The EMBO Journal vol.4 no.6 pp.1523–1528, 1985

Sequence-specific *trans*-activation of the adenovirus EIIa early promoter by the viral EIV transcription unit

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Communicated by C.Kédinger

The contribution of adenovirus early genes, other than that of the well-documented EIa immediate early gene, to the transcriptional regulation of the viral EIIa early transcription unit was examined. HeLa cells were transfected with EIIa-containing plasmids and co-transfected with distinct plasmids bearing one of the viral regions EIa, EIII or EIV. Co-transfection with the EIV-recombinants, but not the EIII constructs, stimulated specific transcription from the major EIIaE start site (EIIaE1) by 5- to 15-fold, as concluded from quantitative S1 nuclease analysis of cytoplasmic RNA and *in vitro* nuclear 'run-on' transcription assays. The extent of the EIV-induced stimulation was similar to that achieved by EIa under identical conditions. However, in contrast to our observations for EIa-mediated stimulation, where no unique EIIaE1 promoter elements were implicated, maximal induction by EIV requires sequences between positions −48 and −19 (with respect to the EIIaE1 start site).

Key words: RNA polymerase B/transient expression/transcriptional control

Introduction

Adenovirus provides a convenient model system for the study of eukaryotic gene regulation. The viral genome is transcribed by the cellular transcription machinery and it is likely that the control mechanism of adenovirus gene transcription reflects those of cellular genes. Six transcription units (EIa, EIb, EIIa, EIII, EIV, L1) are activated with different kinetics early in infection (for review, see Flint, 1982 and references therein). Products of the first early region to be transcribed, the immediate early region EIa, are necessary for efficient transcription of the other early regions (Jones and Shenk, 1979; Berk *et al.* 1979; Nevins, 1981; Persson *et al.*, 1981; Gaynor and Berk, 1983; Cross and Darnell, 1983; Winberg and Shenk, 1984) and are also able to stimulate expression of endogenous cellular genes like those coding for the human heat-shock proteins (Kao and Nevins, 1983) and β-tubulin (Stein and Ziff, 1984). Transcription of early region EIa is followed sequentially by maximal transcription from early regions EIV, EIII and finally, ~6 h post-infection by maximal transcription from the early regions EIb and EIIa (Nevins *et al.*, 1979).

This temporal regulation suggests that factors other than those encoded by region EIa may be involved in control of viral early gene expression. Therefore we examined, in short-term transfection experiments, the possibility that early regions EIII or EIV play a role in control of expression from the early promoter (EIIaE) of the EIIa transcription unit, which encodes a 72 000 dalton DNA-binding protein (72-K DBP) involved in viral DNA replication. Plasmids bearing early regions EIII and EIV were transfected into HeLa cells together with recombinants containing the adenovirus type 2 (Ad2) EIIaE promoter. The results obtained, based on quantitative S1 nuclease analysis of cytoplasmic RNA, show that the EIV, but not the EIII, transcription unit is capable of increasing cytoplasmic levels of EIIaE specific transcripts. Nuclear 'run-on' transcription experiments demonstrated that the stimulation corresponded to an increased rate of transcription. In addition, use of a series of linker-scanning and internal-deletion mutants in the EIIaE promoter revealed that sequence elements located between −48 and −39, and −33 and −19, are essential for maximal EIV-mediated stimulation of EIIaE transcription.

Results

Stimulation of levels of cytoplasmic RNA initiated from the EIIaE promoter by plasmids containing the EIV transcription unit in transfected HeLa cells

To examine the possibility that adenovirus early regions other than EIa activate gene expression, a recombinant containing the Ad2 EIIaE promoter (positions −250 to +37) fused to the rabbit β-globin coding sequences (pEIIG) was transfected into HeLa cells either alone or together with plasmids bearing both the EIII and EIV transcrption units, (pEIII/EIV) or only the EIII transcription unit (pEIII; see legend to Figure 1 for details of construction). Cytoplasmic RNA was isolated 36 h after transfection, hybridized to an excess of the [^{32}P]5′-end-labelled DNA probe specific for EIIaE transcripts (see Figure 1) and digested by S1 nuclease. The S1 nuclease-resistant hybrids were then denatured and subjected to electrophoresis and autoradiograhy (Materials and methods). Transcription from the EIIaE promoter initiates both at major start sites around position +1 (EIIaE1) and at minor start sites at position −26 (EIIaE2) both *in vivo* and *in vitro* (see Mathis *et al.*, 1981; Elkaim *et al.*, 1983 and Figure 4). We have previously shown that transcription initiates accurately from the EIIaE promoter in the transient expression system used here (Leff *et al.*, 1984). The basal level of EIIaE-specific RNA in HeLa cells transfected with pEIIG alone is shown in Figure 2A, lane 1. When plasmid pEIII/EIV was co-transfected with pEIIG a 10-fold stimulation in the level of EIIaE-specific RNA was observed (compare lanes 1 and 2 in Figure 2A). However, no significant stimulation was apparent if pEIIG was co-transfected with plasmid pEIII, from which the EIV transcription unit is deleted, but which retains the entire EIII region (compare lanes 1 and 3 in Figure 2A). A recombinant (p233.37) containing a deletion (extending from position −233 to −37) in the EIII promoter region that virtually abolishes transcription from the EIII promoter (Leff *et al.*, 1985), but which contains intact EIII coding sequences and the entire EIV transcription unit, was also able to induce expression of EIIaE-specific transcripts when co-transfected with pEIIG (compare lanes 1 and 4 in Figure 2A).

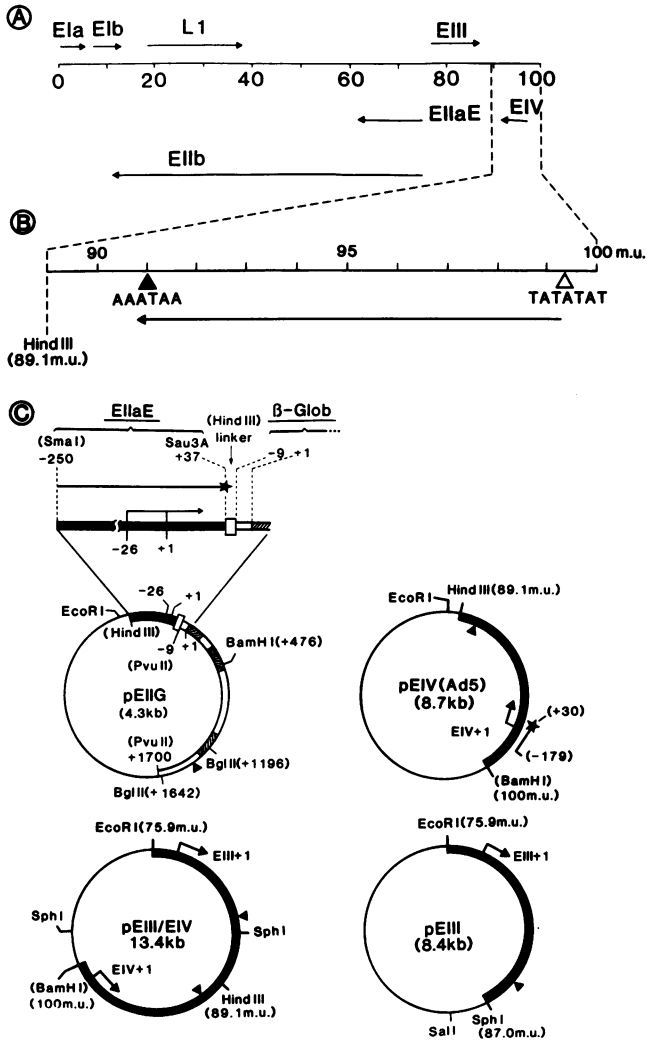

Fig. 1. Organization of early adenovirus transcription units and construction of recombinants. (**A**) Genomic map of adenovirus DNA showing the early viral transcription units (taken from data in Tooze, 1981). (**B**) Expanded map of early region EIV between map units (m.u.) 89.1 and 100 (from data in Tiggs and Raskas, 1984). The position of the TATA box and polyadenylation signal are indicated by the open and closed triangles, respectively. The arrow represents the position and orientation of the EIV primary transcript. The *Hind*III site at 89.1 m.u. is indicated. (**C**) Plasmid pEIIG contains the Ad2 EIIaE promoter region from position −250 to +37 linked *via* a *Hind*III linker to rabbit β-globin sequences (with exons hatched) extending from position −9 to +1700. Plasmid LS WT (not shown, see Zajchowski *et al.*, 1985) is essentially the same as pEIIG, but with EIIaE sequences extending from position −250 to +719. The recombinant pEIII/EIV contains Ad5 sequences extending from the *Eco*RI (75.9 m.u.) site to 100 m.u. inserted between the *Bam*HI (repaired) and *Eco*RI sites of pBR322. Plasmid pEIII was derived from pEIII/EIV by deletion of the *Sph*I (87 m.u.)-*Sph*I (pBR322) fragment. Plasmid p233.37 (not shown) contains a deletion extending from the *Eco*RI site at position −233 to the *Sma*I site at position −37 with respect to the EIII cap site (Leff *et al.*, 1985). The pEIV(Ad5) construct comprises Ad5 DNA extending from 100 m.u. to the *Hind*III site (89.1 m.u.) inserted between the *Bam*HI (repaired) and *Hind*III sites of pBR322. Plasmid pEIV(Ad2) contains the *Eco*RI C fragment (89.9−100 m.u.) of Ad2 inserted between the *Eco*RI and *Pvu*II sites of pBR322 (not shown). The DNA probe used for S1 nuclease mapping of EIIa transcripts is the coding strand of the *Sma*I (−250)-*Sau*3A (+37) fragment labeled at its 5′-end with ^{32}P. The DNA probe for EIV extended from the *Taq*I site at position +30 to position −179 with respect to the major EIV cap site and was [^{32}P]5′ end-labeled at the *Taq*I site. In all cases the filled triangles indicate the positions of polyadenylation signals and the restriction sites in brackets are those which have been lost during the cloning procedures.

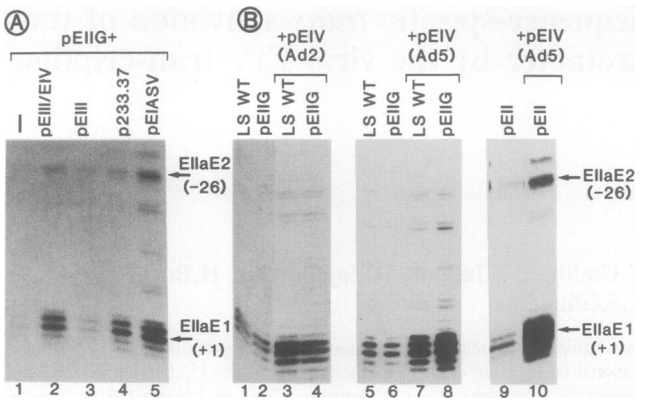

Fig. 2. Induction of EIIaE expression by EIV or EIa products in transfected HeLa cells. (**A**) Quantitative S1 nuclease analysis of 10 μg cytoplasmic RNA isolated from cells transfected with 5 μg pEIIG alone (**lane 1**), or co-transfected with either 10 μg pEIII/EIV (**lane 2**), 7 μg pEIII (**lane 3**), 10 μg p233.37 (**lane 4**) or 5 μg pEIASV (**lane 5**) per dish. (**B**) Quantitative S1 nuclease mapping of 10 μg cytoplasmic RNA isolated from cells transfected with either 5 μg LS WT (**lanes 1, 3, 5** and **7**), 5 μg pEIIG (**lanes 2, 4, 6** and **8**), or 15 μg pEII (**lanes 9** and **10**) alone, or co-transfected with either 7.5 μg pEIV(Ad2) (**lanes 3** and **4**) or 7.5 μg pEIV(Ad5) (**lanes 7, 8** and **10**) per dish. Plasmids LS WT, pEIIG, pEIII/EIV, pEIII, pEIV(Ad5) and pEIV(Ad2) are described in Figure 1C. Plasmic pEII (see Zajchowski *et al.*, 1985) contains the entire EIIa transcription unit between the *Sma*I (75.8 m.u.) and *Bam*HI (59.5 m.u.) sites of Ad2, inserted between the *Sma*I and *Bam*HI sites of pACYC 117. Plasmid pEIASV (described in Leff *et al.*, 1984) contains the left-most *Hpa*I fragment of the Ad2 genome, ligated to the 135-bp SV40 *Hpa*I-*Bam*HI fragment containing the SV40 early and late polyadenylation signals, and inserted into pML2, a derivative of pBR322. The single-stranded EIIa DNA probe used is described in the legend to Figure 1C. Arrows indicate the probe fragments protected by specific transcripts discussed in the text.

These results demonstrate that early region EIV, but not early region EIII, is able to increase transcription from the EIIaE1 start site. Although transcription from the EIIaE1 and EIIaE2 start sites is controlled by two overlapping promoters (Zajchowski *et al.*, 1985), we observed that the extent of the EIV-mediated stimulation of EIIaE2 expression was variable and always lower than that of EIIaE1. Hence we have not further analysed the induction of EIIaE2 expression by EIV.

To compare the stimulation of expression from the EIIaE promoter by pEIII/EIV and p233.37 with the level of induction mediated by the immediate early EIa gene products, pEIIG was also co-transfected with a plasmid bearing the EIa transcription unit, pEIASV (lane 5, Figure 2A). From this, and other experiments, it is clear that EIa and EIV have a similar capacity to increase expression from the EIIaE promoter. In the preliminary experiments described above, the EIV-containing recombinants comprised as much as 25% of the right-most portion of the viral genome. Furthermore, these sequences were taken from Ad5, a serotype homologous to but different from the Ad2 serotype from which the EIIaE sequences were derived. To examine more directly the ability of early region EIV to increase the level of EIIaE-specific transcripts, the EIV transcription unit was subcloned from pEIII/EIV into pBR322 to give plasmid pEIV(Ad5) (see Figure 1). A similar plasmid containing the EIV region (*Eco*RI C fragment) from Ad2 [pEIV(Ad2)] was also constructed (see legend to Figure 1C). Both pEIV(Ad5) and pEIV(Ad2) were co-transfected with plasmid pEIIG (Figure 2B). Quantitative S1 nuclease analysis of cytoplasmic RNA showed that expression from pEIIG was significantly stimulated by co-transfection with plasmids contain-

ing only the EIV transcription unit and whether EIV was derived from Ad2 or Ad5. In this particular experiment the stimulation was ~5-fold (compare in Figure 2B, lanes 2 and 6 with 4 and 8, respectively), but in other experiments ranged between 5- and 15-fold.

To rule out the possibility that the EIV-caused stimulation of pEIIG expression was peculiar to this latter plasmid construct, the effect of pEIV co-transfection on EIIaE-specific expression from two different EIIa-recombinants was analysed: plasmid LS WT, which is similar to pEIIG, but contains EIIaE sequences from positions −250 to +719 fused to the β-globin sequences, and plasmid pEII which contains no globin sequences, but the entire EIIa transcription unit from position −250 to the *Bam*HI site at 59.5 m.u. As shown in Figure 2B (compare lanes 1, 5 and 9 with 3, 7 and 10 respectively), similar levels of stimulation of EIIaE expression by EIV were obtained upon co-transfection with plasmids containing various lengths of EIIa-specific sequences, but sharing the EIIaE promoter region.

Analysis by Southern blotting of total nuclear DNA showed that the copy number of pEIIG was not elevated when co-transfected with pEIV (data not shown), demonstrating that the induction of EIIaE expression described above was not due to variations in plasmid uptake but rather to differences in expression from the templates themselves.

The stimulation of EIIaE expression by pEIV corresponds to an increased rate of transcription

The increase in steady-state cytoplasmic EIIaE RNA mediated by EIV, as detected by quantitative S1 nuclease analysis, could have been a result of an increased rate of transcription or a post-transcriptional event. To determine whether EIV acts at the level of transcription we used a nuclear 'run-on' transcription assay to measure directly the transcription on the transfected templates. Nuclei isolated from cells transfected with plasmid LS WT, alone or co-transfected with pEIV, were analysed under transcription conditions whereby initiation by free RNA polymerase molecules is prevented. In such an assay, the radioactivity incorporated into nascent RNA reflects the number of RNA polymerase molecules actively transcribing *in vivo*. The labeled RNA produced from the LS WT recombinant was quantitated by hybridization to dot-blots of single-stranded recombinant M13 phage DNA containing the coding strand (mG^+) or non-coding strand (mG^-) of the *Bam*HI-*Bgl*II (+476 to +1196; see Figure 1C) globin-specific fragment (see Leff *et al.*, 1984 for details of construction). In the experiment shown in Figure 3, nearly equal amounts of run-on transcripts hybridized both to the globin coding and non-coding strand specific probe. This observation indicates that a significant amount of non-specific transcription occurs, which is probably not limited to the non-coding strand. However, transcripts hybridizing to the coding strand were increased ~5-fold in nuclei isolated from cells co-transfected with pEIV, while those hybridizing to the non-coding strand were increased only 2-fold, compared with cells transfected with LS WT alone (compare columns 1 and 2, rows B and C). The analysis of the cytoplasmic RNA for this experiment revealed a corresponding 6-fold increase in EIIaE1-specific RNA in cells co-transfected with pEIV (not shown). Altogether, these results suggest that pEIV causes an increase of transcription and that the EIIaE promoter is more sensitive to this effect than is the general non-specific transcription occurring on the plasmid.

Specific EIIaE promoter sequences are required for efficient induction of EIIaE transcription by EIV

Having established that EIV stimulates EIIaE expression at the level of transcription, it was of interest to determine whether a specific region of the EIIaE promoter was required for this effect. We have recently constructed and used a series of linker-scanning (LS) and internal deletion (Δ) mutants in the EIIaE promoter to precisely map sequence elements important for constitutive and EIa-induced transcription (Zajchowski *et al.*, 1985). Selected mutants (Figure 4) were therefore transfected into HeLa cells either alone or with pEIV and the cytoplasmic RNA specific for EIIaE and EIV was quantitated by the S1 nuclease assay as described above. The results of such an experiment are shown in Figure 5 and results from this and from similar experiments, using different plasmid preparations, summarized in Figure 6.

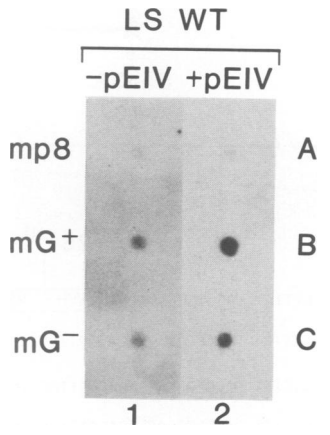

Fig. 3. Stimulation of EIIaE-specific transcription by pEIV. Labeled nuclear run-on transcripts, were isolated after *in vitro* incubation of nuclei (see Materials and methods) prepared from cells transfected with 5 μg LS WT alone (**column 1**) or co-transfected with 7.5 μg pEIV(Ad2) (**column 2**) per dish. The RNA was hybridized against dot-blots of either single-stranded (ss) M13 mp8 DNA (**row A**) or ss M13 mp8 DNA containing the coding strand (mG^+) or non-coding strand (mG^-) of the *Bam*HI-*Bgl*II (+476 to +1196) fragment of the β-globin sequences (**rows B** and **C**, respectively). Plasmids pEIV(Ad2) and LS WT are described in the legends to Figures 1C and 2, respectively. Quantitation of the amount of labeled RNA hybridized to the coding or non-coding strand of the probe DNA was performed (see Materials and methods) and non-specific hybridization to mp8 DNA was subtracted.

The effects of the mutations on constitutive EIIaE1 expression (compare open bars in Figure 6) were essentially as reported (Zajchowski *et al.*, 1985). That is, Δ-9162 reduced transcription to barely detectable levels, while the mutations covered by LS-4839, LS-3829, LS-3323 and LS-2719 each significantly reduced transcription levels. The LS-6052 and LS-1304 mutants were each expressed as well as the LS WT recombinant. When co-transfected with pEIV(Ad2) or pEIV(Ad5), expression from the EIIaE LS WT promoter is stimulated an average of 9.4-fold (over five experiments). Transcription stimulation by EIV of the LS-6052, LS-3829 and LS-1304 mutants is similar to that of LS WT (compare black bars in Figure 6) while that of the deletion mutant Δ-9162 appeared even greater. In contrast, the induction of LS-4839, LS-3323 and LS-2719 was only between 2.5- and 4-fold (stimulation of LS-2719 was generally the most variable), demonstrating the involvement of the EIIaE promoter sequences in the EIV-mediated stimulation of EIIa expression.

Quantitative S1 nuclease analysis of cytoplasmic RNA from these experiments, using the EIV-specific probe shown in Figure 1C, revealed that EIV expression varied by no more than 2-fold (lanes 8−14, Figure 5B). Thus, the reduced induction of mutants LS-4839, LS-3323 and LS-2719 was caused neither by variation in EIV expression nor by an inability of EIV to act when EIIa constitutive expression was low (Δ-9162 was efficient-

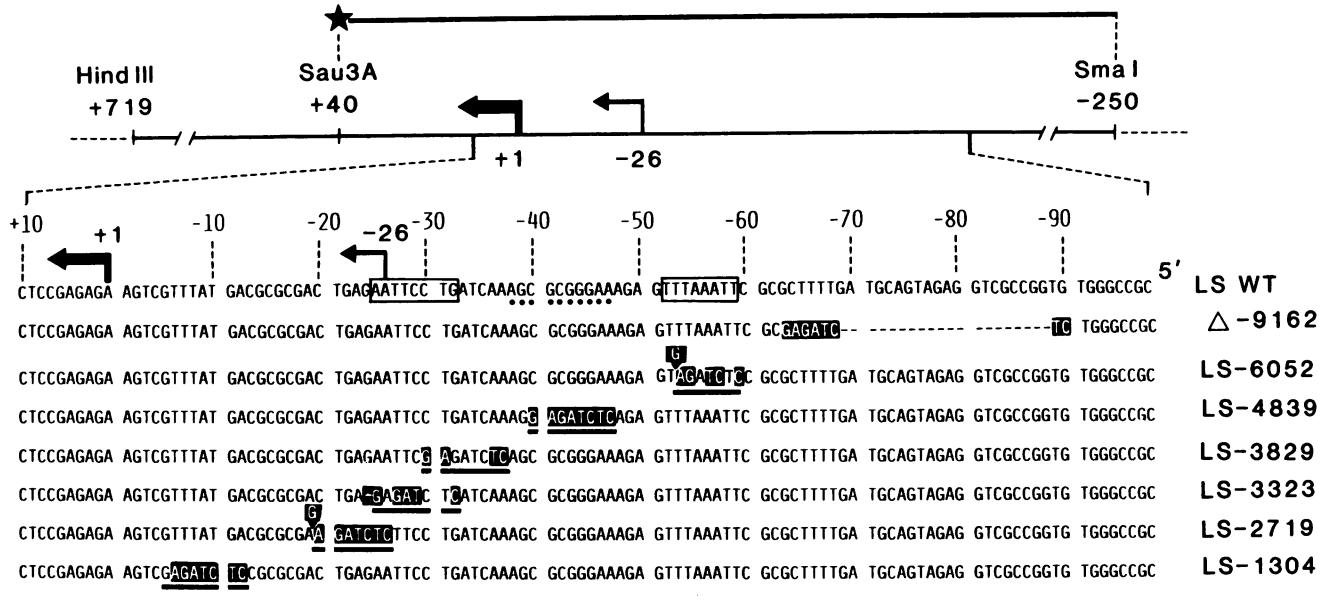

Fig. 4. Nucleotide sequence of the LS and △ EIIaE promoter mutants. The scheme depicts the *Sma*I (position −250)- *Hind*III (position +719) fragment of the EIIaE transcription unit present in the LS and △ mutants (see Zajchowski *et al.*, 1985 and legend to Figure 1C). The arrows point to the direction of transcription and mark the position of the major (+1) and minor (−26) EIIaE RNA start sites. The probe, 5′ ^{32}P-labeled at the *Sau*3A site, used for S1 nuclease analysis, is indicated. The nucleotide sequence between −98 and +10 of the wild-type EIIaE fragment (LS WT) and the corresponding fragment of the LS and △ series is represented with the two putative TATA box elements boxed in the wild-type sequence. A region of homology with other adenovirus promoters (see text) is underline with dots. In the LS series, the *Xba*I linker sequence is underlined and those nucleotides which have been altered from the wild-type sequence are stressed by shaded boxes. Single nucleotide insertions (LS−6052 and LS−2719) or deletions (LS−3323) are arbitrarily indicated on the 3′ border of the linker. The internal deletion mutant (△−9162) shows the entire *Xba*I linker boxed between the boundaries of the deletion. The nomenclature of the various mutants is derived from the position of the nucleotides between which the linker sequence has been inserted.

ly induced) but rather to a requirement for specific promoter sequences for efficient EIV-mediated stimulation of EIIa expression.

In agreement with the dot analysis shown in Figure 3, the observation that alteration of EIIaE promoter sequences strongly reduced the EIV-mediated stimulation enforces the conclusion that EIV action is at the transcriptional level. In addition, the significant stimulation of non-specific transcription induced by EIV co-transfection (see previous section and Figure 3), as well as the residual extent of stimulation of EIIaE expression from the LS-4839, LS-3323 and LS-2719 mutants (see Figure 6), suggest that EIV could have a more general activating effect on transcription.

Discussion

By co-transfecting plasmids bearing the Ad2 or Ad5 early region EIV with recombinants containing the Ad2 EIIaE promoter and either the EIIa or β-globin coding sequences, we have shown, in a transient expression system, that pEIV is able to increase the accumulation of transcripts from the EIIaE promoter. In addition, nuclear run-on experiments demonstrated that transcription of the globin-coding sequences in the LS WT recombinant was correspondingly stimulated in cells co-transfected with pEIV. This result, coupled with the S1 nuclease analysis data, strongly suggests that the increased levels of EIIaE-specific cytoplasmic RNA detected by S1 nuclease mapping are the result of an increased rate of transcription from the EIIaE promoter.

By using a series of EIIaE promoter mutants we were able to examine the sequence requirements for responsiveness to EIV. Strikingly, deletion of the EIIaE upstream promoter elements did not reduce the ability of EIV to induce EIIaE transcription, despite the fact that constitutive expression of the Δ-9162 mutant was virtually undetectable. In contrast, the mutation of either of two regions, between positions −48 and −39, and −33 and −19, effectively reduced the responsiveness to EIV. Both of these regions correspond to promoter elements required for efficient constitutive transcripion from the EIIaE1 start site (Zajchowski *et al.*, 1985) and the present results also demonstrate their involvement in the EIV-induced transcriptional stimulation. Although the molecular mechanism by which EIV achieves this stimulation is unknown, it is tempting to speculate from this observation that the promoter sequences defined by the LS-4839, LS-3323 and LS-2719 mutations correspond to binding sites for specific transcription factors, and that an EIV-encoded product(s) substitutes for, or activates (qualitatively or quantitatively) a pre-existing, rate-limiting cellular transcription factor(s). Analysis of the Ad2 EIV transcription unit has revealed a coding capacity for at least seven unique polypeptides (Rigolet and Galibert, 1984; Virtanen *et al.*, 1984). Our preliminary observation, that a plasmid which contains the EIV promoter region with only the first open reading frame fails to stimulate EIIaE-specific expression (result not shown), rules out the contribution of the corresponding polypeptide in the stimulation process described here. Identification of the EIV-coding sequence responsible for the effect awaits systematic dissection of the EIV transcription unit.

The limited availability of viral mutants within EIV, combined with difficulties in interpreting results obtained using such mutants has not yet allowed a detailed analysis of the role of EIV in viral infection. The ability of EIV to act as helper for adeno-associated virus is greatly reduced by a deletion spanning 82.5−95 m.u., d1807, which also reduces levels of some, but not all, late adenovirus proteins (Challberg and Ketner, 1981; Carter *et al.*, 1983). A mutant virus lacking sequences between 92 and 97 m.u., d1808, is defective, but the specific step at which growth is blocked remains unknown (Weinberg and Ketner, 1983). On the other hand, some evidence is available to indicate that the presence of EIV sequences enhances the expression of

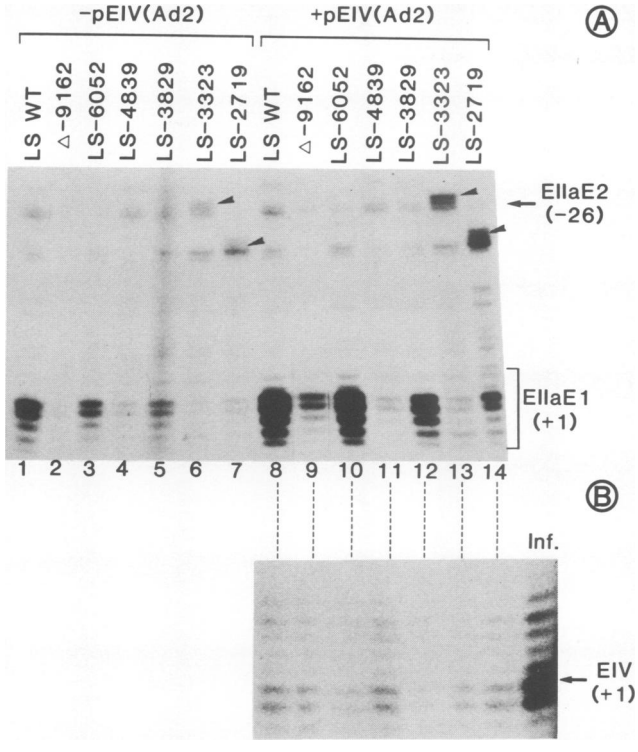

Fig. 5. Quantitative S1 nuclease analysis of cytoplasmic RNA from cells transfected with LS and Δ EIIaE mutants in the presence or absence of co-transfected pEIV. HeLa cells were transfected with 2 μg per dish of the LS- and Δ-mutants alone (**lanes 1–7**) or co-transfected with 7.5 μg pEIV(Ad2) (**lanes 8–14**). Cytoplasmic RNA was isolated and analysed for EIIaE-specific transcripts (**panel A**) as in Figure 2. The arrow-heads in **lanes 6, 7** and **13, 14** point to bands corresponding to transcripts initiated at or upstream from the linker substitution. Cytoplasmic RNA from the cells co-transfected with pEIV were also analysed for the presence of EIV-specific transcripts (**panel B**), using the EIV DNA probe described in Figure 1C. Cytoplasmic RNA extracted from cells 6 h post-infection with Ad2 at 100 p.f.u./cell (Mathis *et al.*, 1981), was analysed in parallel for EIV-specific transcripts with the same probe (**lane Inf.**).

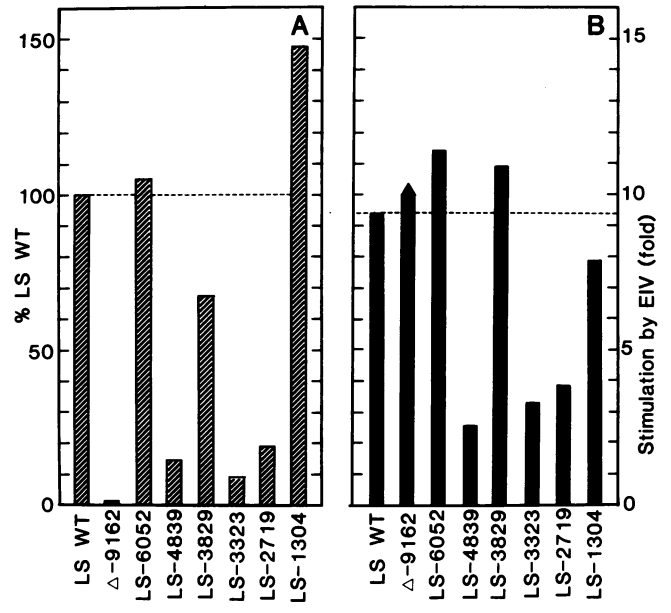

Fig. 6. Relative expression and stimulation of the LS and Δ EIIaE promoter mutants. Transcription from the EIIaE1 sites was quantitated by S1 nuclease mapping in experiments similar to and including that shown in Figure 5, by determining the intensity of the specific bands bracketted in Figure 5 (see Materials and methods). The results for each recombinant transfected alone are expressed relative to the values observed for the LS WT in each experiment (**panel A**). The extent of stimulation of the EIIaE1 transcripts obtained from each recombinant by co-transfection with pEIV is shown in **panel B**. Because of the extremely low level of constitutive expression from Δ-9162, its extent of stimulation is represented as a minimal estimate. The height of each column corresponds to the mean of five experiments, except for the LS-1304 recombinant which has been tested in two experiments. Standard deviations correspond to ~25% of each value.

early region EIb in transformed cells (Shiroki *et al.*, 1984). The fact that a high proportion of adenovirus transformed cell lines contain EIV sequences (Visser *et al.*, 1979; Esche, 1982; Downey *et al.*, 1983) suggests, in addition, that EIV may facilitate transformation by early region EI, perhaps by raising the level of transcription of a specific set of genes. This possibility is supported by the observation of Shiroki *et al.*, (1984) that transformation by the Ad12 early region EI was enhanced by the presence of the EIV transcription unit.

If both EIV and EIa transcription units are able to stimulate transcription from the early viral promoters, the mechanisms involved appear to be different, at least in the case of the EIIaE1 transcriptional induction. Indeed, no single sequence element has as yet been identified for efficient stimulation of this promoter by EIa (Zajchowski *et al.*, 1985). Furthermore, preliminary experiments in which EIa, EIV and EIIa-containing recombinants were mixed and co-transfected together (C.Goding, unpublished results) suggest that the effects of EIa and EIV on EIIaE transcription may be additive.

We recently observed (D.Zajchowski and C.Goding, unpublished experiments) that the expression of another EIa-inducible adenovirus transcription unit, EIb, is also augmented after co-transfection with EIV. It will be of interest to determine whether the other EIa-inducible viral promoters are similarly stimulated by EIV, particularly in view of the strong homology between the GC-rich sequence between position −47 and −38 in the EIIaE promoter (see Figure 4) and elements in analogous positions within the adenovirus EIb, EIII, EIV and major late promoters (Bos and ten Wolde-Kraamwinkel, 1983).

The stimulatory function of EIV described here may account for the sequential induction of the adenovirus early gene transcription during lytic infection (see Introduction) if indeed the prior expression of both EIa and EIV units is required for maximal transcription of the early EIIa and EIb units. Both *in vivo* (Nevins and Winkler, 1980) and *in vitro* (Handa *et al.*, 1983) studies have shown that increasing amounts of the EIIa gene product (the 72-K DBP) suppress transcription from the EIV promoter. Such a 'feed-back' type of regulation has not yet been examined in our system, but does not seem to play a major role under our transfection conditions since the EIV-mediated transcriptional stimulation from the EIIaE promoter occurred whether the EIIa coding sequences were present or not.

The approach used in this study to analyse the effect of EIV on transcription (i.e., co-transfection of cloned transcription units), can now be used to answer these questions and will help to unravel the intricate functional relationship existing between the various viral transcription units during lytic infection and virus-induced transformation.

Materials and methods

HeLa cells grown in monolayers to ~80% confluence were transfected by the calcium-phosphate co-precipitation technique as described (Banerji *et al.*, 1981) with a total of 20 μg of DNA per 10 cm Petri dish (see figure legends), using as carrier DNA either M13 RF DNA or pBR322. Cytoplasmic RNA was purified 12–16 h after removal of the calcium phosphate DNA precipitate. Cells were

lysed with 0.5% Nonidet P-40 and S1 nuclease mapping was carried out as described (Wasylyk *et al.*, 1980). For transcription experiments with isolated nuclei, nuclei were prepared (Groudine *et al.*, 1981) and nuclear transcription performed as previously described (Leff *et al.*, 1984). Quantitation of results was achieved by scanning multiple exposures of autoradiograms.

Acknowledgements

We wish to thank P.Chambon for useful discussions and critical reading of the manuscript. We also thank T.Leff for gifts of plasmids and suggestions. We are most grateful to Mrs. C.Hauss for expert technical assistance, to Mrs. M.Acker and L.Heydler for tissue culture and to J.Kaibach, C.Aron, C.Werlé and B.Boulay for preparing the manuscript. This work was supported by the CNRS (ATP 3582 and 6984) and the Ministère de l'Industrie et de la Recherche (82V1283 and 84V083). C.G. was in receipt of a Royal Society European Exchange Fellowship.

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Received on 11 March 1985; revised on 9 April 1985