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An enhancer element is located 340 base pairs upstream from the adenovirus-2 E1A capsite

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ABSTRACT

A chimeric recombinant, containing the 270 bp left-terminal fragment of Adenovirus-2 (Ad2) inserted upstream from the -34 to +33 Ad2 major late promoter (Ad2MLP) element, has been used to characterize the transcription stimulatory element which is located at least 231 bp upstream from the E1A capsite in the left-end of Ad2 (Ref. 1). We demonstrate that this element, which acts in _cis_, possesses several properties characteristic of transcriptional enhancers. Firstly, it potentiates initiation of transcription from the capsite of the heterologous Ad2MLP and from "cryptic" sites often preceeded by TATA box-like sequences. Secondly, although there is no critical distance requirement between the enhancer element and the Ad2MLP, the extent of stimulation decreases as the distance between the two element increases. However, in contrast to the other known viral or cellular enhancers which are bidirectional, the Ad2 enhancer is unidirectional, i.e. it potentiates the Ad2MLP element only when it is inserted in its "natural" orientation with respect to the direction of transcription. Using two convergent series of deletions, we have localized the Ad2 enhancer element within a 24 bp segment located at approximately 160 bp from the Ad2 left-end, i.e. 340 bp upstream from the E1A capsite. This 24 bp segment contains a sequence which exhibits a striking homology with the consensus sequence of several viral and cellular enhancers.

INTRODUCTION

The promoter region which controls initiation of transcription by RNA polymerase B (II) consists of at least three functional domains located within approximately 100 bp upstream from the capsite (the capsite itself, the TATA box element and the upstream elements - for Refs., see 2-6). In some cases additional cis-acting elements, that appear to be crucial for promoter function, have been identified and termed enhancers. Enhancer elements have been shown to be essential for the expression of a number of viral genes that are transcribed by RNA polymerase B and expressed "constitutively" early in infection (for Refs., see 7, 8). More recently, enhancers have been found in some immunoglobulin genes (9-12). Enhancers exhibit some striking properties that have no known counterparts in prokaryotes (see 7-9, 13, 14). They can be "naturally" located upstream, within,

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or downstream from a transcription unit, and remain active even when they have been separated from promoter elements by several kb. Their enhancing efficiency decreases as the length of the interposed sequences is increased, and proximal promoter elements are activated in preference to more distal ones. In addition, all enhancer elements which have been characterized up to now appear to function bidirectionally, i.e. their efficiency is independent of their orientation with respect to the activated promoter elements. Studies at the transcriptional level, have revealed that the canonical SV40 enhancer is a potentiator of initiation from TATA box-dependent and -independent homologous or heterologous promoter elements (3, 14-17). Finally, some enhancers exhibit a marked species- or cell-specificity (9, 10, 18-20), suggesting that they could interact with specific regulatory proteins (21).

We have shown in the accompanying paper (1) that sequences located more than 231 bp upstream from the main capsite are required for efficient expression of the Adenovirus-2 (Ad2) E1A transcription unit. We demonstrate here that most of these stimulatory sequences are situated between 320 and 344 bp upstream from the E1A main capsite. The Ad2 E1A stimulatory element, which presents some sequence homology with the enhancer core sequence, exhibits all of the properties of the canonical SV40 enhancer, except the bidirectionality.

MATERIALS AND METHODS

Construction of pSVA34 and its derivatives (see Fig. 2).

pSVA34 (3) contains a fragment (-34 to +33) of the Ad2 major late promoter (Ad2MLP) linked to the SV40 early coding region (coordinates 5227-2533). pLA34 was derived from pSVA34 by inserting the EcoRI-BalI fragment of pEIA-A34 (see accompanying paper and Fig. 1B) between the EcoRI and SstI sites of pSVA34. pLIA34 contains the same fragment, but inserted in the opposite orientation. pLPA34 and pLPIA34 were derived from pSVA34 by inserting the EcoRI-BalI fragment into the PvuI site of pBR322 in both orientations. $pL^{-}A34$ and $pL^{-}IA34$ are similar to pLA34 and pLIA34, except that the first 105 bp from the left-end to the HphI site have been deleted.

Construction of the dl recombinant series (see Fig. 3).

pLA34 (Fig. 1B) was successively cut at the EcoRI site, incubated for various lengths of time with Bal31 nuclease, cut at the SphI site of pBR322 and ligated in the presence of BamHI linkers. This generated a family of unidirectional deletions extending from the EcoRI site towards the BalI site. The end-points of the deletions were established by DNA sequencing (22).

Construction of the dlS recombinant series (see Fig. 4).
pEIA-A34 (Fig. 1B) was successively cut at the SacII site, incubated with Bal31 nuclease for various lengths of time, and cut with EcoRI. The resulting families of fragments were purified on sucrose gradients and inserted between the EcoRI and the SacII sites of pEIA-A34 in the presence of BamHI linkers. This generated a family of unidirectional deletions extending from the SacII site towards the EcoRI site. The end-points of the deletions were established by DNA sequencing (22).
All other Materials and Methods were described in the accompanying paper (1).

RESULTS

A) The left terminal 270 bp of the Adenovirus-2 genome stimulates transcription from a heterologous promoter element.

We have shown in the accompanying paper (1) that, after transfection of

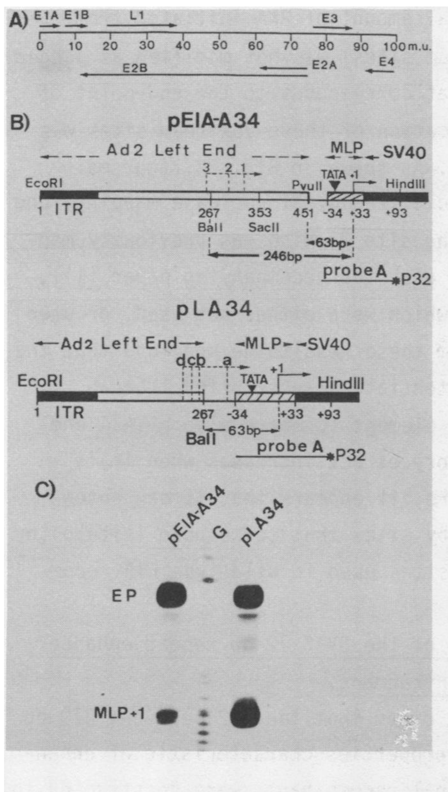

Fig. 1 : A) Schematic representation of the Adenovirus-2 early transcription units; m.u. corresponds to map unit. B) pEIA-A34 and pLA34 recombinants (Materials and Methods). In pEIA-A34 (see also ref.1) the first 451 bp of the Ad2 left-end were inserted in pSVA34 (fig. 2) 63 bp upstream from the Ad2ML capsite (+1 and arrow indicating the direction of transcription). 1, 2 and 3 correspond to the upstream initiation sites previously described (27, 1 and Fig. 6). The extent of probe A, [γ-^{32}P]-labelled at the SV40 HindIII site, used for quantitative S1 nuclease analysis is indicated. pLA34 is similar to pEIA-A34 except that the BalI-PvuII fragment (positions 267 to 451 with respect to the left-end) has been deleted. The locations of upstream initiation sites a, b, c and d shown in fig. 3B and 6 and discussed in the test are indicated. ITR (filled box at the left-end) represents the Ad2 inverted terminal repeat sequence (bp 1 to 102). C) Quantitative S1 nuclease analysis of RNA extracted from HeLa cells transfected with pEIA-A34 and pLA34 using probe A (panel B). MLP+1 corresponds to RNA initiated at the Ad2MLP. EP is the end-point of homology between the RNA transcripts and probe A. G corresponds to a sequence ladder of the probe (G reaction).

the chimeric recombinant pEIA-A34 (Fig. 1B) into HeLa cells, the left-terminal 461 bp EcoRI-PvuII fragment of Ad2 can potentiate transcription from the otherwise inactive -34 to +33 Ad2 major late promoter (Ad2MLP) element. Furthermore, sequences located within the left-terminal 270 bp EcoRI-BalI fragment appeared to be crucial for this stimulatory effect. To determine if this fragment alone could potentiate transcription from the Ad2MLP element, we constructed the chimeric recombinant pLA34 in which the EcoRI-BalI fragment is inserted 63 bp upstream from the Ad2 major late (Ad2ML) capsite of pSVA34 (see Fig. 1B and 2A, and Materials and Methods). pLA34 was transfected into HeLa cells, and the RNA produced was analyzed by quantitative S1 nuclease mapping using either probe A (Fig. 1B) or probe B (Fig. 3A). It is clear from the results shown in Fig. 1C and from similar experiments (not shown), that deletion of the BalI-PvuII fragment, which brings the EcoRI-BalI fragment 183 bp closer to the Ad2ML capsite (Fig. 1B), resulted in an approximately ten-fold stimulation of transcription initiated from this capsite. At the same time, the total amount of RNA initiated from sites upstream from this capsite (upstream sites) was not modified as judged by the relative intensity of the band that corresponds to the end-point of the probe (EP in Fig. 1C). The exact location of these upstream sites was mapped in pLA34 using probe B (Fig. 3A). As shown in Fig. 3B, four main upstream sites, a, b, c and d were characterized by S1 nuclease mapping. One of these sites (site b) corresponds to the site 3 which was previously mapped in pEIA-A34 [Fig. 1B and Fig. 6, see also the accompanying paper (1)], whereas sites a, c and d were new sites which were either not used, or used much less efficiently, in pEIA-A34. From these results we conclude that the Ad2 left-terminal 270 bp fragment can potentiate transcription from an otherwise inactive heterologous promoter element (compare also pSVA34 and pLA34 in Fig. 2B), and that its stimulatory effect increases when it is moved closer to this element. Furthermore, it appears that it can potentiate transcription from additional nearby sites that correspond (site b) or do not correspond (sites a, d and c) to sites used in wild-type E1A recombinants.

B) Comparison of the stimulatory effect of the SV40 72 bp repeat enhancer and the Adenovirus-2 left-end 270 bp fragment.

From the preceeding results, it was clear that the Ad2 left-end 270 bp terminal fragment exhibited some of the properties characteristic of enhancer elements (see Introduction). Additional recombinants were constructed to determine if the properties of this fragment were in other respects similar

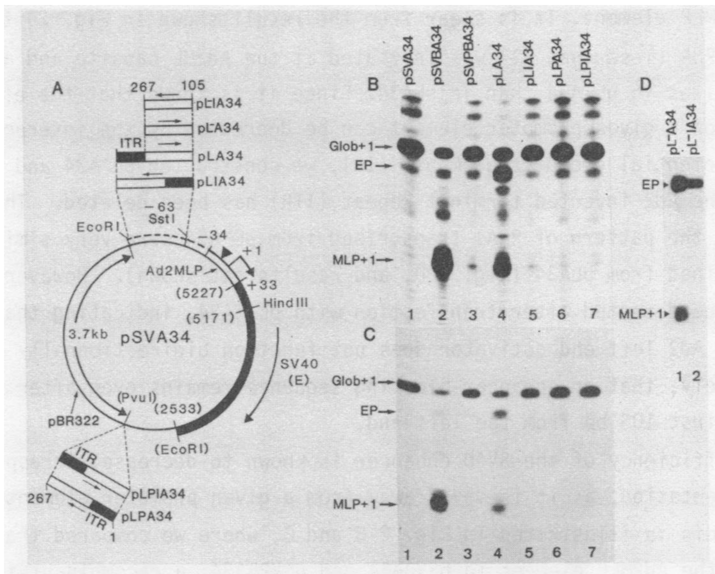

Fig. 2 : A) Schematic representation of pSVA34, pLA34, pLIA34, pL⁻A34, pL⁻IA34, pLPA34 and pLPIA34 (see Materials and Methods and text). Symbols are as in Fig. 1B. B), C) and D) Quantitative S1 nuclease analysis of RNA extracted from HeLa cells transfected with the various recombinants shown in panel A, using probe A (Fig. 1B). Panel C is a shorter exposure of panel B. In the experiments shown in B and C, the reference β-globin recombinant pβ(244^{+})β (1) was cotransfected. Glob+1 corresponds to RNA initiated at the β-globin capsite. MLP+1 and EP are as in the legend to fig. 1.

to those of the SV40 72 bp repeat enhancer. RNA transcribed from pLA34 was compared, using quantitative S1 nuclease analysis, to that produced from recombinant pSVBA34 (3) that is similar to pLA34 but contains the 72 bp repeat region inserted in place of the Ad2 left-end sequence. As shown in Fig. 2, B and C, the SV40 enhancer was approximately 8-fold more effective than the Ad2 left-end fragment at potentiating transcription from the Ad2ML capsite (after correcting for transcription from the cotransfected reference globin recombinant). However, it should be noted that much more RNA was initiated from sites located upstream from the Ad2ML capsite in pLA34 than in pSVBA34 [compare in Fig. 2 B and C, the relative intensity of the bands corresponding to the end-point of the probe (EP) and of other bands located between EP and MLP+1, in pSVBA34 and pLA34].

Bidirectionality is one of the characteristic properties of enhancer elements (see Introduction). pLIA34 is similar to pLA34, except that the Ad2 left-end 270 bp fragment is inserted in the inverse orientation with respect

to the Ad2MLP element. It is clear from the result shown in Fig. 2B that much less RNA (less than 1%) was initiated at the Ad2ML capsite and at upstream sites in pLIA34 than in pLA34. Since it is known that the effect of enhancers on a given promoter element can be decreased by the interposition of other potential promoter elements (14), we constructed $pL^{-}A34$ and $pL^{-}IA34$ in which the Ad2 inverted terminal repeat (ITR) has been deleted. The amount and the pattern of RNAs transcribed from $pL^{-}A34$ were very similar to those obtained from pLA34 (Fig. 2D, and results not shown). However, no RNA could be detected after transfection with $pL^{-}IA34$, indicating that either the Ad2 left-end activator does not function bidirectionnally or, alternatively, that an enhancer-blocking sequence remains even after deleting the first 105 bp from the left-end.

The efficiency of the SV40 enhancer is known to decrease, irrespective of its orientation, as it is moved away from a given promoter element (3, 13, 14). This is illustrated in Fig. 2 B and C, where we compared transcription from the Ad2MLP element in pSVBA34 and pSVPBA34, a recombinant in which the SV40 enhancer is inserted in the PvuI site of pSVA34, 3.7 kb from the Ad2MLP (3 and Fig. 2A). Transcription initiated from the Ad2ML capsite in pSVPBA34 is approximately 2% of that observed for pSVBA34. In the corresponding Ad2 left-end fragment constructions, pLPA34 and pLPIA34, no transcription initiated at the Ad2ML capsite could be detected, indicating that in this respect, the SV40 enhancer and the Ad2 left-end sequence behave similarly.

C) <u>Localisation of the stimulatory sequences in the Adenovirus left-end 270 bp fragment</u>.

Two series of deletion mutants were constructed to delimit the sequences which are responsible for the stimulatory effect of the Ad2 left-end fragment. The dl series, which was derived from pLA34, corresponds to deletions which extend from position 1 (EcoRI site of pLA34) towards the Ad2MLP (see Fig. 3A and Materials and Methods). RNA produced after transfection of the various dl recombinants into HeLa cells was analyzed by quantitative S1 nuclease mapping (probe B, Fig. 3A). The β-globin recombinant $p\beta(244^{+})\beta$ was used as a cotransfected reference gene. Such an experiment is shown in Fig. 3B and the results of several identical experiments are summarized in Fig.3A in which the relative amount of RNA initiated from the Ad2ML capsite is expressed in percent of the "wild type" pLA34 recombinant. Deletion of the first 131 bp from the left-end (dl131, not shown in Fig. 3B) did not decrease the amount of RNA initiated from the Ad2ML capsite nor from sites

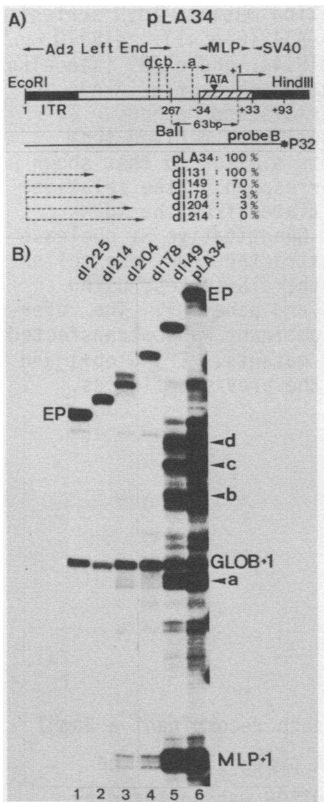

Fig. 3 : A) Deletion mutants (dl series) derived from pLA34 (fig. 1). The deletions extend from the left-end (position 1) to positions 131, 149, 178, 204 and 214 in mutants dl131, dl149, dl178, dl204 and dl214, respectively (see Fig. 6 and Materials and Methods). Figures (in percent) were derived from several experiments similar to that shown in panel B and correspond to the relative amount of RNA initiated from the Ad2ML capsite (+1). B) Quantitative S1 nuclease analysis of RNA extracted from HeLa cells transfected with the deletion mutants shown in panel A. The single-stranded probe B (panel A), [γ -^{32}P]-labeled at the HindIII site and extending to the EcoRI site was used. The reference - globin recombinant (see legend to fig. 2) was cotransfected with each deletion mutant. Glob+1 and MLP+1 are as in fig. 2. EP is the end-point of homology between the RNA transcripts and probe B. a, b, c, d are the upstream initiation sites indicated in panel A and Fig. 6.

(a to d) located further upstream, in keeping with the results obtained with pL^{-}A34, in which the first 105 bp were deleted (see above). A moderate decrease of RNA synthesized from both the Ad2ML capsite and upstream sites was observed when the first 149 bp were deleted (dl149). In contrast, a deletion extending to position 178 (dl178) resulted in a drastic decrease in RNA synthesized from both the Ad2ML capsite and upstream sites. Further deletion to position 204 (dl204) had no significant additional effect, whereas deletion to position 214 (dl214) caused a complete disappearence of RNA synthesized from both the Ad2ML capsite and upstream sites. From these results we conclude that the key sequences responsible for the stimulatory effect are located between positions 149 and 178 and that the same sequences are responsible for stimulation of transcription of RNA initiated at the Ad2ML capsite and at the upstream sites.

The deletion series dlS derived from pEIA-A34 corresponds to deletions which extend from position 353 (SacII site of pEIA-A34) towards the Ad2

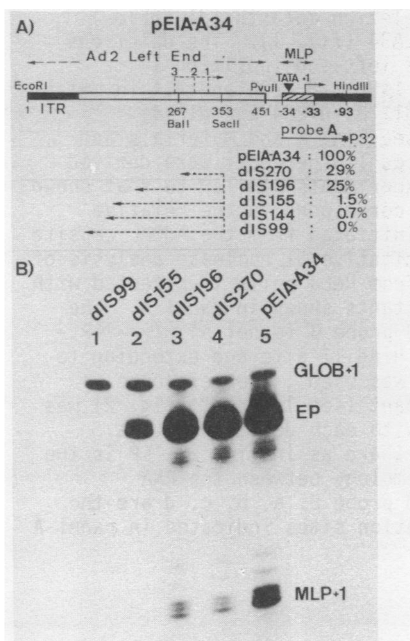

Fig. 4 : A) Deletion mutants (dlS series) derived from pEIA-A34 (Fig. 1). dlS270, dlS196, dlS155, dlS144, and dlS99 (see also Fig. 6) were constructed from pEIA-A34 as described in Materials and Methods. Figures (in percent) were derived from several experiments similar to that shown in panel B and correspond to the relative amount of RNA initiated from the Ad2ML capsite (+1). B) Quantitative S1 nuclease analysis of RNA extracted from HeLa cells transfected with the dlS series, using probe A (Fig. 1B and panel A). The reference β-globin recombinant was cotransfected with the deletion mutants. EP, Glob+1 and MLP+1, are as in the previous figures.

left-end (see Fig. 4 and Materials and Methods). In each recombinant a BamHI linker was inserted in the place of the deletion. RNA produced from the deletion mutants dlS270, dlS196, dlS155, dlS144 and dlS99 was analyzed by quantitative S1 nuclease mapping (probe A, Fig. 4A). Typical autoradiograms are shown in Fig. 4B and 5A and the results of several such experiments are summarized in Fig. 4A. These results clearly show that the key stimulatory sequences present in the Ad2 left-end 270 bp fragment are located between position 196 and 155. As already noted in the accompanying paper (1), deletion of the BalI-SacII fragment resulted in a 3-fold decrease of the amount of RNA initiated from the Ad2ML capsite, whereas the total amount of RNA initiated from upstream sites (EP) was not significantly affected (compare pEIA-A34 and dlS270 in Fig. 4B). A deletion extending to position 99 (dlS99) completely abolished transcription initiated at the Ad2ML capsite and at upstream sites (compare dlS155 and dlS99 in Fig. 4B). From these results and those obtained with the dl series, we conclude that the sequences crucial for the stimulatory effect of the Ad2 left terminal 270 bp fragment are located between positions 155 and 178 (overlined in Fig. 6) and that additional minor stimulatory sequences may be located between positions 131 and 155 and between positions 204 and 214.

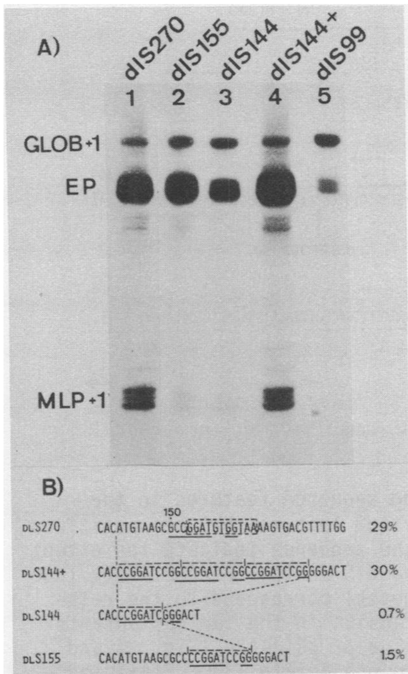

Fig. 5 : A) S1 nuclease analysis (probe A, Fig. 4) of RNA extracted from HeLa cells transfected with mutant dlS270, dlS155, dlS144, dlS144$^+$ and dlS99. dlS144$^+$ is identical to dlS144 but contains three tandemly repeated BamHI linkers instead of a single one (see below and Materials and Methods). Other symbols, are as in the previous figures. B) Sequence comparison showing homologies (underlined) between the BamHI linker and the wild type sequence (dlS270). 150 indicates the base position with respect to the Ad2 left end. The boxed sequence in dlS270 corresponds to the SV40 homology sequence (see Fig. 6 and 7). Figures (in percent) correspond to the relative amount of RNA initiated from the Ad2MLP (MLP+1) in panel A.

One of the dlS mutants (dlS144$^+$) with a deletion end-point identical to that of dlS144 gave an unexpected result, since the amount of RNA initiated from both the Ad2ML capsite and upstream sites was identical to that obtained for dlS270 which contains the entire EcoRI-BalI fragment (Fig. 5A). Sequencing of the dlS144$^+$ mutant across the deletion end-point has shown that this mutant contains three tandemly repeated BamHI linkers in place of the deletion (see Fig. 5B, and unpublished results). A possible explanation accounting for the unexpected behavior of this mutant is discussed below.

DISCUSSION

The results presented in this paper demonstrate that there is a transcription stimulatory element located between positions 155 and 178 from the Ad2 left-end and that this stimulatory element exhibits several properties characteristic of enhancers (see Introduction). Firstly, this Ad2 element potentiates initiation of transcription in *cis* (1) from the capsite of a heterologous promoter (the -34 to +33 Ad2MLP element) as well as from "new" sites which are not part of the "natural" E1A promoter elements (sites a, c

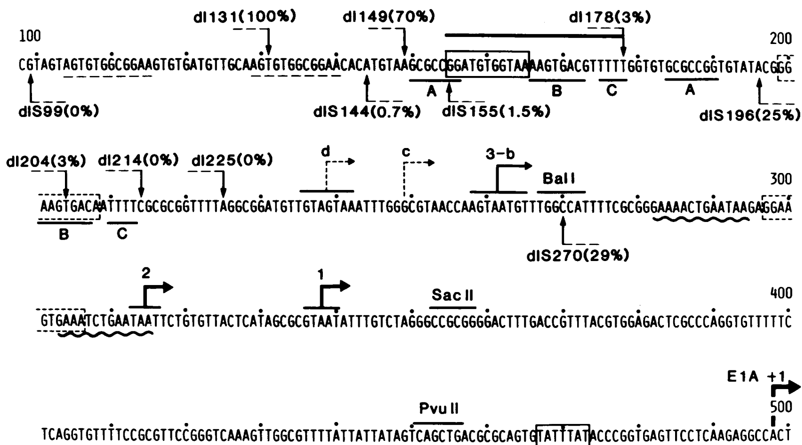

Fig. 6 : Location of the enhancer element and sequence features in the left-end of Adenovirus-2. The sequence of the Ad2 left-end between positions 98 and 500 is shown. Arrows above and below the sequence indicate the extent of the deletions in recombinants of the dl and dlS series, respectively (see fig. 3 and 4). Figures in percent (in parentheses) correspond to the relative amount of RNA initiated from the Ad2ML capsite in the various mutants. In the dlS series, 100% corresponds to the value of pEIA-A34 (Fig. 4 and 5), while in the dl series 100% corresponds to pLA34 (fig. 3). E1A+1 indicates the E1A main capsite. Arrows 1, 2 and 3 correspond to the early E1A upstream initiation sites observed with pEIA-A34 (see text, Fig. 1B, and 26), while b, c and d correspond to the upstream initiation sites observed with pLA34 (see fig. 3; site b is identical to site 3). The region of the Ad2 left-end which is responsible for most of the enhancer activity is overlined (positions 155 to 178). The boxed sequence (155 to 166) is homologous to the SV40 enhancer core sequence (Fig. 7). The two sequences boxed with dashed lines have been pointed out by Hearing and Shenk as possible enhancer sequences (26). Sequences which are repeated are underlined with dashed or wavy lines (see text).

and d in pLA34 - Figs. 3 and 6). As previously observed in the case of the SV40 enhancer-conalbumin promoter recombinants (14), these "new" sites often appear to be preceeded by AT-rich TATA-like sequences located approximately 30 bp upstream (see Fig. 6, upstream from the "new" sites c and d). In this respect, it is interesting to note that the "natural" E1A upstream sites 1 and 2 are also preceeded by AT-rich TATA-like sequences (see Fig. 6, the two regions underlined with wavy lines). In addition, as in the case of the SV40 enhancer-conalbumin promoter recombinants (14), the transcription machinery can clearly discriminate, within a given region, betweeen the various sites that are potentiated by the stimulatory element. There was indeed much more RNA initiated from sites c and d when the region containing the upstream

sites 1 and 2 was deleted [see Fig.1 and 3 and also Fig. 4 in the accompanying paper (1)]. Secondly, there is no critical distance requirement between the Ad2 stimulatory element and the promoter, although the stimulation decreases as this distance increases. In this respect, the behavior of the Ad2 stimulatory element is also very similar to that of the SV40 enhancer. The stimulation is 10-fold higher in pLA34, where the Ad2 activator is located approximately 170 bp upstream from the Ad2ML capsite, than in pEIA-A34 where it is about 200 bp further upstream. In similar SV40 enhancer recombinants [SV40-Ad2MLP (23) and SV40-conalbumin promoter (14)], the extent of stimulation was decreased 10 to 20-fold when the SV40 enhancer was moved from about 100 bp to 300 bp upstream from the capsite of the promoter element. Moreover, increasing the distance between the Ad2 stimulatory element and the promoter element to 3.7 kb, is accompanied by a drastic reduction in the extent of stimulation, as previously observed for the SV40 enhancer (3, 14, see also pSVPBA34 in the present study).

Although the Ad2 stimulatory element exhibits most of the properties which have been used to define enhancer elements, its stimulatory effect does not appear to be orientation-independent. This may be an intrinsic property of a different class of enhancers to which the Ad2 stimulatory element would belong. Alternatively, as mentioned above, it may reflect the blocking interference of Ad2 left-end sequences which are still interposed between the enhancer element and the Ad2MLP in recombinant pL⁻IA34 (see Fig. 2). Studies, with shorter fragments, are in progress to distinguish between these possibilities.

In spite of this lack of bidirectionality, the Ad2 stimulatory element, located between positions 155 and 178, could belong to the family of the already known enhancer elements. This is suggested by the presence of a sequence identical to the enhancer consensus sequence within the Ad2 stimulatory element (boxed sequence in Fig. 6, and Fig. 7 ; see 24, 25). It is noteworthy that this sequence is conserved in Adenovirus-2, 7 and 12, whereas the flanking sequences exhibit very little homology. A comparison of the effects of deletions dl131 and dl149, and dl204 and dl214 (Fig. 6), suggests, however, that sequences located outside of the consensus sequence could also play some role in the enhancer activity. The possible importance of other sequences that exhibit characteristic features within this region, like the repetition of sequences A, B and C (Fig. 6), remains to be determined.

The observation that the deletion mutant dlS144^{+} contains an element

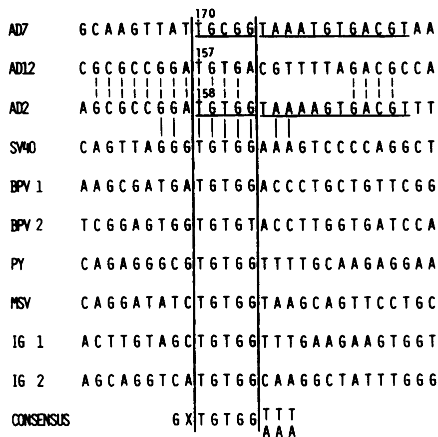

Fig. 7 : Sequence homologies between the Adenovirus-2 enhancer and other known enhancer sequences. The reference for the sequences are as follows : Adenovirus-2, 7 and 12 (Ad2, Ad7 and Ad12) (28, 29) ; SV40 (24, 30) Bovine papilloma virus (BPV) (31, 32) ; Polyoma (PY) (25, 33) ; Murine sarcoma virus (MSV) (25, 34) ; Mouse Immunoglobulin heavy chain (Ig) (9, 10). Numbers in Ad2, Ad7 and Ad12 indicate the position of the sequence with respect to the left-end (in bp). The sequence homology between Ad7 and Ad2 is underlined. The vertical dashed lines show the homology between Ad12 and Ad2 and the vertical lines correspond to the homology between Ad2 and SV40.

which is almost as efficient in stimulating transcription as the wild-type enhancer sequence, is puzzling. From the sequence comparison shown in Fig. 5B, it appears that there is some similarity between the consensus sequence, which is common to the Ad2 and SV40 enhancers (fig. 7), and the BamHI linker sequence repeated three times in dlS144$^+$. Since this sequence is also found in the corresponding region of dlS144 and dlS155 (Fig. 5), which are almost inactive in transcription, one is left with the hypothesis that it is the repetion of the partially homologous sequence that is responsible for the stimulatory effect in dlS144$^+$.

The location of the enhancer element characterized here is clearly different from that of the Adenovirus enhancer element recently reported by Hearing and Shenk (26). These authors have mapped a stimulatory element between positions 195 and 353 of Adenovirus-5 and they have proposed that a sequence which is repeated in this region (dashed boxes in Fig. 6) could play a key role (the left-end 500 bp fragment of Ad5 is 99% identical to that of Ad2). This repeated sequence is clearly outside of the enhancer element that we have localised between positions 155 and 178. However, it is likely that the 4-fold decrease in the amount of RNA produced from dlS196 (Figs. 4 and 6) reflects the deletion of the stimulatory element identified by Hearing and Shenk (26). It is possible that these authors did not notice the effect of mutations that delete the enhancer element described here because a completely different experimental system was used. Hearing and Shenk have studied the effect of deletion mutations during lytic infection

using a reconstructed virus that contains all of the adenovirus genome including the E1A structural sequences. The origin of the discrepancy may well reside in the presence of these latter sequences, since we have shown that the requirement for our enhancer element is much more stringent in their absence [see in the accompanying paper (1), the comparison between pEIASV that contains the E1A structural sequences, and pEIA$^-$ that does not]. It is also remarkable that after deletion of all the sequences located upstream from position +10 of the E1A transcription unit, Hearing and Shenk (26) found that the amount of E1A RNA produced decreased by only 85%. One is thus led to speculate that the expression of the E1A transcription unit is in some way stimulated by the presence of its own structural sequences. As already discussed in the accompanying paper (1), this stimulation could be related either to the presence of an additional cis-acting element within the E1A structural sequences and/or to trans-activation of the E1A promoter by the E1A products. This latter possibility is attractive, since it raises the possibility that the enhancer element characterized here could play a crucial role very early in infection, before any Adenovirus protein has been made, at a time when a single E1A promoter must successfully compete with all of the surrounding cellular promoters.

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ABBREVIATIONS

Ad2ML and Ad2MLP : Adenovirus-2 major late and Ad2ML promoter, respectively; bp : base pair ; SV40 : Simian Virus 40.

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