Involvement of Simian Virus 40 (SV40) Small t Antigen in *trans* Activation of SV40 Early and Late Promoters

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We have previously found that simian virus 40 (SV40) small t antigen (small t) can *trans* activate the E2A and VA-I genes of adenovirus in plasmid DNA-transfected cells (M. R. Loeken, I. Bikel, D. M. Livingston, and J. Brady, Cell 55:1171–1177, 1988). To determine whether *trans* activation by small t might be involved in the SV40 productive infection cycle, we examined the effects of cotransfecting plasmids encoding small t with plasmids containing the chloramphenicol acetyltransferase (CAT) gene linked to the SV40 early or late promoter. Small t increased three- to fivefold the expression of a CAT plasmid linked to the SV40 early promoter and enhancer. Small t expression had no effect by itself on CAT activity directed by the SV40 late promoter, but small t enhanced the effect of a suboptimal concentration of a plasmid expressing large T up to 10-fold. When the concentration of the plasmid expressing large T was increased to a level at which large T alone stimulated the late promoter ninefold, the enhancement by small t was only twofold. The effects of small t on both the SV40 early and late promoters depended on sequences within the small t-unique domain, since a plasmid expressing only the first 82 amino acids common to both large T and small t was inactive. The effects of small t on early- and late-promoter-directed CAT enzyme activity was reflected in increased CAT mRNA as measured by S1 analysis. These results suggest that SV40 small t may play a role in viral infection by increasing transcription from the early promoter and from the late promoter at times when large T levels are low.

In contrast to the large body of knowledge that exists regarding regulation of cellular and viral gene expression by simian virus 40 (SV40) large T antigen (large T), there are very few experimental data suggesting an explanation for the involvement of small t during productive viral infection. Our observation that SV40 small t can *trans* activate promoters transcribed by RNA polymerase II (the adenovirus E2A promoter) and III (the adenovirus VA-I promoter) (19) and that polyomavirus small t can also *trans* activate these promoters (18) suggests the possibility that *trans* activation may be an essential small t function which has been retained during papovavirus speciation. It is possible that transcription of cellular genes, viral genes, or both occurs in response to small t and that this is important for a successful viral infection.

On the other hand, small t is not strictly required for viral infection or transformation in tissue culture. Viruses containing mutations within small t coding regions are not markedly defective but grow slowly (28). SV40-transformed cultured cells can be produced in the absence of small t, and small t in the absence of large T will not transform cells (22). However, small t appears to be required for transformation when cellular concentrations of large T are low (2, 4). We considered that, similarly, small t may be important only during a certain portion of a viral infection, perhaps when the concentration of large T in cells is low. If this is the case, it may be difficult to observe a requirement for small t during infection of cultured cells if the concentration of large T in cells rises quickly.

In previous experiments, *trans* activation of SV40 early and late genes has been attributed solely to large T (3, 7, 8, 13, 32, 33). However, the involvement of small t, which was also present in these studies, in *trans* activation of the SV40

early and late promoters has not been examined. Large T clearly appears to be required for *trans* activation of the late promoter, since expression of small t alone is ineffective (19), and some mutations within large T that do not affect small t can completely block *trans* activation (33). This suggests that certain structures specific to large T are essential for *trans* activation of the SV40 late promoter. However, it is possible that small t participates with large T in the regulation of the SV40 late promoter. Whether small t alone affects the SV40 early promoter has not previously been examined. In the experiments presented here, we utilized plasmids encoding only large T or small t and found that small t can participate in the regulation of SV40 early and late promoters linked to chloramphenicol acetyltransferase (CAT) plasmids. Possible mechanisms that might explain how small t is involved in transcription regulation are discussed.

MATERIALS AND METHODS

Cell culture and transfections. CV-1 cells were cultured and transfected with calcium phosphate-coprecipitated DNA as described previously (19, 20), with the following modifications. Transfections for CAT enzyme and RNA assays were performed with cells cultured in 35-mm plates. Cells were transfected with 2 μg of CAT plasmids and, unless otherwise indicated, 2 μg of plasmids expressing large T, small t, or mutations. Calf thymus DNA (Clontech) was added to the DNA mixture to bring the total DNA concentration to 15 μg, a DNA concentration which we had determined to be optimal under our transfection conditions. For experiments in which CAT mRNA was to be assayed, six plates were transfected in parallel for each experimental condition. One plate was used for the CAT enzyme assay, and RNA was harvested from the remaining five plates, pooled, and assayed for CAT mRNA. For Western blot

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(immunoblot) analysis, 60-mm plates and a total DNA concentration of 40 μg were used. All plasmids used for transfections were purified by two successive gradient centrifugations in CsCl-ethidium bromide (21).

CAT assays. CAT assays were performed as described previously (20). Cell extracts were used at dilutions which would generate CAT activity within the linear range of enzyme activity (1 to 60%). Percent CAT activity is defined as the percent of total chloramphenicol which becomes acetylated in the enzyme reaction, and CAT activity is detected by separation of acetylated and unacetylated [^{14}C]chloramphenicol species by thin-layer chromatography. Each transfection was performed on at least three separate occasions with plasmid DNA from at least two separate preparations. CAT activity from a representative experiment is displayed.

RNA analysis. Total RNA was prepared with the commercial reagent RNAzol (Cinna Biotecx, Friendswood, Tex.), which involves a modified guanidinium thiocyanate-phenol extraction procedure (6). S1 analysis was performed as described previously (20) with 20 μg of whole-cell RNA by using M13-derived probes, with the following modifications. Probes were uniformly labeled by using 100 μCi of [^{32}P]dCTP (3,000 Ci/mmol) and 3.25 U of Sequenase version 2.0 T7 DNA polymerase (U.S. Biochemical Corp.) at 37°C for 30 min. Probes were liberated by digestion with HincII (M1310CAT2) or HindIII (M13mp19LN-CAT) and heating to 95°C for 3 min; digestion was followed by purification on a 5% polyacrylamide–8 M urea gel. Hybridization was performed at 42°C overnight with a 100,000-cpm probe.

Western blot analysis. A 125-μg sample of protein from each plate was applied to a 10% polyacrylamide-sodium dodecyl sulfate gel and analyzed as described previously (3). Large T and small t proteins were detected with monoclonal antibody Pab 419 (12) and alkaline phosphatase-linked goat anti-mouse immunoglobulin G (Promega).

Plasmid constructions. pRSV-T/t, which contains the entire SV40 early coding region linked to the Rous sarcoma virus long terminal repeat, was constructed by Bruce Howard and has been described previously (20). pRSV-TEX contains the SV40 large T cDNA linked to the Rous sarcoma virus long terminal repeat and has been described previously (19). pRSV-t/cDNA was constructed by inserting a HindIII fragment (SV40 nucleotides [nt] 4002 to 5171) from pW2-t/cDNA (kindly provided by Kathleen Rundell) into pRSV.3BglII (which was obtained from Eric Long and which has been described previously [19]). pRSV-T/t common (which expresses only the 82 amino-terminal residues which are common to both large T and small t) was constructed by inserting a BamHI fragment (corresponding to SV40 nt 2533 to 5151, in which the HindIII site at nt 5171 has been replaced with a BamHI site and valine 83 has been mutated to a termination codon) from pSV-T/t common (19) into pRSV.3BglII. pL16N-CAT contains SV40 control sequences and a mutant origin of replication with the late transcription start sites oriented toward the CAT coding sequence (14). It was kindly provided by James Alwine. pSV2CAT contains the SV40 control sequences with early transcription start sites oriented toward the CAT coding sequence (10). The M13-derived template used to synthesize a probe to assay CAT mRNA controlled by the SV40 early promoter (M1310CAT2) has been described previously (20). An M13-derived template used to synthesize a probe to assay CAT mRNA controlled by the SV40 late promoter (M13mp19LN-CAT) was constructed by inserting a HindIII-EcoRI fragment from pL16N-CAT into M13mp19.
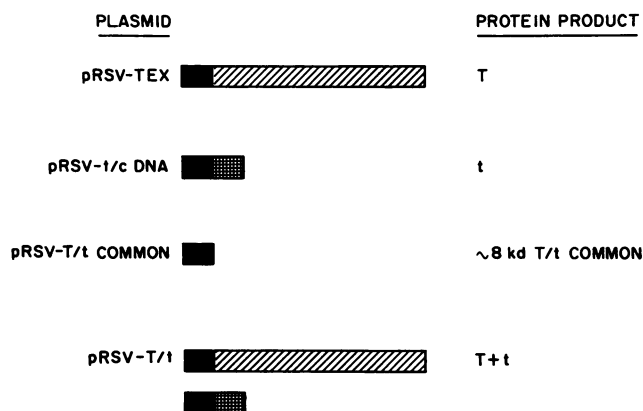

FIG. 1. Diagrams of protein products of large T and small t expression plasmids. The region of identity between large T and small t, the amino-terminal 82 residues, is indicated by the solid region, and the unique regions, which arise as a result of differential spice donor sites, are indicated by the diagonally hatched region (large T) and the cross-hatched region (small t). pRSV-T/t contains the entire early SV40 coding sequence, including splice donor and acceptor sites, and so expresses both large T and small t.

RESULTS

SV40 small t complements trans activation of the SV40 late promoter by limiting concentrations of large T. Plasmids containing cDNA sequences which would express only large T or only small t proteins were employed to test the ability of small t, either by itself or with large T, to affect CAT activity controlled by the SV40 late promoter (pL16N-CAT). The expression plasmids used to express large T, small t, or the 82-residue amino-terminal common domain are described in Materials and Methods, and the protein products of the expression plasmids are illustrated in Fig. 1. We first determined by Western blot analysis that increased amounts of transfected plasmid resulted in proportionate increases in steady-state levels of large T or small t protein (Fig. 2A). As displayed, all concentrations of transfected large T expression plasmid produced detectable levels of large T protein that increased as more plasmid was transfected. Small t protein was not detected following transfection of the two lowest concentrations of small t expression plasmid, but higher concentrations of plasmid produced proportionate increases in small t protein. We then examined whether various concentrations of large T and small t expression plasmids, alone or together, might affect CAT activity directed by the SV40 late promoter. As displayed in Fig. 2B, by using a fixed amount (2 μg) of pL16N-CAT and carrier DNA to keep total DNA concentrations constant, transfection of a plasmid expressing small t at two concentrations of DNA (0.1 and 5 μg) had no effect by itself on CAT activity driven by the late promoter. Indeed, in several experiments in which the small t expression plasmid was transfected over a wide range of concentrations (1 ng to 10 μg), no effect on CAT activity was seen (data not shown). Large T by itself, however, could activate the CAT plasmid driven by the late promoter when enough large T expression plasmid was transfected (Fig. 2B). Yet when large T and small t expression plasmids were cotransfected with the CAT plasmid linked to the late promoter, greater trans activation could be obtained than from large T alone. Indeed, in the presence of 1 μg of a plasmid expressing large T, a concentration which by itself was insufficient to cause a measurable effect,
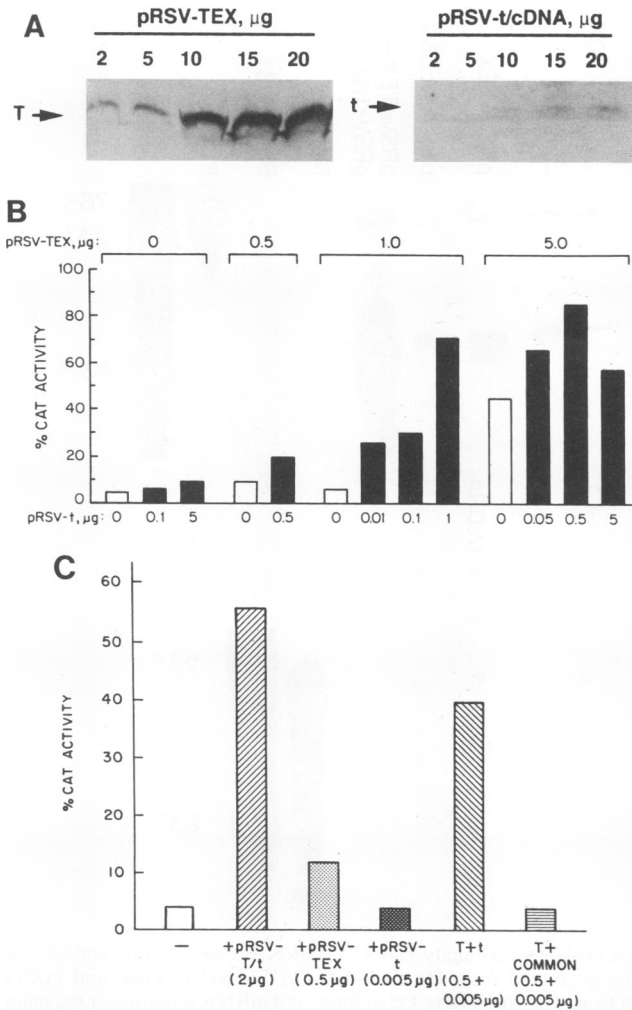
FIG. 2. (A) pRSV-TEX and pRSV-t/cDNA plasmids (plus carrier DNA) were transfected into CV-1 cells at the indicated concentrations. Cell extracts were analyzed by Western blot analysis as described in Materials and Methods. (B) CAT activity directed by the SV40 late promoter (pL16N-CAT) when cotransfected with pRSV-TEX, pRSV-t/cDNA, or both. CAT activity in the absence of small t is indicated by open bars, and that in the presence of small t is indicated by solid bars. The concentrations of transfected pRSV-TEX and pRSV-t/cDNA (pRSV-t) are indicated. The total DNA concentration was kept constant with carrier DNA as described in Materials and Methods. Transfection and CAT assay were performed as described in Materials and Methods. (C) Requirement for small t-unique sequences. pL16N-CAT was transfected alone (open bar) or with plasmids which express large T plus small t (pRSV-T/t), large T alone (pRSV-TEX), small t alone (pRSV-t/cDNA [pRSV-t]), large T plus small t (pRSV-TEX plus pRSV-t/cDNA [T+t]), or large T plus the 82-residue large T-small t common domain (pRSV-TEX plus pRSV-T/t common [T+ common]).
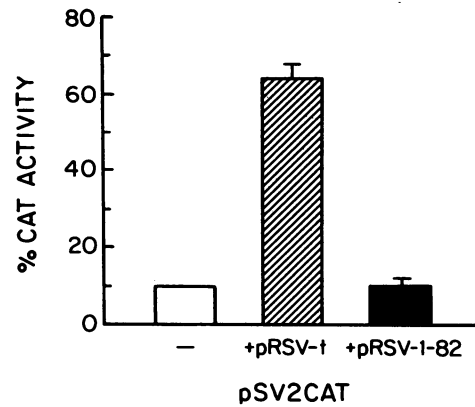

FIG. 3. CAT activity controlled by the SV40 enhancer/early promoter (pSV2CAT) when transfected alone (open bar) or with plasmids expressing small t (hatched bar) or the large T-small t 82-amino-acid common domain (solid bar). Results displayed are from a representative experiment in which plates were transfected in duplicate. CAT activity values represent the means and standard deviations of CAT activity levels from duplicate plates.

cotransfection with 0.01 to 1 μg of the small t expression plasmid produced markedly increased levels of CAT activity. In fact, in the presence of the highest concentration of the small t expression plasmid tested, there was 14-fold more CAT activity produced than that produced in response to the large T expression plasmid alone. In contrast, at the highest concentration of large T expression plasmid, CAT activity was stimulated 10-fold and little effect of cotransfected small t expression plasmid was observed. This result suggests that

small t can potentiate the ability of low concentrations of large T to *trans* activate the SV40 late promoter. At higher concentrations of large T, efficient *trans* activation can occur in response to large T alone and no contribution by small t can be measured. We refer to this effect of small t on *trans* activation of the SV40 late promoter by large T as a complementation effect, because it resembles small t's complementation of cellular transformation in the presence of limiting concentrations of large T (1, 2).

To determine whether this complementation of *trans* activation was due to a unique function of small t or to an increased cellular concentration of the 82-residue amino-terminal domain shared by large T and small t, we examined whether a plasmid encoding only the 82-amino-acid common domain under the control of the Rous sarcoma virus long terminal repeat (pRSV-1-82/common) could substitute for small t. In previous experiments, we have found that this coding sequence produces a stable protein which does not *trans* activate the adenovirus E2A promoter (19). As expected, a control plasmid which coexpresses both large T and small t directed by the Rous sarcoma virus long terminal repeat (pRSV-T/t) stimulated late-promoter-driven CAT activity approximately 13-fold (Fig. 2C). A limiting concentration (0.5 μg) of large T expression plasmid stimulated CAT activity twofold, but 5 ng of small t expression plasmid had no effect. When transfected together, 0.5 μg of large T expression plasmid and 5 ng of small t expression plasmid produced approximately 10-fold *trans* activation. However, when 5 ng of the plasmid expressing the 82-amino-acid large T-small t common domain was transfected along with 0.5 μg of the large T expression plasmid, no *trans* activation was observed. Therefore, it appears that the complementation effect requires sequences within the small t-unique domain.

SV40 small t increases gene expression linked to the SV40 early promoter. If small t is involved in the regulation of SV40 late genes, it is possible that it may affect transcription in the early direction as well. To test this, we examined the effect of small t on the activity of pSV2CAT, in which the CAT coding sequence is controlled by the SV40 enhancer and early promoter (10). We found that cotransfection of the plasmid expressing small t stimulated CAT activity five- to eightfold (Fig. 3). This stimulation required sequences within
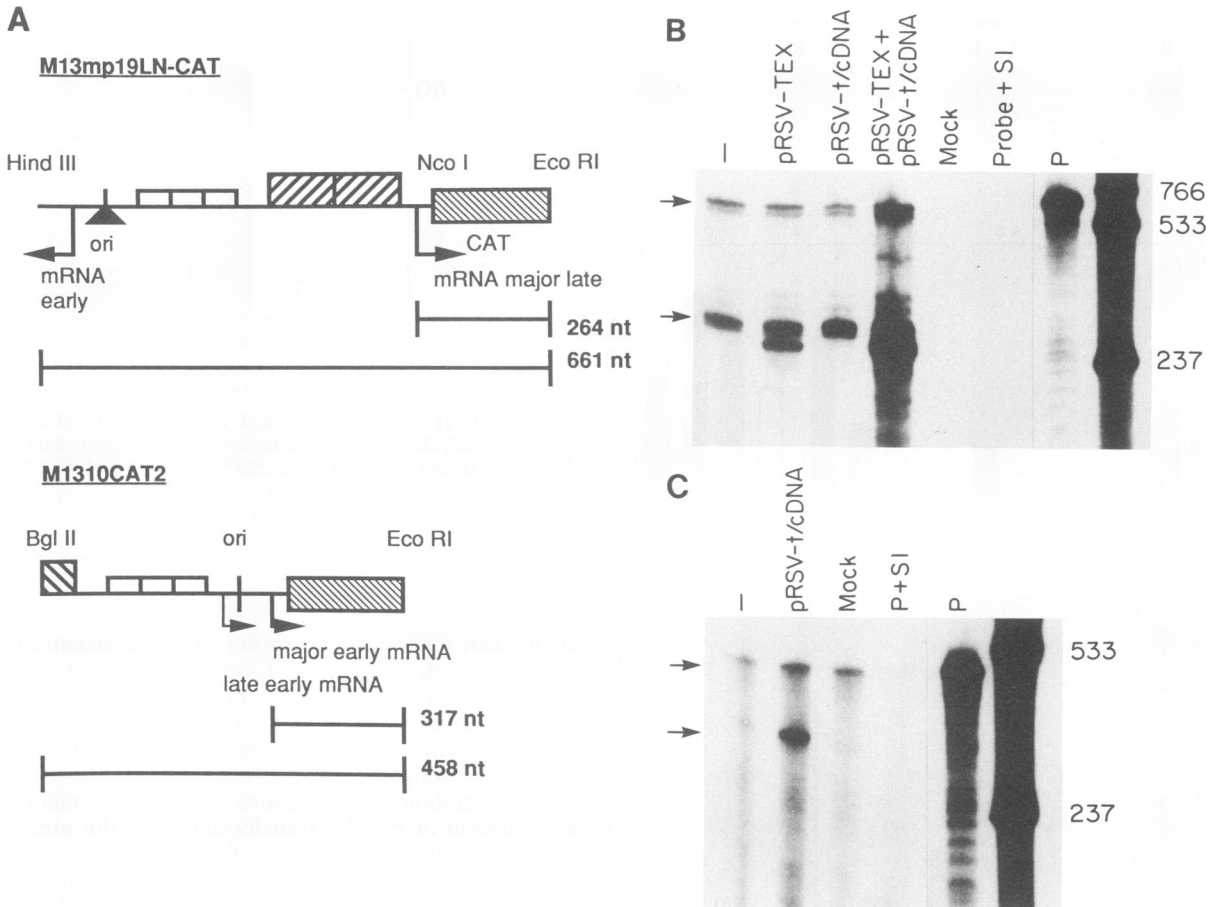
FIG. 4. (A) Diagrams of templates used to generate single-stranded DNA probes for S1 analysis of CAT mRNA. Construction and labeling of probes are described in Materials and Methods. As indicated, a probe generated from M13mp19LN-CAT is 661 nt long, and mRNA initiating at the major late start site would protect 264 nt. A probe generated from M1310CAT2 is 458 nt long, and mRNA initiating at the major early start site would protect 317 nt. (B) S1 analysis of CAT RNA controlled by the SV40 late promoter. S1 analysis was performed as described in Materials and Methods. RNA was assayed following transfection with 2 μg of pL16N-CAT alone (—) or with 1 μg of pRSV-TEX, 1 μg of pRSV-t/cDNA, or 1 μg each of pRSV-TEX and pRSV-t/cDNA. S1 analysis was performed with 20 μg of RNA from mock-transfected plates (Mock) and with 20 μg of tRNA (Probe + S1). Lane P contains 10,000 cpm (1/10 the amount added to hybridization reactions mixtures) of intact probe. The positions of the intact probe and probe protected by CAT RNA initiating in the absence of large T or small t are indicated by arrows. (C) S1 analysis of CAT RNA controlled by the SV40 early promoter. RNA was assayed following transfection with 2 μg of pSV2CAT alone (—) or with 2 μg of pRSV-t/cDNA. Mock and Probe + S1 lanes were prepared as for panel B. Lane P contains 10,000 cpm of intact probe (1/10 the amount added to hybridization reactions mixtures). The positions of the intact probe and the probe protected by CAT RNA are indicated by arrows.

the small t-unique domain, since expression of the 82-amino-acid large T-small t common domain did not elicit this effect.

Small t stimulation of CAT activities is reflected in stimulation of CAT mRNA levels. Although we previously demonstrated that effects of small t on *trans* activation of the adenovirus E2A promoter appear to be at the level of transcription (19), it was possible that effects on SV40-linked CAT activities could be also at the level of translation or selection of the site of transcription initiation. To address these possibilities, we examined the levels of CAT mRNA levels by S1 analysis with probes which would span the 5' ends of CAT mRNA initiating from SV40 early and late start sites. Diagrams of the M13 constructions used to generate these probes are shown in Fig. 4A. M13mp19LN-CAT contains a 661-nt *Hind*III (SV40 nt 5171)-*Eco*RI (256 nt into CAT coding sequences) fragment inserted into M13mp19. M1310CAT2 contains a 458-nt *Bgl*II (corresponding to the

*Sph*I site at SV40 nt 128)-*Eco*RI (256 nt into CAT) fragment derived from pA10CAT2 inserted into M13mp9 (20).

The results from S1 analysis of CAT mRNA transcribed from the SV40 late promoter are shown in Fig. 4B. With the late CAT probe, which is 661 nt long, CAT RNA initiating at the major late start site (SV40 nt 325) would protect a 264-nt fragment. Transcription under basal conditions appeared to initiate at two closely located sites. When cotransfected with a limiting concentration of large T expression plasmid, the same two start sites and an additional downstream start site were observed. There was no effect of small t alone on the level or the start site of the CAT RNA. However, cotransfection with both of the plasmids expressing large T and small t increased by approximately three- to fivefold the levels of all mRNA species observed in the presence of large T alone. In addition to the initiation sites detected in the vicinity of the major late start site, there also appeared to be

transcripts which resulted in two S1-resistant bands which were approximately the same length as the intact probe. These were most likely produced as a result of read-through transcripts and/or transcripts initiating near the origin of replication, since they were not detected in the mock or probe plus S1 control lanes, and increased in amount under conditions in which the late promoter was most potently *trans* activated. Thus, it appears that the effect of small t of complementing large T *trans* activation of the SV40 late promoter is not at the level of translation. Since the mRNA species produced in the presence of small t plus large T initiate at the same sites as those stimulated by large T alone, it appears that small t may potentiate the ability of large T to *trans* activate the SV40 late promoter.

With regard to the SV40 early promoter, a 458-nt probe was generated, 317 nt of which would be protected by CAT mRNA initiating at the SV40 major early start site. As shown in Fig. 4C, a single band of approximately 317 nt is protected by RNA produced by the SV40 early CAT plasmid transfected alone. When cotransfected with the small t expression plasmid, a significant increase in the level of the mRNA initiating at the same start site was observed. Again, it appears that the stimulation of CAT activity linked to the SV40 early promoter by small t is not due to translation or change of site of mRNA initiation but may be at the level of transcription.

DISCUSSION

Our previous observation that SV40 small t could *trans* activate the adenovirus E2A and VA-I promoters prompted us to examine whether this function of small t might affect expression of SV40 genes. Whereas there is not a strict requirement for small t during infection of cultured cells, in some cell types SV40 mutants expressing defective small t proteins grow slowly (28, 29). We considered that there might be many ways, both direct and indirect, by which a *trans* activation function by small t could assist viral infection. For example, small t might *trans* activate cellular genes with promoter elements similar to the E2A or VA-I promoters. These cellular gene products could affect transcription of SV40 genes. In this regard, it is possible that small t is involved in the induction by SV40 early genes of mRNA for Sp1, a transcription factor that participates in regulation of the SV40 early promoter (25). It is also possible that small t activates cellular genes whose products promote progression of the lytic cycle or viral particle assembly. Finally, small t may associate with transcription factors, or proteins that control transcription factors, which regulate the SV40 early and late promoters. For example, it has been reported that small t associates with the cellular enzyme type 2A phosphatase (24, 31). Type 2A phosphatase appears to be an important component for SV40 DNA replication reactions in vitro (16, 30), and it may control the level of large T phosphorylation and subsequent DNA binding during SV40 replication (23, 27). Furthermore, since small t appears to inhibit type 2A phosphatase activity (26), it is possible that small t may affect the phosphorylated state of large T or cellular transcription and replication factors. Thus, small t could associate with a complex that simultaneously regulates SV40 replication and early and late gene transcription.

We had previously found that small t by itself did not stimulate CAT activity linked to the SV40 late promoter but that large T by itself did (19). Therefore, we postulated that if *trans* activation by small t played a role in regulation of the late promoter, it might require the presence of large T for

activity. As reported here, this indeed was the case. Furthermore, an effect of small t was observed only when the concentration of the large T plasmid used was insufficient to stimulate the SV40 late promoter by itself. In this respect, the potentiation of the effect of large T on *trans* activation resembles the small t complementation effect on transformation by suboptimal levels of large T (1, 2). It is interesting to speculate that the similarities between the complementation of *trans* activation and transformation may indicate that during cellular transformation, small t interacts with some cellular proteins which are also involved in activation of the SV40 late promoter. In both cases, small t increases the efficiency of large T activities, perhaps by producing a specific posttranslational form of large T or by activating cellular functions more efficiently than does large T alone.

It should be noted that the helper effect of small t on activation of the SV40 late promoter was not dramatic. The greatest effect we observed of small t was about 14-fold, but it routinely was only about 4-fold. We can only speculate that in an infected animal, the rate of accumulation of large T might be such that assistance from small t may be advantageous to successful infection. During infection of cell culture, however, large T levels may rise quickly enough that a requirement for small t would not be observed. This may explain why an obvious effect of small t during SV40 viral infection in tissue culture has not been apparent in previous studies.

Wildeman has observed that coexpressed large T and small t can *trans* activate the SV40 early promoter in addition to *trans* activating the late promoter (32). We presented evidence here that small t by itself can increase SV40 early gene expression and that this effect was dependent on small t structures in its unique domain. We considered that an effect of small t on early-promoter-directed CAT activity might not be due to an increase in the rate of transcription but could be due to increased utilization of the SV40 early-early, or major early, transcription start site. It has previously been suggested that if transcription initiates at start sites upstream of the early-early start site, translation initiating at the downstream AUG (that used to synthesize CAT enzyme) might not occur (5, 9, 11, 15). However, S1 analysis demonstrated that the transcription start site did not change in the presence of small t but the abundance of the CAT mRNA did increase. This result indicates that the effects of small t on early gene expression are not at the level of translation or determination of the mRNA initiation site but may be at the rate of transcription. The importance of stimulation of SV40 early gene expression is unclear. During infection, large T binds to SV40 DNA, inhibits expression of the early genes, stimulates replication, and activates late gene expression (reference 17 and references therein). It is possible that if early gene expression were shut off too soon, there would not be enough large T protein produced to continue replication of SV40 DNA. Small t may prevent the total shutoff of the early genes to allow continued synthesis of adequate levels of large T.

The experiments presented here provide evidence that SV40 small t can regulate SV40 early and late promoters in transfected cells. Although small t does not appear to be essential for infection of cultured cells, it is possible that papovavirus small t structures evolved to provide a *trans* activation function which may be important during infection in animals. The complementation of SV40 late gene expression by small t bears remarkable similarities to its transformation helper effect. In both cases, small t is inactive on its own but potentiates the effects of suboptimal levels of large

T on these events. It is possible that proteins which mediate small t effects on transcription are also involved in cell cycle control and may become deregulated during cellular transformation.

REFERENCES

1. Bikel, I., H. Mamon, E. L. Brown, J. Boltax, M. Agha, and D. M. Livingston. 1986. The t-unique coding domain is important to the transformation maintenance function of the simian virus 40 small t antigen. Mol. Cell. Biol. 6:1172–1178.
2. Bikel, I., X. Montano, M. E. Agha, M. Brown, M. McCormack, J. Boltax, and D. M. Livingston. 1987. SV40 small t antigen enhances the transformation activity of limiting concentrations of SV40 large T antigen. Cell 48:321–330.
3. 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.
4. Brown, M., M. McCormack, K. G. Zinn, M. P. Farrell, I. Bikel, and D. M. Livingston. 1986. A recombinant murine retrovirus for simian virus 40 large T cDNA transforms mouse fibroblasts to anchorage-independent growth. J. Virol. 60:290–293.
5. Buchman, A. R., M. Fromm, and P. Berg. 1984. Complex regulation of simian virus 40 early-region transcription from different overlapping promoters. Mol. Cell. Biol. 4:1900–1914.
6. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162:156–159.
7. Gallo, G. J., G. Gilinger, and J. C. Alwine. 1988. Simian virus 40 T antigen alters the binding characteristics of specific simian DNA-binding factors. Mol. Cell. Biol. 8:1648–1656.
8. 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.
9. Ghosh, P. K., and P. Lebowitz. 1981. Simian virus 40 early mRNA's contain multiple 5' termini upstream and downstream from a Hogness-Goldberg sequence; a shift in 5' termini during the lytic cycle is mediated by large T antigen. J. Virol. 40:224–240.
10. Gorman, C. M., L. F. Moffat, and B. H. Howard. 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. 2:1044–1051.
11. Hansen, U., D. G. Tenen, D. M. Livingston, and P. A. Sharp. 1981. T antigen repression of SV40 early transcription from two promoters. Cell 27:603–612.
12. 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.
13. 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.
14. 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.
15. Khalili, K., J. Brady, and G. Khoury. 1987. Translational regulation of SV40 early mRNA defines a new viral protein. Cell 48:639–645.
16. Lawson, R., P. Cohen, and D. P. Lane. 1990. Simian virus 40 large T-antigen-dependent DNA replication is activated by protein phosphatase 2A in vitro. J. Virol. 64:2380–2383.
17. Livingston, D. M., and M. K. Bradley. 1987. The simian virus 40 large T antigen: a lot packed into a little. Mol. Biol. Med. 4:63–80.
18. Loeken, M. R. Simian virus 40 small t antigen trans activates the adenovirus E2A promoter by using mechanisms distinct from those used by adenovirus E1A. J. Virol., in press.
19. Loeken, M. R., 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.
20. 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.
21. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. Martin, R. G., V. P. Setlow, C. A. F. Edwards, and D. Vembu. 1979. The roles of the simian virus 40 tumor antigens in transformation of Chinese hamster lung cells. Cell 17:635–643.
23. Mohr, I. J., B. Stillman, and Y. Gluzman. 1987. Regulation of SV40 DNA replication by phosphorylation of T antigen. EMBO J. 6:153–160.
24. Pallas, D. C., L. K. Shahrik, B. L. Martin, S. Jaspers, T. B. Miller, D. L. Brautigan, and T. M. Roberts. 1990. Polyoma small and middle T antigens and SV40 small t antigen form stable complexes with protein phosphatase 2A. Cell 60:167–176.
25. 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.
26. Scheidtmann, K. H., M. C. Mumby, K. Rundell, and G. Walter. 1991. Dephosphorylation of simian virus 40 large-T antigen and p53 protein by protein phosphatase 2A: inhibition by small-t antigen. Mol. Cell. Biol. 11:1996–2003.
27. Scheidtmann, K. H., D. M. Virshup, and T. J. Kelly. 1991. Protein phosphatase 2A dephosphorylates simian virus 40 large T antigen specifically at residues involved in regulation of DNA-binding activity. J. Virol. 65:2098–2101.
28. 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.
29. Topp, W. C. 1980. Variable defectiveness for lytic growth of the dl 54/59 mutants of simian virus 40. J. Virol. 33:1208–1210.
30. Virshup, D. M., M. G. Kauffman, and T. J. Kelly. 1989. Activation of SV40 DNA replication in vitro by cellular protein phosphatase 2A. EMBO J. 8:3891–3898.
31. Walter, G., R. Ruediger, C. Slaughter, and M. Mumby. 1990. Association of protein phosphatase 2A with polyoma virus medium tumor antigen. Proc. Natl. Acad. Sci. USA 87:2521–2525.
32. Wildeman, A. G. 1989. Transactivation of both early and late simian virus 40 promoters by large tumor antigen does not require nuclear localization of the protein. Proc. Natl. Acad. Sci. USA 86:2123–2127.
33. 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.