ihara, K., Kuroda, S., Kibauchi, K., and Hakoshima,
T. (1999) J. Struct. Biol. 126, 166–170
61. Flynn, P., Mellor, H., Palmer, K., Panayotou, G., and Parker, P. J. (1998)
J. Biol. Chem. 273, 20688–20705
62. Shibata, H., Mukai, H., Inagaki, Y., Homma, Y., Kimura, K., Kibauchi, K.,
Narumiya, S., and Ono, Y. (1996) FEBS Lett. 385, 221–224
63. Yoshinaga, C., Mukai, H., Toshimori, M., Miyamoto, M., and Ono, Y. (1999)
J. Biochem. (Tokyo) 126, 475–484
64. Palmer, R. H., Dekker, L. V., Woscholski, R., Le Good, G. J. A., Gig, R., and
Parker, P. J. (1995) J. Biol. Chem. 270, 22412–22416
65. Mackay, D. J., and Hall, A. (1998) J. Biol. Chem. 273, 20685–20688
66. Hall, A. (1998) Science 279, 509–514
67. Tapon, N., and Hall, A. (1997) Curr. Opin. Cell Biol. 9, 86–92
68. Ridley, A. J. (1995) Curr. Opin. Genet. Dev. 5, 24–30
69. Turner, C. E. (1994) BioEssays 16, 47–52
70. Tong, X., Salgia, R., Li, J. L., Griffin, J. D., and Howley, P. M. (1997) J. Biol.
Chem. 272, 33373–33376
71. Prives, C. (1990) Cell 61, 735–738
72. Chatterjee, A., Bockus, B. J., Gjorup, O. V., and Schaffhausen, B. S. (1997)
J. Virol. 71, 6472–6478
73. Campbell, K. S., Oris, E., Burke, B., Su, W., Auger, K. R., Druker, B. J.,
Schaffhausen, B. S., Roberts, T. M., and Pallas, D. C. (1994) Proc. Natl.
Acad. Sci. U. S. A. 91, 6344–6348
74. Cohen, B., Yoakim, M., Pownca-Worms, H., Roberts, T. M., and Schaffhausen,
B. S. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4458–4462
75. Su, W., Liu, W., Schaffhausen, B. S., and Roberts, T. M. (1995) J. Biol. Chem.
270, 12331–12334
76. Dilworth, S. M., Brewster, C. E., Jones, M. D., Lanfrancone, L., Pelicci, G., and
Pelicci, P. G. (1994) Nature 367, 87–90
77. Kaplan, D. R., Whitman, M., Schaffhausen, B., Raptis, L., Goreea, R. L.,
Pallas, D., Roberts, T. M., and Cantley, L. (1986) Proc. Natl. Acad. Sci.
U. S. A. 83, 3624–3628
78. Talmage, D. A., Freund, R., Young, A. T., Dahl, J., Dawe, C. J., and Benjamin,
T. L. (1989) Cell 59, 55–65
