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Stimulation of the Adenovirus E2 Promoter by Simian Virus 40 T Antigen or E1A Occurs by Different Mechanisms

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We have examined the ability of simian virus 40 T antigen to stimulate transcription from the adenovirus E2 promoter. T antigen, produced from a cotransfected plasmid, stimulated chloramphenicol acetyltransferase enzyme and mRNA production from an E2 promoter-chloramphenicol acetyltransferase fusion plasmid (pEC113) in monkey kidney CV-1 cells. The level of stimulation of E2 transcription by simian virus 40 T antigen was equal to that observed in cotransfections of pEC113 and the adenovirus E1A gene product. Deletion mutations from the 5′ end of the E2 promoter were examined for their ability to express basal, T-antigen, or E1A *trans*-activated promoter activity. In each case, deletion of upstream promoter sequences to −70 base pairs reduced chloramphenicol acetyltransferase expression to approximately 30% of the level observed with the intact E2 promoter. Deletion to −59 base pairs resulted in chloramphenicol acetyltransferase expression that was 3 to 5% of that observed with the intact E2 promoter. At saturating levels of the stimulatory proteins, the chloramphenicol acetyltransferase levels obtained in response to T antigen and adenovirus E1A were additive. COS-1 cells, which are derived from CV-1 cells and constitutively express simian virus 40 T antigen, do not support E2 promoter *trans* activation by T antigen. E1A *trans* activation of the E2 promoter is efficient in COS-1 cells. These results suggest that although promoter sequence requirements are similar, T antigen and E1A *trans* activate the E2 promoter by different mechanisms.

Study of the transcriptional activation of viral genes by viral proteins is important to an understanding of the mechanisms by which cellular genes are regulated. Previously, we and others have demonstrated that simian virus 40 (SV40) late transcription is stimulated by SV40 T antigen (5, 15, 19). The ability of SV40 T antigen to induce SV40 late transcription is independent of its ability to initiate DNA replication. *trans* activation of the SV40 late transcription unit was correlated with the ability of T antigen to bind to binding sites I and II (5, 6), which are known to be important for autoregulation of early gene expression and viral replication (25–27). Additional sequences in the SV40 72-base-pair (bp) tandem repeat are also required for efficient *trans* activation of the SV40 late transcription unit (6, 7, 20). Factors which interact with the 72-bp tandem repeat may be indirect mediators of T-antigen activity.

Like the SV40 late promoter, the adenovirus (Ad) E2 transcription unit is distinguished from most eucaryotic promoters by the absence of a classical TATA sequence (2). However, other sequences between −28 and −21 bp may serve this function (22). The Ad E2 early promoter is positively regulated by the product of the 13S Ad E1A mRNA (4, 14, 23). Deletion of 5′ promoter sequences to within −79 bp of the E2 RNA initiation site retains wild-type basal and E1A-responsive promoter activity (17, 18). In addition, a fragment containing sequences between −262 and −21 is responsive to E1A in a position- and orientation-independent fashion (17, 18). There is no evidence for direct binding of E1A to the E2 promoter (9). Thus, the activation of transcription by E1A may be mediated in part by activation of cellular factors which bind to the E2 promoter or enhancer-like region. The observation that pseudorabies virus immediate early proteins will substitute for E1A suggested that cellular factors may interact with more than one viral *trans*-acting protein to induce gene expression (8, 16). It is not known whether the *trans*-acting proteins from adenovirus and pseudorabies virus interact with the same transcription factors.

To gain insight into the *trans*-activation mechanism of polymerase II transcription, we have studied the action of SV40 T antigen on the adenovirus E2 promoter. Analysis of the action of T antigen on this promoter has advantages over the analysis of the SV40 late promoter, because E2 expression is not linked to viral DNA replication. We found that SV40 T-antigen *trans* activated the E2 promoter as efficiently as the homologous viral protein E1A. Although the same upstream sequences (from −79) are required for either T-antigen or E1A *trans* activation, our results suggest that different mechanisms of activation are involved.

MATERIALS AND METHODS

Cell culture and DNA transfection. CV-1 cells are a continuous line of monkey kidney cells. COS-1 cells were derived from CV-1 cells after stable integration of replication-negative SV40 DNA (10). Cells were plated onto 10-cm dishes 24 h before transfection in Dulbecco modified Eagle medium plus 10% fetal bovine serum. Transfected plasmid DNA was kept constant at 35 μg per dish with test plasmids plus pML2 plasmid DNA and coprecipitated with calcium phosphate in a final volume of 2 ml (13). Cells were incubated with calcium phosphate-DNA mixtures for 12 h, washed, and cultured until 48 h after transfection.

Plasmids. E2 promoter-chloramphenicol acetyltransferase (CAT) plasmids (Fig. 1) were generously provided by Michael Imperiale and Joseph Nevins (17). Briefly, an *Sau*3AI fragment of the Ad type 5 (Ad5) E2 early promoter

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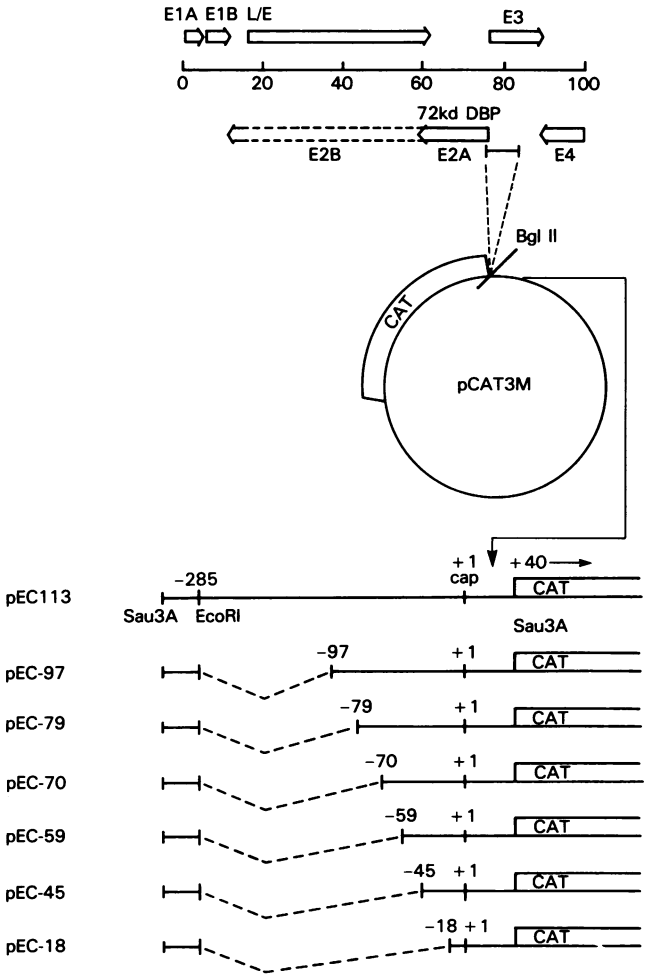

FIG. 1. Map of E2 promoter CAT and E2 promoter deletion plasmids. The entire E2 promoter with 285 bp of 5′ flanking sequences and 40 bp downstream of the mRNA cap site was inserted into pCAT3M to generate pEC113 (17). *Bal* 31 5′ deletion mutations were generated as described previously (17, 18).

containing 285 bp upstream and 40 bp downstream of the RNA cap site were inserted into the *Hin*dIII site of the promoter-negative pCAT3M vector to generate pEC113. Similarly, *Sau*3AI fragments of *Bal* 31 deletion mutants retaining 18 to 97 bp of upstream sequences were inserted into pCAT3M to generate pEC-97, pEC-79, pEC-70, pEC-59, pEC-45, and pEC-18. A plasmid containing an *Eco*RI-*Hin*dIII fragment of Ad2, which encodes E1A (pE1A) was also provided by Imperiale and Nevins (16). Plasmids in which SV40 T antigen (pRSV-T) or the CAT gene (pRSV-CAT) (11) are under the control of the Rous sarcoma virus long terminal repeat were provided by Bruce Howard.

CAT assays. CAT assays were performed as described previously (12), except that samples of cell extracts were diluted in 0.25 M Tris (pH 7.8), in a final volume of 150 μl. Between 1/10 to 1/20 of cell extracts allowed reliable comparisons of basal and *trans*-activated CAT activity.

S1 analysis. Whole cell RNA from two 10-cm plates was prepared by the hot acid-phenol procedure (24). DNA was removed by treatment with 20 μg of DNase I (Worthington Diagnostics) per ml in the presence of RNasin (Promega Biochemicals). Twenty micrograms of RNA was probed for CAT mRNA with a single-stranded DNA probe uniformly labeled with [^{32}P]dCTP (400 Ci/mmol) during strand synthesis from a single-stranded M13 phage. The M13 CAT was kindly provided by L. Laimins. Briefly, a *Bgl*II-*Eco*RI fragment from pA10CAT2 containing 256 bp of CAT-coding sequences was inserted into M13mp9. After synthesis of the [^{32}P]dCTP-labeled probe, a 458-nucleotide single-stranded segment was liberated by *Hin*cII digestion and purified on a 6% polyacrylamide–urea gel. This fragment contained 256 nucleotides homologous to those of CAT. RNA was analyzed by the S1 nuclease procedure as previously described (6).

RESULTS

SV40 T antigen *trans* activates the E2 promoter. We first compared the activity of the Ad E2 promoter (pEC113) in the presence or absence of *trans*-acting viral proteins. The pEC113 plasmid, in which CAT gene expression is under the control of 285 bp upstream and 40 bp downstream (−285 to +40 bp) of the E2 promoter (Fig. 1), was transfected into monkey CV-1 cells alone or with plasmids encoding SV40 T antigen (pRSV-T) or Ad E1A (pE1A). Extracts examined at 48 h after transfection with pEC113 alone contained a low, but detectable, level of CAT enzyme activity during a 60-min reaction (Fig. 2A). In contrast, cotransfection of pEC113 with plasmids encoding either SV40 T antigen or E1A resulted in a 14- to 17-fold enhancement of CAT enzyme levels. An even greater enhancement of basal CAT levels was observed when optimum concentrations of T antigen or E1A were present (Fig. 3A). Similar effects of T antigen and E1A on pEC113 CAT activity were observed in HeLa cells (data not shown).

To establish that the increase in E2 CAT activity was due to elevated levels of CAT mRNA, we examined steady-state E2 CAT mRNA by quantitative S1 nuclease analysis (Fig. 2B). The probe used in the S1 analysis was synthesized from a single-stranded M13 recombinant plasmid (Fig. 2C). The size of the undigested probe was 458 nucleotides (Fig. 2C; Fig. 2B, lane 7), whereas the size of a fragment protected from S1 nuclease digestion by CAT mRNA would be 256 nucleotides. The probe did not include the 37 bases homologous to the E2 sequence between the E2 mRNA cap site and the start of the CAT-coding sequence. Thus, this analysis measures the level of total CAT mRNA, but not the 5′ end. Consistent with the results presented above, CAT mRNA was not detected in mock-transfected cultures or in cultures transfected with pEC113 alone (Fig. 2B, lane 2 or 3, respectively). An S1-protected DNA fragment of 256 bases was detected in cultures transfected with pEC113 plus either pRSV-T (Fig. 2B, lane 4) or pE1A (Fig. 2B, lane 5). The probe alone treated with S1 nuclease is shown in Fig. 2B, lane 6. These results demonstrated that SV40 T antigen and adenovirus E1A stimulate transcription from the E2 promoter in CV-1 cells.

T-antigen and E1A *trans* activation of the E2 promoter is limited by cellular transcription factors. We next examined whether SV40 T antigen and Ad E1A activated the E2 transcription unit by the same or different mechanisms. It was first necessary to determine the plasmid and *trans*-acting protein concentrations where cellular transcription factors were limiting for E2 promoter activation. When increasing concentrations of pEC113 were transfected with a constant amount of either pRSV-T (5 μg) or pE1A (10 μg), maximum CAT enzyme levels were achieved with 15 to 20 μg of pEC113 (Fig. 3A). Higher concentrations of pEC113 did not significantly enhance CAT levels, suggesting that either

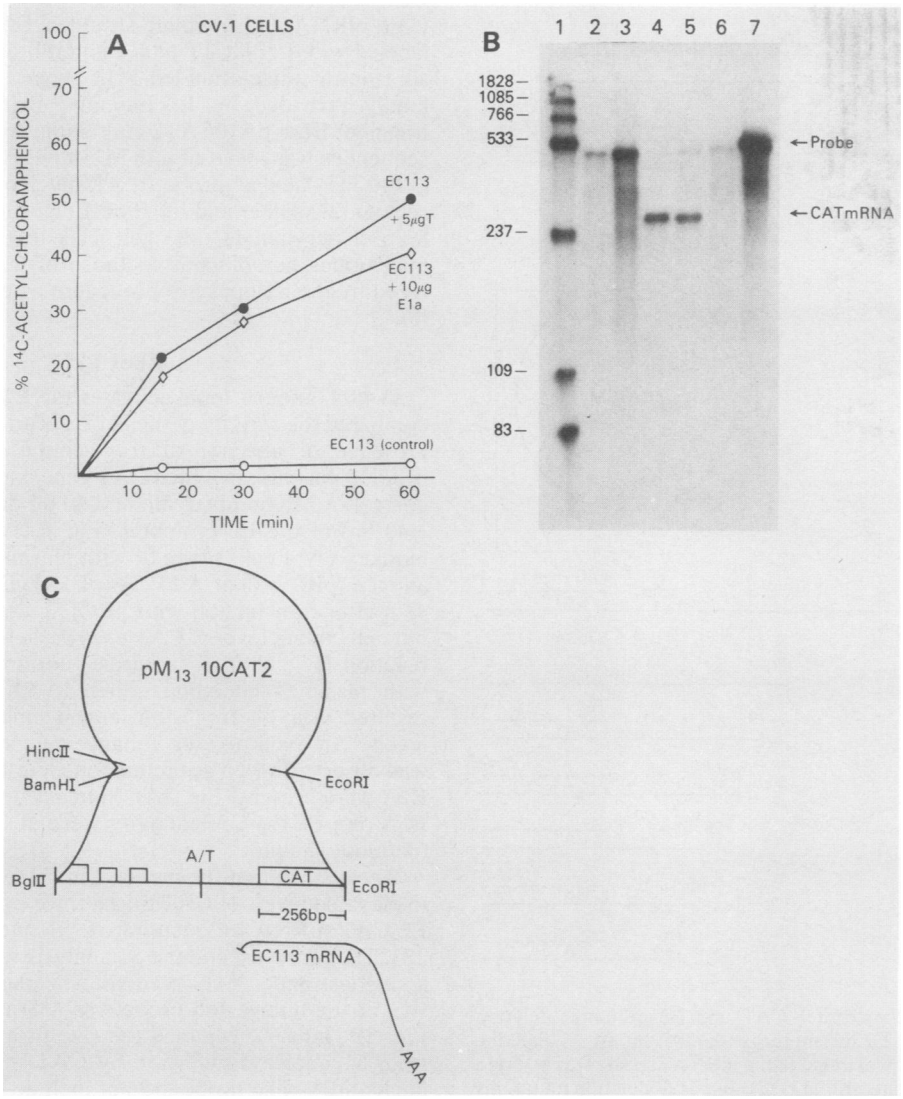

FIG. 2. SV40 T antigen and Ad E1A stimulate transcription from the E2 promoter in CV-1 cells. A, CV-1 cells were transfected with 5 μg of pEC113 alone (○) or with 10 μg of pE1A (◇) or 5 μg of pRSV-T (●). At 48 h, extracts were prepared, and CAT enzyme activity was determined. B, CAT mRNA levels produced in response to T antigen or E1A. Samples (20 μg) of whole cell RNA were probed by S1 nuclease protection analysis for CAT mRNA as described in Materials and Methods. Lanes: 1, molecular weight markers; 2, mock transfection; 3, transfection with 15 μg of pEC113 alone; 4, transfection with 15 μg of pEC113 plus 10 μg of pE1A; 5, transfection with 15 μg pEC113 plus 5 μg of pRSV-T; 6, probe alone digested with S1 nuclease; 7, intact probe. The positions of the intact probe and the DNA fragment protected by CAT mRNA are shown. C, Diagram of pM13-10CAT2 used to generate single-stranded probe for S1 nuclease analysis of CAT mRNA. A 458-bp *Bgl*II-*Eco*RI fragment derived from pA10CAT2 was inserted into M13mp8 as described in Materials and Methods. The single-stranded probe was released by *Hin*cII digestion after labeling with [^{32}P]dCTP. The 256-bp fragment homologous to CAT mRNA is indicated.

trans-acting viral proteins or cellular transcription factors were limiting.

With a subsaturating level of pEC113 (10 μg), increasing concentrations of plasmids encoding T antigen or E1A were added to the transfection mixture (Fig. 3B). Transfection of 2.5 to 20 μg of pRSV-T or pE1A produced increasing levels of T antigen or E1A mRNA as assayed by Northern blot analysis (data not shown). However, maximum pEC113 CAT levels were achieved with 5 μg of pRSV-T or 10 μg of pE1A, the concentrations of *trans*-acting plasmids used in Fig. 3A. Higher concentrations (up to 20 μg) of T antigen- or E1A-encoding plasmids did not produce higher CAT enzyme levels. From these titration experiments, we conclude that transfection of CV-1 cells with 10 μg of pEC113 and either 5 μg of pRSV-T or 10 μg of pE1A sets up conditions such that a cellular transcription factor(s) is limiting for E2 promoter activation.

Additive effect of T antigen and E1A suggests different mechanisms of stimulation of the E2 promoter. When a limiting amount (10 μg) of pEC113 and optimum amounts of pRSV-T (5 μg) and pE1A (10 μg) were transfected together, the CAT enzyme levels observed were approximately the sum of those obtained at saturating concentrations of either *trans*-acting protein alone (Fig. 4). This is not likely to be due to elevated concentrations of T antigen or E1A as a result of stimulation by one viral protein of the production of the other. Although both T antigen and E1A stimulated the expression of each other by five- to sixfold when plasmids

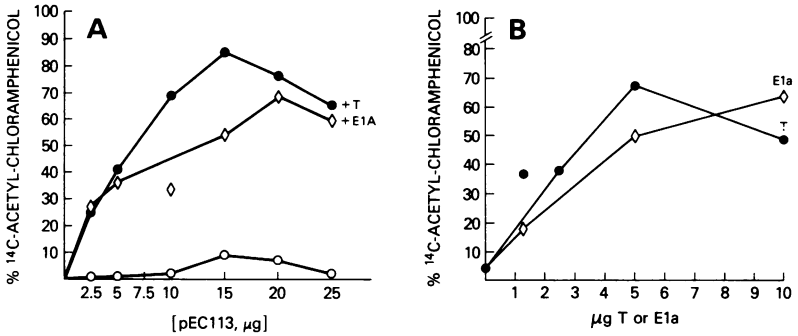

FIG. 3. Effect of plasmid concentration on E2 CAT activity in CV-1 cells. A, Increasing concentrations of pEC113 (2.5 to 25 μg) were transfected either alone (○) or with 5 μg of pRSV-T (●) or pE1A (◇). At 48 h posttransfection, extracts were prepared and analyzed for CAT enzyme activity during a 60-min reaction. B, Increasing concentrations of pRSV-T (●) or pE1A (◇) were transfected with a constant limiting amount (10 μg) of pEC113. In all experimental samples, DNA was brought to a final concentration of 35 μg per 10-cm culture dish with pML2 plasmid DNA. At 48 h posttransfection, extracts were prepared and analyzed for CAT enzyme activity during a 60-min reaction.

were cotransfected, as determined by Northern blot analysis (data not shown), the levels of T antigen and E1A mRNA were within twofold of the levels obtained upon transfection of up to 20 μg of either plasmid.

From the results presented in Fig. 3A and B, at these plasmid concentrations the limiting factor for *trans* activation by either T antigen or E1A is likely to be a cellular transcription factor. The fact that T antigen and E1A stimulate the E2 promoter in an additive manner suggests that the *trans*-acting proteins interact with different cellular transcription factors to activate the E2 promoter. If the cellular factors which mediated the effects of T antigen and E1A were identical, cotransfection of E1A and T antigen would not have increased the E2 promoter activity above that observed with saturating amounts of either T antigen or E1A independently.

***trans* activation of the E2 promoter by T antigen does not occur in COS-1 cells.** Further evidence that T antigen and E1A activate the E2 promoter by different mechanisms was obtained by transfection studies performed in COS-1 cells. COS-1 cells are derived from CV-1 cells and constitutively express SV40 T antigen. Based on the cotransfection experiments presented above, we expected that the E2 promoter would be stimulated by the endogenous SV40 T antigen. We found, however, that pEC113 was not activated by T antigen in COS-1 cells (Fig. 5A). The block in SV40 T-antigen activation of the E2 promoter was apparently not due to insufficient T-antigen concentrations. Comparable levels of T antigen, as determined by immunofluorescence, were observed in COS-1 cells and in CV-1 cells transfected with 5 μg of pRSV-T (data not shown). Furthermore, supplementation of COS-1 T antigen by cotransfection of 5 μg of pRSV-T with pEC113 did not result in an increase in CAT enzyme levels above those of pEC113 alone (Fig. 5A). The Rous sarcoma virus long terminal repeat is functional in COS-1 cells as determined by pRSV CAT expression (data not shown). The T antigen in COS-1 cells is active, since it efficiently stimulates SV40 DNA synthesis and late gene expression (3, 5, 10, 19).

In contrast to the results observed with T antigen, E1A-mediated *trans* activation of the E2 promoter was observed in COS-1 cells (Fig. 5A). The CAT levels obtained by cotransfection of pEC113 and pE1A into COS-1 cells were comparable to those obtained in CV-1 cells (compare Fig. 2A and 5A).

S1 analysis of steady-state CAT mRNA levels in COS-1 cells confirmed the results obtained by assay of CAT enzyme activity (Fig. 5B). CAT mRNA was detected in cells cotransfected with pEC113 plus pE1A (Fig. 5B, lane 4), but not in mock-transfected cells (Fig. 5B, lane 2) or cells transfected with pEC113 alone (Fig. 5B, lane 3).

Promoter sequences required for activity. We next determined whether SV40 T antigen and Ad E1A activate the E2 promoter by recognizing different promoter control sequences. Previously, it had been demonstrated that 79 bp upstream of the mRNA cap site were necessary for full E2 promoter activity in the presence of E1A (17, 18). Deletion to −70, −59, or −45 bp of promoter sequences reduced both basal and *trans*-activated promoter activity. We observed the same pattern of T antigen-stimulated E2 promoter activity when CAT plasmids containing 5′ deletions of the E2

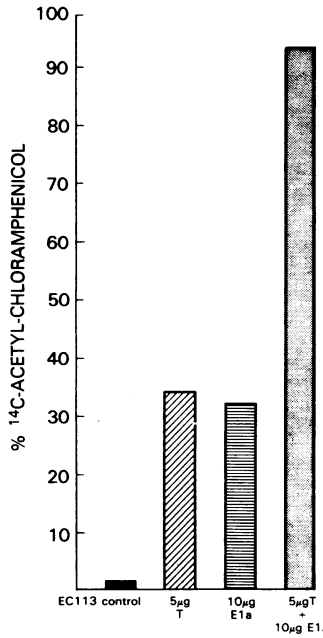

FIG. 4. Effect of SV40 T antigen and Ad E1A together on the E2 promoter in CV-1 cells. A limiting amount of pEC113 (10 μg) was transfected alone (■) or with 5 μg of pRSV-T (▨), 10 μg of pE1A (▤), or 5 μg of pRSV-T plus 10 μg of pE1A (□). In all cases, the final amount of DNA was brought to 35 μg with pML2 DNA. Extracts were prepared 48 h posttransfection and analyzed for CAT enzyme activity during a 60-min reaction.

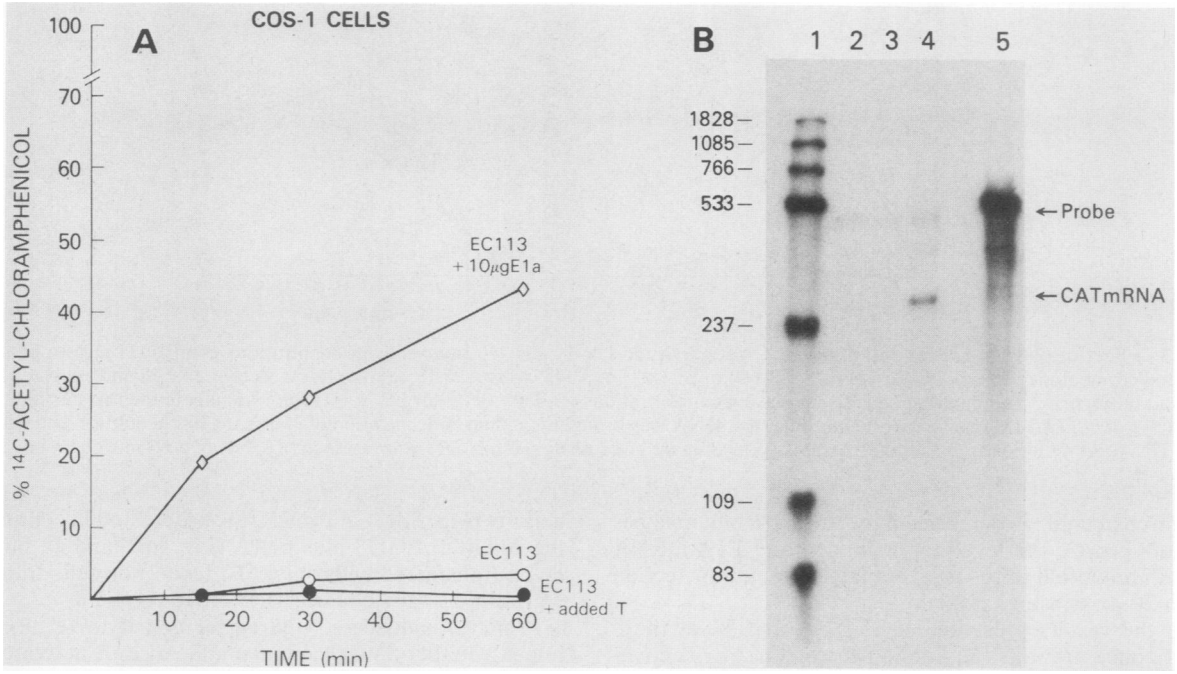

FIG. 5. E1A, but not T antigen, stimulates E2 transcription in COS-1 cells. A, COS-1 cells were transfected with 5 μg of pEC113 alone (○) or with 5 μg of pRSV-T (●) or 10 μg of pE1A (◇). At 48 h posttransfection, CAT activity was determined during a 60-min incubation with the cellular extract. B, CAT mRNA levels were determined by S1 nuclease analysis as described in Materials and Methods. Lanes: 1, molecular weight markers; 2, mock transfection; 3, transfection with 15 μg of pEC113; 4, transfection with 15 μg of pEC113 plus 10 μg of pE1A; 5, intact single-stranded CAT probe.

promoter were examined in CV-1 cells (Fig. 6). Similar CAT levels were obtained from E2 CAT constructs containing 97 or 79 bp of the upstream promoter sequence in response to T antigen or E1A. Deletion to −70 bp decreased both T antigen and E1A *trans*-activated E2 promoter activity to 30 to 50% of that observed with pEC-97. Deletion to −59 bp resulted in CAT levels which were 3 to 5% of that of pEC-97. Further deletion to −45 or −18 bp resulted in CAT levels which were barely detectable. A longer exposure of the CAT assay representing basal E2 promoter activity was qualitatively similar to that observed with SV40 T antigen or Ad E1A (Fig. 6A). Deletion to −70 bp decreased E2 basal promoter activity by approximately 50%. Deletion to −59, −45, or −18 bp resulted in promoter levels that were undetectable by CAT activity.

DISCUSSION

There are several examples of viral regulatory proteins *trans* activating promoter sequences of heterologous viruses. Pseudorabies virus immediate early (IE) proteins have been shown to substitute for Ad E1A functions during Ad infection and specifically to *trans* activate the E2 promoter (8, 16). Similarly, both SV40 T antigen and pseudorabies virus IE proteins increase expression of the Ad E3 promoter and the Rous sarcoma virus long terminal repeat (1). These studies suggest that transcription factors which regulate specific target promoters can be activated or induced by different viral regulatory proteins. It is not known, however, whether the *trans*-acting proteins utilize the same or different transcription factors.

We have shown that SV40 T antigen stimulates CAT enzyme production under the control of the E2 promoter and that CAT enzyme levels are correlated with steady-state CAT mRNA levels. Our studies suggest that the mechanism of promoter activation by SV40 T antigen and by Ad E1A is different, and that a specific promoter might be activated by more than one mechanism. This conclusion is drawn from two observations. First, the effects of saturating levels of T antigen and E1A on the E2 promoter are additive in CV-1 cells. Second, E1A, but not T antigen, can activate the E2 promoter in COS-1 cells.

We have demonstrated that in CV-1 cells the synthesis of CAT enzyme from 10 μg of pEC113 reached a plateau with either 5 μg of pRSV-T or 10 μg of pE1A. This was not due to a limitation in the ability to produce T antigen or E1A. We found that T-antigen and E1A mRNA levels, as determined by Northern blot analysis, continued to increase when 2.5 to 20 μg of T antigen- or E1A-encoding plasmids was transfected into CV-1 cells (data not shown). It seems more likely that the plateau in E2 expression results from the titration of a limiting cellular transcription factor. If SV40 T-antigen and Ad E1A activation of the E2 promoter were mediated by the same transcription factor, cotransfection of pE1A and pRSV-T should not increase the level of E2 expression over that observed in the presence of either T antigen or E1A alone. In contrast, if different cellular factors are required for *trans* activation by T antigen and E1A, cotransfection of pE1A plus pRSV-T might lead to E2 expression which is the sum of the two independent reactions. Clearly, our results are consistent with the latter possibility, suggesting the presence of distinct cellular factors which mediate the indirect effects of T antigen and E1A. Along these lines, it has been shown that it is possible to activate a transfected human β-globin gene by more than one mechanism. Activation may be accomplished by introduction of the SV40 enhancer and transfection into HeLa cells or by transfection of the β-globin plasmid without an enhancer into 293 cells (28). It is reasonable to assume that the

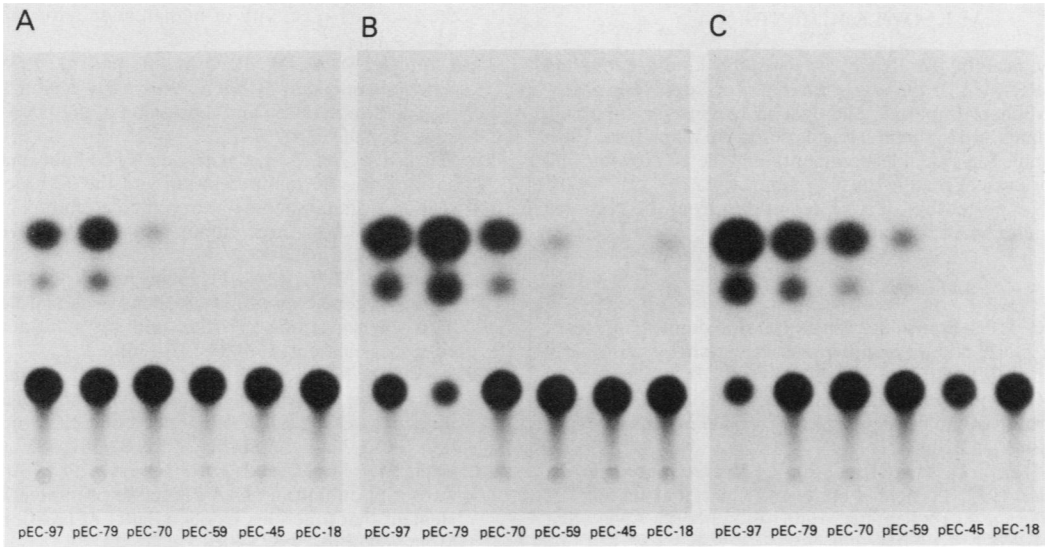

FIG. 6. Effect of 5′ deletion mutations on E2 promoter activity in CV-1 cells. Samples (10 μg) of pEC series plasmids containing 97, 79, 70, 59, 45, or 18 bp of upstream sequences, were transfected alone (A), with 1 μg of pRSV-T (B), or with 1 μg of pE1A (C). (Films were overexposed to enhance visibility of basal CAT activity in panel A. Deletion to −59, −45, or −18 was undetectable even upon prolonged exposure.)

factors which *trans* activate the transfected β-globin gene in 293 cells are different from those which regulate β-globin expression in erythrocytes.

Further evidence that SV40 T antigen and Ad E1A activate the E2 transcription unit by different mechanisms comes from our transfection studies in COS-1 cells. T antigen in COS-1 cells did not *trans* activate the E2 promoter, even when T-antigen levels were supplemented by transfection with pRSV-T. In contrast, cotransfection of pEC113 and pE1A into COS-1 cells resulted in efficient induction of the E2 promoter by E1A. These results suggest that a factor which is required to mediate the T-antigen effect on the E2 promoter is defective or absent in COS-1 cells. It is also possible that a particular posttranslational species of T antigen may be absent in COS-1 cells, even when cells are transfected with an exogenous plasmid encoding T antigen. Another possibility is that high levels of T antigen in COS-1 cells could be repressive to E2 promoter activity. However, we have no evidence of repression of the E2 promoter in CV-1 cells even when elevated levels of T antigen were obtained by cotransfecting up to 20 μg of pRSV-T. That the effects of E1A and T antigen on the E2 promoter can be separated in COS-1 cells suggests that the proteins activate E2 transcription by separate mechanisms.

It is interesting that a function of T antigen which occurs quite efficiently in CV-1 cells is inoperative in COS-1 cells. Other functions attributed to T antigen, such as stimulation of SV40 replication and late gene expression, are intact in these cells (3, 5, 10, 19). This observation suggests that the pleiotropic effects of T antigen as an activator of transcription of different genes can be separated.

The 5′ deletion analysis of the E2 promoter indicates that similar distal transcriptional control sequences are required for E1A and T-antigen *trans* activation as well as basal E2 promoter activity. It is possible, however, that T antigen and E1A require separate sequences downstream from −79 bp. Sequences between −79 and −70 bp may represent the minimal promoter limit. By in vivo competition analysis, we have obtained evidence that sequences downstream from −79 bp bind limiting factors which are responsible for basal and *trans*-activated E2 promoter activity (M. Loeken et al., manuscript in preparation).

It has been suggested that the transcriptional activation observed in response to E1A is nonspecific, since deletion of all but 18 bp of E2 promoter sequences or linker-scanning mutation throughout +9 to −92 bp did not abolish E1A responsiveness (21, 22). In our experiments, we also observed that T antigen or E1A elevated CAT expression from pEC-18. The levels, however, are at least 100 times lower than CAT levels seen from *trans*-activated pEC113. Whereas T antigen and E1A produced up to 80-fold more CAT activity from pEC113, they produced less than two- to fivefold activation of pEC-18 (data not shown). We interpret these results to suggest that although *trans* activation by T antigen or E1A in part involves factors which bind downstream from −18 bp, efficient E2 promoter activity requires to sequences upstream of −18 bp.

It is not clear whether direct binding of T antigen or E1A to the E2 promoter is involved in promoter activation. In the case of T antigen, there is evidence that induction of SV40 late gene expression is correlated in part with binding to high-affinity T antigen-binding sites on SV40 (6, 7). However, there is little apparent similarity between promoters which are activated by T antigen, which include SV40 late (5, 19), Ad E2 (1), Ad E3 (1), Rous sarcoma virus long terminal repeat (1), and Ad major late promoters (J. Manley, personal communication). Thus, for other promoters it is likely that indirect mechanisms are involved. In support of this concept, in vitro assays of T-antigen binding to the E2 promoter have been negative (Myles Brown and D. Livingston, personal communication). In the case of E1A, which does not bind directly to Ad DNA (9), little sequence similarity is observed in the promoters which it activates (2, 4, 23). It seems likely, therefore, that E1A may also activate specific promoters by an indirect mechanism. The evidence presented here suggests that the transcription factors which mediate T-antigen and E1A *trans* activation of the E2 promoter are different.

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