The p53 Complex from Monkey Cells Modulates the Biochemical Activities of Simian Virus 40 Large T Antigen

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We have compared the ATPase, DNA-binding, and helicase activities of free simian virus 40 (SV40) large T antigen (T₀) and T antigen complexed with cellular p53 (T+p53). Each activity is essential for productive viral infection. The T+p53 and T₀ fractions were prepared by sequential immunosorption of infected monkey cells with monoclonal antibodies specific for p53 and T antigen. The immune-complexed T fractions were then assayed in parallel. For ATP hydrolysis, the V_{max} for T+p53 was 143 nmol of ADP per min per mg of protein, or 18-fold greater than for T₀. ATP had no effect on the stability of the T+p53 complex. The T+p53 complex was significantly more active than T₀ in hydrolyzing dATP, dGTP, GTP, and UTP. Of the nucleotide substrates tested, the greatest relative increase (T+p53/T₀) was in hydrolyzing dGTP and GTP. In DNase footprinting assays performed under replication conditions, the T+p53 complex protected regions I, II, and III of origin DNA while equivalent amounts of T₀ protected only regions I and II. Region III is known to contribute to the efficiency of DNA replication and contains the SP1-binding sites of the early viral promoter. The T+p53 fraction was also a more efficient helicase than T₀, especially with a GC-rich primer and template. Thus, the T+p53 complex has enhanced ATPase, GTPase, DNA-binding, and helicase activities. These findings imply that complex formation between cellular monkey p53 and SV40 T antigen modulates a number of essential activities of T in SV40 productive infection.

Simian virus 40 (SV40) large T (T) antigen has multiple roles in the replication of SV40 DNA during lytic infection (for a review, see reference 13). T has at least three activities that appear to be essential for DNA replication: origin-binding, ATPase, and helicase activities. The specific interaction of T with the origin of replication is the first event in DNA synthesis. ATP binding, without hydrolysis, enhances the binding of T to site II within the 64-base-pair core origin (9, 10). Correct origin binding leads to the unwinding of duplex DNA in the origin of replication (14, 45). The helicase activity of T, in conjunction with its ATPase function, then extends the replication forks in both directions (5, 7, 33, 36, 37, 44). ATP binding and hydrolysis may also play roles in the binding of T at replication forks (35) and in synchronizing replication fork movement (38).

Interestingly, T antigen complexes with a cellular p53 protein (T+p53) in productively infected monkey cells and in a variety of SV40-transformed cell lines (22, 23). The p53 cellular protein has long been known to be overproduced in many transformed cells (for a review, see reference 8). In normal cells, expression of the p53 protein is important for the regulation of cell growth (25, 26, 31). However, the role of the T+p53 complexes in productive and transforming infections is not well understood. All known mutants of T antigen defective for p53 binding are unable to replicate viral DNA (K. Peden, A. Srinivasan, J. Farber, and J. Pipas, *Virology*, in press). Tack et al. have shown that the ATPase activity of T antigen is increased in T+p53 complexes (40). Clarke et al. report that p53 also associates with two other ATPases, the *Escherichia coli* dnaK and rat hsc70 proteins (6). Reich and Levine have shown that p53 does not interfere with T binding to a fragment of DNA containing an origin of replication but did not analyze the interaction by DNA-footprinting techniques (29).

In this report, we characterize some activities of the T+p53 complex prepared from lytically infected cells to define better the function of p53 in SV40 productive infection. T+p53 and T without p53 (T₀) were prepared by sequential immunoprecipitation of infected monkey cell extracts and were assayed for their ability to hydrolyze different nucleotide substrates. In addition, we have examined the effect of p53 on the binding of T antigen to the origin of replication under conditions designed for the efficient replication of DNA in vitro. The use of these conditions was important because the presence of ATP strongly enhances the binding of T antigen to the origin and determines the DNase protection pattern of the interaction (2, 9, 10). Finally, we assayed helicase activity by using AT-rich and GC-rich primer-templates under the conditions of Stahl et al. (34).

MATERIALS AND METHODS

Preparation of cell extracts. CV1 cells were grown in Dulbecco modified Eagle medium with 5% fetal calf serum and were infected with SV40 strain wt800 (39). Murine 3T6 cells (clone 7-3 from W. Eckhart) were grown in Dulbecco modified Eagle medium with 5% fetal calf serum. Cells were labeled in vivo with [³⁵S]methionine (1 mCi/15-cm dish; 1,250 Ci/mmol; Amersham Corp.) for 16 h in 10 ml of Dulbecco modified Eagle medium with 0.2 N methionine-3% dialyzed fetal calf serum before lysis. Cell extracts were prepared (at 40 h postinfection for SV40-infected cells) by a hypotonic extraction procedure (10 mM HEPES [N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, pH 7.8]-5 mM KCl-0.5 mM MgCl₂-1 mM dithiothreitol-10 mM potassium phosphate to inhibit phosphatases) and were centrifuged at 20,000 × g for 45 min to remove cell debris (40). About 600 μl of extract was recovered per 15-cm dish.

Immunosorption analysis. Hybridoma supernatants containing monoclonal papovavirus-specific antibodies (PAbS)

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108 (anti-T) and 122 (anti-p53) and NS1 medium (nonspecific control) were prepared as described by Gurney et al. (19, 20). Cell extracts were immunoprecipitated by using saturating amounts of antibody and protein A-Sepharose (Pharmacia, Inc.) at 4°C and then washing three times with NET-N P-40 buffer (100 mM NaCl–5 mM EDTA–50 mM Tris [pH 8.0]–0.05% Nonidet P-40) as described by Tack et al. (39, 40). Sequential immunosorption first with PAb 122 and then followed by reaction of the PAb 122 supernatant with PAb 108 was used to prepare immune complexes containing T+p53 and T depleted in p53 (T_o) (40).

Nucleotide hydrolysis. To measure ATPase activity, immune complexes were washed twice with ATPase buffer (25 mM PIPES [piperazine-*N,N'*-bis(2-ethanesulfonic acid, pH 7.0)]–100 mM NaCl–5 mM $MgCl_2$ –0.01% Nonidet P-40) and were assayed for the rate of conversion of ATP to ADP, using [α - ^{32}P]ATP (New England Nuclear Corp.; 750 Ci/mmol) at 33°C at various ATP concentrations from 1 to 200 μ M as previously described (40). For analysis of altered substrate preference, [α - ^{32}P]GTP, CTP, UTP, dATP, dGTP, dCTP, and TTP (ICN Pharmaceuticals Inc.; 650 Ci/mmol) were analyzed under similar conditions, usually at 2 or 20 μ M nucleotide concentrations. Immune-complexed T and p53 proteins were then analyzed by sodium dodecyl sulfate (SDS) gel electrophoresis, staining with Coomassie blue, and autoradiography. [^{35}S]Methionine-labeled protein was quantified by using an AMBIS Systems beta scanner and an IBM computer (40). The ATPase activity (percent ADP generated in 60 min) was then normalized to the amount of ^{35}S -labeled T present in each sample assayed.

DNase footprinting. After extraction from productively infected CV1 cells, T antigen was purified either by immunoaffinity chromatography (32) or by immunoprecipitation (40). Matched concentrations of immune-complexed T+p53 and T_o were added to an end-labeled fragment of DNA containing the entire origin of SV40 (base pairs 5171 to 160). After binding for 30 min at 37°C in the presence of 4 mM ATP under replication conditions (9, 10), DNase I was added for 5 min at 4°C, and the samples were processed as described by Deb and Tegtmeyer (10).

Helicase. The helicase assay was performed by the method of Stahl et al. (34). The 15-mer universal sequencing primer (5' OH-d[AGTCACGACGTTGTA]-OH 3') was obtained from New England Nuclear. The 17-mer primer (5' OH-d[GTAAACGACGGCCAGT]-OH 3') was obtained from Pharmacia. Both primers were annealed to M13 mp8 DNA, end-labeled with Klenow, and desalted (34). The 17-mer was elongated by using dGTP, [α - ^{32}P]dCTP (Amersham; 3,000 Ci/mmol), and dCTP. The 15-mer was elongated by using dCTP, [α - ^{32}P]dATP (ICN; 650 Ci/mmol), and dATP. Immune-complexed T fractions (0.5 to 5 μ g of T protein) were washed two times in helicase buffer (34) and were then incubated with 10 to 30 ng of annealed primer-template as substrate for times up to 3 h at 37°C. Affinity-purified T antigen (0.5 to 5 μ g) was analyzed in parallel. Reactions were stopped with SDS and EDTA, and samples were loaded onto a 12.5% acrylamide gel in Tris-borate-EDTA buffer (34). After electrophoresis (100 V for 2 h), the gels were dried and autoradiographed. Labeled primer-template and displaced primer bands were quantified with the AMBIS scanning system.

RESULTS

Preparation and ATPase analysis of T+p53 complexes. Hypotonic extracts were prepared from SV40-infected cells

FIG. 1. ATPase activity of T+p53 complexes. Infected cell extracts labeled with [^{35}S]methionine were immunoprecipitated with PAb 122 and 108 in parallel to generate T+p53 (T_+) and total T (T_{tot}) fractions. (A) ATPase assay of immune-complexed T. Parallel precipitates were incubated with [α - ^{32}P]ATP and the rate of labeled ATP production was measured after thin-layer chromatography as described in Materials and Methods. Two time points, 30 and 60 min, are shown for each sample. The negative control (C) sample (beads adsorbed with the nonspecific NS1 antibody) was usually 2% of the total ATPase activity. (B) SDS gel and autoradiographic analysis of immune-complex T fractions. Arrows (lane T_+) indicate 94K and 90K bands.

labeled with [^{35}S]methionine, and different fractions of T were isolated by protein A-dependent immunosorption as described in Materials and Methods. The fraction of SV40 large T antigen complexed with p53 (T+p53) was prepared by using PAb 122 (specific for p53); T depleted in p53 (T_o) was prepared by reacting the anti-p53 supernatant with PAb 108. A portion of the original cell extract was precipitated with PAb 108 to prepare total T. The immune-complexed T fractions were analyzed for their relative ATPase activities as described in Materials and Methods. The results for the T+p53 and total T fractions are shown in Fig. 1A. Hydrolysis of [α - ^{32}P]ATP to [α - ^{32}P]ADP was dependent on the amount of T antigen present and was linear with respect to the time points used (30 and 60 min). SDS gel analysis of the ^{35}S -labeled samples after the ATPase assay is shown in Fig. 1B. The T+p53 fraction (lane T_+) contained approximately equal amounts of labeled T and p53 bands. Total labeled T is shown in the adjacent lane (T_{tot}). The rates of ATP hydrolysis measured (Fig. 1A) were corrected for the amount of T present (Fig. 1B). The T+p53 fraction of T hydrolyzed 38% of the total amount of labeled ADP product and represented 7.5% of the total T, consistent with previous studies (40). Comparison of the relative amounts of Coomassie blue-stained and 16-h ^{35}S -labeled T bands indicated a similar turnover of the different fractions of T (data not shown).

Figure 1B also shows a distinct pair of labeled bands, migrating with apparent molecular weights of 94,000 and 90,000 (94K and 90K), coprecipitated by PAb 122 (lane T_+). These two bands were consistently present in the T+p53 sample. The upper 94K band corresponded to the major species of total T (Fig. 1B, lane T_{tot} , and see Fig. 5A, lane T_o), while the lower 90K band may be an altered form of T or a new cellular protein complexed with T and p53 (L. Tack, J. Wright, and E. Gurney, submitted for publication).

Kinetic analysis: T+p53 complexed had an enhanced V_{max} compared with T_o . Three different fractions of SV40 T

FIG. 2. Kinetic analysis of enhanced ATPase-active T+p53 complexes compared with total T and T_o fractions. Labeled extracts were first immunoprecipitated with PAb 122 to prepare T+p53. One-tenth of a volume of this supernatant was then sequentially precipitated with PAb 108 to prepare T_o ; total T was prepared by using a similar amount of extract and PAb 108 alone. Parallel samples of each T fraction were assayed for ATPase activity at various ATP concentrations as described in Materials and Methods. (A) SDS gel and autoradiographic analysis of labeled T fractions after ATPase analysis. Arrows (lane T+p53) indicate 94K and 90K bands. (B) Lineweaver-Burk reciprocal plot analysis of the rate of ATP hydrolysis with increasing ATP concentrations (1 to 100 μ M).

antigen (total T and T with and without bound p53) were prepared and assayed for ATPase activity by using increasing ATP concentrations (1 to 200 μ M) in parallel as described in Materials and Methods. The results are shown in Fig. 2. Immunosorption with PAb 122 followed by sequential reaction of the anti-p53 supernatant with PAb 108 was used to prepare T+p53 and T_o fractions. So that the relative amounts of protein in each sample were constant, only 1/10 of a volume of the anti-p53 supernatant was precipitated with anti-T. Similarly, 1/10 of a volume of extract was precipitated with PAb 108 (without pretreatment with PAb 122) to prepare total T. The amount of each labeled T fraction after ATPase assay and SDS gel analysis for one experiment is shown in Fig. 2A. Again, the 94K and 90K pair of bands were present in the T+p53 sample.

Lineweaver-Burk analysis of the ATPase data generated in this experiment is shown in Fig. 2B. The results indicated that T+p53 had a V_{max} of 143 nmol of ADP per min per mg of T antigen. This was 18-fold greater than the calculated V_{max} of 8 nmol/min per mg of T for the T_o fraction, which

comprises 94%, on average (40), of the T present in these extracts. This result is similar to the previously determined V_{max} of 10 to 12 nmol/min per mg of protein for total lytic T (39). The K_m for T+p53 was calculated to be 45 μ M, similar to that for the total T and T_o fractions (40 to 50 μ M).

The addition of creatine kinase and creatine phosphate to the ATPase reaction resulted in no net generation of ADP as expected for an ATP-regenerating system. The addition of sodium pyrophosphate also had little effect, but increasing ADP concentrations inhibited the ATP hydrolysis reaction. Pretreatment of the ATPase reaction mixture with sodium vanadate (100 μ M) had little effect on the rate of ATP hydrolysis.

Altered substrate preference: T+p53 complexes had enhanced ATPase and GTPase activities. Giacherio and Hager (17) have previously shown that partially purified SV80 T preferentially hydrolyses dATP and ATP, compared with the other four nucleotide substrates. However, the substrate specificity of lytic T both with and without bound p53 is not known. Therefore, we measured the ability of T+p53 and T_o

TABLE 1. Effect of p53 complex formation on hydrolysis of different nucleotide substrates

Nucleotide substrate	Sp act ^a		(T+p53/T ₀) ^b
	T+p53	T ₀	
dATP	4.90	2.60	1.9 (*)
dGTP	1.00	0.35	2.9 (*)
dTTP	0.44	0.42	1.0
dCTP	0.06	0.08	0.8
ATP	2.05	1.00	2.0 (*)
GTP	1.10	0.34	3.2 (*)
UTP	0.45	0.23	2.0 (*)
CTP	0.06	0.05	1.2

^a Specific activity calculated as relative hydrolysis rate after 60 min incubation at 33°C per µg of T fraction and normalized to the value obtained for the hydrolysis of ATP with T₀ (made equal to 1.0). Data shown are averages of three experiments at 10 to 20 µM nucleotide concentrations.

^b Change in activity was determined as the ratio of the specific activities of the two T fractions. *, Enhanced activity of T fractions containing p53.

immune complexes to hydrolyze various ribo- and deoxyribonucleotides at two different (2 and 20 µM) nucleotide concentrations, as described in Materials and Methods. The results are summarized in Table 1 and Fig. 3. For T+p53, the order of hydrolysis preference was dATP > ATP > dGTP = GTP > dTTP = UTP >> dCTP = CTP. For T₀, the order of preference was dATP > ATP > dTTP = dGTP = GTP = UTP >> dCTP > CTP. In general, both forms of T preferred dATP to ATP. However, T+p53 was significantly better than T₀ at hydrolyzing not only dATP but also ATP, dGTP, GTP, and UTP. In particular, the degree of enhanced hydrolytic activity was greatest for dGTP and GTP. Thus, bound p53 appears to enhance the ATPase and GTPase activities of T antigen.

Altered *ori* DNA binding: T+p53 complexes protected region III. Reich and Levine (29) have shown that T antigen, complexed with murine p53, binds specifically to a fragment of viral DNA that contains an SV40 origin of replication. However, these early studies were done in the absence of ATP and did not include DNase protection techniques to distinguish binding to three distinct regions within the origin DNA. We used DNase footprinting in the presence of ATP, under conditions established for efficient DNA replication *in vitro*, to examine the effect of p53 on origin binding. We used T, purified by elution from immunoaffinity columns, as a positive control and nonspecific NS1 antibody complexed to protein A-Sepharose beads as a negative control. The results are shown in Fig. 4. Affinity-purified and immune-complexed T fractions protected region I and II (10) from DNase in identical patterns. At lower T concentrations, both T₀ and T+p53 protected region I better than region II. Although the results shown in Fig. 4 suggest that T₀ is slightly more efficient than T+p53 at protecting region II, this minor difference reflects a small change in protein concentration (T₀ was more concentrated than T+p53). Analysis of three separate experiments indicates that both T₀ and T+p53 complexes protected region II with similar efficiency. Interestingly, the T+p53 complex bound and protected region III, whereas neither preparation of free T did. Immune-complexed free p53 from 3T6 cells did not protect any region of the DNA (data not shown), consistent with previous results (4, 29). The amount of p53 bound to T in each T+p53 fraction appeared to affect the degree of protection. When different preparations of T+p53 were assayed, those that contained more bound p53 protected region III better (data

FIG. 3. Effect of the p53 complex on the relative rate of nucleotide hydrolysis. The upper panel depicts the results (Table 1) for the different deoxy- and ribonucleotides assayed under the same conditions as described for Fig. 2. The specific activity for the hydrolysis of ATP by T₀ was made 1.0, and the other specific activities were normalized against this value. The activity of each sample was corrected for the amount of T antigen present. The lower panel shows the ratio of the results for T+p53 to the results for T₀ for each nucleotide analyzed. The dotted line represents a theoretical value of 1.0 if no change in hydrolytic activity occurred on comparing T+p53 with T₀.

not shown). We conclude that bound p53 enhances the binding of T to region III of origin DNA.

Altered helicase activity: T+p53 complexes displaced annealed primer DNA more efficiently. T antigen has DNA helicase activity (34), with unwinding occurring bidirectionally from the SV40 origin (14) and preferentially in the 3' to 5' direction (18, 44). We wished to measure the relative helicase activity of T+p53 and T₀ fractions of T by using the unwinding assay of Stahl et al. (34). Two different commercially available M13 primers of various nucleotide compositions were hybridized to M13 DNA and were end labeled by using Klenow and ³²P-labeled nucleotide (see Materials and Methods). One elongated annealed 20-mer primer was 60% GC rich overall, with an 80% GC-rich 3' terminus. The other elongated annealed 19-mer primer was 58% AT rich overall, with an 80% AT-rich 3' terminus. A typical experiment using the two substrates and equal amounts of immune-complexed ³⁵S-labeled T+p53 and T₀ fractions is shown in Fig. 5. Panel A shows SDS gel analysis of the T samples after the helicase assay. Both the 94K and 90K bands characteristic of the T₊ sample were present, while T₀ contained only the 94K band. Panels B and C show typical results with the GC- and AT-rich annealed primer, respectively, as substrates. Nega-

FIG. 4. DNase I footprinting of free and p53-complexed T antigen. Footprinting of T bound to origin DNA was carried out in the presence of 4 mM ATP under in vitro replication conditions as described by Deb and Tegtmeyer (10). Lanes: pure, unbound DNA (C) and DNA bound by T antigen (T) purified by immunoaffinity chromatography as described by Simanis and Lane (32); T+p53 and T_0 , bound matched DNA quantities (1-, 3-, and 10-fold relative increase in amount) of T complexed with p53 and free of p53, respectively. The locations of T-antigen-binding regions I, II, and III are shown on the right.

tive controls included protein A-Sepharose beads complexed with the nonspecific NS1 antibody (lanes C1) or beads alone (lanes C2).

The results of several analyses are summarized in Table 2. T+p53 was consistently more efficient than the T_0 fraction in displacing either the AT-rich or the GC-rich primer from its respective template DNA. In general, the AT-rich primer-template was a better substrate than the GC-rich primer-template, regardless of the form of T used. However, the greatest average increase in activity ($T+p53/T_0 = 2.3$) was with the annealed GC-rich primer as the substrate.

ATP had no effect on the stability of the T+p53 complex. The addition of up to millimolar amounts of ATP to p53 associated with either the *E. coli* dnaK or hsp70 has been reported to reverse the complex (6). Accordingly, we analyzed the lytic T+p53 complex by SDS gel analysis both before and after the addition of up to 4 mM ATP in each of the biochemical assays described above. Our results showed that ATP had no effect on the relative amounts of labeled T and p53 proteins in complex and reactive with either PAb 122 or 108 (Fig. 2 and 5). Thus, the stability of the monkey p53-associated-SV40 T-antigen complex was not affected by the presence of physiological concentrations of ATP.

DISCUSSION

We have analyzed the effect of the bound monkey p53 complex on several biochemical activities of SV40 large T antigen important for productive infection. Sequential immunosorption with monoclonal antibodies specific for p53 and T was used to prepare T+p53 complexes and free-T fractions. Equal amounts of the two T forms were analyzed in nucleotide hydrolysis, DNase-footprinting, and helicase assays. The presence of millimolar concentrations of ATP had no effect on the stability of the SV40 T+p53 (monkey) complex, unlike the effect on p53 complexed with either the heat shock hsc70 or dnaK proteins (6).

ATPase activity and p53 binding related. We have observed that the T+p53 complex has both enhanced ATPase and GTPase activities. The elevated (18-fold) V_{max} and altered nucleotide substrate preference of T+p53 complexes are consistent with a relationship between ATPase activity and p53 binding previously suggested by other studies as follows. (i) T+p53 has an enhanced ATPase specific activity (40); (ii) all subclasses of T that have altered ATPase activity also have altered amounts of bound p53 (40); (iii) the coordinates of the p53 binding site overlap the ATPase domain in SV40 large T (30; Peden et al., in press); and (iv) mutants of T unable to bind p53 are ATPase inactive (L. Tack, K. Peden, and J. Pipas, unpublished observations). Free mouse p53 from 3T6 cells has no detectable ATPase activity (40).

The conformation of the nucleotide-binding site is critical to the function of proteins containing these sites. The ability of such proteins to bind nucleotide may be modulated either by mutations near or within the binding pocket or by protein-protein interactions. The p21 proteins, products of the ras cellular oncogene family, are known GTPases and represent a class of such proteins (1). The GTP-binding site in the N terminus of p21 is homologous to sequences contained in ATP-binding proteins such as RecA, adenylate kinase, and SV40 large T antigen. The interaction of p21 with guanine nucleotides is crucial to the transformation function of this protein. Mutations in a particularly flexible segment of the nucleotide-binding site control access to this site, affect the equilibrium constant for the enzyme, and drastically alter the transforming ability of the p21 protein. The monkey p53 complex may function by interacting with similar elements constituting the ATP-binding site in T or other ATPases. The ability to alter an ATP-binding pocket may be important in p53-T-antigen-mediated cellular events in transformation as well as productive infection.

The self-association of T-antigen molecules may affect the ability of bound p53 to regulate the nucleotide-binding site in T. Much of T forms oligomers of various sizes, some of which are in complex with p53. It is not clear whether complex formation has the same effect on ATPase activity regardless of the size or whether it directly modulates the ATPase site in the T molecule in which it is bound or affects adjoining associated T molecules.

Role for the p53 complex at SV40 ori. DNase footprinting shows that an association of p53 with T antigen has no significant effect on the binding of T to regions I and II but enhances binding to region III of origin DNA. These results confirm and extend the conclusions of Reich and Levine (29). The enhanced binding to region III probably represents a quantitative rather than a qualitative difference because similar binding by T_0 occurs readily under different conditions (11, 42). Perhaps the p53 complex stabilizes or alters the structure of T to increase its binding activity. Region III

FIG. 5. Effect of complexed p53 on the helicase activity of T antigen. Equal amounts of T+p53 (T+) and T₀ samples were prepared by sequential immunosorption as described for Fig. 2 and were analyzed for helicase activity in parallel. (A) SDS gel and autoradiographic analysis of immune-complexed ³⁵S-labeled T fractions after the helicase assay. Arrows indicate 94K and 90K bands. (B) T-dependent helicase activity measured with increasing time (0, 45, 90, and 135 min) at 37°C, using the GC-rich primer. Controls consisted of protein A-Sepharose beads adsorbed with NS1 medium (C1) or beads alone (C2). T₀ and total T samples (Tot) on the far right were prepared by using five times the amount of T as for those analyzed on the left and by assaying for 135 min only. (C) Same as for panel B except that an AT-rich primer was used.

provides an ancillary function in DNA replication and is a major upstream element in the SV40 early promoter; the interplay of events in replication and transcription, if any, remains to be determined (for a review, see reference 12). Thus, the interaction of the p53-T-antigen complex with this DNA regulatory region could modulate initiation of viral DNA replication or transcription or both. Possibly, p53 complex binding to T may affect both protein-DNA and protein-protein interactions at *ori* to modulate and coordinate viral functions. The fraction of total T (about 1.3%) bound to SV40 chromosomes in infected cells is very low (39). Therefore, the amount of T+p53 present is sufficient to directly participate in viral DNA replication.

Complexed p53 and altered helicase activity. In addition to enhanced ATPase activity, the T+p53 complex had an enhanced helicase activity compared with that of T free of p53. In particular, the T+p53 complex was more efficient than T₀ at displacing a GC-rich primer from its template. Higher ratios of p53 to T in the complex generally corresponded to increased helicase specific activity. That T+p53 was more active than T₀ towards a GC-rich primer under

replication conditions may reflect an altered nucleotide preference in the presence of p53. The ability of T+p53 to more effectively unwind both GC-rich and AT-rich DNA compared with T₀ may be important because the SV40 *ori* region is both highly AT and GC rich in localized stretches. T-binding site II lies adjacent to an AT-rich region at *ori*. In contrast, T-binding site III lies within the GC-rich SP1 sites. The p53 complex may allow more efficient binding and unwinding of stable GC-rich DNA in this region. This property could be important for either SV40 replication or transcription.

Monkey p53 complex formation and altered T function. Since the p53-binding and ATPase sites in T overlap, interactions between complexed p53 and T could regulate not only the rate of hydrolysis but also the substrate specificity of this important functional domain. There are several possible mechanisms to explain how the complex affects T function. First, monkey p53 may preferentially recognize an active subclass of T. This implies the existence of a subclass of T that is structurally and functionally distinct, independent of its association with p53. In SV40-infected CV1 cells, only 5 to 10% of lytic T is in complex with monkey p53 (40). In addition, newly synthesized T is ATPase active and preferentially recognized by several monoclonal antibodies (Tack et al., submitted). p53 may recognize some structural element of this subclass and bind to and perhaps even stabilize an active conformation as the rest of T matures into a less active form. Second, p53 complex formation may activate T, in this case, binding to an inactive form. This may or may not be dependent on a subclass of T, but in either case, complex formation per se would activate those molecules of T. If true, then T+p53 complexes formed in vitro should be enhanced for these biochemical properties. It is also possible that another associated factor, such as the 90K

TABLE 2. Effect of p53 complex formation on helicase activity

Annealed primer	Helicase sp act ^a			(T+p53/T ₀) ^b
	T+p53	T ₀	T _{tot}	
GC rich	2.3	1.0	1.2	2.3
AT rich	4.7	3.3	3.9	1.4

^a Rate of displacement of labeled primer from 10 to 20 ng of annealed substrate. Equal amounts (0.5 μg) of the three different T fractions, T+p53, T₀, and T_{tot}, were used. Results shown are averages of three separate experiments.

^b Change in activity was determined as the ratio of the specific activities of the two T fractions.

protein, coprecipitated with the T+p53 complex, accounts for the observed changes in T properties, rather than bound p53. Finally, a differential effect on the properties of T prepared with anti-p53 (PAb 122) compared with that prepared with anti-T (PAbs 101, 108, 419, and KT3) could account for the results. For example, the anti-p53 could enhance or the anti-T could inhibit the activities of T. This is unlikely, as several N- and C-terminal specific monoclonal anti-T antigens were used with similar results; PAb 122 recognizes the C terminus of p53 (43), distant from the location of the T-binding site (41).

What is the role of p53 in the cell? There is evidence to suggest that p53 can either promote or repress several different viral and cellular processes. In the present article, we show that complex formation between monkey p53 and SV40 T antigen enhances several biochemical activities important for SV40 productive infection. Thus, one consequence of complex formation during SV40 infection may be to facilitate viral DNA synthesis in monkey cells. An additional role may be to sequester p53, preventing it from participating in normal cell cycling events and thereby conferring an altered state of permissiveness for virus-specific activities. p53 may thus act at more than one level as a positive host cell factor in SV40 infection. However, in a nonproductive system, p53 may have contrary effects. For example, mouse p53 and DNA polymerase α compete for binding to T antigen in vitro (16). Furthermore, mouse p53 inhibits *ori*-dependent SV40 DNA synthesis (3; Prives and co-workers, unpublished observations). Thus, mouse p53 appears to inhibit and monkey p53 appears to enhance activities important for DNA replication. The amino acid sequence of mouse p53 but not monkey p53 is known. Altered sequences in the two species of p53 may differentially affect T functions.

p53 has oncogenic potential in that overexpression can immortalize cells and cause malignant transformation; it can also cooperate with the ras oncogene in the transformation of primary cells (15, 21, 28). However, other results suggest that p53 may have anti-oncogene or growth suppressor activity in some cells. The p53 genome is rearranged or otherwise inactivated in most Friend virus-induced erythroleukemic cell lines and in a high percentage of human osteogenic sarcomas studied (24, 27). Thus, p53 expressed in different tissues and species may function either positively or negatively with respect to modulating viral replicative and cell transformation processes.

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LITERATURE CITED

1. Barbacid, M. 1987. Ras genes. *Annu. Rev. Biochem.* **56**:779-827.
2. Borowicz, J., and J. Hurwitz. 1988. ATP stimulates the binding of SV40 large T antigen to the SV40 origin of replication. *Proc. Natl. Acad. Sci. USA* **85**:64-68.
3. Braithwaite, A., H. Sturzbecher, C. Addison, C. Palmer, K. Rudge, and J. Jenkins. 1987. Mouse p53 inhibits SV40 origin-dependent DNA replication. *Nature (London)* **329**:458-460.
4. Burger, C., and E. Fanning. 1983. Specific DNA binding activity of T antigen subclasses varies among different SV40-transformed cell lines. *Virology* **126**:19-31.
5. Clark, R., K. Peden, J. M. Pipas, D. Nathans, and R. Tjian. 1983. Biochemical activities of T-antigen proteins encoded by simian virus 40 A gene deletion mutants. *Mol. Cell. Biol.* **3**:220-228.
6. Clarke, C. F., K. Cheng, A. B. Frey, R. Stein, P. W. Hinds, and A. J. Levine. 1988. Purification of complexes of nuclear oncogene p53 with rat and *Escherichia coli* heat shock proteins: in vitro dissociation of hsc70 and dnaK from murine p53 by ATP. *Mol. Cell. Biol.* **8**:1206-1215.
7. Cole, C. N., J. Tornow, R. Clark, and R. Tjian. 1986. Properties of the simian virus 40 (SV40) large T antigens encoded by SV40 mutants with deletions in gene A. *J. Virol.* **57**:539-546.
8. Crawford, L. 1983. The 53,000-dalton cellular protein and its role in transformation. *Int. Rev. Exp. Pathol.* **25**:1-50.
9. Dean, F., M. Dodson, H. Echols, and J. Hurwitz. 1987. ATP-dependent formation of a specialized nucleoprotein structure by SV40 large T antigen at the SV40 replication origin. *Proc. Natl. Acad. Sci. USA* **84**:8981-8985.
10. Deb, S. P., and P. Tegtmeyer. 1987. ATP enhances the binding of simian virus 40 large T antigen to the origin of replication. *J. Virol.* **61**:3649-3654.
11. DeLucia, A. L., B. A. Lewton, R. Tjian, and P. Tegtmeyer. 1984. Topography of simian virus 40 A protein-DNA complexes: arrangement of pentanucleotide interaction sites at the origin of replication. *J. Virol.* **46**:143-150.
12. DePamphilis, M. 1988. Transcriptional elements as components of eukaryotic origins of DNA replication. *Cell* **52**:635-638.
13. DePamphilis, M. L., and M. Bradley. 1986. Replication of SV40 and polyoma virus chromosomes, p. 99-246. In N. Salzman (ed.), *The Papovaviridae*, vol. 1. Plenum Publishing Corp., New York.
14. Dodson, M., F. Dean, P. Bullock, H. Echols, and J. Hurwitz. 1987. Unwinding of duplex DNA from the SV40 origin of replication by antigen. *Science* **238**:964-967.
15. Eliyahu, D., A. Raz, P. Gruss, D. Givol, and M. Oren. 1984. Participation of p53 cellular tumor antigen in transformation of normal embryonic cells. *Nature (London)* **312**:646-649.
16. Gannon, J., and D. Lane. 1987. p53 and DNA polymerase α compete for binding to SV40 T antigen. *Nature (London)* **329**:456-458.
17. Giacherio, D., and L. Hager. 1979. A poly(dT)-stimulated ATPase activity associated with SV40 large T antigen. *J. Biol. Chem.* **254**:8113-8116.
18. Goetz, G., F. Dean, J. Hurwitz, and S. Matson. 1988. The unwinding of duplex regions in DNA by the SV40 large T antigen-associated DNA helicase activity. *J. Biol. Chem.* **263**:383-392.
19. Gurney, E. G., R. O. Harrison, and J. Fenno. 1980. Monoclonal antibodies against simian virus 40 T antigens: evidence for distinct subclasses of large T antigen and for similarities among nonviral T antigens. *J. Virol.* **34**:752-763.
20. Gurney, E. G., S. Tamowski, and W. Deppert. 1986. Antigenic binding sites of monoclonal antibodies specific for simian virus 40 large T antigen. *J. Virol.* **57**:1168-1172.
21. Jenkins, J., K. Rudge, and G. Currie. 1984. Cellular immortalization by a cDNA clone encoding the transformation-associated phosphoprotein p53. *Nature (London)* **312**:651-654.
22. Lane, D. P., and L. Crawford. 1979. T antigen is bound to a host protein in SV40-transformed cells. *Nature (London)* **278**:261-263.
23. Linzer, D., and A. J. Levine. 1979. Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. *Cell* **17**:43-52.
24. Masuda, H., C. Miller, H. Koeffler, H. Battifora, and M. Cline. 1987. Rearrangement of the p53 gene in human osteogenic sarcomas. *Proc. Natl. Acad. Sci. USA* **84**:7716-7719.
25. Mercer, W. E., D. Nelson, A. DeLeo, L. Old, and R. Baserga. 1982. Microinjection of monoclonal antibody to protein p53 inhibits serum-induced DNA synthesis in 3T3 cells. *Proc. Natl. Acad. Sci. USA* **79**:6309-6312.
26. Milner, J., and S. Milner. 1981. SV40-p53K antigen: a possible role for p53 in normal cells. *Virology* **112**:785-788.

27. Mowat, M., A. Cheng, N. Kimura, A. Bernstein, and S. Benchimol. 1985. Rearrangements of the cellular p53 gene in erythroleukemic cells transformed by Friend virus. *Nature (London)* **314**:633–636.
28. Parada, L., H. Land, R. Weinberg, D. Wolf, and V. Rotter. 1984. Cooperation between gene encoding p53 tumor antigen and ras in cellular transformation. *Nature (London)* **312**:649–651.
29. Reich, N., and A. Levine. 1982. Specific interaction of the SV40 T antigen-cellular p53 protein complex with SV40 DNA. *Virology* **117**:286–290.
30. Schmeig, F., and D. Simmons. 1988. Characterization of the in vitro interaction between SV40 T antigen and p53: mapping the p53 binding site. *Virology* **164**:132–140.
31. Shohat, O., M. Greenberg, D. Reisman, M. Oren, and V. Rotter. 1987. Inhibition of cell growth mediated by plasmids encoding p52 anti-sense. *Oncogene* **1**:277–283.
32. Simanis, V., and D. Lane. 1985. An immunoaffinity purification procedure for SV40 large T antigen. *Virology* **144**:88–100.
33. Smale, S., and R. Tjian. 1986. Inhibition of SV40 DNA replication by specific modification of T-antigen with oxidized ATP*. *J. Biol. Chem.* **261**:14369–14372.
34. Stahl, H., P. Droge, and R. Knippers. 1986. DNA helicase activity of SV40 large tumor antigen. *EMBO J.* **5**:1939–1944.
35. Stahl, H., and R. Knippers. 1983. Simian virus 40 large tumor antigen on replicating viral chromatin: tight binding and localization on the viral genome. *J. Virol.* **47**:65–76.
36. Stillman, B., R. Gerard, R. Guggenheimer, and Y. Gluzman. 1985. T antigen and template requirements for SV40 DNA replication in vitro. *EMBO J.* **4**:2933–2939.
37. Strauss, M., P. Argani, I. J. Mohr, and Y. Gluzman. 1987. Studies on the origin-specific DNA-binding domain of simian virus 40 large T antigen. *J. Virol.* **61**:3326–3330.
38. Tack, L., and G. N. Proctor. 1987. Two major subclasses of replicating SV40 chromosomes: T is associated with synchronous replication fork movement. *J. Biol. Chem.* **262**:6339–6349.
39. Tack, L. C., J. H. Wright, and E. G. Gurney. 1986. Free and viral chromosome-bound simian virus 40 T antigen: changes in reactivity of specific antigenic determinants during lytic infection. *J. Virol.* **58**:635–646.
40. Tack, L. C., J. H. Wright, and E. G. Gurney. 1988. Characterization of simian virus 40 large T antigen by using different monoclonal antibodies: T-p53 complexes are preferentially ATPase active and adenylated. *J. Virol.* **62**:1028–1037.
41. Tan, T.-H., J. Wallis, and A. J. Levine. 1986. Identification of the p53 protein domain involved in formation of the simian virus 40 large T-antigen-p53 protein complex. *J. Virol.* **59**:574–583.
42. Tjian, R. 1978. The binding site on SV40 DNA for a T antigen-related protein. *Cell* **13**:165–179.
43. Wade-Evans, A., and J. Jenkins. 1985. Precise epitope mapping of the murine transformation-associated protein, p53. *EMBO J.* **4**:699–706.
44. Wiekowski, M., M. Schwarz, and H. Stahl. 1988. SV40 large T antigen DNA helicase. *J. Biol. Chem.* **263**:436–442.
45. Wold, M., J. Li, and T. Kelly. 1987. Initiation of SV40 DNA replication in vitro: large tumor antigen and origin-dependent unwinding of the template. *Proc. Natl. Acad. Sci. USA* **84**:3643–3647.