

Efficient Transcriptional Activation of Many Simple Modular Promoters by Simian Virus 40 Large T Antigen

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Simian virus 40 (SV40) large T antigen is a multifunctional protein which plays central roles during both lytic and transforming infections by SV40. It is a potent transcriptional activator and increases expression from the SV40 late promoter and from several cellular promoters. To understand better the transcriptional activation activity of large T antigen, we examined its ability to transactivate a set of simple modular promoters containing one of four upstream activation sequences coupled with one of three different TATA box sequences originally constructed and studied by Taylor and Kingston (Mol. Cell. Biol. 10:165–175, 1990). Large T antigen activated transcription from all of these simple promoters. The identity of the TATA box was a more important determinant of the final level of gene expression than was the identity of the upstream activating sequence element. We also determined the ability of a set of mutant SV40 large T antigens to activate a subset of these promoters. Several mutant SV40 large T antigens which had reduced ability to activate the complex SV40 late and Rous sarcoma virus long terminal repeat promoters showed reduced transcriptional activation activity on all of the modular promoters tested. We used a set of promoter derivatives of the human U6 small nuclear RNA promoter containing different TATA boxes and found that wild-type large T antigen could activate transcription from all of them, although to widely different levels of expression.

The simian virus 40 (SV40) large T antigen performs many essential functions during the life cycle of the virus (for a review, see reference 21). Biochemical studies have shown that T antigen binds specifically to sequences within the SV40 origin of DNA replication region and nonspecifically to other DNA molecules. T antigen also possesses ATP-binding and ATPase activities, and both are required for T antigen to function as a helicase. It is through these multiple biochemical activities and interactions with the cellular DNA replication machinery that T antigen catalyzes viral DNA replication in permissive primate cells; T antigen brings about the oncogenic transformation of many mouse and rat cell lines and can also immortalize primary rodent embryo fibroblasts. These alterations in growth control do not require DNA binding, ATP binding, ATPase activity, or helicase activity (66, 69).

T antigen also controls transcription. Through binding to sequences in the origin of DNA replication, T antigen represses transcription from the SV40 early promoter (2, 46, 49, 50). Mutants defective for DNA binding are also defective for this autoregulation. During the lytic infection cycle, T antigen stimulates transcription from the SV40 late promoter in a process that does not require T antigen binding to viral DNA (10, 11, 33, 34). In addition, SV40 infection causes a large increase in the concentration of several cellular proteins involved in nucleotide metabolism and DNA replication, including DNA polymerase alpha, thymidine kinase, thymidylate synthetase, and dihydrofolate reductase (for a review, see reference 60). Transcriptional stimulation by T antigen may underlie these enzyme inductions, which are part of a general program whereby T antigen stimulates resting cells to enter the cell cycle and progress to the S phase so that viral DNA replication can occur.

In its roles as a transcriptional activator, T antigen resembles

the E1A proteins of the human adenoviruses (for a review, see reference 23). Related proteins of 243 and 289 amino acids are the primary products of the adenovirus E1A gene and are encoded by 12S and 13S E1A mRNAs, respectively. Both proteins display transcriptional activation activity. The E1A proteins interact with p105Rb and the related p107 protein (62, 63), each of which also forms complexes with cellular transcription factor E2F (5–7, 13, 16, 52). T antigen and the growth-regulating E7 protein of the human papillomaviruses also form complexes with p105Rb and p107 (18–20, 44). Complex formation between these tumor virus proteins and p105Rb or p107 releases E2F (4), permitting E2F to stimulate transcription of genes containing E2F-binding sites. E2F-binding sites are found in several genes important for cellular growth control, including *c-myc*, *N-myc*, *c-myb*, thymidine kinase, and dihydrofolate reductase (30, 31, 43). In addition, E1A proteins can interact directly with the TATA box-binding protein (TBP), the component of transcription factor TFIID involved in direct binding to the TATA box (37). It is this interaction which may be central to E1A's ability to activate transcription from a range of modular promoters containing different TATA boxes coupled with different upstream transcription factor-binding sequences (57, 58).

Most studies of transcriptional activation by T antigen have used the SV40 late promoter, the Rous sarcoma virus (RSV) long terminal repeat promoter (LTR), or the adenovirus E2 promoter (1, 10, 40, 54, 68). The SV40 late promoter lacks a TATA box, while the others contain this motif. None of the other sequence elements required for efficient transcription of these promoters are shared. Thus, the mechanisms by which T antigen stimulates transcription are not known. Gruda et al. showed that TBP also interacts with SV40 large T antigen (27). Their studies also demonstrated a correlation between the ability of fragments of T antigen to interact with the TBP and their ability to activate transcription from the SV40 late promoter.

We previously examined the ability of a set of SV40 large T antigen mutants to activate transcription from the SV40 late

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and RSV LTR promoters (68). We found that a 138-amino-acid N-terminal fragment of T antigen retained the ability to *trans* activate both promoters at 30 to 50% of the level of transactivation by the wild-type T antigen. Several mutants with lesions within the first 85 amino acids of T antigen were partially defective for *trans* activation of the RSV LTR, and most of these were also partially defective for *trans* activation of the SV40 late promoter. Thompson et al. (59) examined the ability of a set of mutant T antigens lacking 109 to 176 N-terminal residues to activate the adenovirus E3 promoter and found that these mutant T antigens retained *trans* activation activity. This suggests that T antigen contains multiple domains that function to activate transcription. Different activating domains may operate through different mechanisms.

To examine further the transcriptional activation function of T antigen, we measured the responses of several promoter constructs to activation by the SV40 large T antigen. We employed the set of modular promoters used by Taylor and Kingston to examine *trans* activation by E1A (57, 58). These simple promoters contain combinations of one of four different upstream activating sequences (UASs) coupled with one of three different TATA box sequences. We found that T antigen was able to activate all of these constructs and that the identity of the TATA box was more important for the final level of gene expression than was the type of UAS. We also measured the abilities of several mutant T antigens to activate a subset of these modular promoters. Mutants which had reduced ability to activate the SV40 late and RSV LTR promoters also showed reduced *trans* activation activity on all of the modular promoters analyzed. In addition, we used a set of constructs based on the human U6 small nuclear RNA (snRNA) promoter (39). We found that T antigen was able to activate almost all of the promoter variants tested, but a wide range in the level of activation was seen among this set of simple promoters. Possible mechanisms by which T antigen affects transcription are discussed.

MATERIALS AND METHODS

Plasmids and SV40 mutants. Transformation of bacterial cultures (41), analysis of miniprep preparations of DNA (9), and large-scale preparation of purified plasmid DNA (51) were performed by using established methods. All plasmids were propagated in *Escherichia coli* HB101.

(i) **Reporter plasmids and RNase protection probe.** The RNA polymerase II promoter plasmids (see Fig. 1) were kindly provided by I. Taylor and have been described previously (58). In these plasmids, the bacterial chloramphenicol acetyltransferase (CAT) gene is located downstream from a set of modular promoters containing different TATA boxes in combination with different UASs. The plasmid set bearing altered U6 promoters (see Fig. 4) have also been described previously (38, 39), as has the plasmid used to make RNase protection probe pHU6/RA.2/198. These were kindly provided by N. Hernandez.

(ii) **Plasmids that contain wild-type and mutant SV40 early regions.** Most of these plasmids and the mutants they encode have been described previously (53, 61, 67, 68). All of the plasmids containing SV40 genomes, except mutant 153NS (53), had a 6-bp deletion at the *Bgl*I site within the origin of DNA replication, rendering them replication defective without affecting the level of T antigen expression. Mutant *inA2835* contains an insertion of 9 bp causing replacement of thr-85 and tyr-86 with Ile-Ala-Ile-Ala-Asn. Mutant *inA2819* contains a 12-bp insertion at the position of amino acid 346 causing replacement of Ala-346 with the sequence Val-Ala-Ile-Ala-

Thr. Mutant *dIA2834* has a deletion of the central 3 bp of the *Pfl*MI site at nucleotide (nt) 4558. Mutant *dIA2420* has a deletion of 20 bp that causes a frameshift following amino acid (aa) 138 and addition of Leu-Glu-Ser-Cys-Cys-Val following Phe-138. Mutant *dIA2411* has a 12-bp deletion which removes aa 143 to 146. Mutant *dIA2831* has a deletion of aa 4 to 34. The mutant 153NS T antigen contains Ser in place of Asn-153. Mutant *dI888* (14) is a viable mutant with a deletion that removes part of the splice donor site for the early mRNA that encodes small t antigen; it produces only large T antigen. The 153NS point mutation (53) and the *dI888* deletion (14) were combined by exchanging *Pfl*MI fragments, and the resulting mutant is designated 153NS/*dI888*.

Transactivation assays. CV-1 monkey kidney cells were maintained in Dulbecco's modification of minimal essential medium (GIBCO) supplemented with 10% newborn calf serum (Hyclone, Logan, Utah) and antibiotics. Transactivations were performed in duplicate for each condition tested, and each experiment was performed twice. Transactivation assays were performed by cotransfecting cells with 4.0 μ g of a plasmid that encodes wild-type SV40, 0.5 μ g of a reporter plasmid, and 15.5 μ g of salmon sperm DNA. We used the calcium phosphate method to introduce DNAs into cells (25). To monitor transfection efficiency, we included in a parallel plate a control transfection with pSV2APAP DNA (29), which encodes placental alkaline phosphatase. After 45 h, cells expressing placental alkaline phosphatase were detected histochemically by using naphthol AS-MX phosphate alkaline solution and fast violet B (both from Sigma Chemical Co., St. Louis, Mo.) as directed by the manufacturer. At the same time, we also prepared extracts of transfected cells. Cells were washed twice with TS (25 mM Tris HCl [pH 7.5], 137 mM NaCl, 5 mM KCl, 0.6 mM Na₂HPO₄, 0.05 mM MgCl₂, 0.7 mM CaCl₂), scraped off the plates with a cell scraper in the presence of 1.0 ml of TS, centrifuged briefly in a microcentrifuge, suspended in RSB (10 mM Tris HCl [pH 7.35], 10 mM NaCl, 1.5 mM MgCl₂ [pH 7.35]) containing 0.4% Nonidet P-40, and incubated at 0°C for 30 min. The nuclei were pelleted, and the supernatant containing the CAT enzyme was carefully removed.

To assay the amount of CAT enzyme present in each sample, we used a modification of the method of Neumann et al. (45). This assay uses ³H-labeled acetyl coenzyme A (acetyl-CoA) and measures direct diffusion of the labeled product (acetylated chloramphenicol) into water-immiscible liquid scintillation counting fluid (Econofluor; Dupont). Only the acetylated derivatives of chloramphenicol are able to enter this organic phase. Portions of extracts were added to 100 ml of CAT reaction buffer (125 mM Tris HCl [pH 7.8], 2.5 mCi of ³H-labeled acetyl-CoA [200 mCi/mmol; Dupont, Boston, Mass.] per ml, 1.25 mM chloramphenicol). Since chloramphenicol was present in large excess, compared with the concentration of ³H-labeled acetyl-CoA, the initial rate of acetylation of chloramphenicol was proportional to the concentration of ³H-labeled acetyl-CoA and the rate constant was a measure of the amount of the CAT enzyme present in each extract. Because all of the CAT reactions were conducted with the same concentration of ³H-labeled acetyl-CoA, the only measurements required were elapsed time since the acetylation reaction began and amount of radioactivity in the scintillation fluid. In practice, a set of samples was each counted for 0.1 min several times during a 4-h period, and these data were plotted against elapsed time. Background radioactivity was subtracted, and the rate constants were calculated. This method was standardized and validated by use of a range of concentrations of the purified CAT enzyme (Sigma). When using CAT enzyme, the same results were obtained whether or not an

aliquot of an extract from a mock-transfected culture was added to the reaction mixture.

To determine the amount of reporter plasmid DNA present in transfected cell cultures, the nuclear pellet was solubilized by addition of Hirt solution (0.1 M Tris HCl [pH 7.5], 0.6% sodium dodecyl sulfate [SDS], 0.1 M EDTA) (32) and the plasmid DNA was separated from chromosomal DNA by centrifugation at 4°C in an Eppendorf 5413 centrifuge. The supernatant, containing low-molecular-weight episomal DNA, was phenol extracted, depurinated in 0.25 M HCl for 3 to 5 min, denatured in 0.6 M NaOH and 1.5 M NaCl, neutralized with 1 M Tris HCl (pH 7.0)–1.5 M NaCl, and applied to nitrocellulose membranes with a slot blot apparatus (Schleicher & Schuell, Inc., Keene, N.H.). The membrane was baked in vacuo for 1 h at 80°C. The amount of the reporter plasmid DNA in each sample was determined by hybridization of the membrane to a CAT-specific probe made by oligo labeling (22) a twice gel-purified 529-bp *SacI*-*Bam*HI fragment containing part of the CAT protein-coding region. The purity of the probe was tested by hybridization to known quantities of either pURSVCAT (the CAT gene under control of the RSV LTR) or p6-1 (origin-defective SV40 cloned into pUC18). The signal generated with p6-1 was less than 0.5% of the signal generated with an identical amount of pURSVCAT DNA. Autoradiograms of the slot blots were quantitated by using a MasterScan Interpretive Densitometer (Scanalytics, Division of CSP, Inc., Billerica, Mass.).

RNAse protection analysis. Cells were washed with cold RSB and lysed by addition of RSB containing 0.4% Nonidet P-40 and 0.05% deoxycholate. A portion was removed for determination of the reporter plasmid DNA concentration by the Hirt lysis method (32, 68). One milliliter of 2× RNA buffer (500 mM NaCl, 50 mM Tris HCl [pH 8.0], 12 mM EDTA) was added to the remainder of the lysate. This was extracted twice with phenol-chloroform (1:1) and then ethanol precipitated.

To synthesize the probe for RNAse protection, pHU/RA.2/198 DNA was linearized with *Eco*RI and transcribed by using T3 RNA polymerase to produce a transcript of 531 nt. For the sizes of the regions of the probe protected by hybridization with different potential U6 snRNA construct transcripts, see Fig. 4b; they were 393 nt for a transcript which initiates from the U6 promoter and terminates at the L3 3' end [adenovirus L3 poly(A) signal], 198 nt for a transcript which initiates from the U6 promoter and terminates at the run of T residues characteristic of an RNA polymerase III termination site, and 143 nt for a transcript which initiates from the U6 promoter and terminates at the snRNA 3' box.

An experimentally determined excess of the probe was mixed with 20 µg of RNA in a total volume of 30 µl of hybridization buffer [40 mM piperazine-*N,N'*-bis(2-ethanesulfonic acid) PIPES; pH 6.5], 400 mM NaCl, 1 mM EDTA, 80% deionized formamide], heated to 90°C for 10 min, and placed at 61°C overnight. Annealing was halted by addition of 300 µl of cold RNAse digestion buffer (10 mM Tris HCl [pH 7.5], 5 mM EDTA, 300 mM NaCl) containing 200 U of RNAse T₁ per ml, and samples were placed at 37°C for 1 h. Samples were phenol-chloroform extracted, ethanol precipitated, collected, dissolved in sample buffer, and analyzed by electrophoresis on a 4% polyacrylamide–7 M urea gel. After electrophoresis, the gel was dried and autoradiographed.

The amount of the reporter plasmid present in each culture was determined, as described above for the CAT plasmids, by using the same T3 transcript used for RNAse protection analysis as a hybridization probe. The amount of the U6 transcript in each culture was determined by densitometry of the autoradiograph with a MasterScan Interpretive Densitom-

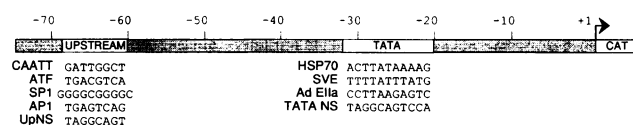


FIG. 1. Structure of the RNA polymerase II modular promoter constructs. The gray bar represents sequences from the human HSP70 promoter, and the white bar represents CAT-coding sequences. The transcription factor recognition sequences that have been placed between -60 and -68 are indicated below the upstream region. The TATA region sequences are indicated below the diagram, between -20 and -31. The arrow indicates the direction of transcription. Ad, adenovirus.

eter, and these values were normalized for the amount of the reporter plasmid present in that culture.

RESULTS

Previous studies of transactivation of transcription by SV40 large T antigen have examined either the wild-type SV40 late and early promoters (11, 33, 34, 64, 68) or reporter genes under control of other complex cellular and viral promoters (1, 68). To begin examining the mechanisms by which T antigen stimulates transcription, we tested the ability of the wild-type large T antigen to transactivate a set of modular promoters generated by Taylor and Kingston (55, 57, 58). The organization of these modular promoters is shown in Fig. 1. They were derived from the human heat shock protein 70 (HSP70) promoter altered to remove upstream sequences including heat shock response elements. This left the basal HSP70 promoter containing an upstream CAATT box and the HSP70 TATA box. Substitutions of this construct were made at both the CAATT box and the TATA box to generate a set containing different upstream elements and different TATA boxes. By combining these elements pairwise, a set of 20 constructs was produced. These contain as the UAS either the HSP70 CAATT box; sites recognized by ATF, SP1, or AP1; or a nonsense sequence not recognized by any known transcription factor. In addition to the HSP70 TATA box, TATA box sequences from the SV40 early promoter and the adenovirus E2A promoter were used, as well as a nonsense sequence in place of any TATA box. Downstream from all 20 constructs was the bacterial CAT gene.

We cotransfected CV-1 monkey kidney cell cultures with a plasmid containing the early region from SV40 and one of the reporter plasmids. Other cultures received only the reporter plasmid plus carrier DNA. CAT activity was determined in extracts prepared 45 h after transfection. We also determined the amount of the reporter plasmid DNA taken up into each culture and used this value to normalize CAT activity data. The results from multiple experiments are displayed in Fig. 2. The bar graphs present CAT activity normalized for the amount of reporter plasmid DNA. Each set of bar graphs presents the CAT activity present in cultures lacking (-) or containing (+) wild-type T antigen. We noted that the SV40 plasmid used in these studies also encodes small t antigen; we saw no effect from small t antigen in transactivation experiments (see Fig. 5; 48). The inset in Fig. 2 presents the average fold induction obtained with the SV40 T antigen.

With all of these constructs, very little CAT activity was detected in the absence of SV40 T antigen. T antigen was able to activate expression from almost all of these modular promoters, but to various degrees. Regardless of which UAS was used, the greatest activity was always seen with the HSP70 TATA box. The adenovirus E2A TATA box could be com-

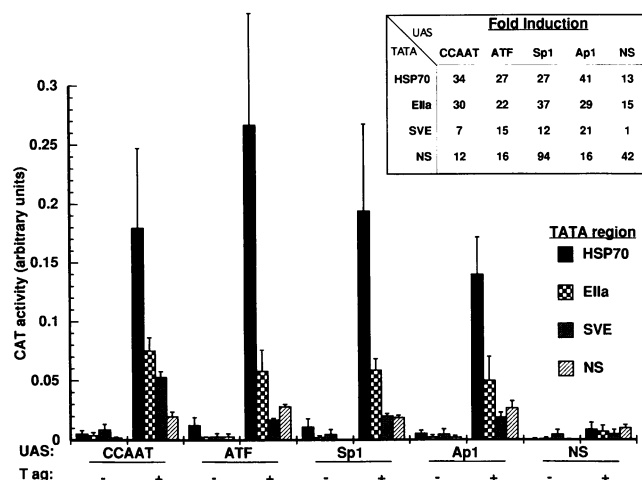


FIG. 2. The level of induction of CAT activity induced by wild-type T antigen (Tag) depends primarily on the TATA region. CAT activity was determined as described in Materials and Methods. The error bars indicate the standard deviation of the mean ($n = 4$). Bar patterns indicate the TATA region in the construct in accordance with the key at the right. The UAS used is indicated along the x axis, and the presence or absence of SV40 large T antigen is indicated by a plus or minus sign, respectively. The inset shows fold induction of CAT activity from each promoter by SV40 large T and small t antigens. Data from the bar graph are represented as fold stimulation of basal activity (i.e., CAT activity of the construct in the presence of SV40 T antigen divided by the uninduced level of CAT activity of that construct).

combined with each of the UASs to yield promoters which could be activated by SV40 T antigen but to CAT levels lower than those obtained with the HSP70 TATA box. In contrast, very little CAT activity was produced when the SV40 early TATA box was coupled with each of the different UAS elements. In fact, CAT activities detected when using the SV40 early TATA box promoters were usually only slightly greater than those obtained with the nonsense TATA sequence. Regardless of the level of CAT detected in cultures lacking SV40 T antigen, there was substantial stimulation of CAT activity in the presence of T antigen. Most of the constructs were stimulated 10- to 40-fold by T antigen. These data indicate that T antigen is able to stimulate expression from a wide variety of modular promoters containing one of four different UASs and one of three different TATA box variants.

We wondered whether mutants that showed a partial defect in activation of complex promoters, such as the SV40 late promoter and the RSV LTR, would show wild-type activity on some simple promoters and a lack of activity on others. Alternatively, mutant T antigens with partial activity on complex promoters might show reduced activity on all simple modular promoters. Therefore, we examined the ability of mutant SV40 T antigens to activate a subset of these modular promoters. We limited this analysis to modular promoters containing the HSP70 TATA box. The results of this study are shown in Fig. 3. Note that the data represent the fraction of the wild-type level of activation seen with each mutant T antigen on each reporter promoter tested. In general, the mutants tested gave the same relative level of activity on all five modular promoters. Thus, mutants which showed a partial defect in transactivation of complex promoters retained that partial transactivation activity when tested on simple promoters.

We extended our studies by testing a set of constructs

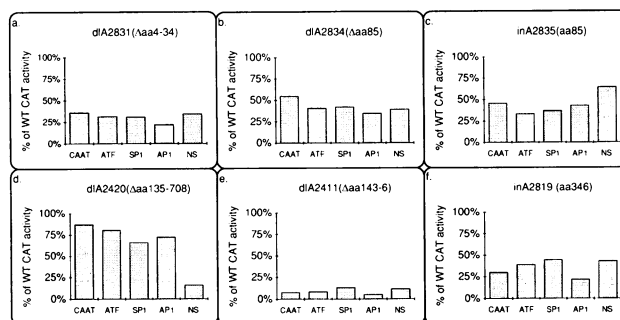


FIG. 3. Transactivation activity on modular promoters of SV40 T antigen mutants. Cells were cotransfected with the subset of modular promoters containing the HSP70 TATA box and different upstream elements. Note that the CAT activity obtained in response to each mutant T antigen is expressed as a percentage of the activity seen with wild-type (WT) large T antigen acting on the same modular promoter.

prepared and used by Lobo et al. to study the human U6 snRNA promoter (39). The structures of these constructs are illustrated in Fig. 4. They contain the U6 TATA box, one of six TATA box variants, or a linker in place of the normal TATA box (LS7). All of the constructs contain the normal U6 snRNA gene sequences upstream of the TATA box region. These promoters drive transcription of an antisense human β -globin RNA which was detected by RNase protection analysis. Downstream of the globin insert are the normal 3' box used for 3'-end formation of snRNAs generated by polymerase II transcription, an RNA polymerase III termination signal (TTTT), and a polyadenylation signal. Depending on which of these elements signals 3'-end formation, the portion of the probe protected from RNase treatment differs, as depicted in Fig. 4b.

We tested activation of these promoters by two different T-antigen constructs. Mutant 153NS was used as a pseudowild-type. It contains a point mutation resulting in a change of Asn-153 to Ser; the 153NS T antigen lacks DNA-binding and viral DNA replication activities (53) but transactivates expression from the RSV LTR and SV40 late promoters, as well as wild-type T antigen (data not shown). It was used in place of wild-type SV40 T antigen to prevent replication of the U6 snRNA constructs, all of which contain a functional SV40 origin of replication. The validity of using this mutant as a pseudowildtype was confirmed by comparing transactivation in cells which received a reporter plasmid and mutant 153NS with those which received the same reporter, a wild-type large T antigen plasmid, and a concentration of cytosine arabinoside (25 μ g/ml) sufficient to inhibit viral DNA replication completely. The same level of transactivation was seen in both cultures (data not shown). Mutant 153NS also encodes a wild-type small t antigen. 153NS/dI888 contains a deletion of the splice donor site for small t mRNA; therefore, it produces pseudowildtype large T antigen (153NS) but no small t antigen.

CV-1 cells were transfected with a U6 snRNA plasmid and either 153NS, 153NS/dI888, or no SV40 plasmid. Total cytoplasmic RNA and low-molecular-weight DNAs were each isolated 45 h after transfection. RNAs were analyzed by RNase protection analysis, and the results are shown in Fig. 5A. Controls were performed to ensure that this analysis was done with an excess of probe. The amount of RNA detected was calculated densitometrically and normalized for the amount of the reporter plasmid in each culture. The wild-type U6 promoter was efficiently activated by T antigen (Fig. 5A,

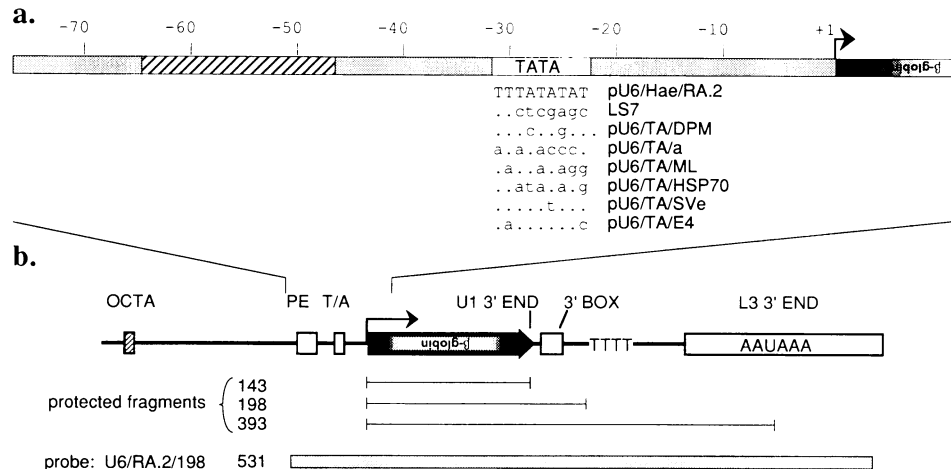


FIG. 4. Constructs derived from the human U6 snRNA gene. (a) Detail of the promoter-proximal region. The gray bars represent U6 sequences. The cross-hatched portion indicates sequences that direct transcription by RNA polymerase III. For the TATA region, mutations are indicated as changed nucleotides below the diagram. The names of the constructs are to the right and indicate the TATA regions that the mutant sequences match. (b) Structural features of the U6 promoter constructs. OCTA is an octamer-binding sequence, PE is the proximal element represented by the hatched box in panel a, and T/A is the TATA region. The RNase protection probe and protected fragments are indicated below the illustration of the construct.

lane 3), and a similar level of activation was detected in the presence of both small and large T antigens (lane 2). The presence of only the 143-nt band indicates that all transcription from the wild-type U6 promoter used the 3' box associated with polymerase II transcription and the normal signal for 3'-end formation of snRNAs. Little expression of RNA or activation by T antigen was seen in constructs containing a linker in place of the TATA box (lanes 4 to 6) or containing two point mutations in the TATA box (lanes 7 to 9). Of the other TATA boxes tested, the greatest activation was seen with the HSP70 TATA box (lanes 13 to 15) and the adenovirus E4 TATA box (lanes 19 to 21). A very low level of activation was seen with the adenovirus major late promoter TATA box (lanes 10 to 12), and a slightly greater level of activation was seen with the SV40 early TATA box (lanes 16 to 18). In some situations, a higher level of activation appeared to occur with SV40 large T antigen alone, while with others, a higher level appeared to occur when both small and large T antigens were present than when only large T antigen was present. We do not believe that these differences are significant. In a repeat of this experiment, we again saw the greatest activation of promoters with the HSP70 and adenovirus E4 TATA boxes, lower activation with the SV40 early and U6 wild-type TATA boxes, little activation of the adenovirus major late TATA box, and very little activity on the LS7 and DPM constructs. In most cases, the activities of large T antigen alone and of small and large T antigens together were similar (data not shown). It has been reported that the small t antigen can assist large T antigen in activation of gene expression under conditions of limiting levels of large T antigen (8). The level of the large T antigen in cells transfected by either 153NS/dl888 or 153NS was near the level obtained in virus-infected cells (data not shown). In several experiments, we have not found reproducible differences in activation of reporter genes by the large T antigen compared with the small t and large T antigens together (48).

DISCUSSION

Our studies indicate that large T antigen is able to activate a variety of modular promoters containing different UASs and

different variants of the TATA box. In comparing different simple modular promoters (Fig. 2), it is important to consider both the level of CAT expression achieved with different promoters and the fold induction by SV40 large T and small t antigens. In all cases, the highest level of induction was seen with the HSP70 TATA box; lower levels were obtained with the adenovirus E2A TATA box. The level of CAT expression obtained with the SV40 early TATA box or when a non-TATA-like sequence replaced the TATA box was very low. Fold induction by T antigen followed a different pattern. When the very inefficient SV40 early TATA construct containing a nonsense sequence was used in place of a UAS as a baseline, all other constructs were activated by T antigen, with a range of 7- to 94-fold. Among those constructs with the highest fold induction were some which contained a non-TATA sequence in place of the TATA box. While we believe that T antigen does activate these constructs, the very low levels of expression in the absence of a transactivator make the fold induction numbers less accurate. Similarly, the fold induction observed with many of the other constructs reflects as much the differences in basal levels of expression as it does the differences in final levels of CAT expression. We conclude that T antigen is able to activate a variety of simple modular promoters.

The critical question that needs to be answered is the mechanism of transcriptional activation by SV40 T antigen. Since T antigen activates promoters containing many different UAS elements, we think it unlikely that T antigen transactivation is mediated primarily by direct interactions between T antigen and proteins that bind to UAS elements, since this would require that T antigen be able to interact with multiple proteins containing different types of activation domains. More likely, transactivation by T antigen involves interactions between T antigen and components of the basic transcription machinery. T antigen could affect interactions between TBP and DNA, since the T antigen is known to bind TBP (27). Alternatively, T antigen could affect the recruitment to or retention in the transcription complex of some factor other than TBP. Gruda et al. have suggested that T antigen mediates transcriptional activation by interacting both with components of the basal transcription machinery and with factors that

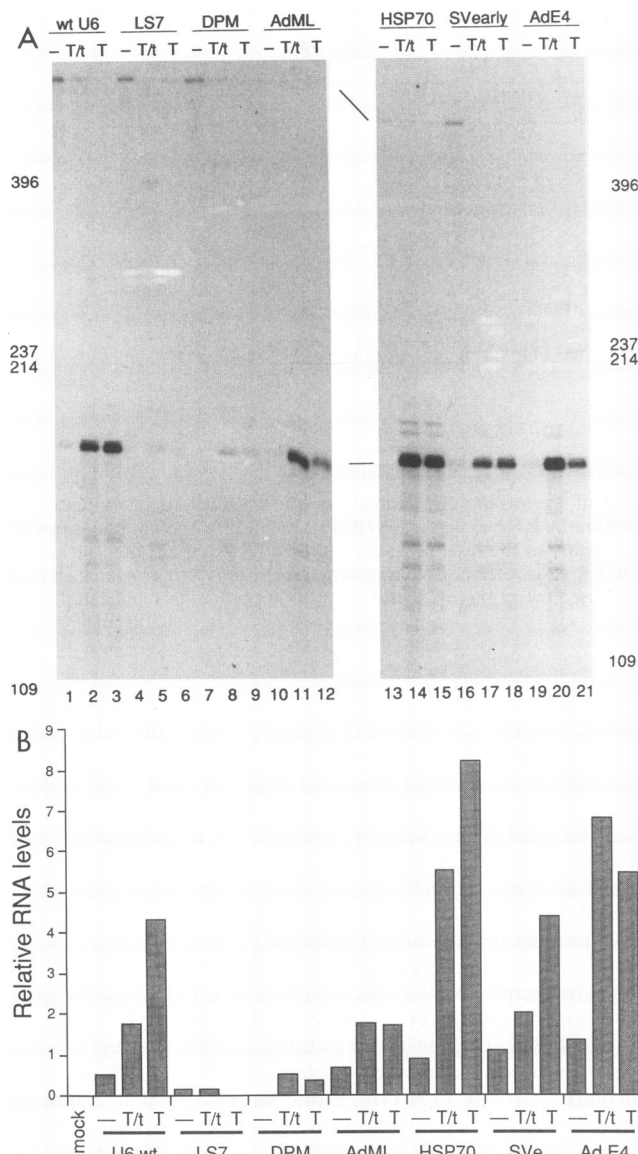


FIG. 5. RNase protection analysis of transcription from pHU6 promoter constructs. (A) Autoradiograph of a 4% polyacrylamide gel showing RNase protection analysis of transcription from U6 promoter constructs. The positions of size markers are shown at the left and right. Lanes marked with minus signs contained cultures which received only a U6 snRNA construct. Lanes T/t also contained pseudo-wild-type mutant 153NS. Lanes T also contained SV40 mutant 153NS *dl*888. (B) Histogram showing normalized values for RNA levels from the autoradiograph. The autoradiograph in panel A was analyzed densitometrically, and the resulting data were normalized for the amount of the reporter plasmid present in each culture. wt, wild type; Ad, adenovirus; ML, major late promoter.

recognize UASs and, in so doing, may stabilize the transcription complex (27). Our data are consistent with this mechanism.

It is not known whether T antigen-TBP complexes form following binding of the TBP to a TATA box or whether a T antigen-TBP complex forms in solution and subsequently binds to the TATA box. If T antigen binds to TBP already bound to

DNA, this interaction could stabilize TBP binding to different extents with different TATA boxes. Since TBP is thought to remain bound to DNA through multiple rounds of initiation by RNA polymerase II, the rate of dissociation of TBP from DNA could be a significant factor in determining how many rounds of transcription are initiated following assembly of the basal transcription complex. However, it is not known whether binding of TBP to DNA is the rate-limiting step in initiation nor whether TBP binds different TATA boxes with different affinities. Since a wide range of sequences can function as TATA boxes, at least in *Saccharomyces cerevisiae* (ref), it is likely that TBP has different affinities for different TATA boxes. For example, TBP might have higher affinity for the HSP70 TATA box than for the others examined in our studies. The probability that T antigen would encounter the TBP bound to the HSP70 TATA box would then be greater than that it would encounter the TBP bound to the other TATA boxes used in these experiments. T antigen bound to TBP could then stimulate recruitment to the transcription complex of whatever factor is rate limiting.

When we examined the ability of a set of mutant T antigens to activate these modular promoters, we focused on several mutants with lesions in the N-terminal half of T antigen. Previously, we showed that these mutants activate the SV40 late promoter, the RSV LTR promoter, or both less efficiently than did the wild-type T antigen (68). Each of the mutants examined here (Fig. 3) activated each of the promoters tested to about the same fraction of the level of activation achieved with wild-type large T antigen. While it is likely that T antigen activates different cellular promoters by different mechanisms, reflecting T antigen's interactions with a multiplicity of transcription factors and other proteins, we think it is likely that the mechanism of activation of these modular promoters is similar or identical. Had different mutants displayed high levels of activation on some promoters and low levels on others, this would have been more consistent with multiple mechanisms of activation of different simple promoters. Gruda et al. showed that T antigen interacts with the TBP through sequences within the first 172 aa of the large T antigen (27). Most of the mutations that reduced the transcriptional activation activity of large T antigen are located within this region. In particular, three of T antigen mutants tested showed absolutely no activation of any of the promoters tested (47, 68). These are mutants *dl*A2411 (lacking aa 143 to 146), *in*A2815 (insertion at aa 168), and 187HR (substitution of His for Arg-187; reference 53). It is possible that these three mutations prevent interaction between T antigen and the TBP. This hypothesis is being tested.

While T antigen is known to activate expression of some cellular genes, it is not known for most of these genes whether this activation is direct or indirect. Most likely, a diverse set of mechanisms underlie changes in cellular gene expression following SV40 infection. Since T antigen forms complexes with p105Rb resulting in dissociation of transcription factor E2F, expression of cellular genes responsive to E2F is likely to be affected by T antigen binding to p105Rb. E2F sites are found in many of the genes induced by serum and by T antigen. Other transcription factors are likely to form complexes with proteins to which T antigen binds, and it is expected that expression of genes responsive to these factors would be induced when T antigen binds the cellular protein to which these factors bind. In some cases, formation of multiprotein complexes containing T antigen may give those complexes enhanced transcriptional activity because of the presence of large T antigen. Still other cellular genes may be induced by T antigen as a consequence

of T antigen binding to tumor suppressor proteins which normally repress expression of those genes. Roles for both p53 and p105Rb in transcriptional repression have been reported (28, 35, 36).

Evidence that T antigen participates directly in transcriptional activation comes from *in vitro* studies with the SV40 late (17) and human HSP70 (56) promoters. The most likely primary target for direct transcriptional stimulation by T antigen is the SV40 late promoter. Late in infection, this promoter is transcribed from amplified viral templates to produce high levels of SV40 late mRNAs. T antigen also binds transcription factor TEF-1 (27). Since TEF-1-binding sites are important for SV40 late transcription (12, 15, 24, 26, 34, 42), T antigen-TEF-1 interactions could play a role in making the SV40 late promoter a particularly good substrate for T antigen activation. The SV40 late promoter lacks a TATA box, but among sequences surrounding the major late transcription initiation site at nt 325 and important for late transcription are some which resemble Inr elements. The TBP is known to be important for *in vitro* transcription of promoters which lack obvious TATA boxes (12, 15, 24, 26, 34, 42) and may also bind directly to Inr elements (65).

We suggest that an additional mechanism of transcriptional activation by T antigen could involve the chromosomal status of the DNA. The only episomal DNA present during an SV40 infection is SV40 DNA. All of the reporter genes which we have examined were part of episomal DNA molecules. If T antigen's activities in transcriptional activation were directed in some manner towards episomal DNA, the normal consequences of this rather non-gene-specific mechanism would be increased transcription from the promoters present in the SV40 genome. Since large T antigen directly represses transcription of the SV40 early promoter, the SV40 late promoter would be the promoter most stimulated by such a mechanism. We conducted a preliminary experiment to examine how T antigen affects episomal versus chromosomal genes. We isolated a cell line containing a stably integrated RSV-luciferase reporter by transfecting cells with an RSV-luciferase construct and pSV2NEO and screened many G418-resistant clones to find any that expressed even a basal level of luciferase. One such cell line was cotransfected with RSV-CAT and a plasmid that encodes SV40 large T antigen. We found that the CAT level was stimulated by T antigen (compared with transfection of the same cell line with RSV-CAT alone), while the level of luciferase was reduced when T antigen was present (47). Additional experiments are required to confirm and extend this finding.

Whether or not nucleosomes are present over the promoter of a particular gene is an important factor for transcriptional activation, since nucleosomes are known to prevent transcription of some genes. Some, but not all, transcriptional activators are able to cause displacement of nucleosomes, a requirement for activation of some promoters when they are present within cellular chromatin but not when they are introduced into cells by transient transfection (3). The studies described here indicate that T antigen is able to activate a wide variety of promoters carried on episomal DNA molecules. The mechanism for this activation is likely to be important for understanding how T antigen stimulates the SV40 late promoter.

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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. Archer, T. K., P. Lefebvre, R. G. Wolford, and G. L. Hager. 1992. Transcription factor loading on the MMTV promoter: a bimodal mechanism for promoter activation. *Science* **255**:1573-1576.
4. Bagchi, S., P. Raychaudhuri, and J. R. Nevins. 1990. Adenovirus E1A proteins can dissociate heteromeric complexes involving the E2F transcription factor: a novel mechanism for E1A transactivation. *Cell* **62**:659-669.
5. Bagchi, S., R. Weinmann, and P. Raychaudhuri. 1991. The retinoblastoma protein copurifies with E2F-1, an E1A-regulated inhibitor of the transcription factor E2F. *Cell* **65**:1063-1072.
6. Bandara, L. R., J. P. Adamczewski, T. Hunt, and N. B. La Thangue. 1991. Cyclin A and the retinoblastoma gene product complex with a common transcription factor. *Nature (London)* **352**:249-251.
7. Bandara, L. R., and N. B. La Thangue. 1991. Adenovirus E1a prevents the retinoblastoma gene product from complexing with a cellular transcription factor. *Nature (London)* **351**:494-497.
8. 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.
9. Birnboim, H. C., and J. Doly. 1979. A rapid alkaline procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* **7**:1513-1523.
10. Brady, J., J. B. Bolen, M. Radonovich, N. P. 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.
11. Brady, J., and G. Khoury. 1985. *trans* activation of the simian virus 40 late transcription unit by T-antigen. *Mol. Cell. Biol.* **5**:1391-1399.
12. 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.
13. Cao, L., B. Faha, M. Dembski, L.-H. Tsai, E. Harlow, and N. Dyson. 1992. Independent binding of the retinoblastoma protein and p107 to the transcription factor E2F. *Nature (London)* **355**:176-179.
14. Carbon, J., T. E. Shenk, and P. Berg. 1975. Biochemical procedure for production of small deletions in simian virus 40 DNA. *Proc. Natl. Acad. Sci. USA* **72**:1392-1396.
15. 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.
16. Chellappan, S. P., S. Hiebert, M. Mudryj, J. M. Horowitz, and J. R. Nevins. 1991. The E2F transcription factor is a cellular target for the Rb protein. *Cell* **65**:1053-1061.
17. 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.
18. 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.
19. Dyson, N., K. Buchovich, P. Whyte, and E. Harlow. 1989. The cellular 107k protein that binds to adenovirus E1A also associates with the large T antigens of SV40 and JC virus. *Cell* **58**:249-255.
20. Dyson, N., P. M. Howley, K. Munger, and E. Harlow. 1989. The

- human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. *Science* **243**:934–936.
21. **Fanning, E., and R. Knippers.** 1992. Structure and function of simian virus 40 large tumor antigen. *Annu. Rev. Biochem.* **61**:55–85.
 22. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132**:6–13.
 23. **Flint, S. J., and T. E. Shenk.** 1989. Adenovirus E1a protein: paradigm viral transactivator. *Annu. Rev. Genet.* **23**:141–161.
 24. **Gallo, G. J., M. C. Gruda, J. R. Manuppello, and J. C. Alwine.** 1990. Activity of simian DNA-binding factors is altered in the presence of simian virus 40 (SV40) early proteins: characterization of factors binding to elements involved in activation of the SV40 late promoter. *J. Virol.* **64**:173–184.
 25. **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.
 26. **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.
 27. **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.
 28. **Helin, K., and E. Harlow.** 1993. The retinoblastoma protein as a transcriptional repressor. *Trends Cell Biol.* **3**:43–46.
 29. **Henthorn, P., P. Zervos, M. Raducha, H. Harris, and T. Kadesch.** 1988. Expression of a human placental alkaline phosphatase gene in transfected cells: use as a reporter for studies of gene expression. *Proc. Natl. Acad. Sci. USA* **85**:6342–6346.
 30. **Hiebert, S. W., M. Blake, J. Azizkhan, and J. R. Nevins.** 1991. Role of the E2F transcription factor in E1A-mediated *trans* activation of cellular genes. *J. Virol.* **65**:3547–3552.
 31. **Hiebert, S. W., M. Lipp, and J. R. Nevins.** 1989. E1A-dependent *trans*-activation of the human MYC promoter is mediated by the E2F factor. *Proc. Natl. Acad. Sci. USA* **86**:3594–3598.
 32. **Hirt, B.** 1967. Selective extraction of polyoma DNA from infected mouse cultures. *J. Mol. Biol.* **26**:365–369.
 33. **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.
 34. **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.
 35. **Kley, N., R. Y. Chung, S. Fay, J. P. Loeffler, and B. R. Seizinger.** 1992. Repression of the basal c-fos promoter by wild-type p53. *Nucleic Acids Res.* **20**:4083–4087.
 36. **Lechner, M. S., D. H. Mack, A. B. Finicle, T. Crook, K. H. Vousden, and L. A. Laimins.** 1992. Human papillomavirus E6 proteins bind p53 in vivo and abrogate p53-mediated repression of transcription. *EMBO J.* **11**:3045–3052.
 37. **Lee, W. S., C. C. Kao, G. O. Bryant, X. Liu, and A. J. Berk.** 1991. Adenovirus E1A activation domain binds the basic repeat in the TATA box transcription factor. *Cell* **67**:365–376.
 38. **Lobo, S. M., and N. Hernandez.** 1989. A 7 bp mutation converts a human RNA polymerase II snRNA promoter into an RNA polymerase III promoter. *Cell* **58**:55–67.
 39. **Lobo, S. M., J. Lister, M. L. Sullivan, and N. Hernandez.** 1991. The cloned RNA polymerase II transcription factor selects RNA polymerase III to transcribe the human U6 gene in vitro. *Genes Dev.* **5**:1477–1489.
 40. **Loeken, M. R., G. Khoury, and J. Brady.** 1986. Stimulation of the adenovirus E2 promoter by simian virus T antigen or E1A occurs by different mechanisms. *Mol. Cell. Biol.* **6**:2020–2026.
 41. **Mandel, M., and A. Higa.** 1970. Calcium-dependent bacteriophage DNA interaction. *J. Mol. Biol.* **53**:159–162.
 42. **May, E., F. Omilli, E. Ernoul-Lange, M. Zenke, and P. Chambon.** 1987. The sequence motifs that are involved in SV40 enhancer function also control SV40 late promoter activity. *Nucleic Acids Res.* **15**:2445–2461.
 43. **Mudryj, M., S. H. Devotol, S. W. Hiebert, T. Hunter, J. Pines, and J. R. Nevins.** 1990. A role for the adenovirus inducible E2F transcription factor in a proliferation dependent signal transduction pathway. *EMBO J.* **9**:2179–2184.
 44. **Munger, K., B. A. Werness, N. Dyson, W. C. Phelps, E. Harlow, and P. M. Howley.** 1989. Complex formation of human papillomavirus E7 proteins with the retinoblastoma tumor suppressor gene product. *EMBO J.* **8**:4099–4105.
 45. **Neumann, J. R., C. A. Morency, and K. O. Russian.** 1987. A novel rapid assay for chloramphenicol acetyltransferase gene expression. *BioTechniques* **5**:444–447.
 46. **Reed, S. I., G. R. Stark, and J. C. Alwine.** 1976. Autoregulation of simian virus 40 gene A by T antigen. *Proc. Natl. Acad. Sci. USA* **73**:3083–3087.
 47. **Rice, P. W., and C. N. Cole.** Unpublished data.
 48. **Rice, P. W., J. Zhu, J. Chen, and C. N. Cole.** Unpublished data.
 49. **Rio, D. C., A. K. Robbins, R. M. Myers, and R. Tjian.** 1980. Regulation of SV40 early transcription *in vitro* by a purified tumor antigen. *Proc. Natl. Acad. Sci. USA* **77**:5706–5710.
 50. **Rio, D. C., and R. Tjian.** 1983. SV40 T antigen binding site mutations that affect autoregulation. *Cell* **32**:1227–1240.
 51. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
 52. **Shirodkar, S., M. Ewen, J. A. DeCaprio, J. Morgan, D. M. Livingston, and T. Chittenden.** 1992. The transcription factor E2F interacts with the retinoblastoma product and a p107-cyclin A complex in a cell cycle-regulated manner. *Cell* **68**:157–166.
 53. **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.
 54. **Srinivasan, A., K. W. Peden, and J. M. Pipas.** 1989. The large tumor antigen of simian virus 40 encodes at least two distinct transforming functions. *J. Virol.* **63**:5459–5463.
 55. **T'Ang, A., J. M. Varley, S. Chakraborty, A. L. Murphree, and Y.-K. T. Fung.** 1988. Structural rearrangement of the retinoblastoma gene in human breast carcinoma. *Science* **242**:263–266.
 56. **Taylor, I. W., Solomon, B. M., Weiner, E. Paucha, M. Bradley, and R. E. Kingston.** 1989. Stimulation of the human heat shock protein 70 promoter *in vitro* by simian virus 40 large T antigen. *J. Biol. Chem.* **264**:16160–16164.
 57. **Taylor, I. C. A., and R. E. Kingston.** 1990. Factor substitution in a human HSP70 gene promoter: TATA-dependent and TATA-independent interactions. *Mol. Cell. Biol.* **10**:165–175.
 58. **Taylor, I. C. A., and R. E. Kingston.** 1990. E1a transactivation of human HSP70 gene promoter substitution mutants is independent of the composition of upstream and TATA elements. *Mol. Cell. Biol.* **10**:176–183.
 59. **Thompson, D. L., D. Calderon, A. Smith, and M. Tevethia.** 1990. Dissociation of Rb-binding and anchorage-independent growth from immortalization and tumorigenicity using SV40 mutants producing N-terminally truncated large T antigens. *Virology* **178**:15–34.
 60. **Tooze, J.** 1981. DNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 61. **Tornow, J., and C. N. Cole.** 1983. Intracistronic complementation in the simian virus 40 A gene. *Proc. Natl. Acad. Sci. USA* **80**:6312–6316.
 62. **Whyte, P., K. Buchovich, J. Horowitz, S. Friend, M. Raybuck, R. Weinberg, and E. Harlow.** 1988. Association between an oncogene and an anti-oncogene; the adenovirus E1A proteins bind to the retinoblastoma gene product. *Nature (London)* **334**:124–129.
 63. **Whyte, P., N. M. Williamson, and E. Harlow.** 1989. Cellular targets for transformation by the adenovirus E1A proteins. *Cell* **56**:67–75.
 64. **Wildeman, A. G.** 1989. Transactivation of both early and late simian virus promoters by large tumor antigen does not require nuclear localization of the protein. *Proc. Natl. Acad. Sci. USA* **86**:2123–2127.
 65. **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.
 66. **Zhu, J., M. Abate, P. W. Rice, and C. N. Cole.** 1991. The ability of

- simian virus 40 large T antigen to immortalize primary mouse embryo fibroblasts cosegregates with its ability to bind to p53. *J. Virol.* **65**:6872-6880.
67. **Zhu, J., and C. N. Cole.** 1989. Linker insertion mutants of simian virus 40 large T antigen that show *trans*-dominant interference with wild-type large T antigen map to multiple sites within the T-antigen gene. *J. Virol.* **63**:4777-4786.
68. **Zhu, J., P. W. Rice, M. Chamberlain, and C. N. Cole.** 1991. Mapping the transcriptional transactivation function of SV40 large T antigen. *J. Virol.* **65**:2778-2790.
69. **Zhu, J., P. W. Rice, L. Gorsch, M. Abate, and C. N. Cole.** 1992. Transformation of a continuous rat embryo fibroblast cell line requires three separate domains of simian virus 40 large T antigen. *J. Virol.* **66**:2780-2791.