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Sequence-Specific Activation of Transcription by Adenovirus EIa Products Is Observed in HeLa Cells but Not in 293 Cells

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The adenovirus EIa gene products activate transcription from the viral EIII and EIIaE promoters. We studied the mechanism of this stimulation by constructing a series of chimeric promoter recombinants containing the upstream regions of the EIII and EIIaE promoters linked to the TATA box-start-site regions of the viral major late and EIIa late promoters. By introducing these recombinants into HeLa cells together with recombinants producing the EIa gene products, we demonstrated that the induction of EIII and EIIaE transcription by EIa 13S and 12S mRNA products is dependent on sequences located in the upstream region (approximately −40 to −250) of these promoters. In addition, we showed that the major late and EIIa late upstream promoter regions do not contain such EIa-responsive sequence elements. In contrast, after transfection of these chimeric promoter recombinants into 293 cells (which constitutively express the EIa proteins), we found that their relative levels of transcription are similar and markedly different from those observed when they are cotransfected into HeLa cells with EIa protein-producing recombinants. We conclude that the efficiency of transcription from a given promoter in 293 cells is not necessarily related to the presence of a specific EIa-responsive element.

The adenovirus type 2 EIa transcription unit encodes the 12S and 13S mRNA products that, either singly or in combination, carry out several interesting functions. First, they positively regulate transcription from other adenovirus early genes (3, 19), as well as from a variety of heterologous genes (8, 11, 15, 17, 28). Second, they negatively regulate expression from promoters containing viral (4, 29) and cellular (R. Hen, E. Borrelli, and P. Chambon, Science, in press) enhancer elements, and finally, they are involved in immortalizing primary cell lines (14). Although it is not clear how these various activities are related to each other, an understanding of the mechanism by which EIa gene products stimulate transcription will be important for understanding the pleiotropic effects of these proteins.

The observation that EIa products are able to stimulate transcription from a wide variety of genes (8, 11, 15, 17, 28) has suggested that they act as general nonspecific stimulators of transcription. This hypothesis is supported by the failure to identify sequences in the promoters of adenovirus early genes specifically required for stimulation by EIa products (9, 20, 21, 25, 28, 30). Despite extensive mutagenesis, putative EIa-responsive elements could not be separated from the sequences required for promoter function in the absence of EIa products. However, this may reflect in some cases the inability of linker-scanning mutation analyses to alter a sequence large enough to completely abolish EIa stimulatory activity. In other cases, deletions including potential regulatory sequences may also delete elements necessary for basal promoter function and thus reduce transcriptional activity to very low levels. To avoid these problems, we took the alternative approach of constructing chimeric promoter regions consisting of the upstream promoter elements of the EIa-inducible adenovirus early region III (EIII) and early region IIa (EIIaE) transcription units, inserted into the EIIa late (EIIaL) or major late (ML) promoter regions deleted for their own upstream promoter elements. These two adenovirus late promoters were chosen because, as shown below, they are not stimulated by EIa products in the transient expression system used here. By transfecting the chimeric constructions into HeLa cells, together with plasmids that produce EIa proteins, we demonstrated that the sequence elements sufficient for induction of transcription by both the EIa 13S and 12S mRNA products reside in the upstream region (approximately −35 to −250) of the EIII and EIIaE promoter region and that such element are absent from the EIIaL and ML promoters.

By introducing these recombinants into 293 cells, which constitutively express EIa proteins (10), and comparing the pattern of expression with that which occurs in HeLa cells in the presence or absence of EIa, we demonstrated that promoter constructions which are not stimulated by the EIa products in HeLa cells are expressed in 293 cells at about the same level as those which are specifically stimulated in HeLa cells. We conclude that the high level of transcription in 293 cells is not due solely to the effect of the endogenously produced EIa proteins on specific EIa-responsive promoter elements. Therefore, this cell line may not be a good model to specifically study the effect of EIa gene products on EIa-responsive promoters of transduced genes.

MATERIALS AND METHODS

Recombinants. pL (Fig. 1A) contains, between the *Eco*RI and *Pvu*II sites of pBR322, the *Fnu*DII fragment of adenovirus type 2 containing the EIIaL promoter from −237 to +32 inserted into the repaired *Xba*I site of a polylinker located immediately 5′ to the rabbit β-globin-coding sequences (−9 to about +1650). pL37 is pL deleted between an *Sma*I site in the polylinker (located just outside the EIIaL sequences at −243) and a *Dde*I site at −37. pLE2E is pL37 containing the EIIaE promoter upstream sequences (−250 to −33) inserted between the *Sma*I and *Dde*I sites. The EIIaE upstream insert is an *Sma*I-*Xba*I (repaired) fragment derived from the EIIaE linker-scanning mutation LS-3323 (30). pLE3

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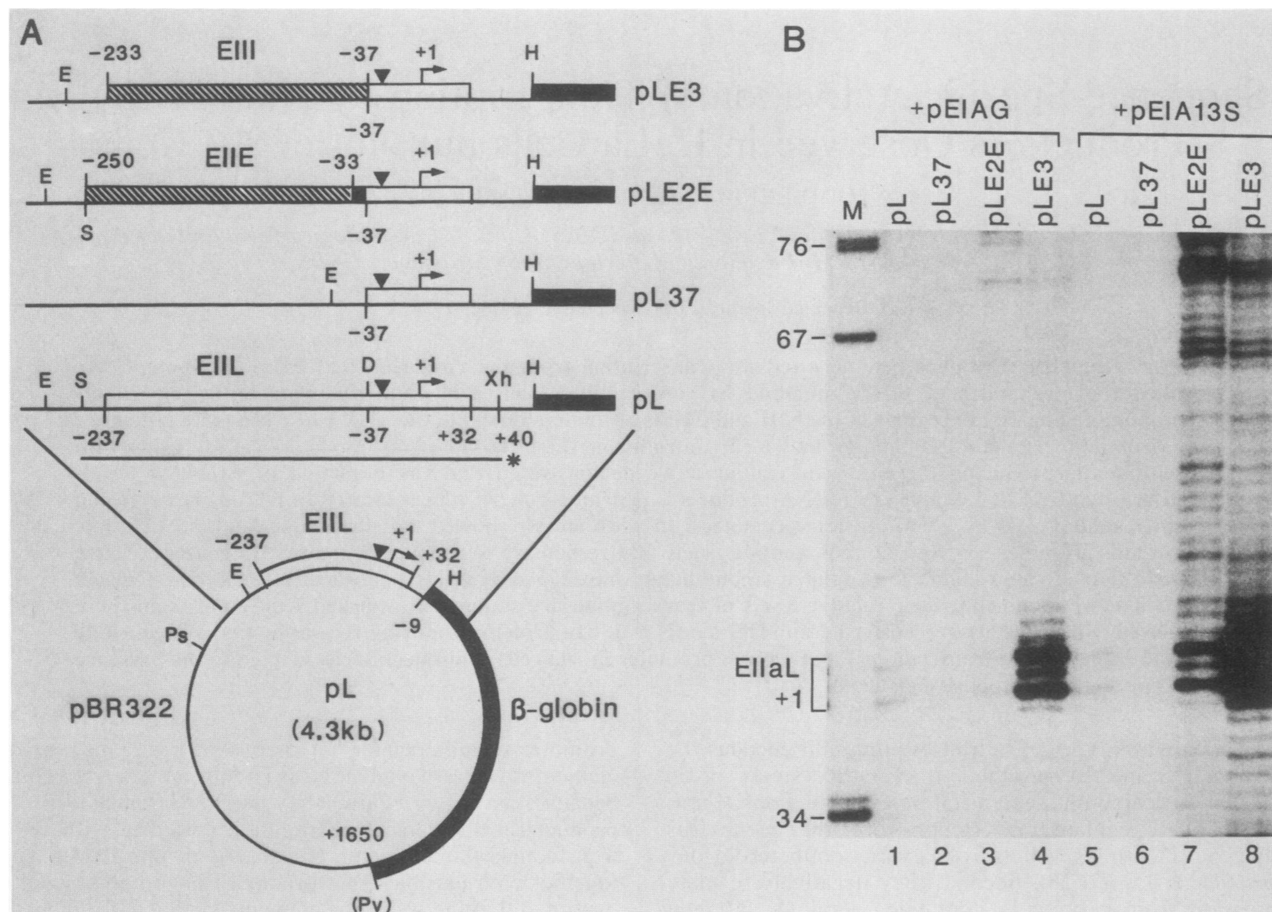

FIG. 1. Stimulation of transcription from chimeric EIIa late (EIIaL) promoter regions by the EIa 13S RNA product. (A) Schematic representation of the EIIaL series of recombinants. The parent construction (pL) is shown below, with the promoter regions of the derivative constructions shown in detail above. Globin sequences are represented as filled boxes, EIIaL sequences are shown as open boxes, and the various upstream regions derived from either the EIII or EIIaE promoters are shown as hatched boxes. The filled black triangles represent the location of the TATA box sequence. Vector sequences, either pBR322 or a poly-restriction enzyme site, are shown as a fine line. The numbers of the detailed maps indicate either positions relative to the EIIaL +1 start site (below each line) or the positions relative to either the EIIaE or EIII +1 start sites (above each line). The ^{32}P 5'-end-labeled DNA probe used for S1 nuclease mapping is the *XhoI-SmaI* fragment (+40 to −245) labeled at the *XhoI* site (indicated by an asterisk). Restriction enzyme sites are as follows: B, *Bam*HI; D, *DdeI*; E, *Eco*RI; H, *Hin*dIII; Ps, *PstI*; Pv, *PvuII*; S, *SmaI*; Xh, *XhoI*. Sites in parentheses were lost during cloning. The filled square in the pLE3 construction represents sequences derived from a repaired *XbaI* linker. The construction of these recombinants is described in Materials and Methods. kb, Kilobases. (B) Quantitative S1 nuclease mapping of cytoplasmic RNA isolated from HeLa cells transfected with 2μg of EIIaL recombinants (as indicated at the top of each lane) and either 0.2 μg of pEIAG (lanes 1 to 4) or 0.2 μg of pEIA13S (lanes 5 to 8). Probe fragments protected by RNA initiated from the EIIaL transcription start site are indicated as EIIaL +1. The additional bands that appear in lanes 7 and 8 are artifacts of incomplete S1 nuclease treatment. Size markers (lane M) are ^{32}P-end-labeled *MspI* restriction fragments of pBR322 and are indicated on the left in nucleotides.

is pL37 with, inserted between the *SmaI* and *DdeI* sites, an *Eco*RI-*SmaI* (repaired) fragment from the EIII promoter (−233 to −37) derived from pEIII (21).

pSVA677 (Fig. 2A) is the adenovirus type 2 ML promoter fragment from −677 to +33 linked directly to the simian virus 40 T-antigen-coding sequences from 5235 to 2533 and inserted into the *Eco*RI site of pBR322 (13). A derivative of pSVA677, pS, containing two point mutations that have created two *SmaI* sites in the ML promoter sequences at −52 and −124 (24) was used to construct pSΔ which is missing the sequences between these two sites. pSE2E, pSE3, and pSE2L were derived from pSΔ by insertion of various upstream-region fragments into the *SmaI* site of pSΔ. pSE2E contains the repaired *SmaI-XbaI* EIIaE promoter fragment (−250 to −48) derived from the linker-scanning mutant LS4839 (30). pSE3 contains the repaired *Eco*RI-*SmaI* EIII promoter fragment (−233 to −37) derived from pEIII (21), and pSE2L contains the repaired *Fnu*DII-*DdeI* (−237 to −35) fragment derived from pL (Fig. 1).

Cell transfection and RNA preparation. HeLa cells at 40 to 50% confluence were transfected by the calcium phosphate coprecipitation technique (2) with either 5 or 2 μg of the appropriate ML or EIIaL recombinant per 10-cm petri dish and either 0.5 or 0.2 μg of pEIA13S, pEIA12S, or pEIAG, and in some cases 2 μg of pβ2x (see figure legends). The total amount of DNA transfected was adjusted to 20 μg by the addition of M13 mp10 double-stranded DNA. 293 cells were transfected under the same conditions, but without cotransfected EIa recombinants. The precipitate was washed off the cells, the medium was changed 18 h posttransfection, and

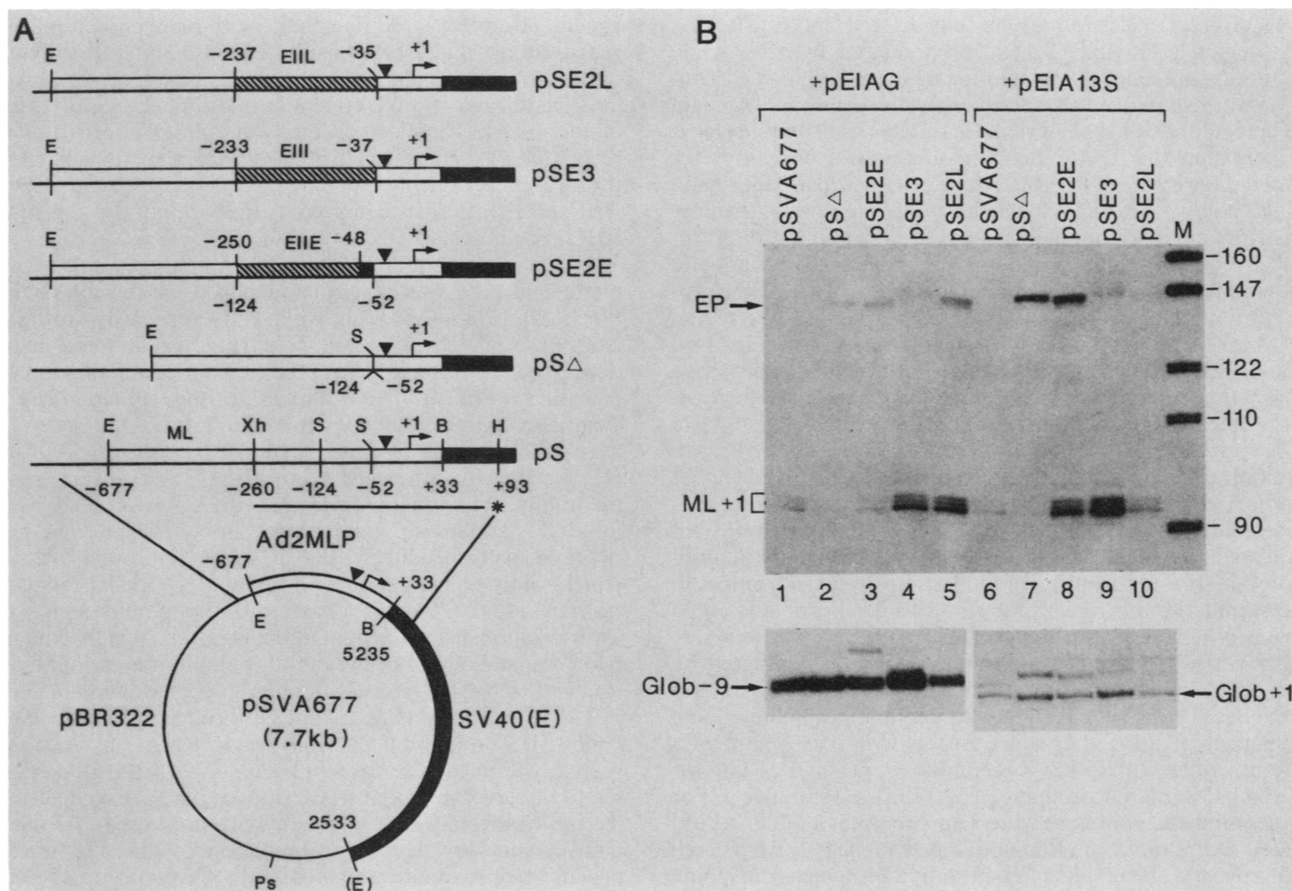

FIG. 2. Stimulation of transcription from chimeric ML promoter regions by the EIa 13S gene products. (A) Schematic representation of the ML series of recombinants. The parent construction (pSVA677) is shown below, with the promoter regions of the derivative constructions shown in detail above. Filled boxes represent simian virus 40 early region [SV40(E)] sequences, open boxes are ML promoter sequences, and hatched boxes are upstream regions from the EIII, the EIIaE, or the EIIaL promoter. Vector pBR322 sequences are indicated as a fine line. The numbers given above each of the detailed maps indicate the boundaries of the inserted upstream elements. The ^{32}P 5'-end-labeled probe used for S1 nuclease mapping is the *Hin*dIII-*Xho*I fragment (+93 to −260) labeled at the *Hin*dIII end (indicated by an asterisk). The filled square in the pSE2E construction represents sequences derived from a repaired *Xba*I linker. All other symbols are as described in the legend to Fig. 1. The construction of these recombinants is described in Materials and Methods. Ad2MLP, Adenovirus type 2 ML promoter; kb, kilobases; SV40, simian virus 40. (B) Quantitative S1 nuclease mapping of cytoplasmic RNA isolated from HeLa cells transfected with 5 μg of ML recombinants, as indicated at the top of each lane, and either 0.5 μg of pEIAG (lanes 1 to 5) or 0.5 μg of pEIA13S (lanes 6 to 10). In addition, the transfections shown in lanes 6 to 10 contained 2 μg of the globin reference recombinant pβ2x. The upper panel displays the results of hybridizations to the ML probe, while the lower panels show the results of hybridizations to the globin probe described by de Villiers and Schaffner (6). ML and globin probe fragments (see above) protected by RNA initiated from the ML and globin transcription start sites are indicated as ML+1 and Glob+1, respectively. Globin RNA synthesized from the pEIAG construction is initiated from a site nine nucleotides upstream from the normal start site and is indicated as Glob−9. The endpoint of homology between the ML probe and RNA synthesized from chimeric promoters is indicated as EP. Size markers (lane M) are as in Fig. 1.

the cells were harvested 36 h posttransfection. Cytoplasmic RNA was isolated by the Nonidet P-40 lysis technique as described previously (13) and quantitated by optical density at 260 nm. All recombinants were initially transfected into both HeLa and 293 cells at various amounts of input DNA (2.0 to 20 μg per petri dish). The transcriptional activity of the transfected recombinants was linear with respect to input DNA over the range of 2.0 to 15 μg per plate.

RNA analysis by quantitative S1 nuclease mapping. The single-stranded ^{32}P 5'-end-labeled probes are described in the legends to Fig. 1 and 2. An excess of the appropriate probe was hybridized to 10 μg of total cytoplasmic RNA in 10 μl of 10 mM PIPES (piperazine-*N*,*N*'-bis[2-ethanesulfonic acid]) (pH 6.5)–400 mM NaCl at 68°C for 14 h. S1 nuclease digestions were carried out by diluting the hybridization reactions into 200 μl of 300 mM sodium acetate (pH 4.5)–3 mM $ZnSO_4$–400 mM NaCl (final concentrations) containing 400 U of nuclease S1 (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) and incubating at 25°C for 2 h. The samples were precipitated with ethanol and analyzed on 8% polyacrylamide–8.3 M urea sequencing gels. Quantitation of specific bands was done by densitometric scanning of autoradiograms exposed for various periods of time.

RESULTS

Sequence-specific activation of transcription by the EIa 13S mRNA product. Previous transcriptional analysis of mutations in the adenovirus type 5 EIII promoter did not reveal any sequence uniquely required for stimulation by the EIa

gene products (21). Mutations that deleted only the TATA box region (−36 to −15) were stimulated by Ela, while mutations that entirely deleted the upstream region (−233 to −37) were so poorly transcribed that no specific RNA could be detected, even in the presence of Ela. Thus, while it was evident that the TATA box region was not necessary for stimulation by Ela, the role played by the upstream region could not be determined. To test the possibility that the upstream region is involved in the stimulation of transcription by the Ela gene products, we constructed a chimeric promoter containing the upstream region of the EIII promoter (−233 to −37) linked to the TATA box-capsite region (−37 to +32) of the adenovirus EIIaL promoter (Fig. 1A). The EIIaL promoter was chosen because it is not active during the early phase of viral infection when the majority of the other viral promoters are positively regulated by Ela (27), suggesting that it is insensitive to stimulation by Ela. In addition, as shown below and as previously observed (12; C. Goding, personal communication), the EIIaL promoter was not stimulated by the Ela 13S mRNA product in a transient expression system. Therefore, by using this chimeric construction, the contribution of the EIII upstream sequences to the stimulation of transcription by Ela gene products could be easily tested in transfected HeLa cells.

To verify that the EIIaL promoter is not stimulated by Ela, a recombinant plasmid containing the EIIaL promoter sequences (−237 to +32) linked to the rabbit β-globin-coding sequences (pL; Fig. 1A) was transfected into growing HeLa cells together with either a recombinant plasmid coding for the Ela 13S mRNA product (pEIA13S; see reference 22) or a recombinant containing the Ela promoter (−500 to −9) linked to the rabbit β-globin-coding sequences (pEIAG; see reference 4). The results of quantitative S1 nuclease mapping of EIIaL-specific RNA extracted from these cells demonstrate that the presence of the Ela 13S mRNA product does not stimulate the level of transcription from the EIIaL promoter (Fig. 1B, lanes 1 and 5). Similar results were obtained for a deletion mutant of the EIIaL promoter missing upstream sequences between −237 and −37 (pL37; Fig. 1A). This promoter segment, while transcribed about 10-fold less efficiently than the wild-type pL, was also completely unaffected by the presence of cotransfected pEIA13S (Fig. 1B, lanes 2 and 6; data not shown). This eliminates the possibility that the EIIaL promoter could appear unresponsive to the Ela gene products by virtue of a sequence element in the upstream region that would mask the stimulatory effect of Ela.

The above results are in sharp contrast to those obtained when the upstream region of the EIII promoter (−237 to −37) was linked to the EIIaL TATA box-capsite region (pLE3; Fig. 1A) and cotransfected into HeLa cells with either pEIAG or pEIA13S. Transcription from this chimeric promoter was stimulated about sixfold by the Ela 13S mRNA product (Fig. 1B, lanes 4 and 8). Similar results were obtained when the EIIaE upstream region (−33 to −250) was linked to the same EIIaL fragment (pLE2E; Fig. 1A). In this case cotransfection with pEIA13S increased EIIaL-specific transcription by about 10-fold (Fig. 1B, lanes 3 and 7). Unlike the pLE3 construction, the basal level of transcription (i.e., in the absence of Ela) was about the same for the chimeric (pLE2) as for the wild-type (pL) construction. In contrast, the pLE3 recombinant exhibited a much higher basal level of transcription. Since the pL series contains the β-globin-coding sequences, it was not possible to utilize the β-globin gene-containing reference recombinant described below to normalize the results. Each series of transfections was therefore repeated four times with independent plasmid preparations, and after densitometric scanning of the autoradiograms the results (which in all cases were ±25%) were averaged (Table 1). We conclude that the stimulatory effect of the Ela 13S mRNA product on transcription from the pLE3 and pLE2E chimeric promoter constructions is due to the presence of Ela-responsive sequence elements in the EIII and EIIaE upstream regions that are not present in the EIIaL promoter.

To generalize the above results, a similar series of chimeric plasmids was constructed based on the adenovirus type 2 ML promoter which, while active primarily during the late phase of viral infection, is also active to some degree during the early phase (7). A recombinant containing the ML promoter (−677 to +33) linked to the simian virus 40 T-antigen-coding sequences (pSVA677; Fig. 2A; see reference 13 for details of construction) was cotransfected into HeLa cells with either pEIAG or pEIA13S. Transcription of the rabbit β-globin recombinant pEIAG was used as an internal standard in experiments in which no Ela gene products were produced (Fig. 2, lanes 1 through 5, lower panel, Glob−9). It encodes a globin RNA that is initiated from position −9 with respect to the globin start site. Transcription from the recombinant pβ2x, which contains two complete copies of the rabbit β-globin transcription unit (6), was used as an internal standard in experiments in which pEIA13S was used (Fig. 2, lanes 6 through 10, lower panel, Glob+1). The amount of ML-specific RNA was then corrected, within each series (+pEIAG or +pEIA13S) according to the level of globin transcription. The average normalized values from four experiments are presented in Table 1. Only a very low level of stimulation by the 13S mRNA product (approximately twofold) was observed for the ade-

TABLE 1. Relative transcriptional activity of chimeric promoter recombinants transfected into HeLa and 293 cells

Recombinants	Relative transcriptional activity in[a]:			
	HeLa cells[b]			293[c] cells
	+pEIAG	+pEIA13S[d]	+pEIA12S	
EIIaL				
pL	1	1 (1)	1	1
pL37	0.1	0.1 (1)	0.1	0.1
pLE2E	1.7	20 (11.8)	23	1
pLE3	50	240 (4.8)		3
ML				
pSVA677	1	1.9 (1.9)		1
pSΔ	0.05	0.1 (2)		0.1
pSE2E	0.5	5.2 (10.4)		0.8
pSE3	2	14 (7)		0.2
pSE2L	8	3 (0.4)		0.7
Wild type[e]				
pEIII	1	10 (10)		
pEIIa	1	10.3 (10.3)		

[a] All values (±25%) given represent the average of four different experiments carried out with independent plasmid preparations.
[b] Within the EIIaL and ML series all values are relative to pL and pSVA677, respectively, in the absence of Ela products (+pEIAG).
[c] Within the EIIaL and ML series all values are relative to the transcriptional activity of pL and pSVA677, respectively, in 293 cells.
[d] The numbers in parentheses represent the average level of stimulation observed when the transcriptional activity of the recombinant in the presence of cotransfected pEIA13S is compared with its transcriptional activity in the presence of cotransfected pEIAG.
[e] From reference 22.

novirus type 2 ML promoter (Fig. 2B, lanes 1 and 6; Table 1) in agreement with a recent study (26) in which the ML promoter was stimulated two- to threefold by EIa. These results are in contrast with a previous report (27) which demonstrated that the EIa gene was required early in the infection cycle for efficient transcription from the ML promoter. One possible explanation for this apparent discrepancy is that the effect of EIa expression on the ML promoter function during infection involves additional events which are not reproduced when the isolated EIa transcription unit is cotransfected with an ML promoter-containing recombinant.

It has been previously demonstrated that the adenovirus type 2 ML promoter upstream sequences required for efficient transcription in vivo lie between −52 and −124 (R. Hen, Ph.D. thesis, Université Louis Pasteur, Strasbourg, France, 1985; R. Hen and N. Miyamoto, personal communication). When deleted for these sequences (pSΔ; Fig. 2A), the ML promoter remained unresponsive to the EIa 13S gene product (Fig. 2B, lanes 2 and 7). This was also the case for a mutant promoter with a more extensive upstream deletion (−372 to −34) (data not shown). When the ML upstream element (−124 to −52) was replaced with upstream sequences from the EIII or the EIIaE promoter (pSE3 and pSE2E; Fig. 2A), ML-specific transcription was strongly stimulated upon cotransfection with pEIA13S (Fig. 2B, lanes 3, 4, 8, and 9; Table 1), in sharp contrast to the results obtained when the EIIaL upstream fragment (−35 to −237) was inserted (pSE2L; Fig. 2A). While this chimeric promoter recombinant exhibited a basal level of transcription several times higher than that of the wild-type recombinant pSVA677, its activity was not increased upon cotransfection with pEIA13S (Fig. 2B, compare lanes 5 and 10; Table 1). This is in agreement with the observation that the EIIaL promoter is insensitive to stimulation by the EIa 13S mRNA product and that it does not contain EIa-responsive sequence elements. It should be noted that the relative strength of the various upstream elements is not related to their inducibility by EIa products. Both the EIII and EIIaL upstream regions are stronger than that of the ML promoter, but only the EIII chimeric promoter was stimulated (compare pSE3, pSE2L, and pSVA677). These results support the conclusion that there is sufficient sequence information in the upstream regions of the EIII and EIIaE promoters for stimulation of transcription by the EIa 13S mRNA product.

EIa 12S RNA product activates transcription from chimeric promoters. We have previously reported that the product of EIa 12S mRNA stimulates EIIaE and EIII transcription when cotransfected into HeLa cells (22). To test the effect of the 12S mRNA product on the chimeric EIIaL constructions described above, pL, pL37, and pLE2E (Fig. 1A) were cotransfected with a recombinant that produced only the 12S mRNA product (pEIA12S; see reference 22). While the pL and pL37 constructions were insensitive to both the 12S and the 13S mRNA products (Fig. 3, lanes 1, 2, 5, 6, 9, and 10; Table 1), the pLE2E chimeric promoter construction was stimulated about 10-fold by either of the EIa products (Fig. 3, compare lanes 3, 7, and 11; Table 1). For comparison, a plasmid containing the entire EIIaE promoter region linked to the rabbit β-globin gene coding sequences (pEIIG) was cotransfected with pEIAG, pEIA12S, and pEIA13S (Fig. 3, lanes 4, 8, and 12). The EIIaE-specific RNA initiated at both the +1 and −26 capsites (23) was stimulated by the pEIA13S or the pEIA12S product to roughly the same level as pLE2E, which is in agreement with our previous report (22). While the insensitivity of the EIIaL promoter to stimulation by the

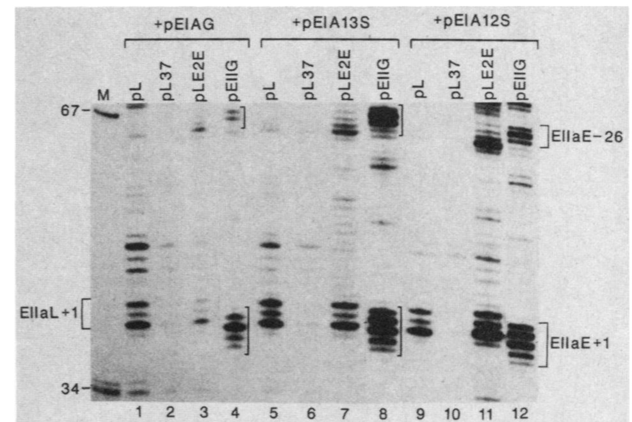

FIG. 3. EIIaE upstream region is responsive to both the EIa 12S and 13S RNA products. Quantitative S1 nuclease mapping of cytoplasmic RNA isolated from HeLa cells transfected with 5 μg of the plasmid indicated at the top of each lane and 0.5 μg of pEIAG (lanes 1 to 4), pEIA13S (lanes 5 to 8), or pEIA12S (lanes 9 to 12). The single-stranded DNA probes are as described in the legend to Fig. 1. The major and minor EIIaE transcription start sites (23) at +1 and −26 (EIIaE +1 and EIIaE −26, respectively) are indicated by brackets (in lanes 4, 8, and 10). The EIIaL transcription start site is indicated as EIIaL +1. The additional bands that appear in lanes 1, 5, 7, and 11 are artifacts of incomplete S1 nuclease treatment. Size markers (lane M) are as in Fig. 1.

13S mRNA product is in agreement with previous observations (12; Goding, personal communication) and with virological evidence (5), the observation that the 12S mRNA product has no effect on EIIaL transcription is in contrast to a recent report (12) in which it appears to repress transcription from the EIIaL promoter. This may be due to differences in either the cell types or the EIa 12S and 13S mRNA-producing plasmids used in the two laboratories.

High levels of transcription in 293 cells are not solely related to the presence of EIa-responsive promoter elements. The 293 cell line is an adenovirus-transformed human cell line that constitutively expresses the viral EIa proteins (10). Transfection of DNA into these cells has often been used as a model to study the effects of EIa proteins on the expression of specific viral and cellular genes. The two families of recombinants described above exhibit a large range of EIa-induced responses and thus can be used to test the suitability of 293 cells to analyze induction of transcription by the EIa gene products.

Both the EIIaL and the ML series of plasmids were transfected into 293 cells as described above for HeLa cells. It is striking that all of the EIIaL series recombinants (Fig. 4A; Table 1) were expressed at approximately the same level in 293 cells. This differs both from the HeLa cell basal level pattern (in which pLE3 was 25- to 50-fold stronger than pL and pLE2E) and from the EIa-stimulated pattern (in which pLE2E was 20-fold higher and pLE3 over 200-fold higher than pL). Similar striking differences were apparent when the ML series was examined. pSE2L had a basal level of transcription in HeLa cells that was eightfold higher than that of the wild-type pSV677 and fourfold higher than that of pSE3 (Table 1; Fig. 2B). In the presence of cotransfected pEIA13S, pSE2L was expressed in HeLa cells at about the same level as the wild-type pSVA677 and more than fourfold lower than pSE3 (Fig. 2B; Table 1). In contrast, pSE3 was the weakest and pSVA677 was the strongest of the three recombinants in 293 cells (Fig. 4B; Table 1). It should be

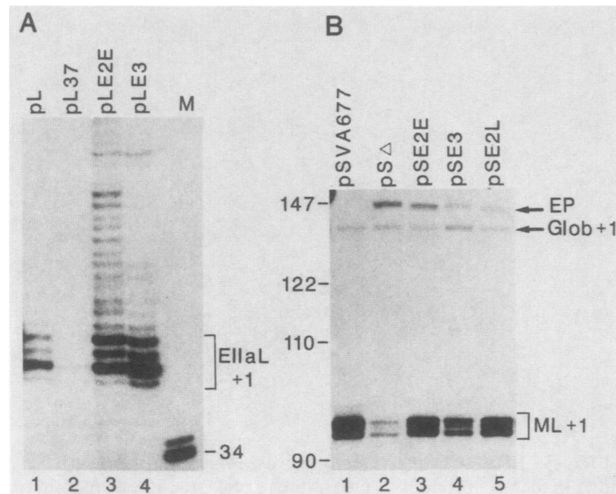

FIG. 4. Transcriptional activity of chimeric promoter regions in 293 cells. Quantitative S1 nuclease mapping of cytoplasmic RNA isolated from 293 cells transfected with 5 μg of either EIIaL recombinants (A) or ML recombinants (B) as indicated at the top of each lane. All single-stranded DNA probes are as described in the legends to Fig. 1 and 2. The transfection represented in panel B also contained 2 μg of the reference recombinant pβ2x (Glob+1). Protected probe fragments are indicated as in Fig. 1 and 2. All symbols are as in Fig. 1 and 2.

noted that the deleted recombinants pL37 and pSΔ were as defective for transcription in 293 cells as in HeLa cells (Table 1). It is important to stress that all transfection experiments presented here were carried out at levels of input DNA far below those required to saturate transcription in both HeLa and 293 cells. Thus, we conclude that the relative transcriptional efficiency of the two chimeric promoter recombinant families in 293 cells is not related to their efficiencies in HeLa cells either in the presence or in the absence of the EIa gene products.

DISCUSSION

EIa-responsive elements are present in the upstream promoter regions of genes strongly induced by the EIa products. The adenovirus EIa proteins activate transcription from viral EIII and EIIaE promoters during the early phase of infection (3, 19). We show here that in transfected HeLa cells, sequences located in the upstream regions of the EIII and EIIaE promoters are responsible for the stimulation of transcription by the EIa gene products. When these upstream regions were linked to the TATA box-capsite region of the EIIaL promoter (that is itself unaffected by EIa), the chimeric promoter constructions were stimulated by EIa to approximately the same extent as the promoter from which the upstream region is derived (Table 1). This was also true for chimeric constructions containing the EIII or EIIaE upstream regions linked to the TATA box-capsite element of the ML promoter. Such EIa-responsive sequence elements are not present in the upstream region of the EIIaL promoter, since transcription of an equivalent construction containing its upstream region was not stimulated by the EIa products.

Our present results raise the question of whether the EIa-responsive sequences in the upstream regions of the EIII and EIIaE promoters represent the unique determinants of EIa inducibility of these promoters or only a subset of the EIa-responsive elements. We have previously shown that, in the case of the EIII promoter, the TATA box region (−40 to −18) is fully dispensable for stimulation by the EIa products (21). In the case of the EIIaE promoter, the TATA box-capsite region (−33 to +34) is not (or only weakly) stimulated by the EIa gene products (D. Zajchowski and H. Boeuf, manuscript in preparation). These results, taken together with those reported here, indicate that the EIa-mediated stimulation of the EIIaE and EIII promoters is due to sequence elements located uniquely in their upstream regions.

While the exact identity of these EIa-responsive sequence elements in the −48 to −250 fragment of the EIIaE promoter has not been determined, their location can be inferred from previous results which have identified an EIa-inducible enhancer element located between positions −21 and −262 (16, 18) and shown to map essentially between −27 and −111 (P. Jalinot and C. Kédinger, personal communication). The EIII promoter, on the other hand, apparently does not contain a similar enhancer-like element, since a derivative of pSE3 (Fig. 2) that contains the EIII upstream sequence inserted into pSΔ in the reverse orientation was transcribed about 10-fold less efficiently than pSE3 and was not EIa inducible (unpublished data). However, previous studies have shown that a mutated EIII promoter deleted for sequences between −39 and −111 was fully inducible by the EIa gene products (21), suggesting that EIa-responsive sequence elements are located between −111 and −233.

Our present conclusion is in contrast to that of Green et al. (11) who suggested that, in the case of the human β-globin gene, the TATA box element only is required for mediating the stimulatory effect of the EIa products. A possible explanation for this discrepancy is that the β-globin promoter may respond to EIa gene products by a mechanism different from that responsible for the activation of the EIII and EIIaE promoters. Results from our laboratory indicate that the β-globin gene promoter is only weakly stimulated when cotransfected into HeLa cells with plasmids expressing the EIa products (approximately twofold; P. Jalinot, H. Boeuf, and D. Zajchowski, personal communication; unpublished data). The sequence target for this low level of stimulation may include the TATA box since the complete β-globin promoter and a deletion mutant retaining only the TATA box and sequences to +37 are stimulated to approximately the same low extent by the EIa products (D. Zajchowski and H. Boeuf, personal communication), suggesting that, in fact, the upstream elements of the β-globin genes play no specific role in EIa-mediated stimulation. We cannot exclude that a strongly EIa inducible promoter such as EIII or EIIaE when deleted for its upstream elements can still be stimulated to a low level by a mechanism similar to that responsible for the weak stimulation of the β-globin or adenovirus type 2 ML promoters. This may partially explain the failure of some studies (see Introduction) to identify regions of the EIII and EIIaE promoters specifically required for EIa stimulation, since these strongly inducible promoters deleted for their upstream EIa-responsive elements may still show some residual nonspecific stimulation by the EIa products.

Relative level of transcription in 293 cells is not related solely to the presence of specific EIa-responsive elements. The apparent discrepancy between the results reported here and those of Green et al. (11) may also reside in their use of the 293 cell line (10), a line which has often served as a model to study the effect of EIa gene products on transcription of transduced genes. The results presented here and summarized in Table 1 demonstrate that various promoters are

transcribed at approximately the same level in 293 cells, irrespective of the extent of their stimulation by the EIa products in HeLa cells (compare, for example, pL with pLE2E or pSVA677 with pSE2E in Table 1). Together with a recent report demonstrating that transfected DNA is stabilized in the nuclei of 293 cells to a much greater extent than in other cell lines (1), our results indicate that relatively high levels of transcription can be achieved in 293 cells in the absence of specific EIa sequence elements and therefore are not uniquely dependent on the activation of such elements by EIa proteins. It is interesting to note that deletions of the upstream regions of the EIIaL and ML promoters (pL37 and pSΔ, respectively) resulted in similar decreases of transcription in 293 cells and in HeLa cells (Table 1). Thus, while a high level of transcription in 293 cells can be observed in the absence of a specific EIa-responsive promoter element, it appears nevertheless to require a functional upstream promoter sequence.

Several classes of promoter responses to EIa gene products. In conclusion, it appears that promoters can be divided into at least three general classes with respect to the effect of EIa gene products on their transcriptional activity: (i) those that are strongly inducible (such as the EIII and EIIaE promoters); (ii) those that are not stimulated or weakly stimulated (the most common class, e.g., the β-globin, adenovirus type 2 ML, and EIIaL promoters); and (iii) those that are inhibited by the EIa products, such as some viral and cellular enhancer-containing promoters (4, 29; Hen et al., in press). While the sequences responsible for the repression of enhancer-containing promoters appear to reside in the enhancer itself, the results presented here indicate that the sequence targets for EIa-mediated stimulation of the EIII and EIIaE promoters are located in their upstream regions. Since there is only a weak similarity in the upstream sequences of the EIII and EIIaE promoter regions, the EIa proteins (or EIa-induced cellular proteins) may not interact directly with these sequences. In vitro competition studies have demonstrated that there exist several classes of upstream factors that interact with specific sets of promoters (24; N. Miyamoto, personal communication). Thus, the EIa proteins may interact with and increase the activity of a class of transcription factors binding uniquely to EIa-stimulatable promoters, such as those of the EIII and EIIaE transcription units.

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