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Simian Virus 40 (SV40) T-Antigen Transcriptional Activation Mediated through the Oct/SPH Region of the SV40 Late Promoter

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Simian virus 40 (SV40) large T antigen is a promiscuous transcriptional activator of many viral and cellular promoters. The SV40 late promoter, a primary target for T-antigen transcriptional activation, contains a previously described T-antigen-activatable binding site (SV40 nucleotides 186 to 225). The T-antigen-activatable binding site element contains overlapping octamer (Oct)- and SPH (TEF-1)-binding sites (Oct/SPH site). Using this Oct/SPH site as an upstream element in a simple promoter, we show that the SPH sites are necessary for transcriptional activation by T antigen. In addition, we show that when Oct 1 is overproduced, it can eliminate T-antigen-mediated transcriptional activation, as well as basal activity, from the simple Oct/SPH promoter as well as the intact SV40 late promoter. This suggests that one function of T antigen in transcriptional activation of the late promoter is to alter factor binding at the Oct/SPH region to favor binding of factors to the SPH sites.

The simian virus 40 (SV40) early gene product, large T antigen, is a potent viral oncoprotein that interacts with at least five different host proteins, is posttranslationally modified in at least seven different ways, and is capable of a large array of biochemical functions (18). One of these functions is the transcriptional activation of both viral and cellular promoters. This function was first detected through studies of the activation of the SV40 late promoter at the appropriate time during lytic infection (5, 15). The SV40 late genes, which encode the virion structural proteins, remain silent during the early phase of infection. A signal to increase the transcription of the late genes is mediated by T antigen through transcriptional activation of the late promoter (5, 15). Besides the viral late promoter, large T antigen also activates many cellular promoters (1). This promiscuous activation is believed to account, at least in part, for the dramatic alterations in cellular gene expression seen during lytic infection and transformation.

The late promoter has been used as a model system for the analysis of large T-antigen-mediated transcriptional activation. However, the promoter has proven to be very complex. It has no classical TATA element (23), and studies from many laboratories have identified a number of elements in the late promoter which affect its activity (3, 4, 6, 10–13, 16, 17, 21). The definition of so many elements is, in large part, a reflection of the various approaches used by different investigators to examine the late promoter. These variations appear to highlight different facets of either the basal promoter or the promoter active in the presence of T antigen.

We and others (11, 12, 16, 21) have found that mutations in a region of the late promoter from nucleotides 185 to 230 cause a loss in the ability of the late promoter to be activated by T antigen. We call this region TABS for (T-antigen-activatable binding site) because it is recognized by a cellular factor, the band A factor, whose binding characteristics are modified in the presence of T antigen (11, 12). In our studies of T-antigen transcriptional activation we have concluded that T antigen does not need to interact directly with the DNA to cause activation (11, 12, 16). Instead, it functions through the utilization of cellular transcription factors. Hence, these data suggest that T antigen may function through modification or induction of factors binding to TABS.

The TABS element consists of two octamer factor (Oct)-binding sites (OBS I and II [24]), two SPH-binding sites overlapping one of the Oct sites (not to be confused with the restriction enzyme, SPH sites are the binding sites for TEF-I [8]), and an AP-1 site. This complexity of TABS has hindered definition of the promoter elements required for T-antigen transcriptional activation. For this reason we analyzed wild-type and mutant forms of the Oct/SPH region in a simple promoter to determine the specific elements required for T-antigen transcriptional activation. We found that positive and negative effects can be exerted through the overlapping elements in TABS.

MATERIALS AND METHODS

Plasmids and cells. pβ6X20 is a simple promoter plasmid containing the β-globin TATA element with upstream elements consisting of six repeats of the B20 oligomer (Fig. 2). Mutants of pβ6X20, dpm2, dpm7, and dpm8, have specific point mutations in the B20 oligomer (25). pα4X(A+C) is an α-globin RNA-expressing plasmid (26), which is used as an internal control for transfection experiments with pβ6X20, dpm2, dpm7, and dpm8. pRSV-TEX contains the large T-antigen cDNA under the transcriptional control of the Rous sarcoma virus long terminal repeat (19); its parent plasmid, pRSV3-*Bgl*II, contains no T-antigen gene and serves as a filler plasmid in transfection experiments. pCGoct1$^+$ (26) and pOEV1$^+$ (22) express Oct 1 and Oct 2, respectively, under the control of the cytomegalovirus immediate early promoter. A filler plasmid, pOE, used in transfections with the octamer-expressing plasmids, has the Oct 2 sequences removed from pOEV1$^+$. This was accom-

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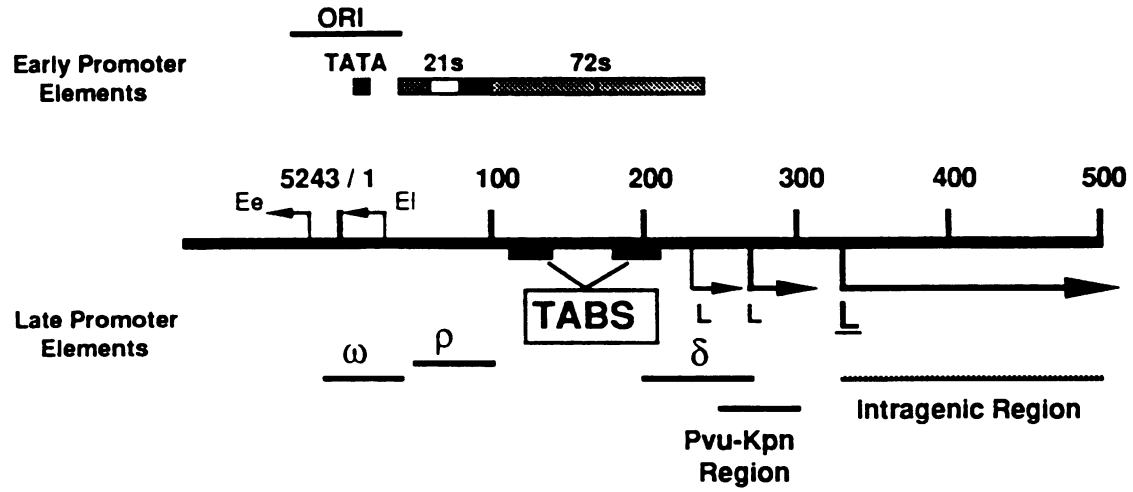

FIG. 1. Promoter region of SV40. Both the early and late promoters are indicated, as well as the origin of replication (ORI). The regions of the late promoter (omega [ω], rho [ρ], TABS, delta [δ], *Pvu-Kpn*, and the intragenic region) are described in the text; all have been shown to affect either the basal late promoter or the late promoter active in the presence of T antigen. The TABS region has been characterized in this laboratory (11, 12, 15) as a significant binding site for cellular factors through which T antigen mediates transcriptional activation.

plished by cleavage with *Kpn*I and religation of the vector fragment. pMSVP16 expresses the herpesvirus immediate-early gene activator protein VP16 under the control of the murine sarcoma virus long terminal repeat. pL16n-cat is a chloramphenicol acetyltransferase (CAT) transient-expression vector containing the late promoter of SV40 (15); the promoter region extends from SV40 nucleotide 5171 through the SV40 origin of replication to nucleotide 333. The SV40 origin of replication has been mutated so that the plasmid cannot replicate in eucaryotic cells in the presence of T antigen. All plasmids were propagated in *Escherichia coli* HB101.

The cell line used for transfection experiments was the established African green monkey kidney line CV-1. Cells were propagated in Dulbecco's minimal essential medium supplemented with 7.5% fetal bovine serum at 37°C in 5% CO_2.

Transfections. Cells (3×10^5 to 5×10^5) were plated and grown overnight. Monolayers at approximately 80% confluence were transfected by using the calcium phosphate procedure, except that *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)-buffered phosphate was replaced with 1× BES (*N,N'*-bis[2-hydroxyethyl]-2-amino ethanesulfonic acid)-buffered phosphate (25 mM BES [pH 6.95], 140 mM NaCl, 0.75 mM Na_2HPO_4) (7). At 40 h after transfection, the cells were harvested for RNA or CAT analysis as described below.

For all transfections, the quantity and quality of the DNA was normalized such that specific sequences or promoters would not vary between samples (see the legend to Fig. 3A). Transfections were done several times to ensure accuracy.

RNA preparation and analysis. Total cellular RNA was prepared as previously described (27). RNase protection assays were performed as previously described (2). Mixed probes of ^{32}P-labeled α- and β-globin antisense RNA were prepared by transcription from linearized pSP6α132 and pSP6β350 (26). The α- and β-globin probes were 220 and 700 nucleotides, respectively. Properly initiated α- and β-globin RNAs protected probe fragments of 132 and 350 nucleotides, respectively (25). Results were quantitated by Phosphorimager (Molecular Dynamics) analysis.

CAT analysis. Cells were harvested as previously described (15), and cell pellets were sonicated in 100 μl of 250 mM Tris-HCl (pH 7.8). Cell debris were removed by centrifugation, and CAT enzyme activity was determined by either the method described by Gorman et al. (14) or the diffusion-based assay (9).

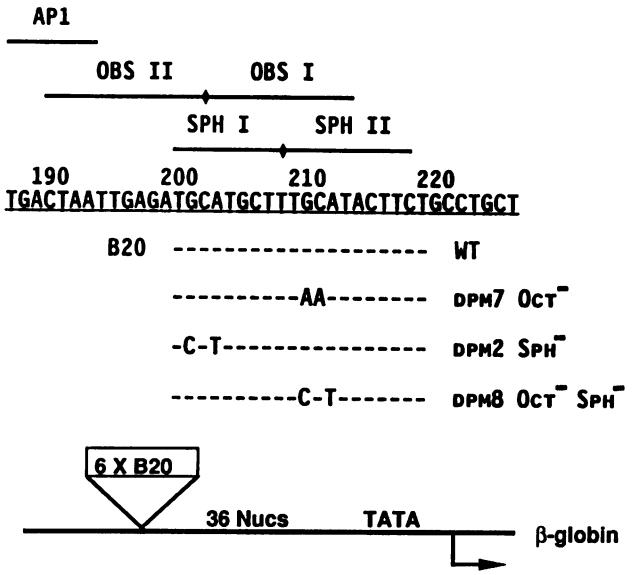

FIG. 2. Sequences of the TABS region and its relationship to the B20, dpm2, dpm7, and dpm8 promoter inserts. The TABS elements includes an AP-1-binding site, two Oct-binding sites (OBS I and II), and two SPH motifs (SPH I and II). The dashed lines below indicate the bases included in the B20 oligomers and mutant oligomers, dpm2, dpm7, and dpm8 (25, 26). The diagram at the bottom of the figure indicates the construction of the test gene by using the wild-type or mutant B20 elements. It contains six tandem copies of the B20 oligomer (or the mutants) upstream of the β-globin TATA element and the β-globin gene.

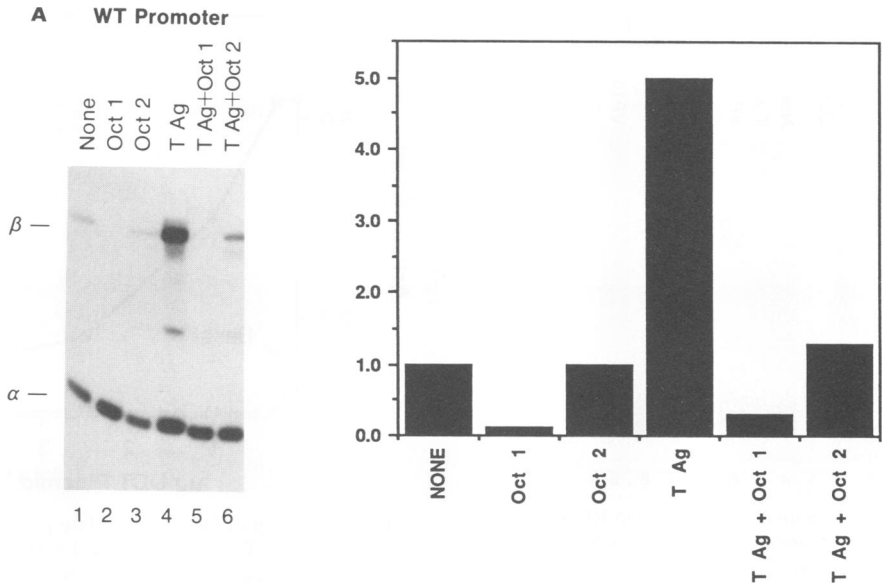

FIG. 3. Nuclease protection analysis and quantitation of β-globin RNA produced by the B20 promoter under a variety of conditions. A) The wild-type B20 promoter plasmid (4 μg) was transfected into CV-1 cells by using the $CaHPO_4$ precipitation technique as described in Materials and Methods. Each transfection also included 2 μg of an α-globin-expressing plasmid as an internal transfection control. A variety of other plasmids were transfected as described below for each lane. Total RNA was harvested 42 h after transfection and analyzed by nuclease protection with ^{32}P-labeled RNA probes for β- and α-globin (see Materials and Methods). Lanes: 1, wild-type (WT) B20 promoter plus 3 μg of the pOE filler plasmid; 2, WT B20 promoter plus 3 μg of Oct 1-expressing plasmid, pCGoct1$^+$; 3, WT B20 promoter plus 3 μg of the Oct 2-expressing plasmid, pOEV1$^+$; 4, WT B20 promoter plasmid plus 3 μg of the large T-antigen-expressing plasmid, pRSV-TEX; 5: WT B20 promoter plasmid plus 3 μg of the large T-antigen-expressing plasmid, pRSV-TEX, and 3 μg of the Oct 1-expressing plasmid, pCGoct1$^+$; 6, WT B20 promoter plasmid plus 3 μg of the large T-antigen-expressing plasmid, pRSV-TEX, and 3 μg of the Oct 2-expressing plasmid, pOEV1$^+$. Each transfection was controlled such that the quantity and type of plasmid DNA was equivalent for each transfection; for example, when the Oct 1-expressing plasmid was used, all other transfections contained the parent plasmid, which does not contain the Oct 1 gene. This ensured that inhibition was not due to competition by the promoter of the T antigen- or octamer-expressing plasmids. (B) The data in panel A were quantitated by using a Molecular Dynamics Phosphorimager, and the amount of β-globin RNA was normalized to the level of α-globin RNA in each sample. The results are shown relative to the level of the wild-type B20 element alone. The effects of T antigen on the levels of α-globin RNA have not been considered here (see text). Abbreviation: T Ag, T antigen.

RESULTS

Large T antigen requires the SPH sites within the TABS region for transcriptional activation. Figure 1 shows a diagram of the SV40 promoter region. Elements of the leftward-transcribing early promoter and the rightward-transcribing late promoter overlap. Work from many laboratories has suggested that there are a number of late promoter elements. Shown are the omega or origin (Ori) region, the rho or 21-bp repeat region, the TABS region, the delta region, the *Pvu-Kpn* region, and an intragenic region (3, 4, 6, 10–13, 16, 17, 21). The region of most interest for the present studies is the TABS region (described in the Introduction).

The TABS region (Fig. 2, top) contains the potential binding sites for AP-1, TEF-1 (which binds to the SPH motifs [25]), and octamer-binding proteins. One octamer site partially overlaps an AP-1 site and the other octamer site overlaps the two SPH motifs. Previous methylation interference footprints in the TABS region (12) suggested that proteins bound to the octamer site might be important targets for T-antigen transactivation (see also Discussion). However, the complexity of overlapping Oct- and SPH-binding sites in the TABS region suggests that multiple interactions may be occurring. To examine the function of the Oct/SPH region of TABS more precisely, we used a simple promoter construction consisting of the β-globin TATA element situated downstream of six repeated B20 Oct/SPH oligomers (25, 26). The sequence of an individual B20 oligomer is indicated by the dashed line in Fig. 2; it represents one Octamer-binding site (OBS I) and the overlapping SPH sites of the TABS. As shown at the bottom of Fig. 2, the B20 promoter directs transcription of the β-globin gene.

The B20 promoter plasmid was transfected into CV-1 cells with and without a cotransfected plasmid which expressed large T antigen. Figure 3A shows results of nuclease protection experiments performed to analyze the β-globin RNA produced by the wild-type B20 promoter. Comparison of lanes 1 and 4 shows that large T antigen causes a marked stimulation of the B20 promoter. A transfection efficiency control plasmid expressing α-globin RNA (26) was also included in each transfection. We have observed that the promiscuous activation mechanism mediated by T antigen had a small stimulatory effect on the α-globin promoter (two- to threefold). This effect must be considered when comparing the normalized quantitative data of β-globin levels with α-globin levels in Fig. 3B for the determination of activation or inactivation. Therefore, the stimulation of the wild-type B20 promoter by T antigen may actually be closer to 10- to 12-fold. In agreement with previous reports (26), the overexpression of Oct 1 did not affect α-globin expression under our conditions. All experiments were performed at least twice to confirm the results. These data show that the B20 region is a significant upstream element for T-antigen-mediated transcriptional activation from a simple promoter.

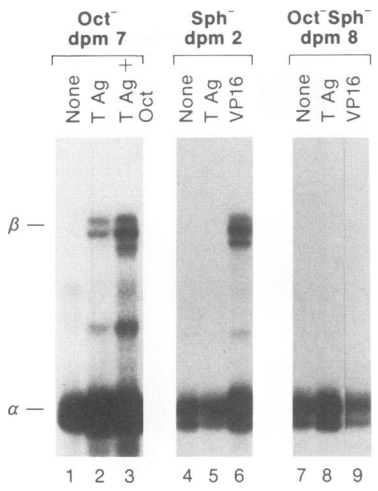

FIG. 4. Nuclease protection analysis of β-globin RNA produced by the mutant B20 promoters under a variety of conditions. CV-1 cell transfections and RNA analysis were as described in the legend to Fig. 3. Lanes: 1, 3 μg of dpm7 promoter plasmid plus 3 μg of filler plasmid, pOE; 2, 3 μg of dpm7 promoter plasmid plus 3 μg of the large T-antigen-expressing plasmid, pRSV-TEX; 3: 3 μg of dpm7 promoter plasmid plus 3 μg of the large T-antigen-expressing plasmid, pRSV-TEX, and 3 μg of the Oct 1-expressing plasmid, pCGoct1⁺; 4, 3 μg of dpm2 promoter plasmid plus 3 μg of filler plasmid, pOE; 5, 3 μg of dpm2 promoter plasmid plus 3 μg of the large T-antigen-expressing plasmid, pRSV-TEX; 6, 3 μg of dpm2 promoter plasmid plus 3 μg of the VP16-expressing plasmid, pMSVP16; 7, 8, and 9, same as lanes 4, 5, and 6, except that 3 μg of the dpm8 promoter plasmid was used. As explained in the legend to Fig. 3, each transfection was controlled such that the quantity and type of plasmid DNA were equivalent for each transfection. Abbreviation: T Ag, T antigen.

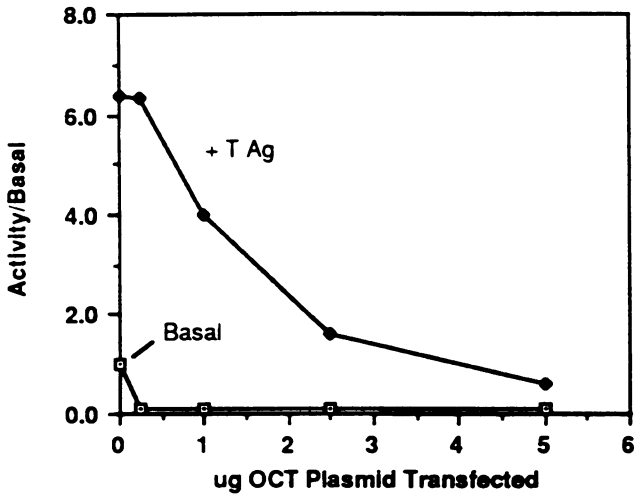

FIG. 5. Effect of Oct 1 on the late promoter in the presence and absence of large T antigen. CV-1 cells were transfected by using the $CaHPO_4$ technique. Two different sets of transfection mixes were prepared; each contained 4 μg of the late promoter plasmid, pL16n, and a variable amount (0, 0.25, 1, 2.5, and 5 μg) of the Oct 1-expressing plasmid, pCGoct1⁺. In each case the difference between the amount of pCGoct1⁺ and 5 μg was made up with the parent plasmid of pCGoct1⁺, which contains all the same sequences but lacks the Oct gene. One set of transfection mixes contained 6 μg of the T-antigen-expressing plasmid, pRSV-TEX; the other set contained 6 μg of an identical plasmid but lacking the T-antigen gene. Cells were harvested 40 h after transfection and assayed for CAT activity. Similar results were seen in multiple experiments.

In addition to the wild-type B20 promoter, identically constructed promoters (25, 26) containing three mutant forms of the B20 element were tested (Fig. 2). In mutant dpm7, the Oct function has been inactivated but function mediated through the SPH sites remains intact. Mutant dpm2 eliminates the function of the SPH sites but Oct function remains intact. Mutant dpm8 eliminates both SPH and Oct functions.

The mutant B20 promoter plasmids were transfected into CV-1 cells with and without the large T-antigen-expressing plasmid. Figure 4 shows the nuclease protection analysis of the resulting β-globin RNA. The Oct⁻ mutant dpm7 can still be activated in the presence of large T antigen (lanes 1 and 2). However, with the SPH⁻ mutant, dpm2, T antigen can no longer activate transcription (lanes 4 and 5). Cotransfection of a plasmid expressing the herpesvirus transactivating protein VP16 results in transcriptional activation of dpm2 (lane 6). This confirms that the Oct site is functional in dpm2, since VP16 functions through interaction with the octamer protein bound to DNA. Mutation of both Oct and SPH functions, as in dpm8, results in no activation by either large T antigen or VP16 (lanes 7 to 9). These data strongly suggest that within the overlapping Oct/SPH region, the factors binding to the SPH sites, not those binding to the overlapping Oct site, are important for large T-antigen transcriptional activation.

Octamer factor expression negatively affects TABS element transcriptional activity. It has been previously observed that the basal activity of the B20 promoter is eliminated when a plasmid expressing Oct 1 is cotransfected (26). This result is shown in Fig. 3A, in which cotransfection of an Oct 1-expressing plasmid eliminates basal promoter activity (compare lanes 1 and 2). Interestingly, cotransfection of the Oct 1-expressing plasmid eliminates the transcriptional activation mediated by T antigen (compare lanes 4 and 5). A similar experiment with Oct 2 showed only a small decrease in basal promoter activity (compare lanes 1 and 3), but still showed a marked diminution of T-antigen activation (compare lanes 4 and 6). These data indicate that binding of a factor to the Oct sites can block the effect of transcriptional activation mediated through the overlapping SPH sites. This is supported by the observation that overexpression of Oct 1 does not decrease T-antigen-mediated transcriptional activation under conditions where the Oct site is mutated, as in mutant dpm7 (Fig. 4, lane 3). The apparent increase in activity seen in this experiment was not consistently observed.

We asked whether this negative effect of overexpression of Oct 1 could be seen in the context of the full late promoter. The late promoter-CAT plasmid, pL16n (16), was used in these experiments. It contains the SV40 late promoter sequences between nucleotides 5171 and 333 (through the origin of replication [Fig. 1]), attached to CAT. The SV40 origin of replication has been mutated such that in the presence of T antigen, plasmid replication cannot occur. pL16n, either alone or with a T-antigen-expressing plasmid, was transfected into CV-1 cells along with increasing amounts of the Oct 1-expressing plasmid. The quantity and quality of plasmid were equalized in all transfections. Figure 5 shows that the basal activity of the late promoter is eliminated at very small amounts of transfected Oct 1-expressing plasmid. In the presence of T antigen, the activity of

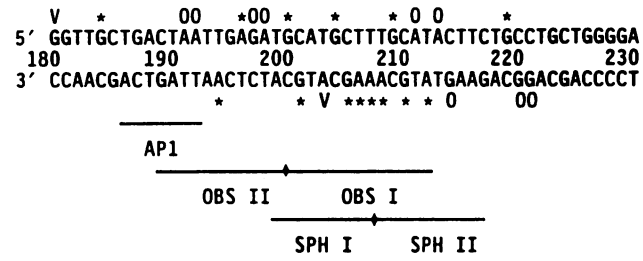

FIG. 6. Methylation interference footprint over the TABS region produced by crude nuclear extracts from either COS or CV-1 cells. Symbols: *, bases where interference was noted in both CV-1 and COS cell extracts; V, bases which interfered only in CV-1 extracts; O, bases which interfered only in COS extracts. Data are from reference 12.

pL16n was increased between six- and sevenfold in the absence of Oct 1. However, overexpression of Oct 1 eliminated T-antigen-mediated transcriptional activation.

DISCUSSION

Our data suggest that T-antigen transcriptional activation of the late promoter is mediated through factors that bind to the SPH sites, possibly TEF-1 (8). This activation could occur by a number of mechanisms. One possibility is that T antigen acts to increase the activation potential of TEF-1. However, our analysis of the TABS region also suggests that the overlapping nature of Oct/SPH sites may provide a positive and negative control mechanism for late promoter activity. The factors binding the SPH sites impart a positive effect mediated by the transcriptional activation function of T antigen. In addition, our data suggest, but do not prove, that Oct factor binding may impart a negative effect on the late promoter. We have observed that overproduction of Oct 1 will repress basal activity as well as T-antigen-mediated transcriptional activation of both the B20 promoter and the late promoter. Mutation of the Oct site, as in the dpm7 promoter, does not cause dramatic up regulation, suggesting that T antigen may function to increase the ability of factors binding the SPH sites to recognize these sites. However, since the dpm7 mutation weakens, but does not abolish, Oct-1 binding in gel retardation assays (20, 25), we cannot rule out the possibility that T antigen plays an additional role in the active displacement of octamer factors from the Oct sites. That factor induction or modification may occur is suggested by our previous studies of TABS-binding factors (11, 12). Figure 6 shows a summary of previously presented methylation interference footprints of factor binding over the TABS region mediated by crude extracts from CV-1 and COS cells (12). An asterisk indicates nucleotides which are detected in the footprint from each extract, an O indicates nucleotides unique to the footprint produced by COS extract, and a V indicates nucleotides unique to the footprint produced by the CV-1 extract. COS cell extracts produce a more complicated footprint with changes in the upstream Oct site and striking changes in the SPH region. In particular, the interference by the G at nucleotide 214 is specific for the SPH-binding factor TEF-I (8). These data suggest that binding at the SPH sites is more favored in the T-antigen-containing COS cells than in the parent cell, CV-1.

In conclusion, the data presented indicate that the TABS element appears to be an upstream element for the SV40 late promoter through which positive effects of T antigen are mediated by factors binding at the SPH sites, possibly TEF-1. In addition, negative effects may occur through octamer factor binding at the overlapping Oct site.

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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. **Ausubel, F. A., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.).** 1990. Current protocols in molecular biology, unit 4.7. Greene Publishing and Wiley-Interscience, New York.
3. **Ayer, D. E., and W. S. Dynan.** 1988. Simian virus 40 major late promoter: a novel tripartite structure that includes intragenic sequences. Mol. Cell. Biol. **8**:2021–2033.
4. **Beard, P., and H. Bruggmann.** 1989. Control of transcription *in vitro* from simian virus 40 promoters by proteins from viral minichromosomes. Curr. Top. Microbiol. Immunol. **144**:47–54.
5. **Brady, J., and G. Khoury.** 1989. *trans*-activation of the simian virus 40 late transcription unit by T antigen. Mol. Cell. Biol. **5**:1391–1399.
6. **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.
7. **Chen, C., and H. Okayama.** 1987. High-efficiency transformation of mammalian cells by plasmid DNA. Mol. Cell. Biol. **7**:2745–2752.
8. **Davidson, I., J. H. Xiao, R. Rosales, A. Staub, and P. Chambon.** 1988. The HeLa cell protein TEF-1 binds specifically and cooperatively to two SV40 enhancer motifs of unrelated sequence. Cell **54**:931–942.
9. **Du Pont, NEN Research Products.** 1989. Technical publication S 2333R. Du Pont, NEN Research Products, Boston.
10. **Fromm, M., and P. Berg.** 1982. Deletion mapping of DNA regions required for SV40 early promoter function in vivo. J. Mol. Appl. Genet. **1**:457–481.
11. **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.
12. **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.
13. **Gong, S. S., and K. N. Subramanian.** 1988. Functional anatomy of the simian virus 40 late promoter. Virology **163**:481–493.
14. **Gorman, C. M., L. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2**:1044–1051.
15. **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.
16. **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.
17. **Kim, C. H., C. Heath, A. Bertuch, and U. Hansen.** 1987. Specific stimulation of simian virus 40 late transcription *in vitro* by a cellular factor binding the simian virus 40 21-base-pair repeat

promoter element. Proc. Natl. Acad. Sci. USA **84**:6025–6029.
18. **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.
19. **Loeken, M., I. Bikel, D. M. Livingston, and J. Brady.** 1988. Transactivation of RNA polymerase II and III promoters by SV40 small t antigen. Cell **55**:1171–1177.
20. **Manuppello, J., and J. Alwine.** Unpublished observation.
21. **May, E., F. Omilli, E. Ernoult-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.
22. **Muller, M. M., S. Ruppert, W. Schaffner, and P. Matthias.** 1988. A cloned octamer transcription factor stimulates transcription from lymphoid-specific promoters in non-B cells. Nature (London) **336**:544–551.
23. **Piatak, M., K. N. Subramanian, P. Roy, and S. M. Weissman.** 1981. Late messenger RNA production by viable simian virus 40 mutants with deletions in the leader region. J. Mol. Biol. **153**:589–618.
24. **Sturm, R., T. Baumruker, B. R. Franza, and W. Herr.** 1987. A 100-kD HeLa cell octamer binding protein (OBP 100) interacts differently with two separate octamer-related sequences within the SV40 enhancer. Genes Dev. **1**:1147–1160.
25. **Tanaka, M., V. Grossaiklaus, W. Herr, and N. Hernandez.** 1988. Activation of the U2 SnRNA promoter by the octamer motif defines a new class of RNA poly II enhancer elements. Genes Dev. **2**:1764–1778.
26. **Tanaka, M., and W. Herr.** 1990. Differential transcriptional activation by Oct-1 and Oct-2: interdependent activation domains induce Oct-2 phosphorylation. Cell **60**:375–386.
27. **Villarreal, L. P.** 1981. A perinuclear extract contains a unique set of viral transcripts late in SV40 infection. Virology **113**:663–671.