Modification of an N-terminal Regulatory Domain of T Antigen Restores p53-T Antigen Complex Formation in the Absence of an Essential Metal Ion Cofactor*

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We have discovered that the ability of the tumor suppressor protein p53 to bind to the viral large T antigen (TAg) oncogene product is regulated by divalent cations. Both proteins were purified from an insect cell line infected with the appropriate baculovirus expression vector. In a two-site capture enzyme-linked immunosorbent assay, complex formation between the purified proteins is strictly dependent on the addition of specific concentrations of divalent metal ions, notably zinc, copper, cadmium, cobalt, manganese, and nickel. In the presence of zinc the pattern of proteolytic fragments obtained when TAg was subjected to proteolysis by endoproteinase Glu-C (V8) was strikingly different, supporting the idea that a conformational change in TAg associated with ion binding is required for it to complex with p53. Monoclonal antibody analysis provides supporting evidence for a conformational change. When TAg was captured onto an enzyme-linked immunosorbent assay plate coated with PAb419 as opposed to many other anti-TAg antibodies, complex formation was completely independent of the presence of additional divalent cations. Our results suggest that the ability of p53 and TAg to form a stable complex in vitro is dependent upon a regulatory domain residing in the N terminus of TAg, zinc ions or the binding of a specific monoclonal antibody (PAb419) provoking a conformational change in TAg that facilitates and supports complex formation.

The large T antigen (TAg) of the DNA papova virus SV40 is a 94-kDa multifunctional phosphoprotein composed of 708 amino acids that is essential for viral replication and transformation of infected cells (1,2). The repertoire of biochemical activities that characterize TAg, and that mediate replication of SV40, include its ability to bind specifically to viral DNA, coordinate the assembly of the replication apparatus, and act as a helicase at the replication fork and the ability to regulate transcription of viral genes (3,4). However, it is the capacity of TAg to bind to and inactivate endogenous cellular proteins encoded by the p53 and retinoblastoma (Rb) tumor suppressor genes that is considered to be a critical event in transformation of infected cells (5–8).

Loss of wild type p53 activity is frequently identified in human tumors (8). This loss of functional p53 is commonly the result of a mutation within the gene, but complex formation with other endogenous or exogenous (viral encoded) proteins has the same effect (9). In the absence of wild type p53, which has been described as the “guardian of the genome,” the cell is predisposed to acquire genetic abnormalities and thus to neoplastic transformation (10). Therefore, it is important to define the biochemical mechanisms that regulate the interaction of p53 with other proteins that may inactivate it. The large TAg of the SV40 virus is the archetype of p53 binding proteins, and indeed it is the ability of p53 and TAg to form a stable complex that originally enabled p53 to be identified (5,6). The identification of mechanisms that determine the ability of p53 and TAg to bind to each other may provide further insight into the regulation of events that permit viral transformation of cells. Furthermore, since putative TAg-like proteins have also been identified in human tumor cell lines the biochemical basis and pathophysiological significance of these interactions may be elucidated (11).

The analysis of mutant or truncated forms of TAg has demonstrated that the various biochemical functions of TAg can be assigned to specific domains within the molecule. It has therefore been possible to examine the dependence of the various biological functions of TAg on its specific biochemical activities. Thus, it has been shown that the ability to support viral replication and the capacity to transform cells are independent functions of TAg (12). However, complex formation with p53 is not an essential prerequisite for TAg to support these activities (13,14). The introduction of certain point mutations within a hydrophobic region of TAg between amino acids 570 and 590 may impair the ability of TAg to bind to p53. However, despite being unable to bind to p53 these mutants are still competent in some cell transformation assays (15). Zhu et al. (16) have also observed that loss of the ability to bind to either p53 or the retinoblastoma protein need not impair the transactivation activity of TAg to any significant extent (16). Conversely, replication and transformation may be defective without affecting the ability of TAg to bind to p53 (17). However in the immortalization of primary cell lines the capacity of TAg to bind to and inactivate p53 appears to be critical (18).

In these studies we have purified TAg and wild type human p53 in a biochemically active state. The purified TAg was able to direct viral DNA synthesis in a standard SV40 replication assay, and this activity could be inhibited with the addition of p53. We sought to determine potential mechanisms that may regulate the interaction of TAg and p53 using a two-site cap-
Regulation of TAg-p53 Complex Formation

MATERIALS AND METHODS

Purification of TAg and p53 (21–23)—Sf9 cells were infected for 2 h with baculovirus, containing either the expression vector for wild type human p53 or TAg, in Ex-Cell 400 tissue culture medium, after which the virus-containing medium was replaced with fresh medium. The infected cells were then incubated for a further 48 h in a humidified incubator at 25 °C before being harvested.

The cells expressing TAg were then pelleted and lysed on ice for 15 min in lysis buffer (0.3 M NaCl, 10% (v/v) glycerol, 0.5% (v/v) Nonidet P-40, 5 mM MgCl2, 1 mM EDTA, 0.2 μg/ml leupeptin, 0.2 μg/ml aprotinin, and 1 mM DTT). Following lysis, the cell debris was pelleted by centrifugation at 4 °C for 30 min at 14,000 rpm, and the supernatant was dispensed into sterile Falcon tubes and neutralized with the addition of half the volume of TAg lysis neutralization buffer, pH 6.8 (0.3 M NaCl, 10 mM Tris-HCl, pH 8.1, 10% (v/v) glycerol, 1 mM EDTA, 0.1 mM PMSF, 0.2 μg/ml leupeptin, 0.2 μg/ml aprotinin, and 1 mM DTT) before being loaded onto a DEAE-Sepharose column with a bed volume of 20 ml that had initially been equilibrated with 10 column volumes of 0.3 M NaCl loading buffer (0.3 M NaCl, 20 mM Tris-HCl, pH 8.0, 10% (v/v) glycerol, 1% (v/v) Nonidet P-40, 1 mM EDTA, 0.1 mM PMSF, 0.2 μg/ml leupeptin, 0.2 μg/ml aprotinin, and 1 mM DTT). The column was then washed with 10 column volumes of 0.3 M NaCl loading buffer after no further protein (as determined by Bradford assay (24)) was present in the flow-through. The flow-through was then run on a protein A-Sepharose column with a bed volume of 1 ml that had been equilibrated as described above. The flow-through from this column was collected and loaded onto a PAb 419 immunoaffinity column prepared by binding and cross-linking 1 mg of the purified anti-TAg monoclonal antibody PAb 419 to protein A-Sepharose beads. This column was equilibrated as described above after all unbound 419 antibody had been removed from the column by washing the column with 5 column volumes of TAg elution buffer (20 mM Tris-HCl, pH 8.5, 1 M NaCl, 1 mM MgCl2, 1 mM EDTA, 10% (v/v) glycerol, 55% (v/v) ethylene glycol, 0.1 mM PMSF, 0.2 μg/ml leupeptin, 0.2 μg/ml aprotinin, 0.2 μg/ml glycerol, and 1 mM DTT). Prior to elution of TAg bound to the 419 column the column was washed with 10 column volumes of 1 M NaCl buffer (50 mM Tris-HCl, pH 8.0, 1 M NaCl, 10% (v/v) glycerol, 1 mM EDTA, 0.1 mM PMSF, 0.2 μg/ml leupeptin, 0.2 μg/ml aprotinin, 0.2 μg/ml glycerol, and 1 mM DTT) and 10 column volumes of 10% ethylene glycol with 0.5 M NaCl wash buffer (50 mM Tris-HCl, pH 8.5, 0.5 M NaCl, 10% (v/v) ethylene glycol, 10% (v/v) glycerol, 1 mM EDTA, 0.1 mM PMSF, 0.2 μg/ml leupeptin, 0.2 μg/ml aprotinin, and 1 mM DTT). The TAg bound to the column was then eluted with TAg elution buffer. Prior to use in any of the subsequent assays the TAg eluate was dialyzed in 2 × 1000 ml of dialysis buffer (10 mM Tris-HCl, pH 8.0, 10 mM NaCl, 1 mM EDTA, 50% (v/v) glycerol, and 1 M NaCl, pH 7.4, 10 mM EDTA, 50% (v/v) glycerol, and 1 M NaCl, pH 8.0), and the supernatant was diluted 5-fold in Buffer B (95% (v/v) glycerol, 25 mM HEPES, pH 7.6, 10 mM NaCl, 1 mM MgCl2, 5 mM DTT, 1 mM PMSF, 5 mM leupeptin, 5 mM aprotinin, 5 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride) before filtration through a 0.45-μm filter. Thereafter, the sample was loaded onto a 5-ml heparin-Sepharose Hi-Trap column (Pharmacia Biotech Inc.) and eluted with a linear KCl gradient from 0.05 to 1 M in Buffer B. This purification procedure resolves three forms of p53 that are produced in Sf9 cells; two forms are in an activated state (forms I and II), and one is in a latent state (form III) for sequence-specific DNA binding.

SV40 Replication Assay—The functional integrity of the purified TAg was determined by the SV40 replication assay (modified from that described previously by using tritiated thymidine as the label) (26). Furthermore the effect of titrating purified p53 into the reaction was investigated by incubating the p53 and TAg at room temperature for 30 min prior to the addition to the reaction mix; this ensured that the activity of the p53 could be inhibited by the TAg and, therefore, that the two proteins would be able to interact with each other under these particular experimental conditions.

ELISA—A standard ELISA using the conformation-dependent monoclonal anti-p53 antibodies 240 and 1620 (specific for p53 in the mutant and wild type configuration, respectively) established that the purified p53 was in the wild type configuration (27, 28).

The ability of p53 to form stable complexes with TAg was further...
investigated using a standard ELISA modified as follows (29). The anti-p53 antibody DO-1 was used to bind p53 (1 μg/ml in a buffer solution containing 100 mM NaCl, 10 mM HEPES, pH 7.5, 5 mM KCl, and 0.01% (v/v) Nonidet P-40 in milli-Q water) to the ELISA plate overnight at 4 °C. After the plate had been washed, TAg diluted to 1 μg/ml in the same buffer as the p53 was added to each well, and the incubation was continued for 3 h at 4 °C. After further washes, bound TAg was detected by probing with the rabbit polyclonal anti-TAg antibody 115, horseradish peroxidase-conjugated swine anti-rabbit immunoglobulins, and 3,3',5,5'-tetramethylbenzidine developing solution as described previously. The development reaction was arrested with the addition of an equivalent volume of 1 M H2SO4, and the absorbance of the reaction products at 450 nm was recorded by a Dynatech 5000 plate reader.

The influence of zinc ions on the ability of p53 and TAg to form stable complexes in the ELISA was investigated by titrating Zn2+ (from the chloride salt) into the reaction. The dependence of complex formation on the presence of Zn2+ was further established by titrating EDTA into the reaction. Since it has been shown that other divalent cations may bind to p53, the influence of other such ions (Ca2+, Cd2+, Co2+, Cu2+, Mg2+, Mn2+, and Ni2+) on the formation of TAg-p53 complexes in this ELISA was investigated in the same manner as for Zn2+; all ion solutions were prepared from the chloride salt (30).

Regulation of TAg-p53 Complex Formation

![Graph](image-url)
A further series of assays was undertaken in which the ELISA plate was coated with PAb 101, 204, 210, 211, 219, 221, 251, 268, 414, and 419, which recognize epitopes along the length of the N-terminal and central domains of TAg (19, 20, 31), with PAb s 421 and 1620, which bind to epitopes along the length of the p53 molecule different from that recognized by DO-1, and with DO-7, an independent antibody that binds to the same region as DO-1 (28, 32). The dependence of TAg-p53 complex formation on Zn$^{2+}$ in these systems was investigated in the same manner as described above. In this instance detection of complex formation was as described above with the exception of those instances where the plate had been coated with antibodies to TAg, in which case the primary antibody used in the detection reaction was the anti-p53 polyclonal serum CM-1 (33).

FIG. 4. Titration of EDTA into the reaction inhibits the effect of zinc ions on restoring the ability of TAg and p53 to form a stable complex. Furthermore, titration of EDTA into the reaction once complex formation has occurred also impairs the ability of the two proteins to form a stable complex (data not shown).

The pattern of proteolytic fragments of TAg generated by the enzyme is different from that obtained in the absence of zinc (-). In contrast, the size of fragments of p53 generated in the same way is not affected by zinc ions. However, in the presence of zinc it does appear that the efficiency of the enzyme is altered, as the intensity of the signal of particular paired bands in the samples derived from digestion of p53 is not the same. In each case the results illustrated were obtained when 100 ng of purified protein was incubated with 625 ng of enzyme at 30°C for 1 h prior to arresting the reaction as described under “Materials and Methods.”

Regulation of TAg-p53 Complex Formation—Strikingly, in a two-site capture ELISA, biologically active purified TAg and p53 did not form a complex (Fig. 2). This result was surprising, since p53 was active at inhibiting TAg-dependent SV40 viral DNA replication (Fig. 1B). These data indicate that p53 is not denatured or unfolded in the assays described in this report. The PAB 1620+ form of p53, which is competent for sequence-specific DNA binding was also active in the inhibition of TAg-dependent replication of SV40 origin containing DNA (Fig. 1C).

The biochemically active forms of p53 were separated chromatographically from latent forms of p53 as described previously (22, 23). The forms of p53 produced using this system are folded properly as defined by strong reactivity to PAb 1620 antibody and lack of reactivity to PAb 240 antibody (Fig. 1B). These data indicate that p53 is not denatured or unfolded in the assays described in this report. The PAB 1620+ form of p53, which is competent for sequence-specific DNA binding was also active in the inhibition of TAg-dependent replication of SV40 origin containing DNA (Fig. 1C).

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We therefore examined whether a series of metal ions, which may have been depleted by the presence of EDTA in the purification buffers, could restore TAg-p53 complex formation. Although TAg could not form a stable complex with p53 captured on a plate coated with the anti-p53 monoclonal antibody DO-1, complex formation could be restored by specific concentrations of zinc or copper ions (Fig. 3A). Other divalent cations such as cadmium, cobalt, nickel, and manganese could substitute at higher concentrations for zinc or copper ions, although neither calcium nor magnesium had any demonstrable effect on the formation of the p53-TAg complex (Fig. 3, A and B). Complex formation promoted by 0.1 mM zinc could be inhibited by titrating EDTA into the reaction (Fig. 4).

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The Pattern of Fragments of TAg Generated by Proteolytic Digestion by Endoproteinase Glu-C (V8) Is Altered in the Presence of Zinc Ions, but That of p53 Is Not—The depletion of metal ions from proteins during purification may alter the conformation of the protein and thus its functional properties. To obtain evidence in support of a role for zinc ions in modifying the conformation of purified TAg or p53, each protein was digested with endoproteinase Glu-C (V8) and the products were separated on a 10% SDS-polyacrylamide gel. The protein fragments were stained with Coomassie Brilliant Blue R-250 and analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 5). The molecular masses of the protein fragments were estimated from the positions of standards run on each gel. The patterns of protein fragments generated by endoproteinase Glu-C (V8) digestion were then compared with those generated by endoproteinase V8 digestion, which is not altered by zinc ions.
subjected to proteolytic digestion with endoproteinase Glu-C, which hydrolyzes peptide bonds at the carboxylic terminus of glutamic or aspartic acid residues. The size of fragments produced was analyzed by first resolving them on a denaturing polyacrylamide gel and then probing a Western blot with the polyclonal antibodies 115 and CM-1 for TAg and p53, respectively. The pattern of degradation products of TAg observed on a Western blot was significantly different when 0.1 mM zinc was added to the reaction (Fig. 5A). However, the proteolytic cleavage pattern of p53 was not altered in the presence of Zn$^{2+}$, indicating that the specificity of this protease is unaffected by Zn$^{2+}$ and that it is the conformation of TAg and not that of p53 that is altered with the addition of zinc (Fig. 5B).

The Binding of a Monoclonal Antibody to the N Terminus of TAg Restores Complex Formation in the Absence of a Metal Ion Cofactor—Monoclonal antibodies can often be used to activate enzyme activity allosterically in the absence of essential ligands or cofactors; examples include protein kinase C, HSF-1, and p53 (23, 39, 40). This antibody-mediated activation is not a general effect of antibody binding but is derived from the regulatory domain that is bound by the antibody. Given the large panel of monoclonal antibodies specific for p53 and TAg, we examined whether the binding of any monoclonal antibody to TAg or p53 could mimic the effects of zinc on TAg and restore the ability of TAg and p53 to form a stable complex.

Regardless of which p53-specific antibodies were used to capture p53 onto the ELISA plate, binding to TAg was strictly dependent upon the added presence of zinc ions, (Fig. 6A). When TAg was first captured onto the ELISA plate with any one of nine different TAg-specific monoclonal antibodies (PAbs 101, 204, 210, 211, 221, 251, 268, and 414) complex formation with p53 remained strictly dependent upon the addition of zinc ions (Fig. 6B). Furthermore, when bound to the ELISA plate with these same monoclonal antibodies, TAg was able to form a stable complex with p110 Rb (Fig. 6C), suggesting that the effect of Zn$^{2+}$ is apparently on a domain of TAg specifically involved in the interaction with p53 and not other TAg-binding proteins.

In striking contrast to all other monoclonal antibodies studied, the formation of TAg-p53 complexes was independent of zinc ions when PAb 419, a monoclonal antibody directed against an epitope at the N-terminal of TAg, was used as the capture antibody (Fig. 6B). These results indicate that the effect of zinc ions on restoring the ability of purified p53 and TAg to form a stable complex in this assay system can be recapitulated by specific antibody binding to TAg. Furthermore, given that the epitope of PAb 419 lies within the N terminus of TAg, the conformational change induced by zinc ions may emanate from or nucleate to the N-terminal domain of TAg.

The PAb 419 Epitope Is Lost Following Proteolytic Digestion of TAg by Endoproteinase Glu-C in the Presence of 0.1 mM Zinc—Western blots of the fragments of TAg generated by proteolysis with endoproteinase Glu-C probed with monoclonal

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**Fig. 6.** The effect of using different monoclonal antibodies to capture either p53 or TAg onto the ELISA plate on the ability of the purified proteins to form a complex. A, in addition to using DO-1 to capture p53 on to the 96-well plate, the experiments were repeated using DO-7, 421, and 1620 as the capture antibodies. In each case the ability of p53 and TAg to form a complex remained strictly dependent upon the availability of additional zinc ions. B, the reciprocal experiments in which TAg was first captured onto the 96-well plate using a range of monoclonal antibodies were also carried out. When the monoclonal antibodies 101, 204, 210, 211, 219, 221, 251, 268, and 414 were used, complex formation remained dependent on the addition of zinc ions; the results for PAb 204, 211, 219, and 251 are illustrated and are representative. The use of PAb 419 as the capture antibody renders complex formation completely independent of zinc ions. C, even in the absence of Zn$^{2+}$, TAg can form a stable complex with p110 Rb, although the signal is slightly greater in the presence of 0.1 mM Zn$^{2+}$.
anti-TAg antibodies revealed that the PAb 419 epitope was lost when zinc had been added to the TAg (Fig. 7). The epitope for PAb 219, which contains a cleavage site for endoproteinase Glu-C (41), is lost both with and without added zinc, and the epitope for PAb 251 is preserved in both circumstances. This indicates that a specific effect of the zinc-dependent conformational change in TAg implicates the PAb 419 epitope. These results further strengthen the possibility that an effect of zinc on the conformation of a critical regulatory domain in the N terminus of TAg is the molecular basis of the ability of p53 tetramers and TAg to form a stable complex.

**DISCUSSION**

TAg is a transforming oncogene that is believed to specifically bind and inactivate the biochemical function of two key tumor suppressor proteins, p53 and Rb. Although the domains of TAg that are required for interaction with Rb and p53 have been shown to be different, the mechanisms regulating the assembly of TAg with these tumor suppressor proteins have not been clarified (3, 7, 34–36, 42). In particular, it has been demonstrated that the p53 binding site on TAg lies out with the N-terminus between amino acids 217 and 517 (3, 34), while the region of TAg required for Rb binding is the LXXE motif between amino acids 104 and 111 (43). Interest in the factors regulating the interaction of TAg and p53 may have important relevance for elucidating how mutations alter the conformation of p53 and TAg and determine whether these proteins can form a stable complex. Furthermore, since putative endogenous TAg-like proteins have been identified in human tumor cell lines it is possible that detailed knowledge of the biochemical parameters that govern the interaction between TAg and p53 may be applicable to these other proteins and offer the potential to devise novel strategies to modulate the activity of p53 within cells (11).

We have used highly purified proteins to study the factors affecting the stability of the TAg-p53 protein complex. The novel findings reported in this paper indicate that the amino terminus of TAg contains a motif that specifically regulates the association of TAg with p53 and that factors influencing the conformation of this domain can regulate the binding of these proteins. We have identified two distinct factors that restore TAg-p53 complex formation; one involves the zinc-dependent conformational changes in TAg that activate its binding to p53, and the second is a protein-protein interaction involving monoclonal antibody PAb 419 that is effective in the absence of zinc.

Depletion of zinc ions from p53 during purification has previously been shown to alter the conformation of wild type (PAb 1620+/Z40–), generating a mutant conformation (PAb 1620+/Z240+), which was inactive in a sequence-specific DNA binding assay and would be unable to bind to TAg (44, 45). The consequences of similar depletion of zinc from TAg during purification have not been studied. TAg contains zinc finger motifs, and the presence of EDTA in the buffer solutions used throughout the purification of TAg would provide a basis by which zinc bound to TAg could be depleted, thus providing an explanation of why zinc alters the conformation of TAg and restores p53 binding activity. However, an intriguing observation is that the proposed zinc finger structures in TAg do not affect p53 binding but do inactivate the replicative function of TAg (46). It is possible, therefore, that the zinc requirement seen here for TAg binding to p53 may involve sites in TAg other than the postulated zinc finger.

The hypothesis that an important aspect of the mechanism by which zinc and PAb 419 restore the p53 binding activity of TAg is a conformational change within the N terminus of TAg is strongly supported by the observation that proteolytic cleavage of TAg in the presence of zinc actually occurs within the PAb 419 epitope. Since binding to p110 Rb is independent of zinc ions, these results further suggest that the conformational changes induced in TAg are occurring in a specific domain that serves to facilitate and support complex formation with p53.

The N terminus of TAg has previously been implicated in the regulation of the replicative and transforming activity (16, 47). The novel findings presented in this paper further indicate that the N-terminal contains a motif, probably within the epitope of the PAb 419 (which has been mapped within the amino-terminal 82 amino acids (19, 20), which can negatively regulate the ability of TAg to bind to p53 in a stable complex. The basis of this regulatory function is dependent upon conformational changes within the TAg molecule that can be provoked by metal ions such as zinc and by interactions with other proteins. It is instead possible that similar mechanisms may control the interaction of p53 with other proteins.

**REFERENCES**

1. Rigby, P. W. J., and Lane, D. P. (1983) Adv. Viral Oncol. 3, 31–57
2. Livingstone, D. M., and Bradley, M. K. (1987) Mol. Biol. & Med. 4, 63–80
3. Mole, S. E., Gannon, J. V., Ford, J. M., and Lane, D. P. (1987) Philos. Trans. R. Soc. Lond. B Biol. Sci. 317, 455–469
4. Fanning, E., and Knippers, R. (1992) Annu. Rev. Biochem. 61, 55–85
5. Lane, D. P., and Crawford, L. V. (1979) Nature 278, 261–263
6. Linzer, D. I. H., and Levine, A. J. (1979) Cell 17, 43–52
7. DeCaprio, J. A., Ludlow, J. W., Figg, J., Shaw, J. Y., Huang, C.-M., Lee, W.-H., Marsilio, E., Paucho, E., and Livingston, D. M. (1988) Cell 54, 275–283
8. Donehower, L. A., and Bradley, A. (1993) Biochim. Biophys. Acta 1135, 181–205
9. Lane, D. P. (1989) in Oncogenes (Glover, D. M., and Hames, B. D., pp. 191–213), IRL Press, Oxford
10. Lane, D. P. (1992) Nature 358, 15–16
11. Maxwell, S. A., and Roth, J. A. (1993) Oncogene 8, 3421–3426
12. Peden, K. W. C., and Pipas, J. M. (1992) Virus Genes 6, 107–118
13. Sompayrac, L., and Danna, K. J. (1994) Virology 200, 849–853
14. Sompayrac, L., and Danna, K. J. (1991) Virology 191, 412–415
15. Peden, K. W. C., Srinivasan, A., Farber, J. M., and Pipas, J. M. (1989) Virology 168, 13–21
16. Zhu, J., Rice, P. W., Chamberlain, M., and Cole, C. N. (1991) J. Virol. 65, 2778–2790
17. Ruttila, J. E., Christensen, J. B., and Imperiale, M. J. (1989) Oncogene Res. 4, 303–310
18. Zhu, J., Abate, M., Rice, P. W., and Cole, C. N. (1991) J. Virol. 65, 6872–6880
19. Gannon, J. V., and Lane, D. P. (1990) New Biol. 2, 84–92
20. Lane, D. P., and Gannon, J. V. (1986) Cancer Cells 4, 387–393
21. Smansis, V., and Lane, D. P. (1985) Virology 144, 181–190
22. Hupp, T. R., and Lane, D. P. (1995) J. Biol. Chem. 270, 18165–18174
23. Hupp, T. R., and Lane, D. P. (1994) Curr. Biol. 4, 865–875
24. Hare, J. M., and Lane, D. P. (1976)Anat. Biochem. 72, 249–254
25. Lane, D. P., and Harlow, E. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
26. Li, J., and Kelly, T. J. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 6973–6977
27. Gannon, J. V., Graves, R., Iago, R., and Lane, D. P. (1990) EMBO J. 9, 1595–1602
28. Miller, J., Cook, A., and Sheldon, M. (1987) Virology. 159, 453–455
29. Daniels, D. A., and Lane, D. P. (1994) Mol. Biol. 243, 639–652
30. Chalkley, G. E., Knowles, P. P., Whitehead, P. C., and Coff, A. J. (1994) Eur. J. Biochem. 221, 167–175
31. Garnsey, E. G., Harrison, R. O., and Fenno, J. (1980) J. Virol. 34, 752–763
32. Stephen, C. W., Helminen, P., and Lane, D. P. (1995) J. Mol. Biol. 248, 58–78
33. Midgley, C. A., Fisher, C. J., Bartak, J., Vojtesek, B., Lane, D., and Barnes,
D. M. (1992) J. Cell Sci. 101, 183–189
34. Schmeig, F. I., and Simmons, D. T. (1988) Virology 164, 132–140
35. Chen, J., Tobin, G. J., Pipas, J. M., and Van Dyke, T. (1992) Oncogene 7, 1167–1175
36. Saenez-Robles, M. T., Symonds, H., Chen, J., and Van Dyke, T. (1994) Mol. Cell. Biol. 14, 2686–2698
37. Ludlow, J. W., DeCaprio, J. A., Huang, C-M., Lee, W-H., Paucha, E., and Livingston, D. M. (1989) Cell 56, 57–65
38. Nelbach, M. E., Pigiet, V. P., Jr., Gerhart, J. C., and Schachman, H. K. (1972) Biochemistry. 11, 315–327
39. Makowskie, M., and Rosen, O. M. (1989) J. Biol. Chem. 264, 16155–16159
40. Zimarino, V., Wilson, S., and Wu, C. (1990) Science 249, 546–549
41. Lindner, K. (1994) On the Role of the N-terminus of SV40 Large T Antigen in Cell Transformation and DNA Replication. Ph.D. Thesis, University of Dundee, Dundee, United Kingdom
42. Zhu, J., Rice, P. W., Gorsch, L., Abate, M., and Cole, C. N. (1992) J. Virol. 66, 2780–2791
43. Defoe-Jones, D., Huang, P. S., Jones, R. E., Haskell, K. M., Vuocolo, G. A., Honold, M. G., Huber, H. G., and Oliff, A. (1991) Nature 352, 251–254
44. Pavletich, N. P., Chambers, K. A., and Pabo, C. O. (1993) Genes & Dev. 7, 2556–2564
45. Hainaut, P., and Milner, J. (1993) Cancer Res. 53, 1739–1742
46. Loepfer, G., Stenger, J. E., Ray, S., Parsons, R. E., Anderson, M. E., and Tegtmeyer, P. (1991) J. Virol. 65, 3167–3174
47. Symonds, H. S., McCarthy, S. A., Chen, J., Pipas, J. M., and Van Dyke, T. (1993) Mol. Cell. Biol. 13, 3255–3265
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