6-1995

A TEF-1-independent Mechanism for Activation of the Simian Virus 40 (SV40) Late Promoter by Mutant SV40 Large T Antigens.

Paul Casaz
Harvard University

Phillip W. Rice
Dartmouth College

Charles N. Cole
Dartmouth College

Ulla Hansen
Harvard University

JOURNAL OF VIROLOGY, June 1995, p. 3501–3509 Vol. 69, No. 6
0022-538X/95/$04.00+0

A TEF-1-Independent Mechanism for Activation of the Simian Virus 40 (SV40) Late Promoter by Mutant SV40 Large T Antigens

PAUL CASAZ,[1,2]† PHILLIP W. RICE,[3]‡ CHARLES N. COLE,[3] AND ULLA HANSEN[1,2]*

Committee on Virology and Department of Microbiology and Molecular Genetics, Harvard Medical School,[1] and Division of Molecular Genetics, Dana-Farber Cancer Institute,[2] Boston, Massachusetts 02115, and Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire 03755[3]

Received 22 August 1994/Accepted 3 March 1995

Simian virus 40 (SV40) large tumor antigen (T antigen) stimulates the activity of the SV40 late promoter and a number of cellular and other viral promoters. We have characterized the ability of T antigens with mutations in the DNA-binding domain and within the N-terminal 85 residues to activate the SV40 late promoter. T antigens lacking both nonspecific and sequence-specific DNA-binding activities were able to induce the late promoter. Mutations within the N-terminal 85 residues of T antigen diminished activation by less than twofold. Activation by wild-type and most of the mutant T antigens required intact binding sites for the cellular transcription factor TEF-1 in the late promoter. Curiously, two mutants altered in the N-terminal region and an additional mutant altered in the DNA-binding domain activated a late promoter derivative lacking TEF-1 binding sites, indicating the existence of a TEF-1-independent pathway for activation of the late promoter. A consensus binding site for the TATA binding protein, TBP, was created in variants of late promoters either containing or lacking TEF-1 binding sites. Basal expression was increased by the consensus TBP binding site only when TEF-1 binding sites were present, leading to a reduction in the degree of activation by T antigen. However, activation by a mutant T antigen of the promoter lacking TEF-1 sites was unchanged or slightly enhanced by the consensus TBP binding site. These results suggest that some mutant T antigens can stabilize an interaction between TBP and additional factors bound to the late promoter.

Simian virus 40 (SV40) large tumor antigen (T antigen) is a multifunctional protein required for viral DNA replication and the temporal regulation of viral transcription during SV40 infection. In addition to having a role in the regulation of the SV40 promoters, large T antigen increases the rate of transcription initiation by RNA polymerase II at a variety of cellular and viral promoters in transient transfection assays (1, 6, 9, 15, 26, 34, 40, 56). Induction of this wide range of promoters could result from a single function of T antigen that affects many different promoters, for instance, altering the activity or enhancing the binding of an essential basal transcription factor. Alternatively, it could result from multiple independent functions of T antigen, each affecting the activity of a different promoter-specific transcription factor. Analysis of the functions of T antigen suggests that both models may be true. T antigen contains several activities, described below, that can regulate transcription (2, 12, 13, 16, 22, 30, 31, 35, 37, 42, 43, 45, 60). The activity of several DNA-binding transcription factors can be altered either directly or indirectly by T antigen. At least four cellular transcription factors interact directly with T antigen: the sequence-specific DNA-binding transcription factors AP-2 (38), TEF-1 (22), and p53 (30, 31) and the TATA binding component (TBP) of the basal transcription factor TFIID (22, 35). Although the interaction of T antigen with TBP could theoretically activate all RNA polymerase II promoters, when examined specifically this interaction was found to be able to generate only a fraction of the activity of wild-type T antigen on the SV40 late promoter or a simple promoter dependent on TEF-1 binding sites (22). Interaction of T antigen with non-DNA-binding proteins can also result in the activation of transcription factors. In particular, T antigen binding to the retinoblastoma susceptibility gene product (Rb) (16) leads to the release of E2F from a complex with Rb and to the activation of promoters containing E2F binding sites (12, 13). Because T antigen itself is a sequence-specific DNA-binding protein (42), it might also directly activate transcription from promoters containing its own binding sites. However, binding to DNA must not be absolutely required for activation of the SV40 late promoter, since mutants with truncations that completely remove the DNA-binding domain retain partial transactivation activity (22, 60). Nevertheless, it remained possible that binding to DNA assists in activation. In the context of full-length T antigen, mutants with mutations that interfere with DNA-binding activity have been reported to be defective in activation of the late promoter (6, 26, 60). Finally, mutations in the amino-terminal 85 amino acids of T antigen decrease induction of the Rous sarcoma virus long terminal repeat (RSV LTR) and the SV40 late promoter (60). This region also contains an activity that contributes to the immortalization and transformation of some cell types (39, 41, 59).

The SV40 late promoter is located in a region of the SV40 genome that also includes the SV40 early promoter and the viral origin of DNA replication. T antigen activates the late promoter through a replication-independent stimulation of late promoter activity (6, 26). Mutagenesis of the late promoter has indicated that binding sites for the cellular transcription factor TEF-1 are necessary for activation by T antigen (11, 17,

* Corresponding author. Mailing address: Division of Molecular Genetics, Dana-Farber Cancer Institute, 44 Binney St., Boston, MA 02115. Phone: (617) 375-8211. Fax: (617) 375-8221.

† Present address: Laboratoire de Microbiologie et Génétique Moléculaire du Centre National de la Recherche Scientifique, 31062 Toulouse, France.

‡ Present address: Collaborative Research Inc., Waltham, MA 02154.

27, 28, 47). In addition, these sites are sufficient to confer T-antigen inducibility on heterologous promoters (11, 21, 22). T antigen interacts directly with both TEF-1 and TBP, and induction of the late promoter is abolished with T-antigen fragments that fail to bind either of these factors, with a single exception (22). Although TEF-1 binding sites are clearly necessary for induction of the late promoter, other sequences have been identified that also contribute to activation (7, 17, 18, 27, 36, 47). These include sequences in the enhancer and 21-bp repeats, as well as sequences at the origin of DNA replication. The presence of several elements which can contribute to induction of the late promoter suggests that multiple activities of T antigen are involved in late promoter activation.

Using mutants of T antigen, we have examined the role of the DNA-binding activity and of the N-terminal 85 residues of T antigen in activation of the SV40 late promoter. Our results show that in the context of full-length T antigen, DNA-binding activity is not necessary for activation of the late promoter. Unexpectedly, we found three mutants that strongly activated a derivative of the SV40 late promoter lacking TEF-1 binding sites, indicating the existence of a second pathway for activation of the late promoter.

MATERIALS AND METHODS

Plasmid DNA. All plasmids were propagated in *Escherichia coli* HB101, isolated by an alkaline lysis protocol (4), and purified twice by centrifugation in CsCl-ethidium bromide gradients.

The plasmid pLCATΔ72 was constructed from pL16nCAT (27) by cleavage with *Sph*I, which deleted one of the two 72-bp repeats. Mutations in pLCATΔ72 were made in the TEF-1 binding sites, the TATA box, or both to generate TEFCAT1,2,3 (11), pLCAT3181, and TEFCAT3181, respectively (see Fig. 5 for sequences). Mutagenesis was performed by using a site-directed mutagenesis kit (Amersham) according to the manufacturer's instructions. The template was a single-stranded M13 derivative, including the origin of DNA replication and both the early and late promoters with only a single 72-bp repeat (SV40 nucleotides [nt] 4770 to 874, except for a deletion of nt 107 to 179). Subsequently, DNA fragments containing the mutations were used to replace the analogous wild-type sequences in pLCATΔ72. The parental (pLCATΔ72) DNA and mutant derivatives therefore contain SV40 sequences from nt 5171 to 107 and nt 179 to 333, with the late promoter driving expression of the bacterial chloramphenicol acetyltransferase (CAT) gene. A 6-bp deletion at the origin of replication prevents replication-induced amplification in the presence of wild-type T antigen or replication-competent mutant T antigens (19). The sequence of the DNA in the promoter regions was confirmed for each isolate by the dideoxynucleotide sequencing method (46) with the enzyme Sequenase (United States Biochemicals).

The 2400 and 2800 series of mutant T antigens (60, 61) and the set of point mutations in the DNA-binding domain (50–52) have been described previously. Mutant *dl*888 (49) and RSV t/cDNA (32) express only large T and small t antigens, respectively. All the expression plasmids for large T antigen utilize the SV40 early promoter to promote expression and contain a mutation at the origin of replication to prevent replication in the presence of wild-type T antigen.

Transfection mixtures included plasmids containing the human growth hormone gene under control of either the herpes simplex virus thymidine kinase promoter (pTKGH) or the mouse metallothionein I promoter (pXGH5) as general indicators of transfection efficiency (48). Growth hormone levels were determined by radioimmune assays with a kit from Nichols Institute Diagnostics. The data presented are not normalized by the levels of secreted growth hormone, however, because a correlation was found between the ability of a given T antigen to activate the SV40 late promoter and its ability to activate the promoters in pTKGH or pXGH5 (see also Discussion in reference 18). The pXGH5 and pTKGH promoters were activated from 1.5- to 2-fold and about 4-fold, respectively, by wild-type T antigen. Thus, correction for growth hormone levels would lower the apparent fold activation and minimize differences between the various mutants. We note that the lack of correction for transfection efficiency increases the ranges of the fold activation by wild-type or mutant T antigens when the data from multiple experiments are compared.

Cell culture and transfection assays. CV-1 cells were maintained in Dulbecco's modified Eagle medium (Hazelton) with 10% (vol/vol) newborn calf serum (Gibco), 50 U of penicillin G per ml, and 50 μg of streptomycin (Gibco) per ml. Transfections were performed by the calcium phosphate precipitation method (20), modified as described previously (11). Transfection mixtures generally included 2 μg of CAT reporter plasmid DNA; 0 or 2 μg of either p6-1, to express wild-type T antigen, or plasmids encoding mutant T antigens; 0.5 μg of pXGH5 or pTKGH; and pBluescript SK+ (Stratagene) to bring the total amount of DNA to 20 μg/10-cm-diameter plate. Cells were harvested between 40 and 45 h following the addition of the DNA. Assays for CAT activity were performed as previously described with 50 to 100 μg of extracted protein (11). The amount of protein per assay was constant for each experiment. The conversion of chloramphenicol to acetyl-chloramphenicol was quantitated with a phosphorimager (model 400E; Molecular Dynamics). The percent conversion ranged from 0.5 to 35%, which is within the linear range of this assay.

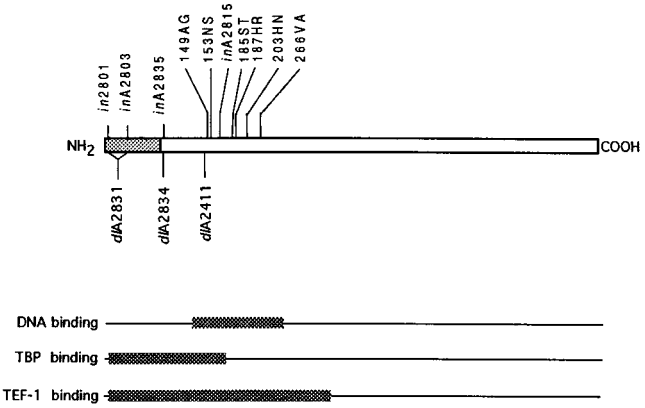

FIG. 1. Location of T-antigen mutations. A schematic diagram of SV40 T antigen and the location of specific mutations is shown. The lightly shaded region in the uppermost rectangle represents the 82-residue sequence shared between large T and small t antigens. The domains of T antigen required for its functions discussed in the text are indicated by shaded boxes below the uppermost rectangle. The minimal region able to bind TEF-1 extends from residues 5 to 383. The region extending from residues 5 to 172 contains at least two independent TBP binding segments: residues 5 to 82 and residues 82 (22) or 100 (25) to 172. The minimal region able to specifically bind DNA extends from residues 131 to 259. Mutants indicated below the uppermost rectangle represent in-frame deletion mutations, and mutants indicated above the rectangle represent in-frame insertion and substitution mutations. Four additional mutants are mentioned in the text, but no data for these are shown. Of these, mutants 204RK, 205VL, and 207RK contain the indicated amino acid substitutions (e.g., K for R) at residues 204, 205, and 207, respectively. Mutant *in*A2817 contains a 4-amino-acid insertion at residue 219.

Western immunoblot analysis. Nuclear extracts from transfected cells were prepared for analysis of the levels of wild-type and mutant T antigens in transfected cells. Transfected cells were washed twice with phosphate-buffered saline and then allowed to swell on ice for 10 min in 2 ml of hypotonic buffer (10 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid], pH 7.9–1.5 mM $MgCl_2$–10 mM KCl–0.5 mM phenylmethanesulfonyl fluoride). The cells were harvested by scraping, transferred to a Dounce homogenizer, and lysed by six strokes with the B pestle. The nuclei were immediately pelleted for 20 s at 5,000 × *g* and resuspended in 50 μl of 20 mM HEPES–1.5 mM $MgCl_2$–0.5 mM dithiothreitol–0.2 mM EDTA–420 mM NaCl–0.5 mM phenylmethanesulfonyl fluoride–25% glycerol. The nuclei were extracted for 20 min on ice and then centrifuged for 5 min at 16,000 × *g*. The supernatant was used for immunoblot analysis. A 10-μg amount of nuclear extract was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the separated proteins were electrophoretically transferred to Immobilon-P filters (Millipore) in 25 mM Tris-HCl, pH 8.0–192 mM glycine–20% (vol/vol) methanol. The filters were blocked for 30 min with 3% bovine serum albumin in TBS (10 mM Tris-HCl, pH 8.0–150 mM NaCl) and then incubated for 5 h with a 1:1,000 dilution of pAb419 or pAb423 (obtained from M. Bradley and J. DeCaprio) (23) in TBS containing 0.05% Tween 20. T antigen was detected with alkaline phosphatase-conjugated goat anti-mouse antisera (Bio-Rad) according to the manufacturer's instructions.

RESULTS

We tested the ability of T antigens carrying mutations in the DNA-binding domain or in the N-terminal 85 residues to activate the SV40 late promoter. Plasmids encoding either wild-type SV40 large T antigen (p6-1) (61) or mutant T antigens were cotransfected into CV-1 cells with CAT reporter plasmids carrying the SV40 late promoter lacking one 72-bp repeat (pLCATΔ72) or the same promoter in which all three binding sites for the cellular transcription factor TEF-1 were mutated (TEFCAT1,2,3) (11). The locations of the mutations in T antigen are diagrammed in Fig. 1. Because wild-type SV40 T

antigen and some of the mutant T antigens will promote replication of DNA containing SV40 origin sequences, the SV40 origin of replication in all the reporter plasmids was mutated (19). The amount of T antigen produced in transfected cells was quantitated by Western blot analysis of nuclear extracts with either antibody pAb419 or pAb423, which bind epitopes near the amino-terminal and carboxyl-terminal regions of T antigen, respectively (23).

DNA-binding activity is not necessary for activation of the SV40 late promoter. T antigen possesses both nonspecific (50, 52) and sequence-specific (42) DNA-binding activities, and the SV40 origin region contains specific binding sites (site I and site II) for T antigen (24, 42, 55). The region including the origin binding sites has been shown to play a role in T-antigen activation of the late promoter provided that sequences within the SV40 enhancer are also present (7, 27). Although T-antigen binding site II at the origin of replication was mutated in the reporter plasmids used in this study, T antigen might still interact either specifically through T-antigen binding site I or nonspecifically. Thus, three types of T-antigen mutants were tested in transfection assays: those defective in sequence-specific binding but which retain nonspecific DNA-binding activity (*in*A2815, *dl*A2411 [60, 61], 153NS, and 204RK [52]), those defective in both specific and nonspecific binding activities (187HR, 149AG, 185ST, 203HN, and 226VA [51, 52]), and several competent for both specific and nonspecific DNA binding (205VL, 207AG [51, 52], and *in*A2817 [60, 61]). We determined the effects of these mutants both on the parental SV40 late promoter (pLCATΔ72) and on this promoter with mutated TEF-1 sites (TEFCAT1,2,3). Initially, we measured the response of the parental late promoter to increasing levels (0.5 to 4.0 μg) of plasmid DNAs encoding either wild-type T antigen or mutant 153NS, which lacks sequence-specific DNA-binding activity but retains the ability to interact nonspecifically with DNA (10). The results we obtained indicated that the levels of DNA transfected were within the linear range of response. A similar titration of plasmid DNA (0.66 to 6.0 μg) encoding the mutant T antigen 149AG, which does not interact specifically or nonspecifically with DNA, indicated that transfected amounts of this DNA were also below saturating levels for the assay (10).

The results of multiple transfection experiments using a fixed amount (2 μg) of DNA encoding several different mutants, in conjunction with either the parental or the mutated SV40 late promoter, are shown in Fig. 2. Levels of CAT activity expressed from the promoters in the presence of each mutant T antigen are normalized to the levels of expression in the presence of wild-type T antigen. Wild-type T antigen (p6-1) induced pLCATΔ72 ninefold in these experiments (Fig. 2A, compare each p6-1 with each −T/t) and did not induce TEFCAT1,2,3 (Fig. 2B), confirming that the induction is dependent on TEF-1 binding sites (11, 18, 28). Mutant 153NS activated the late promoter to roughly the same extent as the wild-type T antigen did, demonstrating that sequence-specific DNA-binding activity is not necessary for efficient transcriptional induction. A similar result was obtained with mutant 204RK (data not shown), whose T antigen lacks specific DNA-binding activity but retains the ability to bind DNA nonspecifically (52). The other two mutants lacking specific DNA-binding activity, *in*A2815 and *dl*A2411, also activated pLCATΔ72, albeit at substantially reduced levels. These last-mentioned data differ from those of Zhu et al. (60), who found that both *dl*A2411 and *in*A2815 failed to induce the late promoter. One possible explanation for this discrepancy is that different mechanisms of transactivation of the SV40 late promoter are utilized in different CV-1 cell subclones (see Discussion).

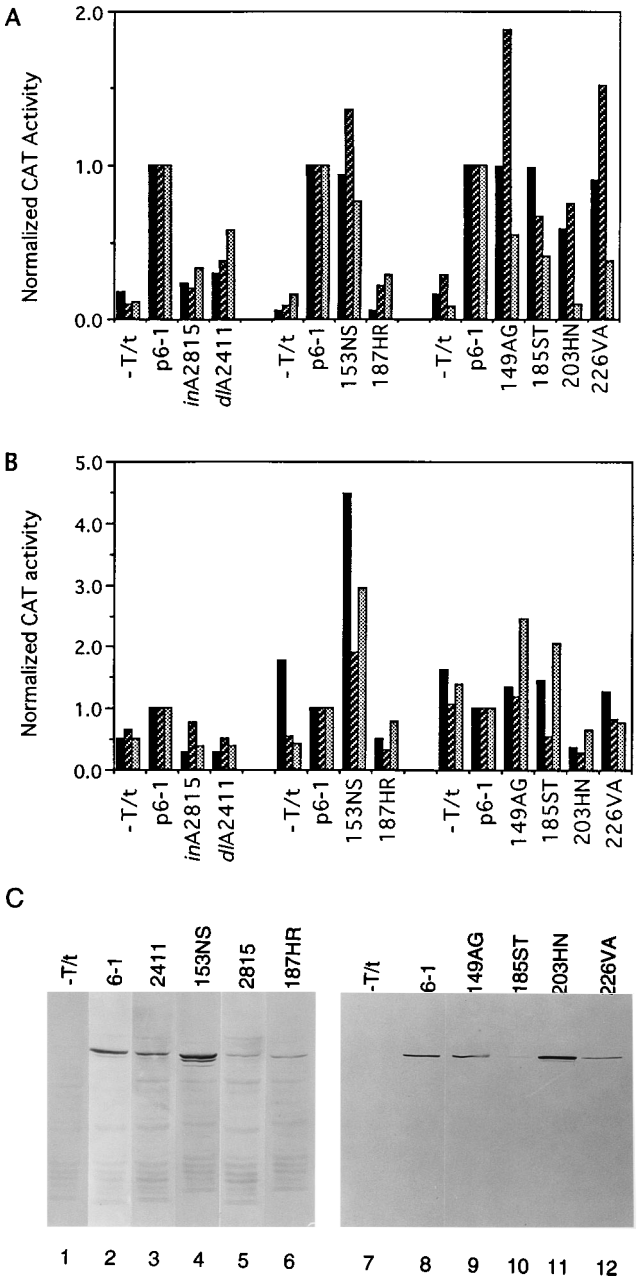

FIG. 2. T-antigen DNA-binding activity is not necessary for activation of the SV40 late promoter. The bars represent CAT activity directed by pLCATΔ72 (A) and TEFCAT1,2,3 (B) reporter plasmids in cells cotransfected with plasmids expressing either wild-type T antigen (p6-1) or mutants lacking DNA-binding activity (see text). For a given mutant, each bar represents the results of a single experiment. Identically shaded bars indicate results from transfections that were performed at the same time. The data have been normalized to the level of CAT activity in extracts from cells transfected with wild-type large T antigen. In these experiments, the average level of activation of pLCATΔ72 by wild-type T antigen (A) was 9.0-fold. The relative consistency of this degree of activation can be discerned by the levels of basal transcription (−T/t) that are indicated. The average basal level (−T/t) of TEFCAT1,2,3 activity was 80% of the average basal level of pLCATΔ72 activity. (C) Levels of wild-type and mutant T antigens expressed in transfected cells. T antigen was detected by Western immunoblot analysis with the monoclonal antibody pAb419. The prominent band in lanes 2 and 8 (p6-1) has an apparent molecular mass of 89 kDa.

Surprisingly, mutant 153NS also induced the mutated promoter TEFCAT1,2,3 by three- to sevenfold in these experiments (Fig. 2B, middle group, compare 153NS with −T/t), an activity not observed with wild-type T antigen. Although the 153NS mutant T antigen is overexpressed relative to wild-type T antigen (Fig. 2C, lane 4), it is unlikely that this accounts for the qualitative change in activity reflected by induction of expression from TEFCAT1,2,3. Mutants *in*A2815 and *dl*A2411 did not induce expression of TEFCAT1,2,3.

Among mutants defective in both nonspecific and specific binding to DNA, 149AG and 226VA effectively induced the parental SV40 late promoter (from six- to sevenfold and five- to sixfold, respectively), showing clearly that nonspecific DNA-binding activity is not necessary for activation (Fig. 2A). Cotransfection with TEFCAT1,2,3 confirmed that this activation required TEF-1 binding sites (Fig. 2B). These data demonstrate conclusively that neither specific nor nonspecific DNA-binding activities of T antigen are required for activation of the late promoter. As anticipated, the T antigens of mutants that fully retain DNA-binding activity activated transcription as well as wild-type T antigen did (205VL, 207AG, and *in*A2817; data not shown).

The mutants *in*A2815 and 187HR activated the wild-type late promoter particularly poorly. To determine whether the mutated proteins were stable, immunoblot analysis was performed with nuclear extracts prepared from transfected cells (Fig. 2C). This analysis showed that the levels of *in*A2815 and 187HR (Fig. 2C, lanes 5 and 6) were reduced compared with levels of wild-type T antigen (p6-1, lane 2). It should be noted, however, that 185ST, which retained the ability to transactivate, was also synthesized at low levels relative to those of wild-type T antigen (Fig. 2C, lane 10).

Novel phenotype of mutants with alterations in the N-terminal region of T antigen. Mutations in the N-terminal 85 residues of T antigen that reduce the ability of T antigen to activate transcription from the RSV LTR and the SV40 late promoter have been identified (60). This region also contains an activity involved in the immortalization and transformation of some types of cells (39, 41, 59). Additional evidence suggests that the transformation function may also regulate transcription (see Discussion). We determined the effect of these mutations on TEF-1-mediated activation of the SV40 late promoter in our cell line. Plasmids encoding insertion mutations at T-antigen residues 5 (*in*2801), 35 (*in*A2803), and 85 (*in*A2835); a deletion of residue 85 (*dl*A2834); or a replacement of residues 5 to 35 with four new residues (*dl*A2831) were cotransfected with either pLCATΔ72 or TEFCAT1,2,3 into CV-1 cells. The levels of expression from these SV40 late promoters are summarized in Fig. 3A and B, respectively. Two different phenotypes were observed. Mutants *in*2801, *dl*A2834, and *in*A2835 induced CAT expression from the parental late promoter (Fig. 3A) but were unable to induce the TEFCAT1,2,3 promoter (Fig. 3B). Thus, these mutants have essentially the phenotype of wild-type T antigen. Surprisingly, mutant *dl*A2831 induced CAT expression not only from the parental late promoter but also from the promoter lacking TEF-1 binding sites (Fig. 3B). Mutant *in*A2803 also displayed this phenotype, although in a less pronounced manner. These effects are not due to overexpression of the mutant proteins, because Western blot analysis indicated that both mutants *dl*A2831 and *in*A2803 are present at levels lower than those of wild-type T antigen (Fig. 3C, lanes 2, 4, and 5). Although the fold activation of TEFCAT1,2,3 with *dl*A2831 was lower than the corresponding activation of pLCATΔ72 (Fig. 3A and B, compare *dl*A2831 data with those for −T/t), these results indicate that a TEF-1-independent

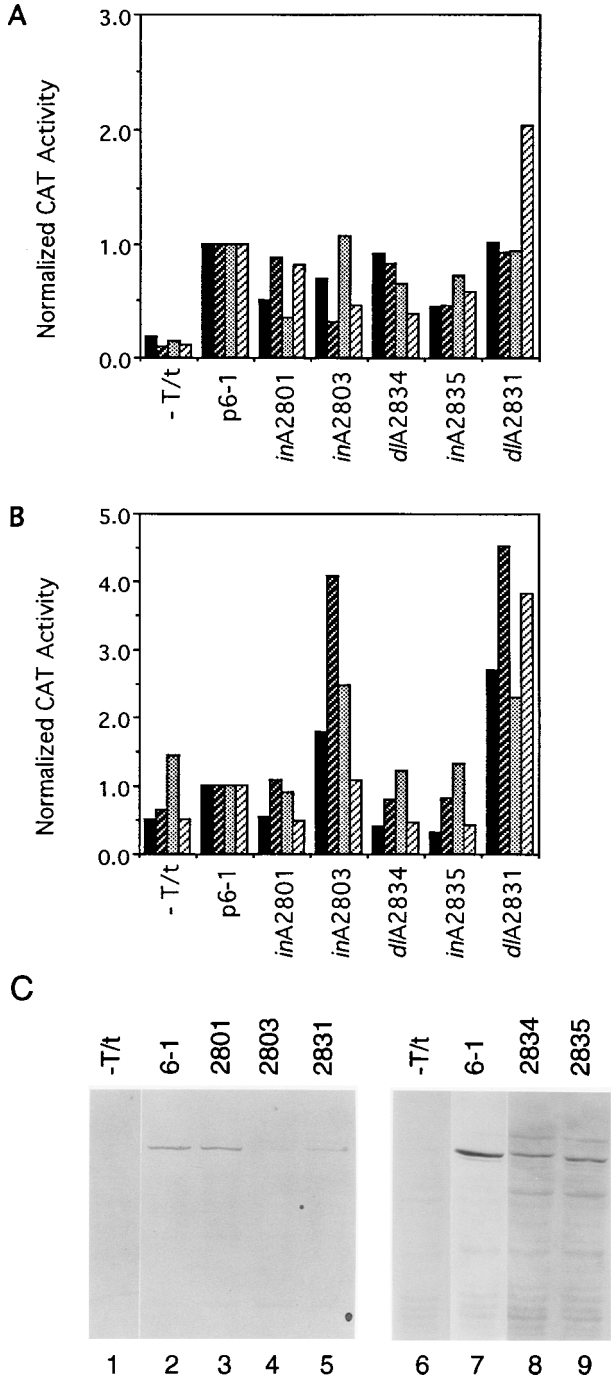

FIG. 3. Activation of the SV40 late promoter via a TEF-1-independent pathway. The bars represent CAT activity directed by pLCATΔ72 (A) and TEFCAT1,2,3 (B) reporter plasmids upon cotransfection with plasmids expressing either wild-type T antigen or T antigen with mutations within the N-terminal region (see text). For a given mutant, each bar represents the results of a single experiment. Identically shaded bars indicate results from transfections that were performed at the same time. Data have been normalized as described in the legend to Fig. 2. Average activation of pLCATΔ72 by wild-type T antigen in these experiments was 7.8-fold. Basal expression from TEFCAT1,2,3 was 90% of the basal expression from pLCATΔ72. (C) Western immunoblot analysis was performed as described in the legend to Fig. 2, except that mutant T antigens encoded by *in*2801, *in*A2803, and *dl*A2831 were detected with the monoclonal antibody pAb423 instead of pAb419.

mechanism exists for activation of the SV40 late promoter by this T antigen.

The mutations in *in*A2803 and *dl*A2831 lie within sequences shared by both large T and small t antigens. Given that t antigen can positively (5, 32) and negatively (57) regulate RNA polymerase II transcription, we sought to determine whether the coincidental mutation of small t antigen could be responsible for the unexpected phenotype of *dl*A2831. Specifically, if small t antigen were a repressor of the late promoter, the mutation in *dl*A2831 might inactivate the repression activity, allowing large T antigen to induce transcription through additional pathways not dependent on TEF-1 binding sites. This model predicts that small t antigen alone would repress both the parental and mutant late promoters and that large T antigen alone would activate transcription even in the absence of TEF-1 binding sites. We therefore measured the individual effects of large T antigen (*dl*888) (49) and small t antigen (pRSV-t/cDNA) (32) on expression from parental and TEF-1-site-mutated late promoters. Large T antigen alone, but not small t antigen alone, induced CAT expression from pLCATΔ72 (Fig. 4A). In agreement with published data (5), we found that small t antigen (expressed from pRSV-t/cDNA) enhanced the activation by large T antigen (expressed from *dl*888) of pLCATΔ72 by two- to fourfold (data not shown). Therefore, wild-type small t antigen is not an inhibitor of the parental late promoter, contradicting the first prediction of the model.

A second prediction of the model is that large T antigen would activate the late promoter independently of TEF-1 sites in the absence of small t antigen. We found that transfection with amounts of *dl*888 DNA sufficient to maximally activate the parental promoter had little effect on the mutant promoter (Fig. 4B). Small t antigen alone caused reduction in TEFCAT1,2,3 activity. This suggests that small t antigen may inhibit T antigen from activating TEFCAT1,2,3 when both large and small T antigens are present. It is likely, however, that mutation of large T antigen is required to obtain the mutant phenotype of *in*A2803 and *dl*A2831, since wild-type T antigen alone did not activate TEFCAT1,2,3.

T antigen can interact directly with TEF-1 and with TBP, the DNA-binding subunit of the general transcription factor TFIID (22, 35). TEF-1 also interacts directly with TBP (22). These observations suggested that T antigen may activate the late promoter by stabilizing or enhancing the interaction between TEF-1 and TBP. To determine whether TEF-1-independent activation by *dl*A2831 could be explained by a similar model, we compared how different TATA elements in the context of the SV40 late promoter would affect activation by *dl*A2831 and wild-type T antigens. The TATA element of the wild-type SV40 late promoter has a low affinity for TBP (58). A TATA element with higher affinity for TBP (up mutant), representing an optimal TBP binding site, was created in both the parental (pLCATΔ72) and mutated TEF-1 binding-site (TEFCAT1,2,3) backgrounds by site-directed mutagenesis. The TATA up mutant used here, 3181 (see Fig. 5C), increases the rate of initiation at the SV40 late promoter in vitro by 20-fold (8, 54). We tested how these mutated promoters responded in vivo to both wild-type T antigen and *dl*A2831 (Fig. 5A and B). In the pLCATΔ72 background, the consensus TATA sequence stimulated basal expression to a degree less than what occurred in vitro, with pLCAT3181 expressing levels only fourfold higher than those expressed by pLCATΔ72 (Fig. 5A, compare −T/t bars). These results suggest, however, that a TATA up mutant does enhance the activity of TFIID at the late promoter, presumably by maintaining a more stable complex for interaction with other transcription factors. Wild-type

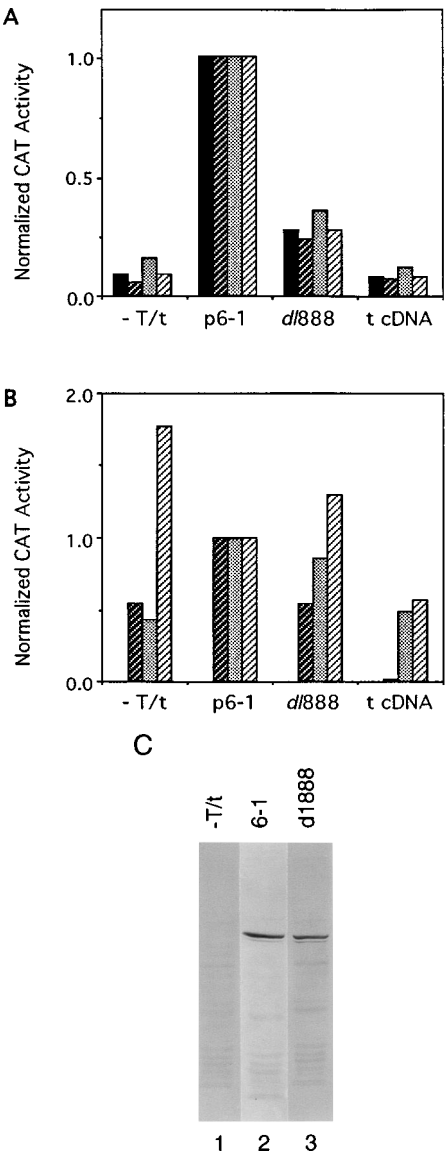

FIG. 4. Large T antigen, but not small t antigen, activates the SV40 late promoter. The bars represent CAT activity directed by pLCATΔ72 (A) and TEFCAT1,2,3 (B) reporter plasmids upon cotransfection with plasmids expressing large T antigen alone (*dl*888), small t antigen alone (t cDNA), or both together (p6-1). For a given mutant, each bar represents the results of a single experiment. Identically shaded bars indicate results from transfections that were performed at the same time. The average activation of pLCATΔ72 by p6-1 in these experiments was 10-fold. Basal expression from TEFCAT1,2,3 (−T/t) was 80% of the basal expression from pLCATΔ72. (C) Western immunoblot analysis of T antigen from cells transfected with *dl*888 was performed as described in the legend to Fig. 2.

T antigen activated pLCATΔ72 an average of sevenfold; this was reduced to fivefold with pLCAT3181 (Fig. 5A and C). Induction by *dl*A2831 was similarly reduced by the TATA up mutation (Fig. 5A and C).

In a TEFCAT1,2,3 background, the 3181 mutation did not affect the basal level of expression (Fig. 5B, compare −T/t bars), indicating that an altered TATA sequence alone is not sufficient to increase expression from this promoter. Wild-type T antigen minimally induced both TEFCAT1,2,3 and TEFCAT3181 in these experiments (Fig. 5B and C). In contrast, activation by *dl*A2831 is observed on both promoters, with a

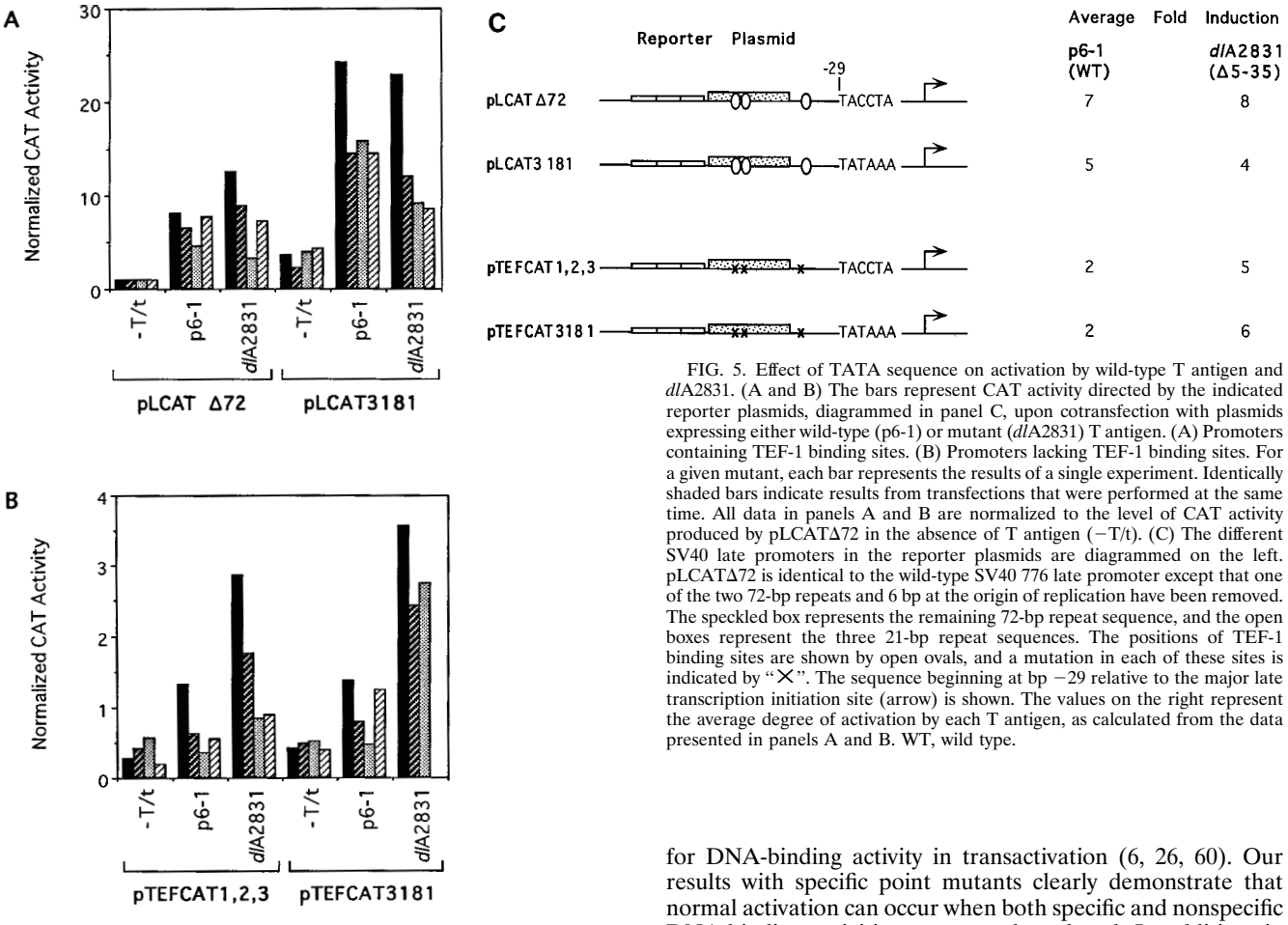

FIG. 5. Effect of TATA sequence on activation by wild-type T antigen and *dl*A2831. (A and B) The bars represent CAT activity directed by the indicated reporter plasmids, diagrammed in panel C, upon cotransfection with plasmids expressing either wild-type (p6-1) or mutant (*dl*A2831) T antigen. (A) Promoters containing TEF-1 binding sites. (B) Promoters lacking TEF-1 binding sites. For a given mutant, each bar represents the results of a single experiment. Identically shaded bars indicate results from transfections that were performed at the same time. All data in panels A and B are normalized to the level of CAT activity produced by pLCATΔ72 in the absence of T antigen (−T/t). (C) The different SV40 late promoters in the reporter plasmids are diagrammed on the left. pLCATΔ72 is identical to the wild-type SV40 776 late promoter except that one of the two 72-bp repeats and 6 bp at the origin of replication have been removed. The speckled box represents the remaining 72-bp repeat sequence, and the open boxes represent the three 21-bp repeat sequences. The positions of TEF-1 binding sites are shown by open ovals, and a mutation in each of these sites is indicated by "×". The sequence beginning at bp −29 relative to the major late transcription initiation site (arrow) is shown. The values on the right represent the average degree of activation by each T antigen, as calculated from the data presented in panels A and B. WT, wild type.

slight increase from fivefold with TEFCAT1,2,3 to sixfold with TEFCAT3181 (Fig. 5B and C). These results suggest that in the absence of TEF-1 sites, the mutant T antigen *dl*A2831 provides an activity that stabilizes the binding of TFIID to the promoter or allows TFIID bound at the promoter to function more efficiently. An interpretation consistent with these data is that *dl*A2831 stabilizes an interaction between TBP and an upstream factor bound to the mutated late promoter.

DISCUSSION

We have shown that induction of the SV40 late promoter by wild-type T antigen does not require the specific or nonspecific DNA-binding activities of T antigen. We have also shown that some T-antigen mutants can induce transcription from a late promoter lacking TEF-1 binding sites, an activity not seen with wild-type T antigen.

Previous studies have shown that activation of the SV40 late promoter does not absolutely require T-antigen DNA-binding activity (22, 60). Similarly, transcriptional activation by T antigen of the SV40 early and late promoters in vitro occurs efficiently with preparations of T antigen unable to specifically bind DNA (14). However, analysis of the late promoter has indicated that the T-antigen binding sites at the origin of replication contribute to activation (7, 27, 36). In addition, several mutations within T antigen that inhibit its DNA-binding activity resulted in lower levels of activation, also suggesting a role for DNA-binding activity in transactivation (6, 26, 60). Our results with specific point mutants clearly demonstrate that normal activation can occur when both specific and nonspecific DNA-binding activities are severely reduced. In addition, in some cases where the level of activation observed with DNA-binding mutants was low (*in*A2815 and 187HR), the level of activation correlated with reduced levels of the proteins in transfected cells. It should be noted, however, that other mutant T antigens expressed at low levels retained transcriptional activation activity. Two simple explanations for the effects of some DNA-binding domain mutations on transactivation could be that sequences in the DNA-binding domain, but not DNA-binding activity itself, are involved in activation and that the DNA-binding domain mutations cause aberrant protein folding. The lack of dependence on the ability of T antigen to bind DNA does not imply that activation occurs without localization of T antigen to the promoter. In this regard, Gruda et al. have demonstrated that T antigen will bind directly to in vitro-translated TBP and TEF-1 and that the regions of T antigen required for these interactions are also necessary for activation of the late promoter in vivo (22). Thus, T antigen may be bound at the promoter indirectly, through protein-protein interactions, which may be necessary for transactivation of the SV40 late promoter.

An unexpected phenotype was observed upon examination of mutants altered in the N-terminal 85 amino acids of T antigen. Previous studies had indicated that these mutants induced the RSV LTR to 40 to 50% of wild-type levels and the SV40 late promoter to 40 to 100% of wild-type levels, therefore showing slight defects in transcriptional activation (60). A comparison between adenovirus E1A and SV40 T antigen provides further support for a role of this region in regulation of transcription. Sequences at the extreme N terminus of E1A are

necessary for transformation of BRK cells in conjunction with an activated *ras* gene and are also required for binding the E1A-associated p300 protein (53, 59). Furthermore, the ability to bind p300 correlates with E1A-mediated inhibition of enhancer activity (53), as well as activation of particular promoters (29). T antigen possesses an activity that complements the transformation defect conferred by a mutation in the p300 binding region of E1A. Complementation was prevented by deletion of T-antigen residues 17 to 27 (59). Thus, by analogy with E1A, the N-terminal region of T antigen may also regulate transcription. Recently, p300 has been shown to be highly homologous to other transcriptional coactivators (3), strengthening the potential importance of the T-antigen–E1A analogy for T-antigen transactivation.

In our experiments, mutant T antigens with insertions of 4 amino acids at residue 5 or 85 or a deletion of residue 85 were able to activate the SV40 late promoter, with activation depending on intact TEF-1 binding sites. However, an insertion at residue 35 or replacement of residues 5 to 35 with four different amino acids resulted in activation of transcription from a promoter lacking TEF-1 binding sites, an activity not observed with wild-type T antigen. A similar activity was observed with the specific DNA-binding mutant 153NS. The difference between the level of induction by these mutant T antigens of a wild-type late promoter (5- to 12-fold) versus one lacking TEF-1 binding sites (3- to 5-fold) suggests that the TEF-1-dependent mechanism is at least partially intact in these mutants. In addition, these mutants can activate the late promoter through a pathway not normally used by wild-type T antigen.

Activation of the late promoter by wild-type T antigen has been proposed to result from the direct interactions between T antigen, TEF-1, and TBP (22). We expect that the mutant T antigens exhibiting a TEF-1-independent phenotype will still bind to TBP. There are two regions of T antigen that are sufficient to bind TBP: one localized to the N-terminal 88 residues (22) and a second that is functional even upon deletion of the first 100 residues of T antigen (22, 25). The role of TBP in TEF-1-independent activation of the late promoter by *dl*A2831 was investigated by creating TBP binding sites of increasing affinities in the context of the SV40 late promoter. We found that a consensus TBP binding site in the context of the parental late promoter increased the basal promoter activity, but when the promoter lacked TEF-1 binding sites, the increased affinity for TBP was not sufficient to increase basal promoter activity. The apparent requirement for TEF-1 binding sites to obtain a response on basal levels of transcription to a strong TBP binding site is consistent with a model in which TEF-1 stabilizes the formation of the preinitiation complex, presumably through direct interaction with TBP. These data also suggest that other factors bound to the late promoter do not stabilize the preinitiation complex in the same manner as TEF-1 does.

With a strong TBP binding site in pLCATΔ72, the absolute levels of promoter activity in the presence of *dl*A2831 and wild-type T antigens were increased but the fold activation was reduced in each case. It is unlikely that the reduced degree of activation, at least for *dl*A2831, is due to the late promoter reaching its maximal activity, because higher CAT expression levels were obtainable with wild-type T antigen. Alternatively, the increased affinity for TBP may partially substitute for a function of T antigen. For example, if the binding of TBP to the weak SV40 late promoter TATA element is stabilized by T antigen in conjunction with TEF-1, a higher-affinity binding site for TBP, in the presence of TEF-1, might suffice to stabilize binding. This would increase basal transcription and thus lower the fold activation by T antigen.

However, increasing the affinity of the TBP binding site in the context of a late promoter lacking TEF-1 binding sites left unchanged or slightly increased the fold activation by *dl*A2831. This is consistent with a model in which *dl*A2831 facilitates the interaction of TBP with another factor bound to the late promoter. The model would necessitate that such a factor would not interact efficiently with TBP in the absence of T antigen, since basal activity of the promoter lacking TEF-1 sites was not increased by a strong TBP binding site. In support of this model, natural promoters lacking TEF-1 binding sites are activated by T antigen through other promoter elements (15, 33). In addition, a series of synthetic promoters containing binding sites for several transcription factors combined with different TATA elements are effectively activated by T antigen (18, 44). Of note, the degree of activation varies considerably with different TATA boxes.

The apparent absence of the TEF-1-independent activity in wild-type T antigen in our experiments could be explained in several ways. First, *dl*A2831 and *in*A2803 could have gained a new function as a result of the insertion of amino acids at residue 35. We find this possibility unlikely since different mutants (*dl*A2831, *in*A2803, and 153NS) demonstrate a similar phenotype. Second, sequences around residue 35 may constitute a regulatory domain which normally masks the TEF-1-independent mechanism. In this scenario, the mutations in *dl*A2831 and *in*A2803 would relieve the inhibition. If this were the case, other transcription regulatory functions of T antigen necessary for TEF-1-independent activation could be mapped by using these mutants. In this regard, Gruda et al. (22) have shown that a C-terminal portion of T antigen from residue 379 through residue 707, fused to a portion of GAL4, could activate transcription from the SV40 late promoter. As a third possibility, these mutations might enhance the normal function of the N-terminal region. For instance, mutations may strengthen the interaction with a cellular protein that normally binds to this region, such as p300.

The TEF-1-independent mechanism may be more consequential in other cell lines. We have found that in another subclone of CV-1 cells (see reference 60), activation of the late promoter by wild-type T antigen was much less affected by mutation of the TEF-1 binding sites. *dl*A2831 also activates both promoters in these cells (10). Thus, wild-type T antigen may in fact possess TEF-1-independent activity, which may be masked in some cell lines.

These results are in accord with the model that several functions of T antigen can lead to activation of transcription. We have found that T antigen binding to DNA is not necessary for activation of the SV40 late promoter in cells where activation is dependent on TEF-1 binding sites. In addition, three mutant T antigens, two mutated near the amino-terminal region and one mutated in the DNA-binding domain, are able to activate the late promoter independently of TEF-1 binding sites. We suggest that these mutants function by promoting the interaction between TBP and a second factor bound to the late promoter. This alternate pathway may be important for regulation of promoters that lack TEF-1 binding sites and are dependent on other promoter elements for induction.

ACKNOWLEDGMENTS

We thank Dan Simmons for supplying mutant T-antigen expression vectors, Peggy Bradley and Jim DeCaprio for supplying anti-T-antigen antibodies, and Janet Mertz for discussions and critical comments on the manuscript. We also thank the members of our laboratory for their support with and advice on this work.

This work was supported by grants from the Council for Tobacco Research (to U.H.) and the National Institutes of Health (to C.N.C.). P.C. was supported in part by a fellowship from Harvard Graduate School of Arts and Sciences. P.W.R. was supported by an NRSA fellowship from the National Cancer Institute.

REFERENCES

1. **Alwine, J. C.** 1985. Transient gene expression control: effects of transfected DNA stability and *trans*-activation by viral early proteins. Mol. Cell. Biol. **5:**1034–1042.
2. **Alwine, J. C., S. I. Reed, and G. R. Stark.** 1977. Characterization of the autoregulation of simian virus 40 gene *A*. J. Virol. **24:**22–27.
3. **Arany, Z., W. R. Sellers, D. M. Livingston, and R. Eckner.** 1994. E1A-associated p300 and CREB-associated CBP belong to a conserved family of coactivators. Cell **77:**799–800.
4. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1989. Current protocols in molecular biology. John Wiley and Sons, New York.
5. **Bikel, I., and M. R. Loeken.** 1992. Involvement of simian virus 40 (SV40) small t antigen in *trans* activation of SV40 early and late promoters. J. Virol. **66:**1489–1494.
6. **Brady, J., J. B. Bolen, M. Radonovich, N. Salzman, and G. Khoury.** 1984. Stimulation of simian virus 40 late gene expression by simian virus 40 tumor antigen. Proc. Natl. Acad. Sci. USA **81:**2040–2044.
7. **Brady, J., M. R. Loeken, and G. Khoury.** 1985. Interaction between two transcriptional control sequences required for tumor-antigen-mediated simian virus 40 late gene expression. Proc. Natl. Acad. Sci. USA **82:**7299–7303.
8. **Brady, J., M. Radonovich, M. Vodkin, V. Natarajan, M. Thoren, G. Das, J. Janik, and N. P. Salzman.** 1982. Site-specific base substitution and deletion mutations that enhance or suppress transcription of the SV40 major late RNA. Cell **31:**625–633.
9. **Cao, S. X., H. Mishoe, J. Elion, P. E. Berg, and A. N. Schechter.** 1989. Activation of the human epsilon- and beta-globin promoters by SV40 T antigen. Biochem. J. **258:**769–776.
10. **Casaz, P., and U. Hansen.** Unpublished data.
11. **Casaz, P., R. Sundseth, and U. Hansen.** 1991. *trans* activation of the simian virus 40 late promoter by large T antigen requires binding sites for the cellular transcription factor TEF-1. J. Virol. **65:**6535–6543.
12. **Chellappan, S., V. B. Kraus, B. Kroger, K. Munger, P. M. Howley, W. C. Phelps, and J. R. Nevins.** 1992. Adenovirus E1A, simian virus 40 tumor antigen, and human papillomavirus E7 protein share the capacity to disrupt the interaction between transcription factor E2F and the retinoblastoma gene product. Proc. Natl. Acad. Sci. USA **89:**4549–4553.
13. **Chittenden, T., D. M. Livingston, and W. G. Kaelin, Jr.** 1991. The T/E1A-binding domain of the retinoblastoma product can interact selectively with a sequence-specific DNA-binding protein. Cell **65:**1073–1082.
14. **Coulombe, J., L. Berger, D. B. Smith, R. K. Hehl, and A. G. Wildeman.** 1992. Activation of simian virus 40 transcription in vitro by T antigen. J. Virol. **66:**4591–4596.
15. **Cox, P. M., and C. R. Goding.** 1992. An ATF/CREB binding motif is required for aberrant constitutive expression of the MHC class II DR alpha promoter and activation by SV40 T-antigen. Nucleic Acids Res. **20:**4881–4887.
16. **DeCaprio, J. A., J. W. Ludlow, J. Figge, J.-Y. Shew, C.-M. Huang, W.-H. Lee, E. Marsilio, E. Paucha, and D. M. Livingston.** 1988. SV40 large tumor antigen forms a specific complex with the product of the retinoblastoma susceptibility gene. Cell **54:**275–283.
17. **Ernoult-Lange, M., F. Omilli, D. R. O'Reilly, and E. May.** 1987. Characterization of the simian virus 40 late promoter: relative importance of sequences within the 72-base-pair repeats differs before and after viral DNA replication. J. Virol. **61:**167–176.
18. **Gilinger, G., and J. C. Alwine.** 1993. Transcriptional activation by simian virus 40 large T antigen: requirements for simple promoter structures containing either TATA or initiator elements with variable upstream factor binding sites. J. Virol. **67:**6682–6688.
19. **Gluzman, Y., R. J. Frisque, and J. Sambrook.** 1980. Origin defective mutants of SV40. Cold Spring Harbor Symp. Quant. Biol. **44:**293–300.
20. **Graham, F. L., and A. J. van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52:**456–467.
21. **Gruda, M. C., and J. C. Alwine.** 1991. Simian virus 40 (SV40) T-antigen transcriptional activation mediated through the Oct/SPH region of the SV40 late promoter. J. Virol. **65:**3553–3558.
22. **Gruda, M. C., J. M. Zabolotny, J. H. Xiao, I. Davidson, and J. C. Alwine.** 1993. Transcriptional activation by simian virus 40 large T antigen: interactions with multiple components of the transcription complex. Mol. Cell. Biol. **13:**961–969.
23. **Harlow, E., L. V. Crawford, D. C. Pim, and N. M. Williamson.** 1981. Monoclonal antibodies specific for simian virus 40 tumor antigens. J. Virol. **39:** 861–869.
24. **Jessel, D., T. Landau, J. Hudson, T. Lalor, D. Tenen, and D. M. Livingston.** 1976. Identification of regions of the SV40 genome which contain preferred SV40 T antigen-binding sites. Cell **8:**535–545.
25. **Johnston, S. D., X.-M. Yu, and J. E. Mertz.** Submitted for publication.
26. **Keller, J. M., and J. C. Alwine.** 1984. Activation of the SV40 late promoter: direct effects of T antigen in the absence of viral DNA replication. Cell **36:**381–389.
27. **Keller, J. M., and J. C. Alwine.** 1985. Analysis of an activatable promoter: sequences in the simian virus 40 late promoter required for T-antigen-mediated *trans* activation. Mol. Cell. Biol. **5:**1859–1869.
28. **Kelly, J., and A. G. Wildeman.** 1991. Role of the SV40 enhancer in the early to late shift in viral transcription. Nucleic Acids Res. **19:**6799–6804.
29. **Kraus, V. B., E. Moran, and J. R. Nevins.** 1992. Promoter-specific *trans*-activation by the adenovirus E1A 12S product involves separate E1A domains. Mol. Cell. Biol. **12:**4391–4399.
30. **Lane, D. P., and L. V. Crawford.** 1979. T-antigen is bound to a host protein in SV40 transformed cells. Nature (London) **278:**261–263.
31. **Linzer, D. H. I., W. Maltzman, 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.
32. **Loeken, M., I. Bikel, D. M. Livingston, and J. Brady.** 1988. Trans-activation of RNA polymerase II and III promoters by SV40 small t antigen. Cell **55:**1171–1177.
33. **Loeken, M. R.** 1993. Multiple, distinct *trans*-activation functions are encoded by the simian virus 40 large T and small t antigens, only some of which require the 82-residue amino-terminal common domain. J. Virol. **67:**7684–7689.
34. **Loeken, M. R., G. Khoury, and J. Brady.** 1986. Stimulation of the adenovirus E2 promoter by simian virus 40 T antigen or E1A occurs by different mechanisms. Mol. Cell. Biol. **6:**2020–2026.
35. **Martin, D. W., M. A. Subler, R. M. Munoz, D. R. Brown, S. P. Deb, and S. Deb.** 1993. p53 and SV40 T-antigen bind to the same region overlapping the conserved domain of the TATA-binding protein. Biochem. Biophys. Res. Commun. **195:**428–434.
36. **May, E., F. Omilli, J. Borde, and P. Scieller.** 1992. Simian virus 40 T antigen activates the late promoter by modulating the activity of negative regulatory elements. J. Virol. **66:**3347–3354.
37. **Mietz, J. A., T. Unger, J. M. Huibregtse, and P. M. Howley.** 1992. The transcriptional transactivation function of wild-type p53 is inhibited by SV40 large T-antigen and by HPV-16 E6 oncoprotein. EMBO J. **11:**5013–5020.
38. **Mitchell, P. J., C. Wang, and R. Tjian.** 1987. Positive and negative regulation of transcription in vitro: enhancer-binding protein AP-2 is inhibited by SV40 T antigen. Cell **50:**847–861.
39. **Montano, X., R. Millikan, J. M. Milhaven, D. A. Newsome, J. W. Ludlow, A. K. Arthur, E. Fanning, I. Bikel, and D. M. Livingston.** 1990. Simian virus 40 small tumor antigen and an amino-terminal domain of large tumor antigen share a common transforming function. Proc. Natl. Acad. Sci. USA **87:**7448–7452.
40. **Pannuti, A., A. Pascucci, G. La Mantia, L. Fisher-Fantuzzi, C. Vesco, and L. Lania.** 1987. *trans*-activation of cellular and viral promoters by a transforming nonkaryophilic simian virus 40 large T antigen. J. Virol. **61:**1296–1299.
41. **Peden, K. W., S. L. Spence, L. C. Tack, C. A. Cartwright, A. Srinivasan, and J. M. Pipas.** 1990. A DNA replication-positive mutant of simian virus 40 that is defective for transformation and the production of infectious virus. J. Virol. **64:**2912–2921.
42. **Reed, S. I., J. Ferguson, R. W. Davis, and G. R. Stark.** 1975. T antigen binds to simian virus 40 DNA at the origin of replication. Proc. Natl. Acad. Sci. USA **72:**1605–1609.
43. **Reed, S. I., G. R. Stark, and J. C. Alwine.** 1976. Autoregulation of the simian virus 40 gene A by T antigen. Proc. Natl. Acad. Sci. USA **73:**3083–3087.
44. **Rice, P. W., and C. N. Cole.** 1993. Efficient transcriptional activation of many simple modular promoters by simian virus 40 large T antigen. J. Virol. **67:**6689–6697.
45. **Saffer, J. D., S. P. Jackson, and S. J. Thurston.** 1990. SV40 stimulates expression of the trans-acting factor Sp1 at the mRNA level. Genes Dev. **4:**659–666.
46. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
47. **Scieller, P., F. Omilli, J. Borde, and E. May.** 1991. Characterization of SV40 enhancer motifs involved in positive and negative regulation of the constitutive late promoter activity; effect of T-antigen. Virology **181:**783–786.
48. **Selden, R. F., K. Burke-Howie, M. E. Rowe, H. M. Goodman, and D. D. Moore.** 1986. Human growth hormone as a reporter gene in regulation studies employing transient gene expression. Mol. Cell. Biol. **6:**3173–3179.
49. **Shenk, T. E., J. Carbon, and P. Berg.** 1976. Construction and analysis of viable deletion mutants of simian virus 40. J. Virol. **18:**664–671.
50. **Simmons, D. T., G. Loeber, and P. Tegtmeyer.** 1990. Four major sequence elements of simian virus 40 large T antigen coordinate its specific and nonspecific DNA binding. J. Virol. **64:**1973–1983.
51. **Simmons, D. T., R. Upson, K. Wun-Kim, and W. Young.** 1993. Biochemical analysis of mutants with changes in the origin-binding domains of simian virus 40 tumor antigen. J. Virol. **67:**4227–4236.
52. **Simmons, D. T., K. Wun-Kim, and W. Young.** 1990. Identification of simian virus 40 T-antigen residues important for specific and nonspecific binding to DNA and for helicase activity. J. Virol. **64:**4858–4865.

53. **Stein, R. W., M. Corrigan, P. Yaciuk, J. Whelan, and E. Moran.** 1990. Analysis of E1A-mediated growth-regulating functions: binding of the 300-kilodalton cellular product correlates with E1A enhancer repression function and DNA synthesis-inducing activity. J. Virol. **64:**4421–4427.
54. **Sundseth, R., and U. Hansen.** 1992. Activation of RNA polymerase II transcription by the specific DNA-binding protein LSF: increased rate of binding of the basal promoter factor TFIIB. J. Biol. Chem. **267:**7845–7855.
55. **Tjian, R.** 1978. The binding site on SV40 DNA for a T antigen-related protein. Cell **13:**165–179.
56. **Wagner, S., and R. Knippers.** 1990. An SV40 large T antigen binding site in the cellular genome is part of a cis-acting transcriptional element. Oncogene **5:**353–359.
57. **Wang, W.-B., I. Bikel, E. Marsilio, D. Newsome, and D. M. Livingston.** 1994. Transrepression of RNA polymerase II promoters by the simian virus 40 small t antigen. J. Virol. **68:**6180–6187.
58. **Wiley, S. R., R. J. Kraus, and J. E. Mertz.** 1992. Functional binding of the "TATA" box binding component of transcription factor TFIID to the −30 region of TATA-less promoters. Proc. Natl. Acad. Sci. USA **89:**5814–5818.
59. **Yaciuk, P., M. C. Carter, J. M. Pipas, and E. Moran.** 1991. Simian virus 40 large-T antigen expresses a biological activity complementary to the p300-associated transforming function of the adenovirus E1A gene products. Mol. Cell. Biol. **11:**2116–2124.
60. **Zhu, J., P. W. Rice, M. Chamberlain, and C. N. Cole.** 1991. Mapping the transcriptional transactivation function of simian virus 40 large T antigen. J. Virol. **65:**2778–2790.
61. **Zhu, J. Y., and C. N. Cole.** 1989. Linker insertion mutants of simian virus 40 large T antigen that show *trans*-dominant interference with wild-type T antigen map to multiple sites within the T-antigen gene. J. Virol. **63:**4777–4786.