Differential activation of RNA polymerase III-transcribed genes by the polyomavirus enhancer and the adenovirus E1A gene products

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ABSTRACT

We have compared the effect of the polyomavirus cis-acting transcriptional enhancer and the adenovirus trans-acting E1A gene on expression of RNA polymerase III-transcribed genes (the adenovirus VAI gene and a bacterial tRNA gene) using DNA transfection and transient expression assays. The polyomavirus enhancer has little effect upon transcription of the VAI gene by RNA polymerase III in any cell type tested (murine, hamster, or human). In contrast, expression of the E1A gene within adenovirus infected cells stimulates transcription of RNA polymerase III-transcribed genes from co-transfected DNAs. Human 293 cells, which constitutively produce adenovirus E1A gene products, also express high levels of RNA polymerase III transcripts from transfected DNAs.

INTRODUCTION

Enhancer sequences are cis-acting regulatory elements that increase expression of linked RNA polymerase II-transcribed genes in an orientation and distance-independent fashion (1-9). Viral enhancer sequences are capable of activating heterologous genes, but show some host cell specificity in their stimulation of transcription (10-14). The mechanism of transcriptional enhancement is unknown, although several models have been proposed. Some of these hypothetical mechanisms would be expected to enhance only RNA polymerase II-transcribed genes. For example, the RNA polymerase II "entry site" model proposed by several investigators (5,6,8,15) would predict that RNA polymerase II or an accessory transcription factor might interact in a sequence-specific manner with the enhancer element. Transcription of genes by RNA polymerase I or III would not be expected to be similarly stimulated. On the other hand, transcriptional activation of genes linked to an enhancer element could be the result of nonspecific activation. For example, the alteration of chromatin structure observed in the SV40 and polyomavirus enhancer regions (16-21, P. Bryan and W. R. Folk, unpublished observations) might allow molecules required for transcription access to linked promoter sequences. Other possibilities for nonspecific transcriptional enhancement

include alteration of the topology of the DNA, hypomethylation of linked sequences, or promotion of an association between DNA and the nuclear matrix. In any of these cases, enhancers might affect transcription by all three RNA polymerases.

An alternative type of transcriptional activation is that induced by the adenovirus E1A gene product (and the herpes virus immediate early gene product, ICP4) (22-31). The E1A gene product stimulates expression of its own gene and the other adenovirus RNA polymerase II-transcribed genes, as well as a number of heterologous genes, in a trans-acting manner (22-31). The mechanism of activation of transcription by E1A is unknown: In several different genes, sites required for transcriptional activity have been localized to sequences upstream of the mRNA initiation sites (26,28-30); however, it is unclear if the E1A gene product acts directly or indirectly at these sites (24,26,28-31). Also, it is unknown whether E1A has any effect on transcription by RNA polymerases I or III.

To help define the mechanism of action of these transcriptional stimulatory elements, we have examined the effects of the cis-acting polyomavirus enhancer and the trans-acting adenovirus E1A gene product on expression of RNA polymerase III-transcribed genes. The genes chosen for this study are the adenovirus VAI gene and a bacterial tRNA gene since no corresponding cellular sequences exist to interfere with analysis of their transcription. We show that the polyomavirus enhancer region does not activate VAI transcription, but that transcription of the VAI and of the bacterial tRNA gene are greatly stimulated in cells expressing the adenovirus E1A gene product.

MATERIALS AND METHODS

Construction of Recombinant Plasmids

pAd5 contains the adenovirus VAI and VAII genes within a SalI-HindIII DNA fragment of adenovirus 5 inserted in pBR322 (32) (Fig. 1b). Between the HindIII and EcoRI sites of this DNA was inserted: a) a fragment of polyomavirus strain A3 DNA extending from StuI (nucleotide 5252) to EcoRI (nucleotide 1575) (Fig. 1a) joined to an MboI fragment extending from nucleotide 2784 to nucleotide 3406, yielding pAdPyE⁻ (Fig. 1c) an analogous fragment of polyomavirus RNA containing in addition, the BclI (nucleotide 5046) to StuI (nucleotide 5252) (Fig. 1a) sequences of polyomavirus, yielding pAdPyE⁺ (Fig. 1c). Sequences of the latter BclI-StuI fragment have been shown to contain polyomavirus enhancer activity (4,7,12,14,33). Both clones were deleted for a portion of the large T-antigen sequences (between nucleotide

Origin and Construction of the pAdPyE⁺ and pAdPyE⁻ DNAs

Figure 1. (A) Schematic representation of the BclI-EcoRI fragment of polyomavirus DNA used in the pAdPyE⁺ construction. Heavy lines indicate polyomavirus sequences, and vertical bars indicate restriction sites. The origin of replication is marked as *ori*. Thin lines refer to M13 DNA vector used to clone the polyomavirus BclI-EcoRI fragment. (B) Map of pAd5. Vertical lines show restriction sites used in the pAdPyE⁺ and pAdPyE⁻ construction. Thin line refers to pBR322 vector DNA. (C) Maps of pAdPyE⁻ and pAdPyE⁺. pAdPyE⁻ was constructed by cleavage of pAd5 with HindIII, followed by fill-in with the Klenow fragment of *E. coli* polymerase; this DNA was cleaved with Eco RI and ligated to the polyomavirus StuI-EcoRI fragment. pAdPyE⁺ was constructed by cleavage of pAd5 with HindIII and EcoRI and ligation to an m13-polyomavirus HindIII-EcoRI fragment. To provide an efficient polyadenylation signal (34) the MboI fragment from map unit 22.7-34.5 was cloned into the EcoRI site (after Klenow fill-in and blunt end ligation).

1575 and nucleotide 2784), thereby preventing DNA replication and the concomitant contribution of increased DNA template concentration to transcription in pAdPyE transfected cells.

Cell transfection

The cell lines used in these experiments were the mouse L-cell line

DAP-3 (35), the baby hamster kidney cell line BHK TK⁻ ts13 (36), the human HeLa cell line, and the human embryonic kidney cell line 293 (37). All cells were grown in Dulbecco's modified MEM containing 10% calf serum.

Plasmid DNAs were purified by banding in CsCl containing ethidium bromide. RNA was removed by chromatography over Biogel agarose 50 M. In most instances, plasmid DNAs were cotransfected with pSVB3, a pBR322-SV40 recombinant (cloned at BamHI sites), by calcium phosphate coprecipitation (38), with modifications (39). At the time cells were harvested for RNA isolation (see below), other cells identically transfected were fixed with ice-cold acetone. Indirect immunofluorescence was used to detect SV40 T-antigen, as a measure of transfection efficiency.

Analysis of RNAs by S₁ nuclease and primer extension

48 hours after transfection whole cell RNA was isolated by extraction with guanidinium isothiocyanate-hot phenol followed by treatment with DNase I, digestion with proteinase K, and ethanol precipitation.

The probe for adenovirus VAI RNA detection was a HaeIII to TaqI fragment (nucleotides 10,550 to 10,671), 5'-end-labeled at the TaqI site. The probe for bacterial tRNA^{TP} was an 80 bp 5'-end-labeled TaqI fragment of plasmid pMY242 (40) which extends 56 nucleotides inside the transcribed region of the tRNA gene. The probes used for detection of polyomavirus early gene transcripts were either a HaeIII to BstNI fragment (nucleotides 120 to 421) or a HinfI to AccI fragment (nucleotides 13 to 383) end-labeled at the BstNI or AccI site. Annealing and S₁ nuclease treatment were performed as described by others (41-43).

The probe for primer extension analysis was prepared by internal labeling of a polyomavirus DdeI to AccI fragment (nucleotides 200 to 383), using the exonuclease and polymerase activities of T4 DNA polymerase. The labeled primer was annealed to RNA and extended using AMV reverse transcriptase essentially as described (40,44).

Analysis of pulse-labeled RNA from α -amanitin-treated 293 cells

Two 100 mm diameter dishes of 293 cells were transfected with pAdPyE⁻ DNA. At 45.5 hours post-transfection, 1 μ g/ml α -amanitin was added to the growth media in one of the dishes. 30 minutes after addition of α -amanitin, both dishes of cells were pulse-labeled with ³H-uridine (1 mCi/ml, 1 Ci/mmol). 2 hours later cellular ³H-RNA was isolated, as described above. ³H-RNA was hybridized to the appropriate strand-specific polyomavirus early gene region-M13mp8 or VAI-M13mp8 DNA, the samples were treated with RNase to remove unhybridized RNA, and the hybrids were collected onto nitrocell-

ulose filters, essentially as described elsewhere (41).

Analysis of bacterial tRNA^{trp} transcription in adenovirus infected cells

Hela cells were mock-infected or infected with adenovirus 5 dl309 (containing a wild-type E1A gene and a small deletion in the early gene 3 region) or dl312 (containing a large deletion in the E1A gene) (45). Cytosine arabinoside (20 µg/ml) was added to the media at the time of infection and at each 12 hour interval thereafter (46). At two hours after infection the three sets of cells were transfected with pMY242. At 48 hours after transfection, the cells were harvested and whole cell RNA was isolated and analyzed as described above.

RESULTS

Activation of early gene transcription by the polyomavirus enhancer

To investigate the effect of the polyomavirus enhancer region on transcription by RNA polymerase III, we analyzed transcription from two clones containing the adenovirus VAI and VAII genes linked to a portion of the polyomavirus early gene region. One of the clones (pAdPyE⁻) lacked sequences from the enhancer region (from BclI to StuI), whereas the other clone (pAdPyE⁺) retained these sequences.

To confirm that these clones were, respectively, enhancer negative and enhancer positive, after transfection of these DNAs into cells the amount of polyomavirus early gene transcription was measured by S₁ nuclease or primer extension analysis (Fig. 2). A significant number of transcripts is apparent in the pAdPyE⁺-transfected DAP-3 mouse cell RNA (panel A, lane 1) whereas no early gene transcripts appear in the pAdPyE⁻-transfected cell RNA (panel A, lane 2). A similar observation was made using RNA from transfected BHK cells (panel B). No polyomavirus early gene transcripts were detected in RNAs from Hela cells transfected by either pAdPyE⁺ or pAdPyE⁻ DNAs (panel C), even after long autoradiographic exposure of the gel. Densitometric scans of these and other films indicates that stimulation of transcription in mouse DAP-3 cells by the enhancer is at least twenty-fold, and the amount of transcription observed in pAdPyE⁺-transfected mouse cells is several-fold greater than that observed in pAdPyE⁻-transfected BHK cells.

To ascertain if the cells were transfected by the pAdPyE⁺ and pAdPyE⁻ DNAs with similar efficiencies, in each case the DNAs were cotransfected with a recombinant clone containing the SV40 genome (pSBV3). At the time cells were harvested for RNA isolation, similarly transfected cells were fixed and stained for SV40 T-antigen. The results of representative analyses are

Figure 2. Effect of the enhancer region on polyomavirus early gene transcription. M = end-labeled HpaII-digested pBR322 DNA fragments in nucleotides. Arrows indicate expected sizes of reaction products in each panel. (A) Primer extension analysis of polyomavirus early gene transcription in mouse DAP-3 cells. Autoradiographic exposure time was 12 hours. lane 1) 15 ug RNA from pAdPyE⁺-transfected cells. lane 2) 15 ug RNA from pAdPyE⁻-transfected cells. lane 3) 15 ug RNA from mock-transfected cells. lane 4) 2 ug RNA from wild-type polyomavirus infected mouse 3T6 cells, as a marker to show correct position of 5' end of polyomavirus early RNA. This lane was taken from a separate gel where electrophoresis conditions were different, but identical markers were used. (B) S₁ nuclease analysis of polyomavirus early gene transcription in BHK cells. Lanes 1, 2 and 3 as in panel A; 20 g BHK RNA was used in each case. Autoradiographic exposure time was 5 days. (C) Primer extension analysis of polyomavirus early gene transcription in Hela cells. Lanes 1, 2 and 3 as in panel A; 15 g Hela RNA was used in each case. Autoradiographic exposure time was 12 hours. (D) α -amanitin sensitivity of polyomavirus early gene transcripts in BHK cells. Autoradiographic exposure time was 5 days. lane 1) 20 ug RNA from pAdPyE⁺-transfected cells grown in normal media. lane 2) 20 ug RNA from pAdPyE⁻-transfected cells grown in normal media. lane 3) 20 ug RNA from pAdPyE⁺-transfected cells grown in media containing 1 ug/ml α -amanitin. lane 4) 20 ug RNA from pAdPyE⁻-transfected cells grown in media containing 1 ug/ml α -amanitin.

given in Table 1. Although the four cell types were not equally transfectable, there was no significant difference detected in the efficiency of transfection by DNAs mixed with the pAdPyE⁺ and pAdPyE⁻ constructs.

Effect of the polyomavirus enhancer upon transcription of the adenovirus VAI gene

The effect of the polyomavirus enhancer region on RNA polymerase III transcription was determined by measuring the adenovirus VAI transcripts in

Table 1
Measurement of Transfection Efficiency
by Detection of SV40 T Antigens in Transfected Cells

Cell Line	Coprecipitation Mixture	Total Cells Counted	% Transfection
Hamster BHK	pAdPyE ⁺ + pSVB3	569	6.9
	pAdPyE ⁻ + pSVB3	511	5.1
Mouse DAP-3	pAdPyE ⁺ + pSVB3	912	1.4
	pAdPyE ⁻ + pSVB3	1734	1.3
Human 293	pAdPyE ⁺ + pSVB3	681	8.4
	pAdPyE ⁻ + pSVB3	731	8.8
Human Hela	pAdPyE ⁺ + pSVB3	940	0.96
	pAdPyE ⁻ + pSVB3	1163	0.86

DNA coprecipitation mixtures contained 22 μ g pAdPyE DNA and 3 μ g of pSVB3 DNA for each ml of CaPO₄ precipitates. Cells were fixed at 48 hours post-transfection, reacted with SV-40 T-antigen antiserum and scored by indirect immunofluorescence.

the same cellular RNAs used to measure polyomavirus early gene transcription. A 5'-labeled fragment complementary to VAI RNA was used as a probe in the S₁ nuclease analysis (Fig. 3).

In contrast to the results with the early gene transcripts, the polyomavirus enhancer appears to have little effect upon VAI gene transcription in mouse DAP-3 cells (Fig. 3, panel A, lanes 1 and 2). Similarly, in BHK cells, VAI gene transcription is virtually the same after transfection by pAdPyE⁺ and pAdPyE⁻ (Fig. 3, panel B, lanes 1 and 2). RNAs from a number of transfection experiments were analyzed using the same techniques; densitometric scans of these films indicate that at most there is a two-fold difference in transcription of the VAI gene from the pAdPyE⁺ DNA relative to the pAdPyE⁻ DNA (Fig. 3), as compared with the approximately twenty-fold difference observed with the polyomavirus early gene transcription (Fig. 2). It is noteworthy that VAI transcripts are present in transfected Hela cells and in mouse DAP-3 cells in similar quantities (Fig. 3, compare panels A and C), in striking contrast to the absence of polyomavirus early gene transcripts in Hela cells (Fig. 2).

α -Amanitin sensitivity of RNA transcripts

To ensure that the transcripts measured in these experiments were the products of RNA polymerase II (polyomavirus early mRNAs) and RNA polymerase

Figure 3. Effect of the polyomavirus enhancer region on adenovirus VAI transcription as measured by S_1 nuclease analysis. M = end-labeled Hpa II-digested pBR322 DNA fragments. Arrows indicate expected sizes of reaction products in each panel. (A) RNA from transfected mouse DAP 3 cells. Autoradiographic exposure time was 12 hours. lane 1) 15 ug RNA from pAdPyE⁺-transfected cells. lane 2) 15 ug RNA from pAdPyE⁻-transfected cells. lane 3) 15 ug RNA from mock transfected cells. (B) RNA from transfected BHK cells. Lanes 1, 2, 3 as in panel A. Lane 4: RNA from adenovirus infected cells, as a marker to show correct position of 5' ends of adenovirus VAI RNA; 5 ug BHK RNA was used in each case. Autoradiographic exposure time was 6 days. (C) RNA from transfected Hela cells. Lanes 1, 2, 3 as in panel A; 15 ug Hela RNA was used in each case. Autoradiographic exposure time was 12 hours. (D) α -amanitin sensitivity of adenovirus VAI gene transcripts in BHK cells. Autoradiographic exposure time was 6 days. lane 1) 5 ug RNA from pAdPyE⁺-transfected cells grown in normal media. lane 2) 5 ug RNA from pAdPyE⁻-transfected cells grown in normal media. lane 3) 5 ug RNA from pAdPyE⁺-transfected cells grown in media containing 1 ug/ml α -amanitin. lane 4) 5 ug RNA from pAdPyE⁻-transfected cells grown in media containing 1 ug/ml α -amanitin.

III (VAI RNAs), their sensitivity to α -amanitin was measured. RNA polymerase II transcription is inhibited by low levels of α -amanitin, while RNA polymerase III transcription is blocked only by high levels of the toxin (47). BHK cells were transfected with pAdPyE⁺ or pAdPyE⁻, the cells were incubated for 24 hours and then 1 ug/ml of α -amanitin was added to the growth media. 24 hours later the cells were harvested, RNA was isolated and assayed with S_1 nuclease for polyomavirus early gene transcripts and adenovirus VAI transcripts.

Low levels of α -amanitin effectively inhibited transcription of the polyomavirus early genes (Fig. 2, panel D, compare lane 1 and lane 3). However, addition of 1 ug/ml α -amanitin to the growth media did not affect adenovirus VAI gene transcription, since equivalent amounts of transcripts are evident in pAdPyE⁺ and pAdPyE⁻-transfected BHK cells incubated in normal media or media supplemented with 1 ug/ml α -amanitin (Fig. 3, panel D, compare lanes 1 and 2 with lanes 3 and 4). This indicates that VAI transcripts are not the result of read-through transcription by RNA polymerase II, nor do the early gene products arise from RNA polymerase III transcription.

Stimulation of RNA polymerase III transcription by the adenovirus E1A gene products

The E1A gene is a trans-acting inducer of RNA polymerase III transcription of adenovirus genes expressed early after infection (22-24,29,30). The E1A gene also activates RNA polymerase II-transcribed genes from heterologous sources (25,26,27). Although its effect is similar to that of the cis-acting enhancers, E1A gene stimulation of transcription is likely to occur by a different mechanism.

To examine the effect of the E1A gene on transcription by RNA polymerase III, we infected Hela cells with adenoviruses differing in their capacity to express the E1A gene, and subsequently transfected the cells with a DNA containing a bacterial tRNA gene, capable of being transcribed by RNA polymerase III (48). (The latter gene was used to measure RNA polymerase III transcription rather than the VAI gene on the pAdPyE DNAs, as the infecting adenoviruses would be expected to encode large quantities of VAI RNAs.) At 48 hours after adenovirus infection the cells were harvested, RNA was prepared and the amount of bacterial tRNA^{t^{RP}} measured by hybridization (Fig. 4). It is apparent that Hela cells express little tRNA^{t^{RP}} from the transfected pMY242 DNA in the absence of adenovirus infection (lane 1). Infection of Hela cells with an adenovirus lacking the E1A region (dl312) followed by transfection with pMY242 showed a similar lack of expression of tRNA^{t^{RP}} (lane 2). However, prior infection by an adenovirus containing a wild-type E1A gene (dl309) followed by transfection with pMY242 results in transcription of tRNA^{t^{RP}} (lane 3). These results demonstrate that the E1A gene stimulates the expression of RNA polymerase III-transcribed genes.

Transcription of the adenovirus VAI gene and the polyomavirus early genes in human 293 cells

We further characterized the E1A gene stimulation of RNA polymerase III transcription by utilizing human 293 cells which have been transformed by a

Figure 4. S_1 nuclease analysis of $tRNA^{trp}$ gene expression in adenovirus-infected HeLa cells. M = end-labeled $HpaII$ -digested pBR322 DNA fragments in nucleotides. Arrow indicates expected size of reaction products. 10 μ g RNA was used in each case. lane 1) RNA from mock-infected, pMy242-transfected cells. lane 2) RNA from dl312-infected, pMy242-transfected cells. lane 3) RNA from dl309-infected, pMy242-transfected cells. lane 4) authentic bacterial tRNA to indicate correct position of 5' end.

segment of adenovirus RNA including the E1A gene region. To ensure that the 293 cells expressed E1A gene products capable of inducing RNA polymerase II transcription we measured the polyomavirus early gene transcripts in cells transfected by pAdPyE⁺ and pAdPyE⁻ DNAs. In contrast to the lack of expression in HeLa cells, 293 cells transfected by both types of DNAs expressed high levels of polyomavirus early gene transcripts (Fig. 5, panel A). Furthermore, the *cis*-acting polyomavirus enhancer region had no effect upon transcription in these cells, since transcripts in pAdPyE⁺-transfected cell RNA are approximately equal to transcripts in pAdPyE⁻-transfected cell RNA (panel A, lanes 1 and 2).

The activation of polyomavirus early gene transcription by the E1A gene is not solely the result of increased transfection into 293 cells. Although 293 cells and BHK cells were transfected with similar efficiencies (Table 1), the polyomavirus early transcripts in 293 cells far exceeded those in BHK cells (compare Figs. 2 and 5). Direct comparison by primer extension analysis of RNAs isolated from pAdPyE⁻-transfected BHK, mouse DAP-3 and 293 cells indicates that polyomavirus early gene transcription is elevated twenty- to fifty-fold in 293 cells (data not shown).

The effect of the *trans*-acting E1A gene product on adenovirus VAI gene

Figure 5. Polyomavirus early gene and adenovirus VAI transcription in 293 cells. M = end-labeled HpaII-digested pBR322 DNA fragments in nucleotides. Arrows indicate expected sizes of reaction products in each panel. (A) Primer extension analysis of polyomavirus early gene transcripts. Autoradiographic exposure time was 12 hours. lane 1) 15 ug RNA from pAdPyE⁺-transfected cells. lane 2) 15 ug RNA from pAdPyE⁻-transfected cells. lane 3) 15 ug RNA from mock-transfected cells. (B) S₁ nuclease analysis of adenovirus VAI gene transcripts. Autoradiographic exposure time was 12 hours. Lane 1, 2, 3 same as panel A; 15 ug RNA was used in each case.

transcription was analyzed in the same pAdPyE⁺ and pAdPyE⁻ transfected 293 cell RNAs as was used for the analysis of polyomavirus early gene transcription. A large amount of VAI transcription occurred in pAdPyE⁻-transfected 293 cells, as is evident in Fig. 5 (panel B); it exceeds that observed in transfected BHK, Hela or mouse DAP-3 cells (compare Figures 3 and 5); furthermore, the polyomavirus enhancer region does not further activate VAI transcription in 293 cells (Fig. 5, panel B, lanes 1 and 2). Thus, for both RNA polymerase II and RNA polymerase III-transcribed genes, the E1A gene products appear to greatly stimulate transcription.

VAI genes transfected into 293 cells are transcribed by RNA polymerase III

To demonstrate that the elevated level of VAI gene transcription detected in 293 cells is the product of RNA polymerase III transcription, the susceptibility of VAI RNA synthesis to α -amanitin inhibition was measured. Separate

Table 2
 α -Amanitin Sensitivity of Pulse-labeled Polyomavirus
Early Gene Transcripts and VAI Gene Transcripts in
pAdPyE⁻-Transfected 293 Cells

<u>Complementary DNA</u>	<u>Percent Hybridizable RNA</u>	
	<u>-α-amanitin</u>	<u>+α-amanitin</u>
M13-polyomavirus	.218	.011
M13-VAI	.255	.370

The total amount of ³H-RNA in each hybridization reaction was $7-8 \times 10^6$ cpm (3 μ g of whole cell RNA). Each hybridization value has been corrected for .050% background hybridization to M13 mp8 DNA. Values given are the percent of input counts that hybridized to complementary DNA.

samples of 293 cells were transfected with pAdPyE⁻; at 45.5 hours after transfection 1 μ g/ml α -amanitin was added to the growth media of one sample of cells, while the second sample was grown in normal media. 30 minutes later both samples of transfected cells were pulse-labeled with ³H-uridine. Cells were harvested at 48 hours post-transfection, ³H-RNA from both samples of cells was isolated and analyzed for the presence of polyomavirus early gene transcripts and VAI gene transcripts by hybridization to complementary single-stranded M13 DNAs.

Treatment of transfected 293 cells with low levels of α -amanitin completely blocked polyomavirus early gene transcription (Table 2). On the other hand, α -amanitin treatment had little effect on VAI gene transcription, since the amount of VAI ³H-RNA remained unchanged from that seen with RNA from cells grown in the absence of α -amanitin. Therefore, the high levels of polyomavirus early gene transcripts in 293 cells must be products of RNA polymerase II transcription and the high levels of VAI gene transcripts are most likely the result of RNA polymerase III transcription.

To further confirm that the elevated expression of the VAI gene in 293 cells was the result of RNA polymerase III transcription, we tested the effect of deletions of the 5' flanking sequences and the 5' intragenic promoter of the VAI gene (49) on transcription in 293 cells. Transcription of the adenovirus VAI gene is dependent upon an intragenic promoter region located between +9 and +72 nucleotides relative to the initiation site of the VAI RNA (32,49). Deletion of the entire 5' flanking region and 8 base pairs at the RNA initiation site (pAd5-dl 9) had no effect on transcription of the VAI gene, since

Figure 6. S₁ nuclease analysis of VAI gene deletion mutant transcription in 293 cells. M = end-labeled HpaII-digested pBR322 DNA fragments in nucleotides. Arrow indicates expected size of reaction product. 10 ug whole cell RNA from cells transfected by indicated DNAs used in each case. Autoradiographic exposure time was 12 hours.

the same quantity of VAI transcripts was present in 293 cells transfected by the VAI wild-type gene (pAd5-wt) or the mutant gene lacking flanking sequences and 8 base pairs of the gene (Fig. 6, lanes 1 and 2). Deletion of an additional 15 base pairs (pAd5-dl10), which removes a portion of the VAI intragenic promoter and eliminates transcription in Hela cell extracts (32), also completely abolishes transcription in 293 cells (Fig. 6, lane 3).

These data indicate that the elevated expression of the VAI gene in 293 cells occurs by a modification of RNA polymerase III-mediated transcription.

DISCUSSION

Transcription by RNA polymerase III is not affected by the cis-acting polyomavirus enhancer. Thus, cis-enhancement appears to occur for only specific classes of genes, lessening the likelihood of mechanisms which involve a generalized activation of chromatin. One attractive hypothesis consistent with our findings is that enhancers act as association sites for RNA polymerase II or its essential transcription factors. As RNA polymerases II and III differ in structure and in their promoter requirements, it is not surprising that a polypeptide required for activity by one polymerase might

not be required by the other. Recently, Shöler and Gruss have shown that enhancement of transcription can be competed by cotransfection with other DNAs (containing enhancers), suggesting that cells contain a limited amount of factors required for enhancer function (50). The nature of this factor is unknown, but it may be a protein required for initiation of transcription solely by RNA polymerase II.

Our experiments corroborate the observations made previously by others, that transcriptional activation by viral enhancers exhibits a cell-type specificity. DAP-3 cells, a mouse L-cell line, provides a more permissive environment for polyomavirus early gene transcription than semi-permissive BHK cells or the non-permissive HeLa cell line. The BHK cells yield approximately the same amount of transcripts as the DAP-3 cells, although the BHK cells are five-fold more transfectable. Although HeLa and mouse DAP-3 cells are equally transfectable, polyomavirus early gene transcription from the pAdPyE⁺ DNA was undetectable in HeLa cells. Other laboratories have also shown that the polyomavirus enhancer does not function well in HeLa cells (7). Conclusions about the host range of enhancers must be tempered, however, by the knowledge that cell lines vary greatly in their properties, some of which may not be attributable to their species of origin. An example of this is the human 293 cell line which contains a fragment of the adenovirus genome encoding the E1A protein. From our transfection data, it is apparent that transcription of the polyomavirus early region proceeds at a remarkably high rate. Thus, the restriction of polyomavirus transcription in human cells need not be absolute.

Surprisingly, the E1A gene products appear to stimulate both RNA polymerase II and RNA polymerase III transcription. What mechanism could account for this ability? The E1A gene product may act indirectly to stimulate RNA polymerase III transcription, through the increased synthesis of essential transcription factors. Although little is known about the limiting steps in RNA polymerase III transcription, stimulation of synthesis of the enzyme itself, or of its associated transcription factors might result in heightened expression of the tRNA and Va genes. Alternatively, the E1A gene product may help catalyze the formation of stable RNA polymerase III transcription complexes, in a fashion similar to that proposed by Gaynor and Berk for its action upon RNA polymerase II activity (51). Since stable transcription complexes are required for efficient transcription by both RNA polymerases (52-55), it is possible that a common element might participate in the assembly of active transcription complexes for both types of genes.

It is apparent, from the differential effect of these activators upon RNA polymerase III transcription, that their mechanisms of action are distinct. Determining how activation occurs, in each case, should reveal much about the control of eukaryotic gene expression.

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