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Association of protein phosphatase 2A with polyoma virus medium tumor antigen

(antibodies against protein phosphatase 2A subunits/protein sequence/simian virus 40 small tumor antigen/cell transformation)

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ABSTRACT **The polyoma virus medium and small tumor antigens, as well as simian virus 40 small tumor antigen, form specific complexes with two cellular proteins designated 61- and 37-kDa proteins. In this report, we demonstrate that the 61- and 37-kDa proteins correspond to the A and C subunits, respectively, of the serine- and threonine-specific protein phosphatase 2A (PP2A). On the one hand, antibodies raised against the 61-kDa protein reacted specifically with the purified A subunit of PP2A. Furthermore, the amino acid sequences of seven tryptic peptides from the A subunit were almost identical to sequences of the 61-kDa protein as deduced from the corresponding cDNA sequence. On the other hand, antibodies against the purified C subunit (catalytic subunit) of PP2A reacted specifically with the medium tumor antigen-associated 37-kDa protein. These data suggest a role of PP2A in cell transformation by polyoma virus and simian virus 40.**

The importance of protein phosphorylation in signaling pathways that control cell proliferation and carcinogenesis is well established (for review, see ref. 1). Many oncogenes and growth factor receptors are tyrosine-specific protein kinases and two oncogenes, *mos* and *raf*, are serine/threonine-specific protein kinases (2, 3). Little is known about the role of protein dephosphorylation in growth control, although it seems likely that reversible regulatory mechanisms involving specific phosphorylation events also require specific mechanisms for dephosphorylation. It has recently been shown that several tyrosine-specific protein phosphatases comprise a family of receptor-linked enzymes, which may be important in attenuating the effects of tyrosine phosphorylation on cell proliferation (for review, see ref. 4). Four major forms of serine/threonine-specific phosphatases have been characterized in mammalian tissues, which are differentiated by substrate specificity, sensitivity to inhibitors, regulatory properties, antibody reactivity, and primary sequence (for review, see ref. 5). Protein phosphatase 2A (PP2A) comprises a significant portion of total activity in many tissues, has a broad substrate specificity, and is active in the absence of divalent cations. It consists of a 36- to 38-kDa catalytic subunit (termed C) and two additional subunits of 60 and 55 kDa, termed A and B, respectively (5). cDNA clones encoding the catalytic subunit have been isolated from a number of tissues and species (6–8). Analysis of the primary amino acid sequence has shown that the type 2A catalytic subunit is highly conserved between species (nearly 100% identity) and that it is a member of a gene family that includes the catalytic subunit of types 1 and 2B protein phosphatases (5, 9). Currently, very little is known about the structure or function of the A and B subunits. The presence or absence of the A, or A and B, subunits alters the activity and substrate preference of the catalytic subunit (10–12).

The transforming proteins of DNA tumor viruses frequently form complexes with cellular proteins. These interactions play an important role in growth control and tumorigenesis (13). The transforming protein of polyoma virus, medium tumor antigen (medium T antigen), associates with $pp60^{c\text{-}src}$, the product of the protooncogene c-*src* (14), and activates its protein-tyrosine kinase activity (15). It also binds to the $pp62^{c\text{-}yes}$ and $pp59^{c\text{-}fyn}$ proteins (16–18), two $pp60^{c\text{-}src}$-related kinases, and to a phosphatidylinositol kinase (19, 20). In addition, medium T antigen associates with three unidentified cellular proteins of 88, 61, and 37 kDa (designated 88-, 61-, and 37-kDa proteins, respectively) whose functions are unknown (21, 22). Genetic studies indicate that they play a role in transformation. Medium T antigen encoded by the nontransforming host range (hrt) mutants (23) does not associate with these proteins or form less stable complexes, whereas medium T antigen of other nontransforming mutants does interact with these proteins (21). Interestingly, medium T antigen encoded by hrt mutants forms a complex with the 73-kDa heat shock protein and not the 88-, 61-, and 37-kDa proteins (24). The pattern of complex formation of the 88-, 61-, and 37-kDa proteins with mutant medium T antigens resembles that described for $pp60^{c\text{-}src}$ and medium T antigen (14, 15, 25). This suggests that binding of medium T antigen to the 88-, 61-, and 37-kDa proteins as well as to $pp60^{c\text{-}src}$ is a necessary but insufficient requirement for transformation. The 61- and 37-kDa proteins bind also to polyoma and simian virus 40 (SV40) small tumor antigen (t antigen) (26, 27).

We have recently isolated and sequenced a cDNA clone encoding the 61-kDa protein. The deduced amino acid sequence of the 61-kDa protein revealed that it consists of 15 nonidentical repeats 38–40 amino acids long and is unrelated to known proteins (28). Based on the finding that the 37-kDa protein is apparently identical by comigration on two-dimensional gels, two-dimensional chymotryptic peptide mapping, and partial amino acid sequencing to the catalytic subunit of PP2A, and that the 61-kDa protein immunologically cross-reacts with the A subunit of PP2A (D. Pallas, personal communication), we compared the polyoma medium T antigen-associated 61- and 37-kDa proteins with subunits of purified PP2A by using specific antibodies and amino acid sequence analysis. In the present communication, we demonstrate that the 61- and the 37-kDa proteins represent the regulatory and catalytic subunits, respectively, of PP2A. These findings suggest that serine- and threonine-specific protein phosphatases play a role in growth control and transformation.

MATERIALS AND METHODS

Cells and Viruses. Human 293 cells (29) were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. They were infected with a hybrid

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Abbreviations: PP2A, protein phosphatase 2A; medium T antigen, medium tumor antigen; t antigen, small tumor antigen; SV40, simian virus 40.

adenovirus type 5, Ad5(pymT), containing the gene for medium T antigen under the control of the late adenovirus promoter (30). Mouse 3T6 cells were grown and infected with the polyoma virus mutant dl8 as described (22).

Labeling of Cells and Preparation of Extracts. The labeling of 293 and 3T6 cells and the preparation of extracts in Nonidet P-40 lysis buffer was carried out as described (21).

Purification of the Medium T Antigen Complex. Medium T antigen–61-kDa protein–37-kDa protein complex from dl8-infected, ^{35}S-labeled 3T6 cells was purified by sequential immunoprecipitation with monoclonal anti-peptide antibodies to medium T antigen and with polyoma tumor serum (26). The medium T antigen complex from Ad(pymT)-infected 293 cells was purified by affinity chromatography with monoclonal anti-peptide antibodies (28). Briefly, the infected cell extract was incubated with Sepharose-coupled antibodies. The medium T antigen complex was eluted from the antibodies at pH 10.8. The eluted proteins were neutralized and concentrated with an Amicon concentrator (Centricon 30).

Antibodies Against the 61-kDa Protein. A 321-nucleotide-long *Eco*RI/*Xba* I fragment of clone P9 was inserted into a pATH vector containing the *TrpE* promoter, and 320 codons of the *TrpE* structural gene, followed by the multiple cloning region of pUC12 (31). The vector was obtained from Joe Lipsick (University of California at San Diego). The fusion protein was overexpressed in *Escherichia coli*, strain JM109 (Stratagene), purified by SDS/PAGE, and injected into rabbits. Antisera were obtained 2 weeks after each booster injection given monthly.

Immunoprecipitation and Immunoblots. One hundred microliters of ^{35}S-labeled 293 cell extract was precipitated with 10 μl of serum and the precipitates were analyzed on a SDS/10% polyacrylamide gel. Immunoblots were prepared as described. ^{125}I-labeled protein A was used to detect the antibodies (32).

PP2A. The two- and three-subunit forms of bovine cardiac PP2A were purified as described (12). The A subunit was purified from the two-subunit form, which was denatured in 6 M urea/10 mM Tris·HCl, pH 7.5/100 mM KCl/0.5 mM dithiothreitol. Then the subunits were separated on a Sephacryl S-200 column (1 × 50 cm) equilibrated as described (33).

Partial Sequence of the A Subunit of PP2A. The A subunit of protein phosphatase was isolated by SDS/PAGE followed by electroblotting to nitrocellulose paper (34). It was localized on the paper by staining and subjected to tryptic digestion. Peptides released from the paper were subjected to reverse-phase chromatography on an Applied Biosystems RP-300 C_8 column (2.1 × 100 mm). Separation was performed in 0.1% (vol/vol) trifluoroacetic acid with a gradient of 0–50% (vol/vol) acetonitrile over 120 min at a flow rate of 50 μl/min. Peaks were collected on 1-cm Whatman GF/c discs. Peptides were reduced, cysteine residues were alkylated (35), and the peptides were subjected to automated Edman degradation on an Applied Biosystems model 470 A sequencer using standard manufacturer's programming and chemicals. Phenylthiohydantoin-derivatized amino acids were identified by a model 120 A HPLC system.

RESULTS

Antibodies Against Medium T Antigen-Associated 61-kDa Protein Coprecipitate 37-kDa Protein. We recently purified the polyoma virus medium T antigen-associated 61-kDa protein and obtained a partial amino acid sequence. Synthetic oligonucleotides were used to screen a cDNA library from human placenta. A clone (designated P16) encoding the 61-kDa protein was isolated and sequenced (28). Antibodies were prepared against a TrpE fusion protein containing a 107-amino acid fragment from a closely related protein (87% similarity) encoded by a second clone (P9) isolated from the human placenta library. The complete size of the P9-encoded protein is presently unknown because P9 is incomplete at the 5′ end. However, based on the available information it must be as large or larger than the P16-encoded 61-kDa protein (G.W., unpublished data). As shown in Fig. 1*A* (lane 2), the antibodies precipitated a protein of 61 kDa from human 293 cells, which comigrated with the medium T antigen-associated 61-kDa protein from polyoma virus-infected mouse 3T6 cells (lane 3). In addition, they precipitated a protein of 37 kDa (lane 2), which comigrated with the medium T antigen-associated 37-kDa protein (lane 3). Antibodies prepared against two other fusion proteins containing different segments of the P9-encoded protein also precipitated proteins of 61 and 37 kDa from 293 cells (data not shown). These results suggest that these proteins are the human homologues of the previously identified medium T antigen-associated 61- and 37-kDa proteins from mouse. In addition, they suggest that the 37-kDa protein is coprecipitated with antibodies against the 61-kDa protein because these two proteins form a complex. We have previously demonstrated that they comigrate during sedimentation on glycerol gradients (21). As judged by the amount of radioactivity, the antibodies against the 61-kDa protein precipitated the 61-kDa protein in molar excess over the 37-kDa protein (lane 2), indicating that not all 61-kDa protein was associated with 37-kDa protein. Approximately equal quantities of the 61-kDa protein and the 37-kDa protein were coprecipitated with an excess amount of medium T antigen (lane 3), suggesting that not all molecules of medium T antigen were associated with 61- and 37-kDa proteins. At the moment we do not know whether these ratios reflect the situation *in vivo*.

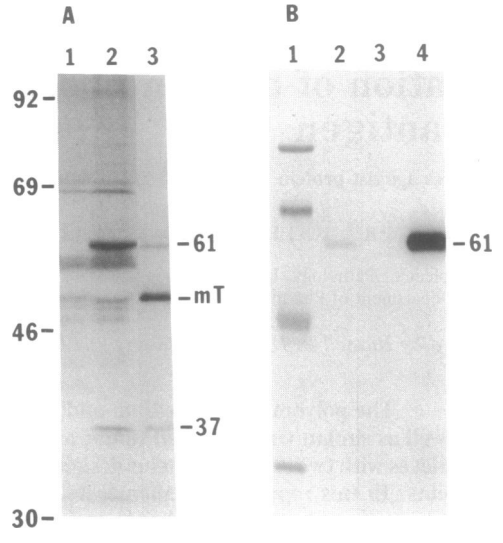

FIG. 1. Antibodies against the medium T antigen-associated 61-kDa protein react with the regulatory subunit A of PP2A. (*A*) Immunoprecipitation of 61- and 37-kDa protein from [^{35}S]methionine-labeled human 293 cell extract with anti-61-kDa protein antibodies. Lanes: 1, preimmune serum; 2, immune serum; 3, affinity-purified medium T antigen (mT)–61-kDa protein–37-kDa protein complex from [^{35}S]methionine-labeled mouse 3T6 cells infected with polyoma deletion mutant dl8. (*B*) Immunoblot of purified subunit A of PP2A. Lanes: 2, amido black-stained subunit A (400 ng); 3, subunit A (100 ng) with preimmune serum; 4, subunit A (100 ng) with anti-61-kDa protein immune serum; 1, amido black-stained molecular mass markers—from top to bottom: 97 kDa (phosphorylase b), 68 kDa (bovine serum albumin), 43 kDa (ovalbumin), 24 kDa (trypsinogen).

Reaction of Antibodies Against 61-kDa Protein with Subunit A of PP2A. To investigate whether the A subunit of PP2A and the medium T antigen-associated 61-kDa protein were structurally related, purified A subunit from bovine heart PP2A was assayed by immunoblotting with the anti-61-kDa fusion

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                                        MAAADGD        7

DSLYPIAVLIDELRNEDVQLRLNSIKKLSTIALALGVER               46

TRSELLPFLTDTIYDEDEVLLALAEQLGTFTTLVGGPE                84
                                    1
                                    ----------
YVHCLLPPLESLATVEETVVRDKAVESLRAISHEHSPSD               123
           2
------P--- ----------
LEAHFVALVKRLAGGDWFTSRTSACGLFSVCYPRVSSA                161

VKAELRQYFRNLCSDDTPMVRRAAASKLGEFAKVLELDN               200
  3                 4
  ------------------------------------
VKSEIIPMFSNLASDEQDSVRLLAVEACVNIAQLLPQED               239

LEALVMPTLRQAAEDKSWRVRYMVADKFTELQKAVGPEI               278
  5
  -------------x-xQ--
TKTDLVPAFQNLMKDCEAEVRAAASHKVKEFCENLSADCRENV           321

IMSQILPCIKELVSDANQHVKSALASVIMGLSPILGKDN               360

TIEHLLPLFLAQLKDECPEVRLNIISNLDCVNEVIGIRQ               399

LSQSLLPAIVELAEDAKWRVRLAIIEYMPLLAGQLGVEF               438

FDEKLNSLCMAWLVDHVYAIREAATSNLKKLVEKFGKEW               477
        6
        -------------
AHATIIPKVLAMSGDPNYLHRMTTLFCINVLSEVCGQDI               516
                                    7
                                    ---------
TTKHMLPTVLRMAGDPVANVRFNVAKSLQKIGPILDNST               555

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LQSEVKPILEKLTQDQDVDVKYFAQEALTVLSLA                    589
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FIG. 2. Amino acid sequence (single-letter code) of the 61-kDa protein. The sequence is deduced from the cDNA of clone P16 (28). It shows 15 repeats, with the most conserved amino acids in boldface letters. The dashed lines numbered 1–7 indicate tryptic peptides from the regulatory subunit of bovine heart protein phosphatase. A dash indicates identity between cDNA-derived sequence and peptide sequence; x indicates that these amino acids were not determined in peptide 5.

protein antibodies. As shown in Fig. 1*B* (lane 4), the antibodies specifically recognized the A subunit.

Sequence Comparison Between 61-kDa Protein and A Subunit of PP2A. Further support that the medium T antigen-associated 61-kDa protein is a regulatory subunit of PP2A came from a sequence comparison of the P16-encoded 61-kDa protein with seven tryptic peptides derived from the A subunit. As shown in Fig. 2, 110 of 112 amino acids were identical between the 61-kDa protein and the tryptic peptides (dashed lines 1–7). The difference of two amino acids, alanine vs. proline (peptide 1) and glutamic acid vs. glutamine (peptide 5), could represent a species difference between the human and bovine proteins. These data indicate that the medium T antigen-associated 61-kDa protein is probably identical to the A subunit of PP2A. Furthermore, they suggest that subunit A of bovine heart PP2A is encoded by a homolog of P16 rather than P9 since the P9-encoded protein differs from the P16-encoded protein in 24 of the 112 amino acids from the seven tryptic peptides (G.W., unpublished data).

Reaction of Antibodies Against Catalytic Subunit of PP2A with 37-kDa Protein. To demonstrate that the medium T antigen-associated 37-kDa protein is the catalytic subunit of PP2A, the 37-kDa protein was purified as a complex with medium T antigen by affinity chromatography with monoclonal antibodies against medium T antigen. Fig. 3 (lane 3) shows that the medium T antigen complex contains the 37-kDa protein, the 61-kDa protein, and medium T antigen. In addition, it contains other proteins, most of which are

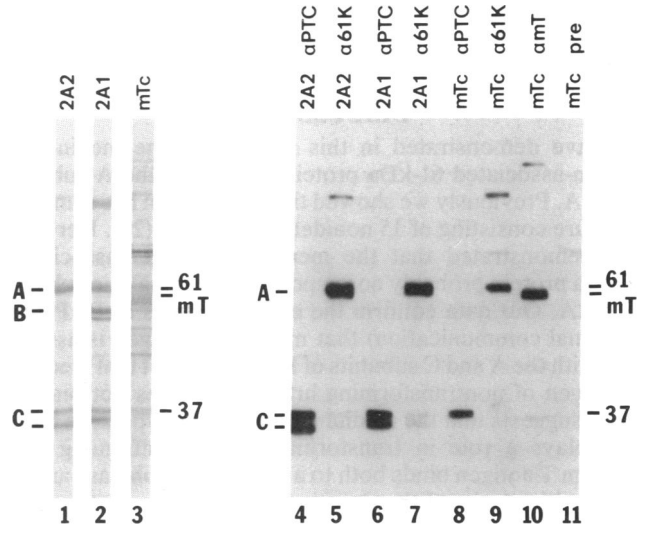

FIG. 3. Antibodies against the catalytic subunit (C) of PP2A react with the medium T antigen (mT)-associated 37-kDa protein. The two-subunit form, PP2A2 (2.5 μg), was loaded on lanes 1, 4, and 5; the three-subunit form, PP2A1 (8 μg), was loaded on lanes 2, 6, and 7. Medium T antigen complex (mTc) (≈15 μg) was loaded on lanes 3 and 8–11. After transfer to nitrocellulose paper, proteins in lanes 1–3 were stained with amido black. Lanes 4–11 were incubated with the following antibodies: lanes 4, 6, and 8, monoclonal antibodies to subunit C (4 μg/ml) (36); lanes 5, 7, and 9, fusion protein antibodies against 61-kDa protein (diluted 1:100); lane 11, corresponding preimmune serum (diluted 1:100); lane 10, monoclonal antibodies against medium T antigen (ascites fluid; diluted 1:100) (21).

bound nonspecifically and can be removed by further purification as shown (21). It is this complex from which the 61-kDa protein was initially isolated to obtain partial amino acid sequences (28). The complex was tested by immunoblotting with a monoclonal antibody against the C subunit of PP2A (anti-PTC) (36). As shown in lane 8, the 37-kDa protein of the medium T antigen-bound proteins reacted specifically, indicating that it represents the catalytic subunit of PP2A. As expected, the fusion protein antibodies against the 61-kDa protein recognized the 61-kDa protein in the complex (lane 9), and monoclonal antibodies against medium T antigen recognized medium T antigen (lane 10). It should be pointed out that medium T antigen shown in Fig. 3 is the wild-type protein, which migrated slightly faster than the 61-kDa protein, whereas the medium T antigen shown in Fig. 1*A* (lane 3) is from the deletion mutant dl8, which is well separated from the 61-kDa protein. As a positive control, two preparations of purified bovine PP2A are also shown; one contains subunits A and C, designated PP2A2 (lane 1), and the other contains subunits A, B, and C, designated PP2A1 (lane 2) (see ref. 5 for nomenclature). Subunit C appears as a doublet due to intramolecular disulfide bond formation in the purified protein and incomplete reduction prior to gel electrophoresis (R. L. Lickteig and M.M., unpublished data). It is also possible that during extended storage the PP2A2 and PP2A1 preparations had undergone some irreversible oxidation of disulfide bonds. The other bands in the enzyme preparations have not been characterized but probably represent contaminants (12). As demonstrated by immunoblots, antibodies to the catalytic subunit recognized only the C subunit (lanes 4 and 6), whereas the fusion protein antibodies recognized the A subunit in both enzyme preparations (lanes 5 and 7). The larger proteins in lanes 5, 9, and 10 have not been further characterized. The 88-kDa proteins shown in lanes 5 and 9 appear to be specific since they are not recognized by the preimmune serum (lane 11). The nature and origin of these proteins is under investigation. The weak signal from the C

subunit with antiserum to the 61-kDa protein (lanes 5 and 7) is considered to be nonspecific and caused by loading a large amount of the purified enzymes.

DISCUSSION

We have demonstrated in this report that the medium T antigen-associated 61-kDa protein represents the A subunit of PP2A. Previously we showed that this protein has a unique structure consisting of 15 nonidentical repeats (28). Here we also demonstrated that the medium T antigen-associated 37-kDa protein probably corresponds to the catalytic subunit of PP2A. Our data confirm the earlier findings of D. Pallas (personal communication) that medium T antigen is associated with the A and C subunits of PP2A. The fact that medium T antigen of nontransforming hrt mutants does not bind to PP2A suggests that the medium T antigen–phosphatase complex plays a role in transformation. It is intriguing that medium T antigen binds both to a protein phosphatase and to protein kinases, and the question arises whether it binds to both enzymes simultaneously or forms distinct complexes with each one. It is conceivable that the kinase and the phosphatase are brought into close proximity by medium T antigen and that they act on the same or neighboring substrates. For example, they might phosphorylate and dephosphorylate a common substrate at a site on the membrane to which medium T antigen binds and which might be involved in membrane signaling and growth control. This mechanism could imply that PP2A, mainly known for its serine and threonine phosphatase activity, also dephosphorylates tyrosine phosphate, a property of PP2A that has been reported to take place *in vitro* (37).

The effects of medium T antigen on protein phosphatase activity remain to be investigated. It could either stimulate or inhibit PP2A or alter its specificity by increasing its activity toward tyrosine phosphate. Medium T antigen might exert these effects by binding to the A or C subunit or to both. It is noteworthy that the B subunit has not been observed in the purified medium T antigen–PP2A complex. Possibly medium T antigen competes with subunit B for binding to the complex of the A and C subunits.

A crucial problem is to determine the substrate(s) for the medium T antigen-associated phosphatase. Since all proteins encoded by protooncogenes are phosphoproteins, medium T antigen might transform by mediating enhanced and constitutive dephosphorylation of potentially oncogenic proteins leading to their activation. In this manner, PP2A could become an oncogene itself. One might also consider mechanisms involving the inactivation of suppressor genes by the medium T antigen–PP2A complex.

The protein-tyrosine kinase activity of $pp60^{c\text{-}src}$ is inhibited by phosphorylation at tyrosine-527 (25, 38–41). Medium T antigen-bound $pp60^{c\text{-}src}$ has a lower degree of phosphorylation at tyrosine-527 than free $pp60^{c\text{-}src}$ and a higher kinase activity (42). It has been proposed that medium T antigen blocks the phosphorylation of tyrosine-527 and thereby prevents the inactivation of the $pp60^{c\text{-}src}$ kinase activity (25, 38). In view of the findings reported here, one might also consider the possibility that the medium T antigen-associated phosphatase maintains $pp60^{c\text{-}src}$ in a state of low phosphorylation at tyrosine-527, resulting in elevated kinase activity. Previous studies indicate that in polyoma-infected cells, the bulk of the medium T antigen–61-kDa protein–37-kDa protein complex (medium T antigen–PP2A) is distinct from the medium T antigen–$pp60^{c\text{-}src}$ complex (43). However, other studies have shown that a small fraction of $pp60^{c\text{-}src}$ is bound to medium T antigen in the same complex with what is now known to be PP2A (22). The latter observation is consistent with the idea that the medium T antigen-bound PP2A would dephosphorylate $pp60^{c\text{-}src}$.

It has recently been demonstrated that PP2A stimulates SV40 DNA replication *in vitro*, which depends on the presence of SV40 large tumor antigen (44). Probably PP2A dephosphorylates some of the six phosphorylated serine residues present in large tumor antigen (45) that were shown to play a role in the initiation of SV40 DNA replication (46, 47).

The 61-kDa protein–37-kDa protein complex (PP2A2) binds to the t antigens of SV40 and polyoma virus (26, 27). Since it does not associate with the SV40 or polyoma virus large tumor antigens, it is likely that the unique sequence in SV40 t antigen and sequences common to the polyoma virus medium T antigens and t antigens are involved in this interaction. This view is further supported by the finding that hrt mutations affecting shared sequences of medium T antigen and t antigen weaken the interaction with PP2A2, as do deletions in sequences unique to the t antigen. Loeken *et al.* (48) demonstrated recently that SV40 t antigen is a transcriptional activator and that the unique sequences are essential for this function. It will be important to investigate whether the transcriptional activation induced by t antigen, as well as its role in transformation, are mediated through binding to PP2A2. Since t antigen does not bind to DNA, it might not be a transcription activator by itself. Instead, it might activate the associated protein phosphatase, resulting in an enhanced dephosphorylation and activation of a transcription activator.

Note Added in Proof. Since this manuscript was submitted, Pallas *et al.* (49) published that the 37- and 61-kDa proteins correspond to the C and A subunits, respectively, of PP2A.

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1. Hunter, T. & Cooper, J. A. (1985) *Annu. Rev. Biochem.* **54,** 897–930.
2. Kloetzer, W. S., Maxwell, S. A. & Arlinghaus, R. B. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 412–416.
3. Moelling, K., Heimann, B., Beimling, P., Rapp, U. R. & Sander, T. (1984) *Nature (London)* **312,** 558–561.
4. Hunter, T. (1989) *Cell* **58,** 1013–1016.
5. Cohen, P. (1989) *Annu. Rev. Biochem.* **58,** 453–508.
6. Green, D. D., Yang, S. I. & Mumby, M. C. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 4880–4884.
7. da Cruz e Silva, O. B., Alemany, S., Campbell, D. G. & Cohen, P. T. W. (1987) *FEBS Lett.* **221,** 415–422.
8. Kitagawa, Y., Tahira, T., Ikeda, I., Kikuchi, K., Tsuiki, S., Sugimura, T. & Nagao, M. (1988) *Biochim. Biophys. Acta* **951,** 123–129.
9. Kincaid, R. L., Nightingale, M. S. & Martin, B. M. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8983–8987.
10. Imaoka, T., Imazu, M., Usui, H., Kinohara, N. & Takeda, M. (1983) *J. Biol. Chem.* **258,** 1526–1535.
11. Pato, M. D. & Kerc, E. (1986) *J. Mol. Biol.* **261,** 3770–3774.
12. Mumby, M. C., Russel, K. L., Garrard, L. J. & Green, D. D. (1987) *J. Biol. Chem.* **262,** 6257–6265.
13. Green, M. R. (1989) *Cell* **56,** 1–3.
14. Courtneidge, S. A. & Smith, A. E. (1983) *Nature (London)* **303,** 435–439.
15. Bolen, J. B., Thiele, C. J., Israel, M. A., Yonemoto, W., Lipsich, L. A. & Brugge, J. S. (1984) *Cell* **38,** 767–777.
16. Kornbluth, S., Sudol, M. & Hanafusa, H. (1987) *Nature (London)* **325,** 171–173.
17. Kypta, R. M., Hemming, A. & Courtneidge, S. A. (1988) *EMBO J.* **7,** 3837–3844.
18. Cheng, S. H., Harvey, R., Espino, P. C., Semba, K., Yamamoto, T., Toyoshira, K. & Smith, A. E. (1988) *EMBO J.* **7,** 3845–3855.
19. Kaplan, D. R., Whitman, M., Schaffhausen, B., Pallas, D. C., White, M., Cantley, L. & Roberts, T. M. (1987) *Cell* **50,** 1021–1029.
20. Courtneidge, S. A. & Heber, A. (1987) *Cell* **50,** 1031–1037.

21. Grussenmeyer, T., Carbone-Wiley, A., Scheidtmann, K. H. & Walter, G. (1987) *J. Virol.* **61,** 3902–3909.
22. Koch, W., Carbone, A. & Walter, G. (1986) *Mol. Cell. Biol.* **6,** 1866–1874.
23. Benjamin, T. L. (1970) *Proc. Natl. Acad. Sci. USA* **73,** 394–399.
24. Walter, G., Carbone, A. & Welch, W. J. (1987) *J. Virol.* **61,** 405–410.
25. Courtneidge, S. A. (1985) *EMBO J.* **4,** 1471–1477.
26. Walter, G., Carbone-Wiley, A., Joshi, B. & Rundell, K. J. (1988) *Virology* **62,** 4760–4762.
27. Pallas, D. C., Cherington, V., Morgan, W., DeAnda, J., Kaplan, D., Schaffhausen, B. & Roberts, T. M. (1988) *J. Virol.* **62,** 3934–3940.
28. Walter, G., Ferre, F., Espiritu, O. & Carbone-Wiley, A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8669–8672.
29. Graham, F. L., Smiley, J., Russell, W. C. & Nairn, R. (1977) *J. Gen. Virol.* **36,** 59–72.
30. Schaffhausen, B. S., Bocus, B. J., Berkner, K. L., Kaplan, D. & Roberts, T. M. (1987) *J. Virol.* **61,** 1221–1225.
31. Dieckmann, C. L. & Tzagoloff, A. (1985) *J. Biol. Chem.* **260,** 1513–1520.
32. Le Beau, J., Wiestler, O. D. & Walter, G. (1987) *Mol. Cell. Biol.* **7,** 4115–4117.
33. Chen, S.-C., Kramer, G. & Hardesty, B. (1989) *J. Biol. Chem.* **264,** 7267–7275.
34. Aebersold, R. H., Laevitt, J., Saavedra, R. A., Hood, L. E. & Kent, S. B. H. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6970–6974.
35. Andrews, P. C. & Dixon, J. E. (1987) *Anal. Biochem.* **161,** 524–528.
36. Mumby, M. C., Green, D. D. & Russel, K. L. (1985) *J. Biol. Chem.* **260,** 13763–13770.
37. Chernoff, J., Li, H.-C., Cheng, Y.-S. E. & Chen, L. B. (1983) *J. Biol. Chem.* **258,** 7852–7857.
38. Cooper, J. A. & King, C. S. (1986) *Mol. Cell. Biol.* **6,** 4467–4477.
39. Kmiecik, T. E. & Shalloway, D. (1987) *Cell* **49,** 65–73.
40. Piwnica-Worms, H., Saunders, K. B., Roberts, T. M., Smith, A. E. & Cheng, S. H. (1987) *Cell* **49,** 75–82.
41. Reynolds, A. B., Vila, J., Lansing, T. J., Potts, W. M., Weber, M. J. & Parsons, J. T. (1987) *EMBO J.* **6,** 2359–2364.
42. Cartwright, C. A., Eckhart, W., Simon, S. & Kaplan, P. L. (1987) *Cell* **49,** 83–91.
43. Grussenmeyer, T., Scheidtmann, K. H., Hutchinson, M. A., Eckhardt, W. & Walter, G. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7952–7954.
44. Virshup, D. M. & Kelly, T. J. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 3584–3588.
45. Scheidtmann, K. H., Echle, B. & Walter, G. (1982) *J. Virol.* **44,** 116–133.
46. Mohr, I. J., Stillman, B. & Gluzman, Y. (1987) *EMBO J.* **6,** 153–160.
47. Graesser, F. A., Mann, K. & Walter, G. (1987) *J. Virol.* **61,** 3373–3380.
48. Loeken, M., Bikel, I., Livingston, D. M. & Brady, J. (1988) *Cell* **55,** 1171–1177.
49. Pallas, D. C., Shahrik, L. K., Martin, B. L., Jaspers, S., Miller, T. B., Brautigan, D. L. & Roberts, T. M. (1990) *Cell* **60,** 167–176.