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Transcriptional analysis of the adenovirus-5 EIII promoter: absence of sequence specificity for stimulation by EIa gene products

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ABSTRACT

To identify the adenovirus-5 EIII promoter sequences that are involved in basal level of transcription, a series of promoter deletion mutants were analyzed in vivo by transfection into HeLa cells and in vitro using a HeLa whole cell extract system. Three regions within the EIII promoter were shown to be important for efficient transcription : the TATA sequence, an upstream element centered at -55/-57, and an additional element located between -111 and -233. In vivo transcriptional analysis of EIII promoter deletions in the presence of adenovirus EIa gene products have demonstrated that the same three regions are required for EIa-stimulated transcription. We conclude that there is no sequence element in the EIII promoter between -15 and -233 that is uniquely required for the stimulation of EIII transcription by EIa gene products.

INTRODUCTION

At early times after adenovirus infection, efficient transcription from all known early promoters (Fig. 1A) requires the expression of the "immediate early" EIa transcription unit (1, 2, 3). The stimulation of early viral transcription by EIa gene products has been reproduced in the absence of viral infection in a variety of transfected (4-8) and micro-injected cells (9, 10). Recently, using a HeLa cell transient expression system and plasmids containing viral early genes, we have demonstrated that the stimulation is due to an increase in the rate of transcription, and that both the 12S and 13S EIa mRNA products are capable of stimulating EIIa and EIII transcription (6). The mechanism of this stimulation, however is not well understood. It is not known for example if EIa gene products act alone or in conjunction with other cellular trans-acting factors, or whether the mechanism involves an interaction of factors with the template or directly with the transcriptional machinery.

To gain further insight into the mechanism of the stimulation of early viral transcription units by EIa gene products, we have examined the sequences in the viral EIII promoter that are required for "basal level"

(i.e. transcription level in the absence of EIa gene products) and EIa-stimulated transcription. The effect of a series of internal deletions in the EIII promoter has been analysed in vivo in transfected HeLa cells. Three sequence elements appear to be important for efficient transcription: the TATA element, a proximal upstream element centered at -55/-57, and a distal upstream element located between -111 and -233. Transcription of mutant promoters in vitro using a HeLa whole cell extract showed that the TATA element was absolutely required for promoter function, and that upstream sequences between -35 and -57 strongly affected the efficiency of transcription. Analysis of the effect of EIII promoter deletions in the presence of EIa products was carried out in HeLa cells by co-transfection with an EIa-containing plasmid, or in 293 cells that constitutively express EIa proteins (11). The relative degree of stimulation of transcription by EIa gene products was the same for the wild-type recombinant and the various deletion mutants. Thus, no sequence element could be identified that was uniquely required for stimulation of EIII transcription by EIa products.

MATERIALS AND METHODS

Generation of deletions in the EIII promoter.

pEIII (Fig. 1A) was linearized at the unique SmaI site (-40 in the EIII promoter, Fig. 1B), and treated with Bal-31 exonuclease as described (12). After digestion at the unique BglII site located at +569 (Fig. 1A), the two fragments were separated on a 5-20% sucrose gradient. The small fragment was ligated to the large SmaI-BglII fragment prepared from the pEIII wild-type recombinant, and the large fragment from the Bal-31 treated plasmid was ligated to the small SmaI-BglII fragment from the pEIII wild-type recombinant. This procedure generated two families of plasmids ; one containing deletions extending from the SmaI site toward the EIII capsite, and the other containing deletions extending from the SmaI site in the upstream direction. The 233.37 deletion was generated by excision of the EcoRI-SmaI fragment (-235 to -40) from the wild-type pEIII recombinant. The deletions were analysed by restriction enzyme digestion and DNA sequencing.

Transcriptional analysis of EIII promoter deletion mutants in transfected cells.

HeLa cells or 293 cells were transfected at 50-70 % confluence with 0.5-20 μg of recombinant plasmid (see Figure Legends) per 10 cm Petri dish as described (13). After 36-48 h cytoplasmic RNA was purified from cells

lysed with 0.3 % Nonidet-P40. Hybridizations were carried out with 25 µg of total cytoplasmic RNA (approximately one half of the RNA from one Petri dish) dissolved in 10 µl of 10 mM Pipes, pH6.5/0.4 M NaCl containing an excess of the appropriate single-stranded DNA probes (see below and Figure legends) and hybridized at 68°C for 12 h. The samples were then diluted into 200 µl of 30 mM NaOAc, pH4.5/3 mM $ZnCl_2$, 400 mM NaCl containing 40 units (BRL) of nuclease S1, and incubated for 2 h at 25°C. Nuclease resistant hybrids were analysed on 8% acrylamide/8.3 M urea sequencing gels (14). The EIII specific S1-nuclease resistant hybrids generated in this way have been analyzed previously, and shown to be identical to those generated by EIII specific RNA synthesized during normal adenovirus infection of HeLa cells (6). The recombinant pβ(244+)β was used in several experiments as an internal control for transfection efficiency (Fig. 2 and Fig. 4, lanes 1-5). In these cases EIII specific RNA was normalized according to the amount of variations in the globin signal. Where the pβ(244+)β recombinant was not used, for example in cases where globin expression would be inhibited by EIa products (15, 16), the experiments were repeated several times (Fig. 4A, lanes 6-10; and Figs. 4B and 5).

Probes for S1 nuclease mapping.

The single stranded DNA probe (shown in Fig. 1A) used to detect EIa specific RNA (Fig. 4) was prepared from pE1ASV and is the coding strand of the EcoRI/Sau3A fragment (-498 to +129) labeled at the 5' end with ^{32}P. The rabbit β-globin probe used in the experiment shown in Fig. 2 and Fig. 4A was prepared from pβ(244+)β as described (17). The EIII specific probe was made using a 5'-^{32}P-end labeled synthetic 19-mer corresponding to the coding strand of the EIII sequence between positions +96 and +115 which was hybridized to a single stranded M13-mp8 phage clone containing the non-coding strand of the EIII EcoRI-PstI(-235 to +115) fragment for 2 h at 65°C in 10 mM $MgCl_2$/10 mM Tris-HCl, pH8 and then elongated in the presence of 0.35 mM of each of the four dNTPs and 3-5 units of the large fragment of E.coli DNA polymerase (BRL). After digestion by EcoRI, the 350 nucleotide long ^{32}P-end labeled single-stranded DNA fragment was isolated on an 8 % polyacrylamide strand separation gel (14).

In vitro transcription.

The in vitro transcription assay (18) and the preparation of HeLa whole cell extract have been described (19). The DNA template for run-off assays was a complete BglII digest of pEIII or of the pEIII deletion mutant derivatives of which 250 ng was used for 25 µl total volume reaction. The

569 nucleotide-long run-off RNA was analyzed on 5 % acrylamide/8.3 M urea gels (14).

RESULTS

Experimental design

To examine the possible involvement of specific EIII promoter sequences in the EIa-mediated stimulation of transcription, a series of EIII promoter mutants were constructed containing small, internal deletions (Fig. 1). The transcriptional activity of these mutants was analysed in vivo in the absence and in the presence of EIa gene products by comparing the amount of EIII-specific cytoplasmic RNA produced from each mutant to the amount produced from the wild-type promoter under the same conditions. A mutant that deletes a sequence uniquely interacting with factors involved in the basal (non-stimulated) level of transcription, can be expected to show the same relative reduction in RNA levels under stimulated and basal level conditions. Conversely, a mutant that deletes an element uniquely interacting with a factor(s) required for EIa stimulation should produce wild-type amounts of RNA in the absence of EIa gene products, but relatively lower amounts in their presence. In the combined case, where an element involved in EIa-mediated stimulation is deleted together with an element involved in the basal level of transcription, the decrease from the wild-type level will be more drastic in the presence of EIa products than in their absence.

In vivo and in vitro analysis of basal level transcription from the EIII promoter deletion mutants.

To establish basal levels of transcription, the recombinant pEIII, which contains the EIII transcription unit (Fig. 1A), and its deletion mutant derivatives (Fig. 1B) were introduced into HeLa cells by the calcium-phosphate co-precipitation technique (13) in the absence of EIa gene products. Cytoplasmic RNA isolated 36-40 h after transfection was analysed by quantitative S1-nuclease mapping using the EIII-specific probe shown in Fig. 1A. In this experiment the reference recombinant pβ(244^{+})β, containing two copies of the rabbit β-globin gene and the polyoma enhancer element (17) was transfected together with the EIII plasmids. β-globin specific RNA was quantified by S1-nuclease mapping (using the probe described in ref. 17) together with EIII-specific RNA. The EIII signal in each lane was normalized according to the variations in the globin signal. The results of several experiments (Fig. 2 and Table I, row 1) demonstrate that the

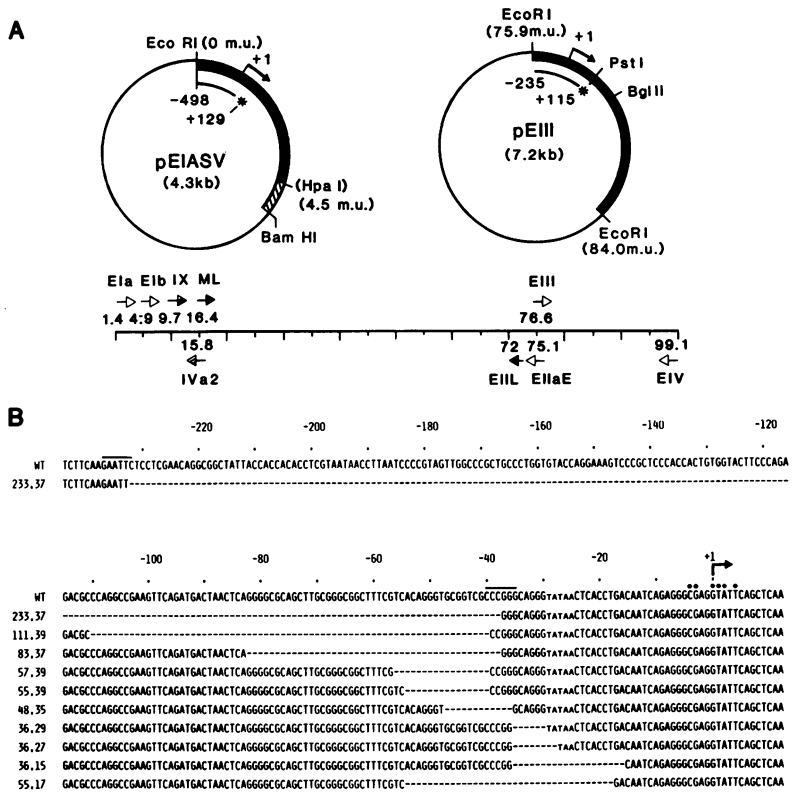

Figure 1 : (A) Organization of adenovirus-5 (Ad5) transcription units, and recombinants pE1ASV and pEIII. The Ad5 transcription map is taken from data in ref. 34. Transcription units active during the early phase of infection are indicated by open arrows, while those active at intermediate or late times are indicated respectively by hatched or filled arrows. The numbers on the Ad5 map indicate the location in map units (m.u.) of the cap site of each transcription unit. pE1ASV contains the left 4.5 % of the adenovirus-2 (Ad2) genome (HpaI E fragment, solid box), ligated to the 135 bp simian virus 40 (SV40) HpaI (2604)/BamHI (2469) fragment (hatched box) containing the SV40 early and late polyadenylation signals, inserted between the BamHI and EcoRI sites of pML2, a derivative of pBR322 (35). pEIII is the Ad5 EcoRI C fragment inserted into the EcoRI site of pBR322. Restriction enzyme sites in parentheses were lost during construction. Asterisks mark the labeled 5' ends of the probes (narrow lines) used for S1 nuclease mapping (Materials and Methods). (B) Sequence of the wild-type (WT) Ad5 EIII promoter (upper line) and the sequences of each deletion mutant. Deleted nucleotides are indicated by broken lines. The EcoRI site (75.9 m.u.) at -235 and the SmaI site at -40 used to generate the deletions are overlined. The name of each deletion mutant is indicated to the left of the sequences. Startsites for transcription are indicated by filled dots and the +1 by and arrow.

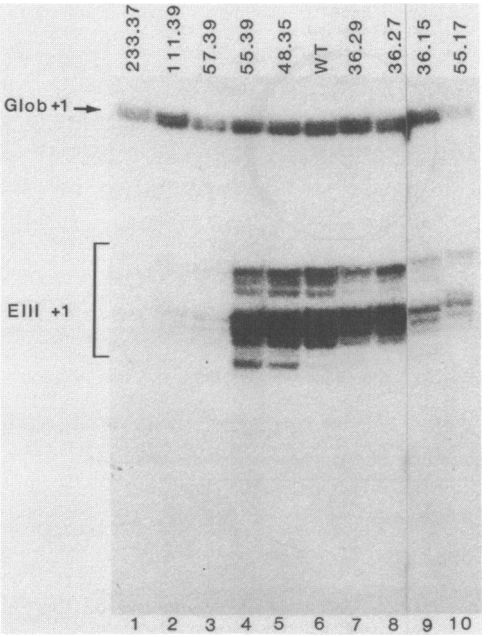

Figure 2 : Transcriptional activity of EIII promoter deletion mutants in HeLa cells. Each pEIII recombinant (15 μg) was transfected together with 0.5 μg of the reference recombinant pβ(244+)β, and the EIII and β-globin specific RNA analysed by quantitative S1-nuclease mapping as described in Materials and Methods. A representative gel of this experiment is presented. The results of this and several similar experiments are presented in Table 1, row 1. The structure of the deletion mutants and of the wild-type pEIII plasmid is described in Fig. 1. EIII +1 indicates the EIII transcription startsites shown in Fig. 1 ; Glob +1 indicates the globin transcription startsite from pβ(244+)β (17). Variations in transfection efficiency are indicated by variation in the β-globin signal.

EIII promoter contains an important upstream element at position -55/-57 that, when deleted (Fig. 2, compare lanes 3 and 4), reduced transcription by about 10-fold. There are no crucial elements located between positions -57 and -111 as deletion of this region (Fig. 2, lanes 2 and 3) resulted in only a slight additional decrease in the amount of EIII RNA. However, the deletion of the entire upstream region (-37 to -233, Fig. 2 lane 1) caused a complete loss of transcriptional activity. This suggests that there is an additional upstream element located between -111 and -233 that affects the efficiency of the EIII promoter.

Deletions extending from -36 toward the transcriptional startsite (+1) show that the TATA region is not absolutely required for expression in vivo. Mutant 36.27 (Fig. 2, lane 8), that deletes the first TA of the TATA sequence, was nearly as efficient (70 %) as the wild-type promoter (Table I, row 1). Mutant 36.15 (Fig. 2, lane 9), that deletes the entire TATA sequence, was decreased to about 20 % of the wild-type level (Table I, row 1). It is interesting that EIII promoters lacking a TATA element (36.27, 36.15 and 55.17) continued to utilize the wild-type transcriptional startsites (Fig. 2, lanes 8 to 10).

The present series of deletion mutants was also analysed in vitro using a HeLa whole cell extract transcription system (19). The results

TABLE I
Effect of EIII promoter deletions on transcriptional efficiency (as percent of wild-type RNA level)

Experimental Conditions	Deletion mutant (a) 233.37	111.39	83.37	57.39	55.39	48.35	36.29	36.27	36.15	55.17
Transfection into HeLa cells	<1	10(5)	23(3)	15(8)	58(28)	84(16)	62(8)	67(16)	20(12)	65(4)
Transfection into HeLa cells together with pEIASV	<1	4(1)	-	8(3)	39(9)	74(10)	60(9)	55(11)	21(6)	71(4)
Transfection into 293 cells	<1	8(2)	7(3)	9(3)	61(15)	67(8)	100(5)	63(17)	8(1)	30(8)
Transcription In vitro	-	3(1)	10(5)	9(4)	23(5)	45(5)	81(1)	2(1)	1(0.5)	-

(a) see Fig. 1B

Table I : Transcriptional activity of EIII promoter deletion mutants in vivo in the presence and absence of EIa gene products and in vitro in a HeLa whole cell extract system. Each row represents cytoplasmic RNA levels as the percent of the amount of RNA produced by the wild-type promoter under the conditions given at the left hand side of the table. Values presented are the average of 3 experiments. Numbers in parentheses represent the largest variation from the average value for each data point. The wild-type level of the series presented in the second row was approximately 5 times higher than that for the series shown in the first row. Quantitation of RNA levels was by densitometric scanning of several exposures of each S1-nuclease mapping gel.

(Fig. 3 and Table I, row 4) demonstrate that both the TATA element and an upstream element were required for optimal levels of transcription in vitro. The clear localization of the upstream sequences observed in vivo (-55/-57) was not apparent in the in vitro analysis. Instead there was a gradual decrease in transcriptional efficiency as sequences extending from -35 to -57 were deleted (48.35, 55.39 and 57.39, Table I). There was a striking difference in the relative contribution of the TATA element to the efficiency of the promoter in vivo and in vitro. Transcription from the 36.27 deletion (missing the first TA of the TATA element) that was nearly as efficient as the wild-type promoter in vivo (Fig. 2, lane 8) was reduced by 50-fold in vitro (Fig. 3, lane 7, Table I).

Transcriptional analysis of EIII promoter mutants in the presence of EIa gene products.

The effect of EIa gene products on transcription of the EIII deletion mutants was examined by co-transfecting EIII plasmids into HeLa cells with

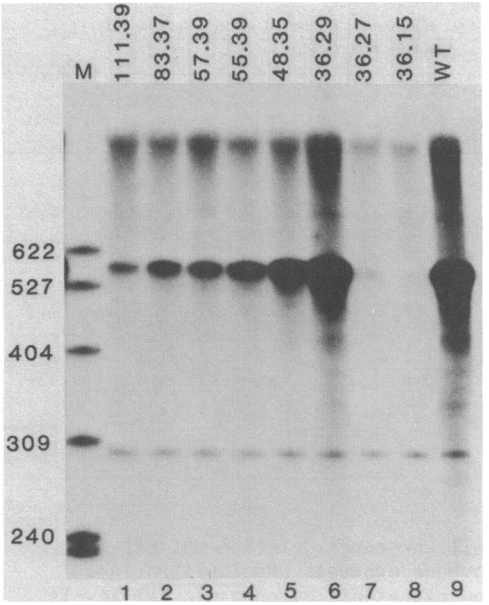

Figure 3 : Transcriptional run-off analysis of EIII promoter deletion mutants in vitro using a HeLa whole cell extract transcription system. Plasmids linearized at the BglII site were used as templates, generating a specific 569 nt run-off transcript. The in vitro transcription conditions are described in Materials and Methods. Lane M : size marker (^{32}P-end-labeled MspI fragments of pBR322).

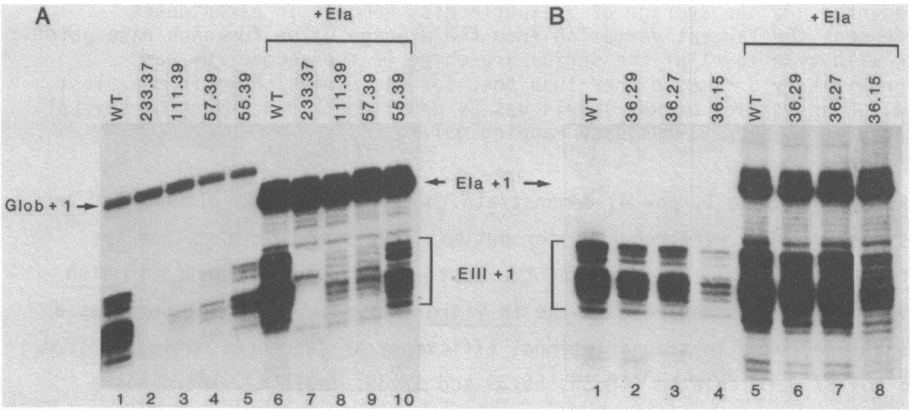

Figure 4 : Transcriptional activity of EIII promoter deletion mutants in HeLa cells in the presence or absence of Ela gene products. 15 μg of each mutant plasmid were transfected into HeLa cells either alone (panel A lanes 1-5 and panel B lanes 1-4) or together with 1 μg of pE1ASV (panel A lanes 6-10 and panel B lanes 5-8). Cytoplasmic RNA was analysed by quantitative S1-nuclease mapping using probes shown in Fig. 1. The transfections shown in panel A lanes 1-5 contained 0.5 μg of the reference recombinant pβ(244+)β. RNA synthesized from this plasmid is indicated by Glob +1. The results of several similar experiments are presented in Table I, rows 1 and 2.

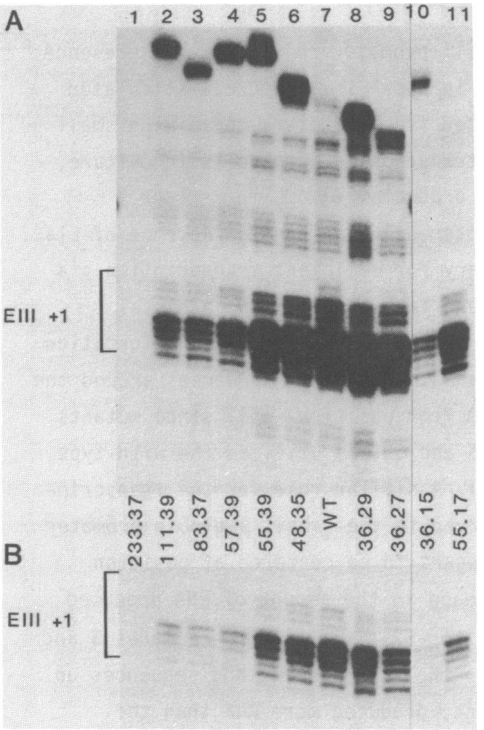

Figure 5 : Transcriptional activity of EIII promoter deletion mutants in 293 cells. EIII recombinants (15 μg) were transfected into 293 cells and the RNA analysed by quantitative S1-nuclease mapping as described in Materials and Methods. Panels A and B are different exposures of the same gel. A representative gel of this experiment is presented here. The results of this and several additional experiments are presented in Table I, row 3. The bands that appear in the upper part of the autoradiogram represent the end-points of homology between the DNA probe and RNA initiated from sites further upstream. Mutants 36.15 and 55.17 never give strong end-point signals, perhaps because of an unusual RNA-DNA hybrid secondary structure in these cases.

pE1ASV (Fig. 1A), a plasmid that produces both the EIa 12S and 13S mRNAs (6). Under these transfection conditions, the level of EIII transcription was stimulated 5-20 fold by the products of the co-transfected EIa gene (6). The results of such experiments (Fig. 4, and Table I, row 2), demonstrate that the effect of each deletion was approximately the same in the presence or absence of EIa products (i.e., transcription from each mutant was stimulated to the same relative degree). The same results were obtained when a derivative of pE1ASV that produces only the 13S mRNA product (pE1A13S, in ref. 6) was co-transfected with the EIII deletion mutants (data not shown).

Transcription of the EIII mutants was also analysed by transfection into 293 cells, an adenovirus-5 transformed human embryonic kidney cell line (11) that constitutively expresses EIa proteins. These results (Fig. 5 and Table I, row 3) show that again each mutant reduced transcription from the EIII promoter by the same degree as in the absence of EIa protein (Table I, compare row 3 to row 1).

DISCUSSION

We have analyzed the adenovirus EIII promoter region for the presence of sequence elements that are involved in basal level and EIa-stimulated transcription. The amount of RNA produced from promoters containing small internal deletions was measured after transfection into cells in culture, in the presence or absence of EIa gene products.

In vivo analysis of the EIII promoter deletions in the absence of EIa products revealed three regions necessary for efficient transcription : a TATA element, a proximal upstream element (at -55/-57), and a distal upstream element (located between -111 and -233). In addition, information necessary for accurate initiation appears to reside in sequences around the transcriptional start-sites (downstream from position -15), since mutants missing the TATA sequence (36.27, 36.15 and 55.17) utilized the wild-type start-sites (Fig. 2, lanes 8, 9 and 10). A similar role for the transcriptional start-site region has been observed in the rabbit β-globin promoter (20). The proximal upstream element appears to be centered at position -55/-57, since there is an abrupt decrease in the amount of RNA produced when the deletion is extended from -55 to -57 (Fig. 2, compare lanes 3 and 4). Mutant 55.17, which is deleted for the TATA element and sequences up to but not including the -55/-57 element, produced more RNA than the promoters containing shorter TATA deletions (36.27 and 36.15, Table I). These observations suggest that the upstream element is more efficient in stimulating transcription when closer to the start-site. The presence of a second more distal upstream element (located between positions -111 and -233) is not uncommon since several upstream elements have been observed in other promoter regions, like those of the rabbit β-globin gene (20) and herpes simplex virus thymidine kinase gene (21).

Comparison of *in vitro* and *in vivo* data demonstrates that the TATA element makes a much larger contribution to the efficiency of the promoter *in vitro* than *in vivo* (Table I). Similar results have been reported for TATA box elements of the chicken conalbumin and rabbit β-globin promoters when *in vitro* and *in vivo* transcription were compared (22-25). The present *in vitro* analysis of EIII mutants demonstrates a stronger dependence on upstream promoter sequences than a previous report (26). This is probably because we have used a whole cell extract rather than an S100 transcription system. It has already been demonstrated that a whole cell extract system more accurately reflects the *in vivo* requirements for upstream promoter elements (27, 28).

Analysis of the EIII transcription in the presence of EIa products was accomplished by co-transfection of the EIII mutants into HeLa cells with a recombinant producing EIa gene products, or by transfection into 293 cells that constituitively express EIa proteins. In both cases the relative effect of each mutation with respect to wild-type levels was approximately the same as in the absence of EIa gene products (Table I). Every mutant that retained some transcriptional activity was stimulated by EIa. These results suggest that there is no sequence element in the EIII promoter (from -233 to -15) that is uniquely required for the EIa-mediated stimulation of transcription. These results are consistent with a model in which the EIa products stimulate the overall activity of the transcription machinery by facilitating, for instance, the assembly of an active initiation complex. Such a general mechanism could operate without a unique "EIa responsive" sequence element in EIa-stimulated promoters.

In addition to the stimulation of transcription of the other early adenovirus genes, the EIa gene products are capable of stimulating transcription of some cellular genes (29-31). Transcriptional analysis of the human β-globin gene transfected into 293 cells (32) suggested that only the TATA element and not the upstream elements, was involved in the EIa-mediated stimulation of transcription. This is clearly in contrast to data presented here demonstrating that the EIII upstream region was required for efficient transcription in the presence of EIa products (Figs. 4 and 5) and that a mutated EIII promoter completely missing the TATA sequence (36.15) was stimulated to the same relative degree as the wild-type promoter (Fig. 4b, compare lanes 4 and 8). A similar conclusion has been reached after a detailed analysis of the adenovirus-2 EIIa promoter region (Zajchowski, D., Boeuf, H. and Kédinger, C. submitted). These apparently contrasting results may be explained by an ability of EIa products to stimulate transcription from different promoters by more than one mechanism. In this respect it is worth noting that there appear to be some promoters that are not stimulatable by EIa products. It has been shown that in co-transfection experiments, transcription from Adenovirus-2 major late and EII-late promoters is not stimulated by EIa gene products (Leff, T. and Goding, C., unpublished results). This is consistent with the observation that during infection, viral intermediate and late promoters are not activated at the same time as the early genes (for review see ref. 33). Experiments are under way to examine the difference between promoters that are stimulatable by EIa gene products and those that are not.

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REFERENCES

1. Jones, N., and Shenk, T. (1979) Proc. Natl. Acad. Sci. USA, 76, 3665-3669.
2. Berk, A.J., Lee, F., Harrison, T., Williams, J., and Sharp, P.A. (1979) Cell, 17, 935-944.
3. Nevins, J.R. (1981) Cell, 26, 213-220.
4. Weeks, D.L., and Jones, N.C. (1983) Mol. and Cell. Biol., 3, 1222-1234.
5. Bos, J.L., and ten Wolde-Kraamwinkel, H.C. (1983) The EMBO J., 2, 73-76
6. Leff, T., Elkaim, R., Goding, C.R., Jalinot, P., Sassone-Corsi, P., Perricaudet, M., Kédinger, C., and Chambon, P. (1984) Proc. Natl. Acad. Sci. USA, 81, 4381-4385.
7. Imperiale, M.J., Feldman, L.T., and Nevins, J.R. (1983) Cell, 35, 127-136.
8. Svensson, C., and Akusjarvi, G. (1984) The EMBO J., 3, 789-794.
9. Jones, N., Richter, J.D., Weeks, D.L., and Smith, L.D. (1983) Mol. and Cell. Biol., 3, 2131-2142.
10. Rossini, M. (1983) Virology, 131, 49-58.
11. Graham, F.L., Smiley, J., Russel, W.C., and Nairn, R. (1977) J. Gen. Virol., 36, 59-94.
12. Benoist, C., and Chambon, P. (1981) Nature (London), 290, 304-310.
13. Banerji, J., Rusconi, S., and Schaffner, W. (1981) Cell, 27, 299-308.
14. Maxam, A., and Gilbert, W. (1980) Methods Enzymol., 65, 499-580.
15. Sassone-Corsi, P., Hen, R., Borrelli, E., Leff, T., and Chambon, P. (1983) Nucleic Acids Res., 11, 8735-8746.
16. Borrelli, E., Hen, R. and Chambon, P. (1984) Nature (London), in the press.
17. de Villiers, J., and Schaffner, W. (1981) Nucleic Acids Res., 9, 6251-6264.
18. Sassone-Corsi, P., Corden, J., Kédinger, C., and Chambon, P. (1981) Nucleic Acids Res., 9, 3941-3958.
19. Manley, J.L., Fire, A., Cano, A., Sharp, P.A., and Gefter, M.L. (1980) Proc. Natl. Acad. Sci. USA, 77, 3855-3859.
20. Dierks, P., Van Ooyen, A., Cochran, M.D., Dobkin, C., Reiser, J., and Weissmann, L. (1983) Cell, 32, 695-706.
21. McKnight, S.K., Kingsbury, R.C., Spence, A., and Smith, M. (1984) Cell, 37, 253-262.
22. Wasylyk, B., Derbyshire, R., Guy, A., Molko, D., Roget, A., Téoule, R., and Chambon, P. (1980) Proc. Natl. Acad. Sci. USA, 77, 7024-7028.

23. Wasylyk, B., Wasylyk, C., Augereau, P., and Chambon, P. (1983) Cell, 32, 503-514.
24. Grosveld, G.C., Shewmaker, C.K., Jat, P., and Flavell, R.A. (1981) Cell, 25, 215-226.
25. Grosveld, G.C., de Boer, E., Shewmaker, C.K., and Flavell, R.A. (1982) Nature (London), 295, 120-126.
26. Lee, D.C., Roeder, R.G., and Wold, W.S.M. (1982) Proc. Natl. Acad. Sci. USA, 79, 41-45.
27. Hen, R., Sassone-Corsi, P., Corden, J., Gaub, M.P., and Chambon, P. (1982) Proc. Natl. Acad. Sci. USA, 79, 7132-7136.
28. Sassone-Corsi, P., Dougherty, J.P., Wasylyk, B., and Chambon, P. (1984) Proc. Natl. Acad. Sci. USA, 81, 308-312.
29. Nevins, J.R. (1982) Cell, 29, 913-919.
30. Treisman, R., Green, M.R., and Maniatis, T. (1983) Proc. Natl. Acad. Sci. USA, 80, 7428-7432.
31. Gaynor, R.B., Hillman, D., and Berk, A.J. (1984) Proc. Natl. Acad. Sci. USA, 81, 1193-1197.
32. Green, M.R., Treisman, R., and Maniatis, T. (1983) Cell, 35, 137-148.
33. Flint, S.J. (1982) Biochem. Biophys. Acta, 651, 175-208.
34. Tooze, J. (ed.) (1981) in DNA Tumor Viruses, Molecular Biology of Tumor Viruses, 2nd ed., Cold Spring Harbor Laboratory, New-York.
35. Lusky, M., and Botchan, M. (1981) Nature (London), 293, 79-81.