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Adenovirus E1a Proteins Repress Expression from Polyomavirus Early and Late Promoters

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We have examined the effects of the E1a products of adenovirus types 5 and 12 on the expression of polyomavirus early and late promoters. In cotransfection experiments in HeLa cells, plasmids expressing the E1a region of adenovirus type 5 or 12 repressed both the early and late promoters of polyomavirus, and deletion analysis indicates that the polyomavirus enhancers were the target of the E1a repression. With mutants lacking enhancer sequences, the polyomavirus early promoter but not the late promoter was *trans*-activated by E1a. Chimeric mutant plasmids with deletions in the regulatory region that contained either the A enhancer or the B enhancer were repressed to the same extent, indicating that E1a can repress both elements. Polyomavirus variant plasmids with rearrangements in the regulatory region conferring activity in embryonal carcinoma stem cells were repressed by E1a as was the wild type, suggesting that the repressor function is quite general. We discuss a model in which the influence of E1a on the transcriptional activity of a gene is the sum of positive and negative effects on promoter and enhancer elements and discuss possible mechanisms of negative regulation of enhancer function.

The E1a proteins of adenovirus are of great interest because of their ability to influence viral and cellular gene expression during infection and in transformed cells. The E1a region of adenovirus type 5 (Ad5) is the first transcription unit active during viral infection, and it encodes two proteins, 289 and 243 amino acids long (34, 36). These two proteins, which have both unique and common biochemical features, differ by a 46-amino-acid internal peptide unique to the larger peptide. The large peptide activates transcription from early viral promoters (2, 27, 33) and certain cellular genes (28, 42) both in their chromosomal location and in cell transfection assays (10, 15, 43). The smaller peptide contributes to viral DNA replication in growth-arrested cells (41). Both proteins are able to immortalize primary cells (20, 38) and cooperate with the E1b region of Ad5 to establish a fully transformed phenotype (9, 14). Recently, we (46) and others (5, 17) have described a novel property of the E1a products, the ability to repress transcription from enhancer-dependent promoters. Both cellular (17) and viral (5, 46) enhancer-dependent genes are subject to this repression.

To analyze more fully the mechanism of E1a enhancer repression, we have examined the effects of the E1a proteins on transcription from the polyomavirus early and late promoters. The polyomavirus early and late promoters are located within a complex transcription regulatory region which contains two enhancer elements, A and B, with different cell specificities (19). Element A shares homology with the Ad2 E1a enhancer, provides the major enhancer activity in mouse fibroblasts, and is preferentially repressed in embryonal carcinoma (EC) cells. Element B has a simian virus 40 (SV40)-type consensus sequence and is less active in mouse fibroblasts. Polyomavirus mutants which have a broader host range than the wild-type virus have been isolated and are characterized by rearrangements of the regulatory region (for a recent review, see reference 1). The sequence changes which extend host range are host specific and are invariably restricted to one of the two enhancer domains. The best examples of such rearrangements are represented by the EC cell polyomavirus variants. Undifferentiated EC cells are not permissive for wild-type polyomavirus growth, but become so after induction of differentiation (4, 44). Polyomavirus mutants selected for their ability to grow in undifferentiated EC cells are characterized by rearrangements in the enhancer region, and these changes are restricted to one of the two enhancer elements and depend on the cell line used for the selection (45). Recently, Gorman et al. (13) suggested that the inactivity of the polyomavirus as well as the SV40 enhancer in EC cells may reflect the presence of a cellular repressor(s) whose activity is lost after differentiation.

The evidence for a cellular repressor of enhancers is reminiscent of the repressor effect of E1a proteins on the enhancers described above and raises the possibility that the E1a products may be related to a negative regulatory factor(s) which operates in undifferentiated cells. This possibility has led us to ask whether both polyomavirus enhancer sequences, the A and B elements, are repressed by the adenovirus E1a gene products. We have therefore used transient expression assays in HeLa cells to analyze the effects of E1a on a series of deletion mutations in the polyomavirus transcriptional control region. We present evidence that E1a represses both the early and late promoters through action on the A and B enhancer elements. These findings indicate that the E1a repressor function is quite general and extends to viral enhancers with different host ranges. We also present evidence that the *trans*-activation function of E1a is capable of inducing expression from the early (but not the late) polyomavirus promoter when it is deprived of its enhancers. We discuss a model for E1a action in which transcriptional control by E1a reflects a balance between transcription stimulation at the promoter and transcription repression at the enhancer element.

MATERIALS AND METHODS

Cell maintenance and DNA transfection. HeLa cells were maintained and transfected as described previously (46). For

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the transfection experiments at 39°C, which is the nonpermissive temperature for the temperature-sensitive large T antigen in mutant plasmid pB2E102, HeLa cells were transfected and maintained for 24 h in the presence of the DNA precipitate at 33°C. After removal of the DNA precipitate and washing, the cells were shifted to 39°C until the time of harvesting.

RNA isolation and analysis and CAT enzyme assay. Cytoplasmic RNA was isolated 40 to 48 h after transfection, and the polyadenylated [poly(A)$^+$] fraction was affinity purified on poly(U)-Sepharose columns and analyzed by the Northern blot technique as described previously (46).

For the chloramphenicol acetyltransferase (CAT) assay, cells were harvested 48 h after transfection. CAT enzyme activity was measured by the assay method of Gorman et al (12). Briefly, the enzyme assay was performed in a final volume of 150 μl of reaction mixture containing 10 μl of cell extracts and 0.1 μCi of [^{14}C]chloramphenicol (50 mCi/mmol; New England Nuclear Corp.) for 10 to 40 min at 37°C. The products of the reaction were spotted on silica gel thin-layer plates and chromatographed; after chromatography they were sprayed with En^{3}Hance (New England Nuclear Corp.) and exposed for autoradiography. Levels of CAT enzyme were expressed as percentage of conversion of [^{14}C]chloramphenicol to the acetylated forms, after determination of the radioactivity of the different forms by liquid scintillation counting.

Plasmids and probes. The construction of pB2E63 has been detailed previously (29). Plasmids pB2E102, pB2E11, and pB2E82 were constructed in the same manner with the previously described (29) plasmids pBE102, pBE11, and pPyNeoBE82 as the respective parental plasmids. The construction of pSVE1a and pSVXL3 has also been described (39). The plasmid p102AE14, which contains a truncated early coding region, was constructed by inserting the polyomavirus early poly(A) addition site fragment (nucleotides [nt] 2763–3387, according to the numbering system of Soeda [40]) with *Eco*RI linkers into the unique *Eco*RI site of pBE102. The plasmid pSVE1–12 contains the E1a region of Ad12: the *Eco*RI fragment (nt 0–1594 of Ad12 DNA) of the plasmid pAD12Acc, a gift from P. Bernard, was inserted in the unique *Eco*RI site of pSVod. pBcB22CAT7 and pBB22CAT6 were constructed by first isolating a 314-base *BclI-BstX*I fragment that spans the polyomavirus origin of replication (nt 5022–168) from plasmid pBE22 (29). The fragment termini were filled in with T4 DNA polymerase, and *Hin*dIII linkers were added. The fragment was then inserted into the *Hin*dIII site of the pSVOCAT vector (12). In one orientation the inserted fragment results in the CAT gene being placed under the control of the polyomavirus early promoter (pBB22CAT6), while in the opposite orientation CAT sequences are linked to the polyomavirus late promoter (pBcB22CAT7). Plasmid p53.A6.6 (25) contains the entire polyomavirus genome cloned at the *Bam*HI site of pAT153. PCC4-204 and F9-i have the corresponding polyomavirus variant genomes (45) cloned at the *Bam*HI site of pBR322 and were a gift of M. Yaniv. Probes are described in the figure legends and were prepared from single DNA fragments excised from the vectors and purified by gel electrophoresis.

RESULTS

Expression from polyomavirus early and late promoters repressed by adenovirus E1a products. We have previously shown that the SV40 early promoter is repressed by the adenovirus E1a products (46). In transient assays in HeLa cells, the E1a products fail to stimulate transcription from the SV40 core promoter in the absence of the enhancer element (46). E1a, however, does repress the enhancer-dependent activity of this promoter, resulting in overall repression. By examining the effect of E1a on the expression of the early and late promoters of polyomavirus, we have extended E1a repression to another member of the papovavirus family. We transfected into HeLa cells a series of plasmids containing wild-type or deletion mutant forms of the polyomavirus early and late promoters, along with the plasmid pSVE1a, which contains the E1a region of Ad5 (Fig. 1B). In the first plasmid, pB2E102 (Fig. 1A), the polyomavirus early region encodes a thermolabile large T antigen, and the polyomavirus late coding region has been replaced with the bacterial neomycin resistance gene (*neo*). When pB2E102 was transfected into HeLa cells and maintained either at the permissive temperature of 33°C or at 39°C, the temperature nonpermissive for the temperature-sensitive large T protein, abundant early and late mRNAs were detected (Fig. 2A), confirming that polyomavirus expression from the late promoter is not dependent on the presence of a functional large T (29). In contrast, both the early and late mRNA levels were dramatically reduced when the polyomavirus-*neo* plasmid pB2E102 was cotransfected with an equimolar amount of pSVE1a DNA.

To confirm that the mechanisms of E1a repression of both early and late transcripts were independent of large T antigen, we examined the effects of E1a on a second polyomavirus-*neo* plasmid, p102AE14, which is identical to pB2E102 but encodes a truncated large T protein. The Northern blot (Fig. 2A) shows that mRNAs from the early and late regions of p102AE14 were well expressed in the absence of E1a but their levels dramatically decreased after pSVE1a cotransfection. Together these experiments showed that the polyomavirus early promoter was repressed, as is

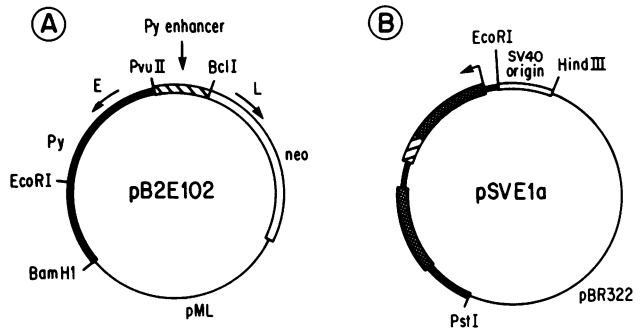

FIG. 1. Diagram of the recombinant expression plasmids. (A) pB2E102 contains the complete early coding region of *ts*a polyomavirus (Py) in pML; the late region has been substituted with the neomycin resistance coding sequence. The slashed box shows the polyomavirus enhancer region. Arrows indicate the directions of early (E) and late (L) transcription. Thick lines represent polyomavirus sequences; open boxed region, *neo* sequences; thin lines, pML DNA. (B) pSVE1a has nt 1 through 1834 of genomic Ad5 DNA, which contain the E1a gene inserted between the *Eco*RI and *Pst*I sites of pSVod. The stippled areas denote the amino acid residues present in the 243-amino-acid-long protein and in common with the 289-amino-acid-long E1a protein. The hatched box indicates the coding sequences for a 46-amino-acid polypeptide unique to the 289-amino-acid protein. Thick lines represent sequences flanking the E1a gene and within the E1a intron; thin lines are vector sequences; open areas are SV40 DNA. The arrow marks the cap site and direction of transcription of the E1a gene.

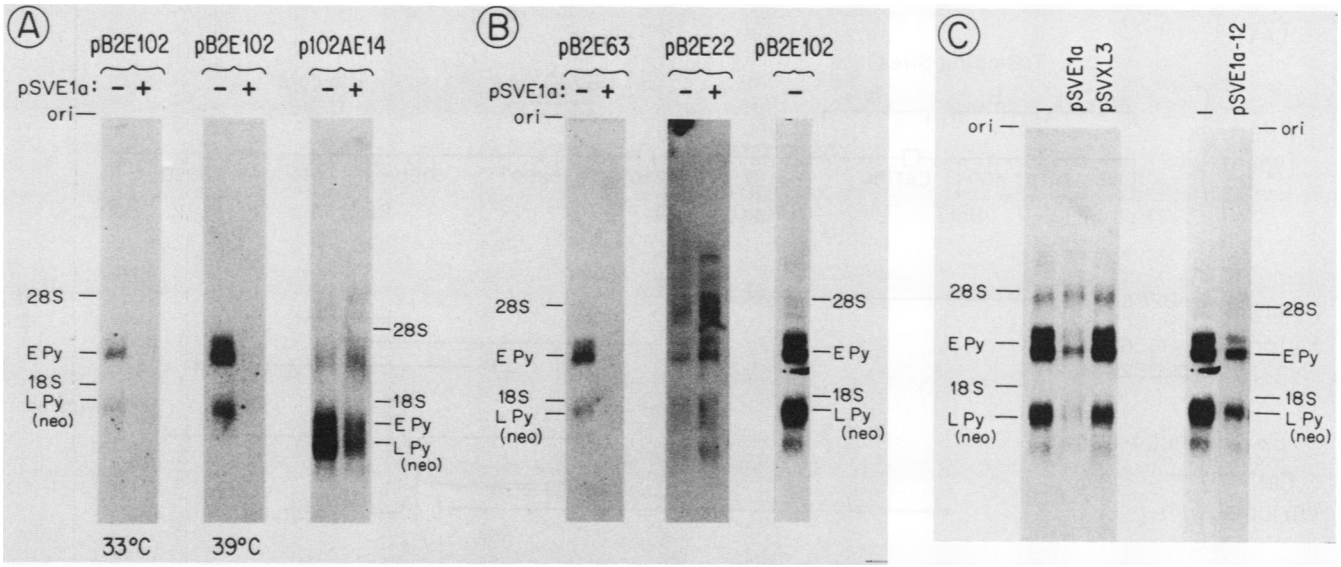

FIG. 2. Effect of E1a on expression from polyomavirus early (E Py) and late (L Py) promoters. (A) Independence from large T antigen of the expression of early and late polyomavirus mRNA and repression by E1a. (B) Northern blot analysis of E1a repression of polyomavirus plasmids with deletions in the controlling region. HeLa cells were transfected with 5 μg of the indicated polyomavirus-*neo* plasmids alone (lanes −) or in the presence of an equimolar amount of pSVE1a (lanes +). In the experiment at the temperature nonpermissive for temperature-sensitive T antigen, HeLa cells were shifted from 33 to 39°C after removal of the DNA precipitate (see Materials and Methods). pB2E102 is the wild-type plasmid which encodes a temperature-sensitive large T antigen. p102AE14 encodes a truncated large T protein. pB2E63 and pB2E22 are mutant plasmids with deletions in the controlling region; pB2E63 lacks the TATA and CAT boxes of the early promoter, as well as the origin of replication (ori). In pB2E22 the deletion spans the A and B enhancer elements. The precise structures of these plasmids are given in Fig. 3A. (C) Effect of different E1a plasmids on the expression of pB2E102. The polyoma virus-*neo* plasmid was transfected alone (lanes −) or in the presence of pSVE1a, the wild-type E1a plasmid, with pSVXL3, the frameshift mutant; or with pSVE1-12, which expresses the Ad12 E1a region. mRNAs from pB2E102 in panel A and pB2E63 in panel B were from cells transfected in single experiment; mRNAs from pB2E22 and pB2E102 in panel B were from a second experiment; mRNAs from p102AE14 were from a third experiment. In all panels, the mRNAs from the early and late promoters were visualized by Northern blot hybridization with a nick-translated 6.7-kb *Bam*HI fragment released from plasmid pB2E102. Positions of the early and late polyomavirus mRNAs and RNA species are indicated.

found for SV40. In addition the late promoter was also repressed by the Ad5 E1a products.

We next examined the effects on E1a repression of deletions in the polyomavirus transcription regulatory region by using two polyomavirus-*neo* plasmids shown in Fig. 3A. pB2E63 contains an intact enhancer region but lacks the core sequences of the viral origin of replication. It also lacks the CAT and TATA boxes and the large T antigen-binding sites in the early promoter region as well. Figure 2B shows that the levels of early and late mRNAs from pB2E63 were not greatly affected by the deletion in the early region (compare pB2E102 in Fig. 2A with pB2E63 in Fig. 2B), but a drastic reduction of the levels of the early as well as the late mRNAs was observed when pB2E63 was cotransfected with pSVE1a. The decrease in late as well as early mRNA caused by E1a is consistent with an enhancer repression mechanism and confirms that the polyomavirus late transcripts are enhancer dependent for their expression (29). Plasmid pB2E22 has a deletion which spans the entire E1a enhancer and part of the SV40 enhancer core sequences. Thus, this plasmid is devoid of most of the enhancer sequences, and constitutive expression from the early and late promoters was drastically reduced, as shown in the Northern blot analysis (Fig. 2B). The basal mRNA level was not further decreased when pSVE1a was cotransfected with pB2E22, a result also in agreement with the enhancer serving as the target of the repression.

Functional E1a proteins are required for repression of the activity of polyomavirus enhancer. Figure 2C shows that plasmid pSVXL3, a mutant E1a plasmid (39) in which a frameshift of the coding sequence severely truncated the E1a peptides, did not affect expression from either the early or the late polyomavirus promoters under conditions in which pSVE1a gave efficient repression. We conclude that a functional E1a product is required for repression and exclude competition between plasmids pB2E102 and pSVE1a for cellular factors as the repression mechanism.

The E1a region of the highly oncogenic adenovirus strain Ad12, although structurally closely related to the E1a of nononcogenic Ad5 (3), renders Ad12-transformed cells tumorigenic in immunocompetent mice (8). To determine whether the difference in transforming ability of Ad12 and Ad5 E1a regions might reflect a different ability of these genes to repress enhancer-dependent expression, HeLa cells were cotransfected with the polyomavirus-*neo* plasmid along with pSVE1a-12, a plasmid containing the Ad12 E1a region. As shown in Fig. 2C, Ad12 E1a was as effective as Ad5 E1a in repressing the early and late promoter of the test plasmid.

Polyomavirus A and B enhancer elements equally repressed by E1a. To determine whether the two enhancer sequences in the noncoding region of polyomavirus show a difference in susceptibility to E1a repression, a series of polyomavirus-*neo* plasmids with deletions in the A or B element (Fig. 3A) were cotransfected with pSVE1a. pB2E82 has a deletion which spans the SV40-like enhancer, the B element. A second plasmid, pB2E11, lacks the E1a-like sequence, the A element. HeLa cells were transfected with wild-type or mutant polyomavirus-*neo* plasmids in the presence or absence of pSVE1a DNA. As shown in Fig. 3B, early and late expression from both pB2E82 and pB2E11 were comparable

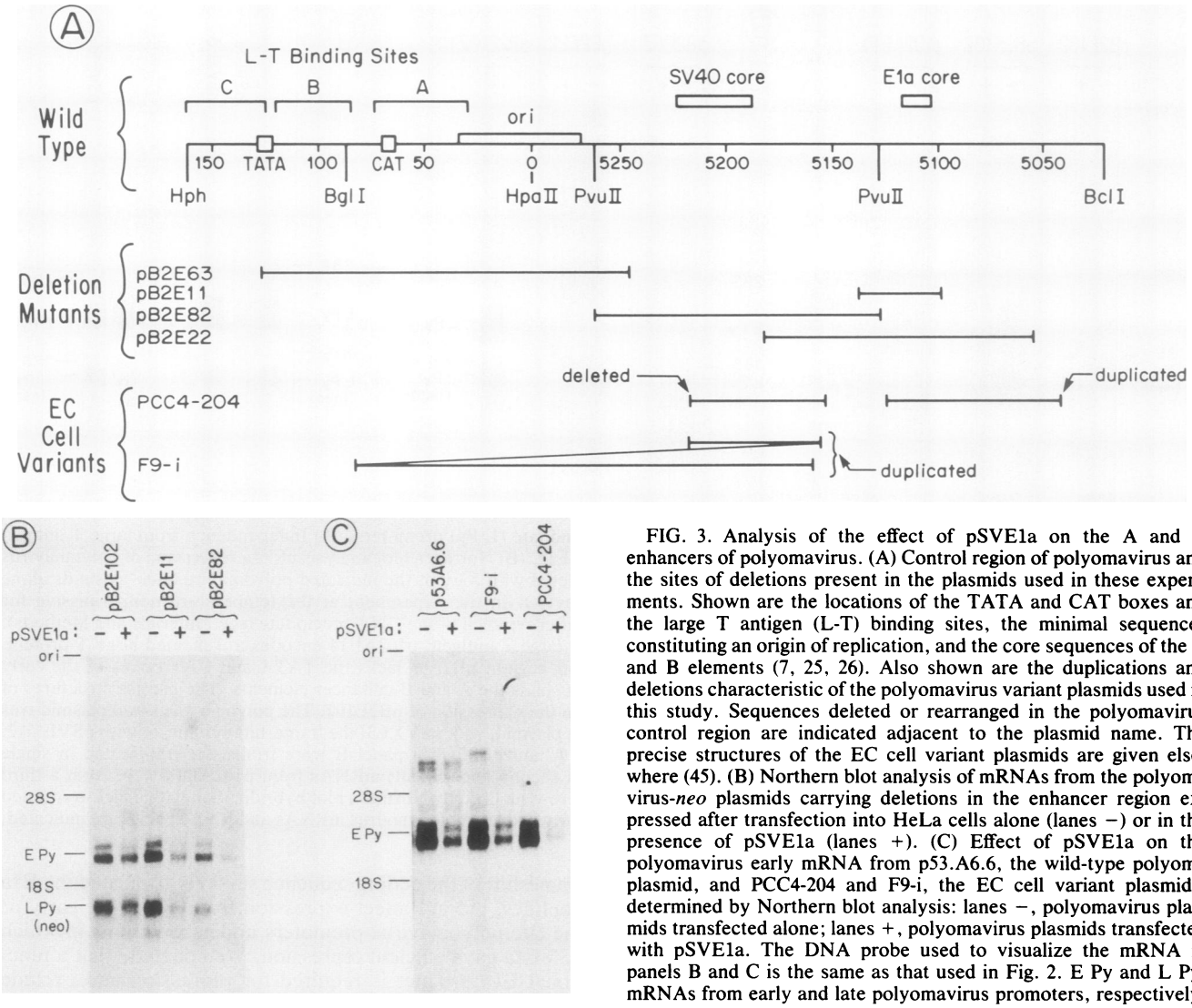

FIG. 3. Analysis of the effect of pSVE1a on the A and B enhancers of polyomavirus. (A) Control region of polyomavirus and the sites of deletions present in the plasmids used in these experiments. Shown are the locations of the TATA and CAT boxes and the large T antigen (L-T) binding sites, the minimal sequences constituting an origin of replication, and the core sequences of the A and B elements (7, 25, 26). Also shown are the duplications and deletions characteristic of the polyomavirus variant plasmids used in this study. Sequences deleted or rearranged in the polyomavirus control region are indicated adjacent to the plasmid name. The precise structures of the EC cell variant plasmids are given elsewhere (45). (B) Northern blot analysis of mRNAs from the polyoma virus-*neo* plasmids carrying deletions in the enhancer region expressed after transfection into HeLa cells alone (lanes −) or in the presence of pSVE1a (lanes +). (C) Effect of pSVE1a on the polyomavirus early mRNA from p53.A6.6, the wild-type polyoma plasmid, and PCC4-204 and F9-i, the EC cell variant plasmids, determined by Northern blot analysis: lanes −, polyomavirus plasmids transfected alone; lanes +, polyomavirus plasmids transfected with pSVE1a. The DNA probe used to visualize the mRNA in panels B and C is the same as that used in Fig. 2. E Py and L Py, mRNAs from early and late polyomavirus promoters, respectively.

to that from pB2E102, the wild-type plasmid, and were repressed to a comparable extent in all cases when pSVE1a was included in the transfection mixture. These data suggest that the two polyoma enhancers are equally repressed by E1a.

Repression of the wild-type polyomavirus enhancers by E1a is reminiscent of the inactivity of these enhancers in EC cells. Variants of polyomavirus that are active in EC cells have been isolated and carry repetitions of a single enhancer element. We then asked whether EC cell polyomavirus variant plasmids were also able to overcome E1a repression. A schematic view of changes in the noncoding region of such polyomavirus variant plasmids is given in Fig. 3A. The F9-i and PCC4-204 strains of polyomavirus (45) were isolated as host range mutants with enhancer rearrangements which rendered them capable of replicating in EC cell lines. In F9-i there was a duplication of the SV40-like B element, without alterations in the E1a-like A element. In PCC4-204 a region spanning the B element was deleted and the E1a-like enhancer was duplicated, providing this mutant with two A elements and no B elements. Figure 3C shows that in control transfections, comparable signals for early mRNA levels were obtained from the polyomavirus wild-type and mutant plasmids and that these levels were similarly reduced by pSVE1a. Thus, the A and B elements are equally repressed by E1a products, confirming the results of Fig. 3B. The multiple copies of the A or B element in these two strains did not accentuate a difference in their response to E1a. This experiment also suggests that mutations which overcome enhancer repression in F9 cells do not overcome E1a repression, as determined under the conditions of our experiment.

Polyomavirus early promoter weakly *trans*-activated by pSVE1a. In Fig. 2B, the Northern blot analysis of early mRNA from pB2E22 suggests that pSVE1a slightly stimulated the polyomavirus early promoter. We investigated this possibility further by using two chimeric constructs, pBB22CAT6 and pBcB22CAT7, in which the CAT gene is driven by the polyoma early and late promoters, respectively, and which contain the same enhancer deletion present in pB2E22 (Fig. 3A). The levels of expression from the early and late promoters, as reflected by CAT activity, were comparable in the absence of E1a (Fig. 4). Cotransfection of pSVE1a with pBB22CAT6 resulted in a two- to fivefold increase in the level of CAT enzyme expressed from the

early promoter, confirming the modest E1a stimulation seen in Fig. 2B. In contrast, the levels of CAT enzyme did not change in cells cotransfected with pSVE1a and pBcB22CAT7, indicating that the polyomavirus core late promoter is unresponsive to E1a.

DISCUSSION

In this report we examine the effects of the Ad5 and Ad12 E1a gene products on the activity of the polyomavirus enhancers and promoters. It was previously shown that polyomavirus enhancers linked to a surrogate promoter in chimeric plasmids are repressed by E1a proteins (5). We have extended this observation by showing that both the early and late promoters of polyomavirus in their normal location in the polyomavirus transcription control region are repressed by the action of E1a proteins. We have used a series of plasmids containing the polyomavirus late promoter linked to a surrogate neomycin resistance gene and the polyomavirus early transcription unit to confirm that the polyomavirus enhancers are the target for E1a repression. Thus, because the mutant plasmid pB2E63 was repressed by E1a, the TATA box and CAT box sequences which are lacking in this plasmid are not required for repression. However, when the enhancer region was deleted, as in pB2E22, the repressive effect of E1a was no longer observed. In fact, in the absence of the enhancer, the E1a plasmid weakly stimulated expression from the early promoter.

A novel finding of our experiments is the ability of E1a to repress transcripts from the polyomavirus late promoter. The activity of the polyomavirus late promoter does not require early gene products, although it can be stimulated by large T antigen (30). As previously shown for polyomavirus late expression from integrated genomes (29), the polyomavirus late promoter is constitutively expressed from transfected plasmids and relies on the polyomavirus enhancer elements. The finding that E1a represses these late transcripts is therefore consistent with the notion of enhancer dependence of the polyomavirus late transcripts.

Although the early and late promoters were similarly repressed by E1a when linked to the enhancer, they responded differently to the *trans*-activation property of E1a when present in enhancer-deleted constructs. In the absence of the enhancer, E1a increased expression from the early promoter but had no effect on the late promoter. However, the modest E1a *trans*-activation did not bring the activity of the early promoter to levels comparable to those obtained with an enhancer-linked early promoter CAT construct (data not shown). The polyomavirus early promoter contains a TATA box and a CAT box, as do many RNA polymerase II promoters. As reported by Green et al. (15), the TATA box seems to be the promoter sequence element which is sufficient for E1a *trans*-activation of the cloned, transfected human β-globin gene. It should be noted, however, that E1a-responsive sequences upstream from the TATA box have been identified (24, 31, 32) in strongly E1a-induced promoters, such as the adenovirus E3 or E2a genes. In the absence of such E1a-specific elements, a more general mechanism may use the TATA box element as target for a weak E1a-mediated stimulation. These considerations would explain both the weak E1a induction of the polyomavirus early promoter and the unresponsiveness of the late promoter. These data support the model that E1a can simultaneously exert both positive and negative effects on a single transcription unit: repression through action on the enhancer and activation through stimulation of a susceptible promoter.

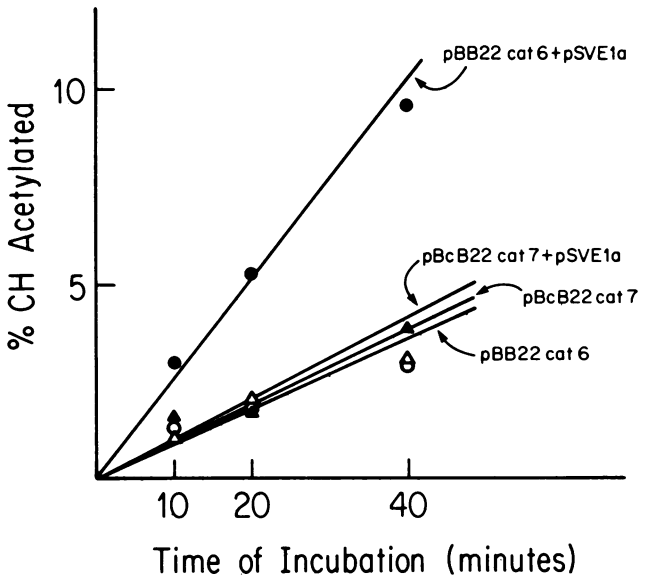

FIG. 4. Transactivation by E1a of the polyomavirus early promoter. A 3-μg amount of Polyomavirus recombinant plasmids in which the CAT gene is expressed from the early (pBB22CAT6) or the late (pBcB22CAT7) polyomavirus promoter were transfected alone or in the presence of 5 μg of pSVE1a. At 48 h posttransfection the CAT enzyme level was assayed as described in the Materials and Methods section. The percent of chloramphenicol (CH) conversion into acetylated forms in the different extracts is plotted as function of time of incubation.

The two polyomavirus enhancer elements show a degree of cell specificity for their activity (19) and a different ability to interact in vitro with cellular proteins (35). Despite this, we could not detect any difference in the response of two plasmids, pB2E82 and pB2E11, which retain the A and B enhancers, respectively, to E1a proteins. In the absence of pSVE1a, these plasmids expressed the same levels of early and late mRNAs as the wild-type plasmid pB2E102, and both the A and B enhancer-specific plasmids were repressed to the same extent as the wild type by E1a.

Rearrangements of DNA sequences in the noncoding region of polyomavirus which generate additional enhancer sequence motifs provide a mechanism for altering virus host range and for conferring the ability to grow in undifferentiated EC cells (1). We asked whether reiteration of different core motifs in the polyomavirus variant plasmids PCC4-204 and F9-i, which were selected for the ability to grow in EC cells, could provide resistance to E1a repression. This was of interest because in vivo enhancer competition experiments in EC cells suggest that undifferentiated EC cells may have a negative regulatory factor(s) which prevents the polyomavirus enhancers from being active (13) and which could be a cellular counterpart of the adenovirus E1a gene products (23). Thus, enhancers which are active in EC cells and escape the block imposed on wild-type polyomavirus enhancers by the proposed cellular E1a counterpart might also escape repression by E1a products themselves. Despite these considerations, such rearrangements did not, in our experiments, relieve the block imposed by E1a. In other experiments, Hen et al. (18) found that variant polyomavirus enhancers transfected into an E1a-transformed mouse L cell line were active, while under the same conditions the wild-

type polyomavirus enhancers were repressed. Perhaps the continuous expression or lower levels of E1a proteins in the transformed cells of Hen et al. (18) more nearly reproduce the conditions of the undifferentiated EC cells. However, our finding of E1a repression of the variant enhancers indicates that the resistance of these enhancers to E1a repression is a matter of degree and that under the appropriate conditions they too may be repressed. It should also be noted that the polyomavirus EC cell mutants are not phenotypically identical and are restricted in their activities to the EC cell line in which they have been selected (45). During the in vitro differentiation process, F9 cells acquire sensitivity to PCC4-adapted mutants earlier than they do to wild-type polyomavirus (11). This suggests that EC cells acquire permissivity in a series of steps on their way to terminal differentiation. This series of steps would reflect changes in the level or activity of cellular E1a-like proteins. Such changes could be analogous to the differences in the conditions used in the current work and in that of Hen et al. (18).

The regulatory properties of the E1a proteins considered above become more complex when E1a activation of the adenovirus E2 promoter is considered. The E2 promoter sequence located between nt −79 and −21 relative to the E2 cap site stimulates promoter activity of a mutant gene, which retains the TATA box but lacks other upstream elements (22). In the absence of E1a this element functions independently of its position and orientation, in the manner of an enhancer. In the presence of E1a, the stimulation is increased. Therefore it seems that E1a proteins can have opposite effects on different enhancers: repression of papovavirus enhancers and stimulation of the E2 enhancer. Differential effects are also evident from additional studies of E2 regulation in which the 289-amino-acid E1a protein stimulates E2 promoter activity through the enhancerlike sequence of the E2 early promoter (21, 24, 37, 47), while the 243-amino-acid E1a protein represses expression from the E2 late promoter (16), which is the active E2 promoter late in infection.

Furthermore, Borelli et al. have recently reported (6) that the E1a gene product can have opposite effects on the activity of the immunoglobulin heavy chain enhancer. In lymphoid cells the enhancer is functional, and its activity is repressed by the E1a proteins (17). In mouse L cells, a nonlymphoid cell line, the enhancer is only slightly active, and the E1a proteins actually stimulate this basal activity. In view of these observations, it is tempting to speculate that cellular regulatory factors analogous to E1a could display positive or negative effects on the activity of enhancers depending on the state of differentiation of the cell or the phase of the cell cycle. The sum total of these effects on the promoter and enhancer elements of a given transcription unit would determine whether, overall, E1a was stimulatory or repressive for that transcription unit.

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