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Adenovirus tripartite leader sequence enhances translation of mRNAs late after infection

(5′ noncoding region/viral mutants/fused genes/translational regulation)

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ABSTRACT A series of adenovirus type 5 variants was constructed to probe the function of the tripartite leader sequence, a 200-nucleotide, 5′ noncoding sequence carried on the majority of late viral mRNAs. Recombinant plasmids were constructed that carried the major late transcriptional control region followed by portions of the tripartite leader sequence fused to the E1A coding region. These modified E1A genes were then rebuilt into intact viral chromosomes, replacing the corresponding wild-type region. The leader segments had no effect on the translation of E1A mRNAs early after infection, but the tripartite leader significantly enhanced (5-fold) the efficiency with which the mRNAs were translated late after infection.

The majority of mRNAs produced late after infection with adenoviruses are derived from the major late transcription unit. This unit produces a long primary transcript that is differentially processed to yield five 3′-coterminal families of mRNAs. These mRNAs all share a common 5′ nontranslated region, 200 nucleotides in length, spliced to the mRNA coding region (1, 2). This 5′ region is termed the "tripartite leader sequence" because it is coded in three spatially separated segments on the viral chromosome that are joined by splicing of the primary major late transcript.

To probe the function of the tripartite leader, we have constructed a series of adenovirus type 5 (Ad5) variants that produce modified E1A mRNAs that carry either none, part of, or most of the leader segment. Although the leader segment has no effect on the translation of mRNAs early after infection, it significantly enhances the efficiency with which mRNAs are translated at late times.

MATERIALS AND METHODS

Plasmids and Construction of Rearrangements. The recombinant plasmid pE1A-WT contains the left-end 1339 base pairs (bp) of the Ad5 genome inserted between the unique *Eco*RI and *Pvu* II sites of pBR322. The source of adenovirus type 2 (Ad2) tripartite leader sequences, pJAW 43, was a gift of J. Sambrook (3); Ad2 major late transcriptional control sequences were from pSmaF, a gift of R. Roeder (4). Rearranged E1A genes carrying major late transcriptional control sequences and 5′ tripartite leader segments were constructed into pE1A-WT. *sub*360-3′-L1,2,3, which carries a leader sequence within the E1A 3′ noncoding region, was constructed by insertion of the segment at sequence position 1572 in pAd5-XhoC [contains the left-end 5580 bp of the Ad5 genome (5)].

Viruses and Cells. Wild-type Ad5 (H5*wt*300) and mutant H5*dl*309 have been described (6). Rearranged genes carrying segments of the tripartite leader at the 5′ end of the E1A coding region were rebuilt into viruses by using the procedure of Stow (7), and *sub*360-3′-L1,2,3 was generated by overlap recombination (8). The 293 cell line (9) was maintained in medium containing 10% calf serum. Spinner cultures of HeLa cells were grown in medium containing 7% calf serum.

RNA Preparation and Analysis. To prepare RNAs, HeLa spinner cells were infected at a multiplicity of 25 plaque-forming units per cell and harvested at either 6 hr after infection (early) or 24 hr after infection (late). RNAs were subjected to blot hybridization analysis (5) using pE1A-WT as probe. S1 endonuclease analysis using phage M13 single-stranded probes was essentially as described by Gaynor *et al.* (10). The M13-E1A probe DNA carried the Ad5 sequences between 0 and 4.5 map units. S1 endonuclease analysis using a 5′-end-labeled probe was as described by Hearing and Shenk (11). The probe was derived from *sub*360-L1,2,3 and extended from Ad5 sequence position 1 to position 623. Primer extension analysis of the 5′ ends of E1A mRNAs (12) utilized a single-stranded, 5′-end-labeled probe extending from Ad5 sequence position 550 to position 619.

Polysome Analysis. For polysome analysis, cells were infected as described above for RNA preparation. At 24 hr after infection, cells were harvested and a cytoplasmic extract was prepared and analyzed by velocity sedimentation in sucrose gradients as described by Lenk *et al.* (13). RNA was prepared from gradient fractions and subjected to blot hybridization analysis.

Analysis of Polypeptides. At either 6 hr (early) or 24 hr (late) after infection at a multiplicity of 25 plaque-forming units per cell, HeLa spinner cells were pulse-labeled for 20 min with [^{35}S]methionine (1100 Ci/mmol, 50 mCi/ml; 1 Ci = 37 GBq). Immunoprecipitations were performed as described by Sarnow *et al.* (14), using an Ad5 E1A-specific monoclonal antibody provided by A. Zantema and A. van der Eb. Extracts for two-dimensional gel electrophoresis were prepared and processed as described by O'Farrell (15).

RESULTS

Construction of Variants. It was not practical to mutate leader sequences at their normal location. Not only could leader mutations potentially compromise function of the entire major late family of mRNAs but also such alterations could inactivate E2B gene products encoded on the opposite DNA strand. As a result, alterations in the regions coding the leader segment would likely render the virus defective and difficult to propagate. Therefore, we chose to append the tripartite leader to the E1A coding region and test its effect on E1A expression. In this case, a defective virus could be propagated in 293 cells, a transformed human line that contains and expresses the Ad5 E1A and E1B genes (9).

Rearranged E1A genes were initially prepared in recombinant plasmids. The source of leader sequences (diagrammed

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Abbreviations: Ad5 and Ad2, adenovirus type 5 and type 2; bp, base pair(s); kDa, kilodalton(s).

in Fig. 1*A*) was the plasmid pJAW-43, which carries a cDNA copy of the Ad2 fiber mRNA 5′ domain (3). The parent to all of the leader constructs is *sub*360-L0. It lacks the E1A transcriptional control sequences located between the enhancer and mRNA 5′ end and carries in their place a 241-bp segment that includes the major late transcriptional control region (Fig. 1*B*). As a result, E1A mRNA can be produced at substantially increased levels as compared to the wild-type configuration late after infection, facilitating analysis of the test RNA and its products. The E1A-specific 5′-noncoding region was removed from *sub*360-L0 and replaced with portions of the tripartite leader sequence. *sub*360-L1,2,3 (Fig. 1*B*) carries nucleotides 1–172 of the tripartite leader. Additional constructions that contain lesser portions of the leader sequence were assembled by using the *Pvu* II, *Hae* III, and *Xho* I cleavage sites indicated in Fig. 1*A*. It is important to note that the constructions do not contain precisely the leader segments indicated by their names, but rather close approximations as dictated by the availability of restriction endonuclease cleavage sites. All of the constructs contain the same 12 nucleotides before the initiator AUG to minimize possible effects from surrounding nucleotides on the efficiency of initiation at the correct AUG (18).

The rearranged E1A genes were rebuilt into viral chromosomes by using the method of Stow (7). In this procedure, plasmid DNA is linearized at the Ad5 *Xba* I cleavage site (sequence position 1339) and ligated to a right-end Ad5 fragment derived from a viable variant, *dl*309, that carries only one *Xba* I cleavage site, at sequence position 1339. The recombined molecules are then used to transfect permissive cells. The resulting viruses contain a normal left end, having lost pBR322 sequences, presumably by copying the right-end terminal repeat during replication or through recombination. Initially, the variants were propagated on 293 cells to complement potential E1A defects, but all of the viruses turned out to grow as well as wild-type virus on HeLa cells.

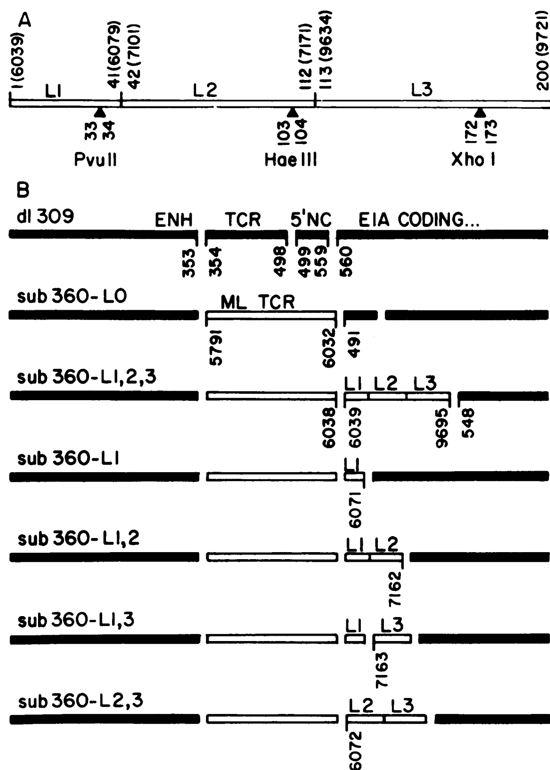

FIG. 1. Diagrams of the tripartite leader sequence and viral variants which contain leader segments appended to E1A coding regions. (*A*) The tripartite leader sequence. The three segments (L1, L2, and L3) are located relative to the start of the leader, and Ad2 nucleotide positions (16) are included in parenthesis. Arrowheads designate cleavage sites utilized for constructions. (*B*) Ad5 variants. Solid blocks designate segments normally present in the Ad5 E1A region, and open blocks designate segments derived from the Ad2 major late transcriptional control region or tripartite leader sequence. Ad2 (16) and Ad5 (17) sequence positions are indicated. ENH, enhancer; TCR, transcriptional control region; 5′NC, 5′ noncoding region; ML, major late.

Leader-Containing Variants Produce Similar Levels of E1A mRNAs. The E1A-specific mRNAs produced by the variants were quantitated by S1 endonuclease analysis. The assay utilized an *in vivo* ^{32}P-labeled M13 probe DNA covering the entire E1A coding region. Late after infection the levels of mRNA produced by the variants containing the major late control region increase substantially over those produced by the wild type (Fig. 2*A*). This indicates that the major late control region is functioning at its new location. The reduction in size of the bands corresponding to the 5′ exons in members of the *sub*360 family carrying leader sequences maps the discontinuity between the wild-type probe DNA and the variant mRNA. All variants produce about the same amount of E1A-specific mRNAs. Slight variations in mRNA levels (2-fold) were evident in individual experiments, but repeated analyses showed no consistent variation. Thus, the 5′ alterations have not differentially affected mRNA stability.

The structures of variant mRNA 5′ ends were confirmed by primer extension analysis (Fig. 2*B*). All variant mRNAs initiated at predicted sites except *sub*360-L0, whose 5′ end was located 5 bp upstream of the normal E1A start site. Analysis of *sub*360-L1,2,3 mRNA generated a series of bands in addition to the expected product. These bands could represent premature stops occurring during the extension reaction or true initiation sites. To distinguish these possibilities, S1 endonuclease analysis was performed on *sub*360-L1,2,3 mRNA, using a homologous, 5′-end-labeled probe DNA (Fig. 2*C*). This experiment revealed a unique 5′ end at the predicted position.

We conclude that the structures of variant mRNAs are in good agreement with DNA sequence predictions, and very similar levels of E1A mRNA species are produced by the variants late after infection. The only difference, then, between variants is the nature of E1A 5′ noncoding sequences.

Tripartite Leader Facilitates Translation Late After Infection. To quantitate the levels of E1A proteins synthesized by the variants, infected cells were briefly labeled with [^{35}S]methionine 24 hr after infection; extracts were prepared and subjected to immunoprecipitation using an E1A-specific monoclonal antibody. The supernatant from the first precipitation was subjected to a second cycle of precipitation to ensure quantitative results. The immunoprecipitates were analyzed by gel electrophoresis (Fig. 3), and the results were quantitated by excising relevant areas from the gel and measuring radioactivity (Table 1). *dl*309 produces less E1A-specific protein than the other variants, reflecting in part its greatly reduced RNA levels. *sub*360-L1,2,3 clearly produces much more of the E1A products than does any other member of the *sub*360 family. Thus, it is clear that the tripartite leader sequence enhances the ability of a mRNA to be translated late after infection. Most variants carrying portions of the leader sequence displayed a modest increase in the level of E1A products as compared to *sub*360-L0, which contains the normal E1A 5′ noncoding region, but none were as effectively translated as *sub*360-L1,2,3 mRNAs.

Further evidence that *sub*360-L1,2,3 mRNAs are translated more efficiently than those encoded by *sub*360-L0 was obtained by monitoring the levels of E1A polypeptides by two-dimensional gel electrophoresis without immunoprecipitation. E1A polypeptides were identified by use of mutants

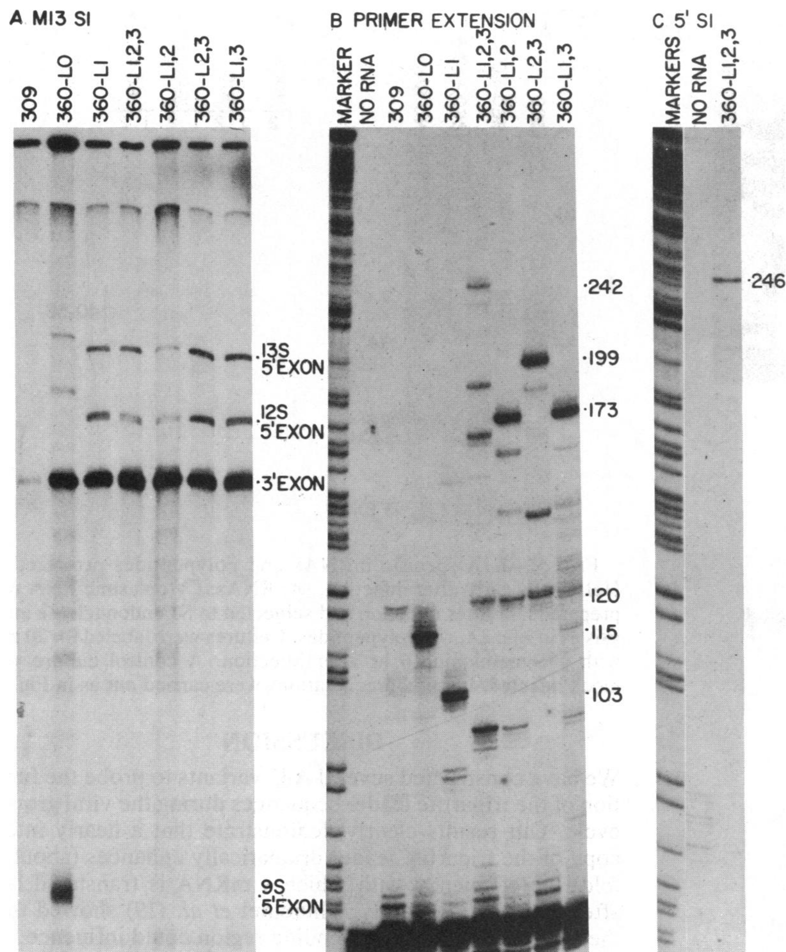

FIG. 2. E1A-specific mRNAs produced in HeLa cells late after infection. (*A*) S1 endonuclease analysis. RNAs were prepared 24 hr after infection and analyzed by using M13-E1A single-stranded DNA as probe. S1 endonuclease-treated products were resolved in a 5% polyacrylamide gel containing 8 M urea. Bands corresponding to 5′ and 3′ exons are identified. (*B*) Primer extension analysis. Poly(A)$^+$ RNAs were studied by primer extension using a single-stranded 5′-end-labeled probe DNA (nucleotides 550–619). Products of the reaction were resolved in an 8% polyacrylamide gel containing 8 M urea. The relative sizes of extended products (numbers on right) were determined by using markers obtained by sequence analysis of a 5′-end-labeled DNA fragment. (*C*) 5′-S1-endonuclease analysis. Poly(A)$^+$ *sub*360-L1,2,3 RNA was studied by hybridization to a 5′-end-labeled probe DNA (nucleotides 1–623). After digestion with S1 endonuclease, the products were subjected to electrophoresis as in *B*.

that produce altered E1A species (data not shown). Fig. 4*A* shows portions of two-dimensional gels on which polypeptides are displayed from *sub*360-L0- and *sub*360-L1,2,3-infected cells labeled with [^{35}S]methionine 24 hr after infection. The E1A species can be clearly identified in the case of *sub*360-L1,2,3 but are difficult to detect in the pattern from *sub*360-L0-infected cells.

A final confirmation of the tripartite leader effect was obtained by examining the position of E1A-specific mRNAs within polysome profiles derived from infected cells. If a mRNA species is more efficiently translated than another RNA of similar size, it should be associated with faster-sedimenting polysomes (more ribosomes per mRNA). This prediction was satisfied when *sub*360-L1,2,3 E1A mRNA was compared to that of *sub*360-L0 (Fig. 4*B*). Whereas mRNA molecules from *sub*360-L0 are mainly associated with one or two ribosomes, those from *sub*360-L1,2,3 are associated with three to six ribosomes.

We conclude that an E1A-specific mRNA carrying the tripartite leader sequence is translated at significantly enhanced levels as compared to a wild-type E1A mRNA late after infection.

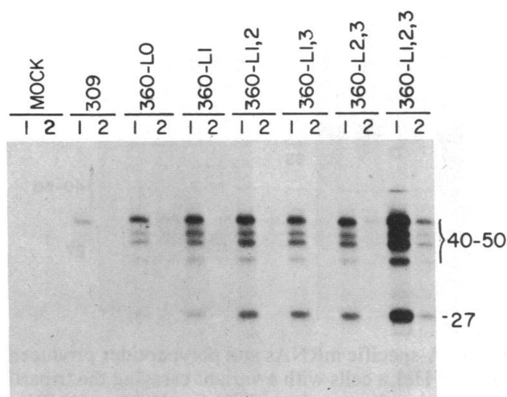

FIG. 3. E1A-specific polypeptides produced in HeLa cells late after infection. Cultures were labeled for 20 min with [^{35}S]methionine 24 hr after infection. Extracts were prepared and subjected to two rounds of immunoprecipitation (labeled 1 and 2) using an Ad5 E1A-specific monoclonal antibody. Electrophoresis was in a sodium dodecyl sulfate-containing polyacrylamide gel. E1A-specific polypeptides are designated and labeled by their mass in kilodaltons (kDa).

Tripartite Leader Does Not Facilitate Translation Early After Infection. Immunoprecipitations of E1A-specific polypeptides were also carried out with extracts prepared 6 hr after infection. At this time, quantities of E1A polypeptides

Table 1. Quantitation of translational enhancement

Variant	E1A polypeptides, ^{35}S cpm × 10^{-4}		
	40–50 kDa	27 kDa	Total
*dl*309	1.0	0	1.0
*sub*360-L0	3.3	0.6	3.9
*sub*360-L1	4.3	0.5	4.8
*sub*360-L1,2	4.9	1.0	5.9
*sub*360-L1,3	4.5	0.9	5.4
*sub*360-L2,3	4.4	1.4	5.8
*sub*360-L1,2,3	16.0	3.8	19.8

Indicated regions were excised from the gel whose autoradiogram is displayed in Fig. 3, and radioactivity was quantitated.

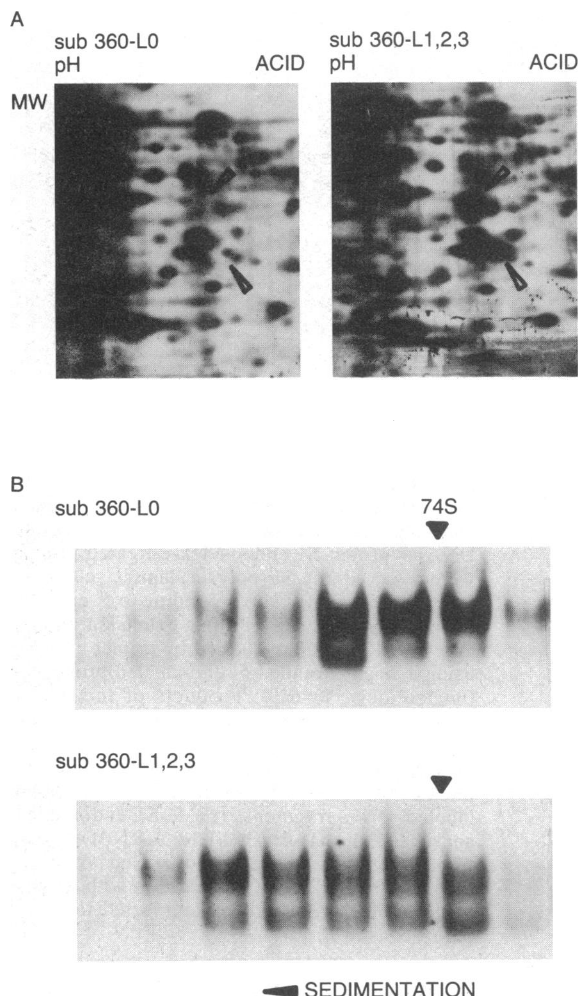

FIG. 4. E1A-specific polypeptides and polysomal RNAs produced in HeLa cells late after infection. (*A*) Two-dimensional gel electrophoresis. Cells were labeled with [^{35}S]methionine 22–24 hr after infection, extracts were prepared, and polypeptides were analyzed. Arrowheads designate E1A-specific polypeptides. MW, molecular weight. (*B*) RNAs obtained from polysomes. Cell extracts were prepared 24 hr after infection, polysomes were resolved on a 15–50% sucrose gradient, and RNA was prepared from each gradient fraction and subjected to blot hybridization analysis using pE1A-WT as probe. The position of 74S monosomes is designated.

parallel the level of E1A-specific mRNA; *sub*360-L1,2,3 mRNA is no more actively translated than the *dl*309 or *sub*360-L0 species (Fig. 5). Therefore, the tripartite leader sequence does not detectably influence the efficiency with which a mRNA is translated early after infection. This result also indicates that the leader does not simply alter the structure of the mRNA to improve its function under all conditions. The enhancing function is evident only late after infection. This conclusion was further strengthened by the fact that *sub*360-L0 and *sub*360-L1,2,3 mRNAs are translated with equal efficiency in reticulocyte cell-free lysates (data not shown).

Tripartite Leader Does Not Function at the 3′ End of a mRNA. To determine whether the tripartite leader sequence could exert its enhancing effect on late translation irrespective of its location on the mRNA molecule, the sequence was inserted into the E1A 3′ noncoding region as diagrammed in Fig. 6*A*. Levels of E1A-specific mRNA and protein were measured late after infection (Fig. 6 *B* and *C*). The tripartite leader exhibited little effect on translational efficiency when present near the 3′ end of the mRNA.

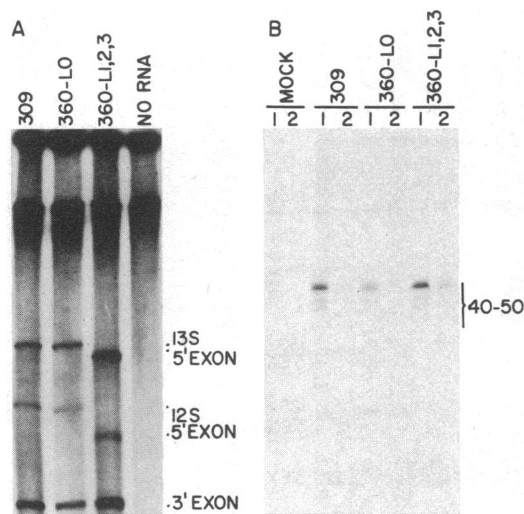

FIG. 5. E1A-specific mRNAs and polypeptides produced in HeLa cells early after infection. (*A*) RNAs. Cytoplasmic RNA was prepared 6 hr after infection and subjected to S1 endonuclease analysis as in Fig. 2*A*. (*B*) Polypeptides. Cultures were labeled for 20 min with [^{35}S]methionine 6 hr after infection. A control culture was mock infected. Immunoprecipitations were carried out as in Fig. 3.

DISCUSSION

We have constructed several Ad5 variants to probe the function of the tripartite leader sequences during the viral growth cycle. Our results clearly demonstrate that a nearly intact copy of the tripartite leader dramatically enhances (about 5-fold) the efficiency with which a mRNA is translated late after infection. Recently, Thummel *et al.* (19) showed that the structure of the 5′ noncoding region could influence the translation of mRNAs in adenovirus-infected cells. Their data were complicated due to complex splicing and varying initiator AUG usage, and they were not able to unambig-

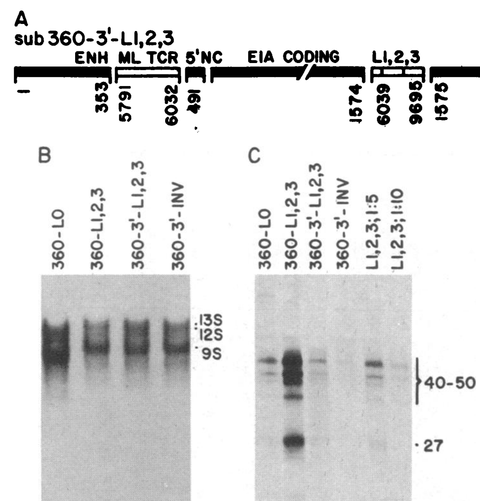

FIG. 6. E1A-specific mRNAs and polypeptides produced late after infection of HeLa cells with a variant carrying the tripartite leader in the 3′ noncoding region of E1A mRNAs. (*A*) Diagram of *sub*360-3′-L1,2,3. This virus carries a portion of the tripartite leader segment in the E1A 3′ noncoding region. Symbols, numbering, and abbreviations are as in Fig. 1. (*B*) RNAs. Poly(A)$^+$ RNAs were prepared at 24 hr after infection. Blot hybridization analysis utilized pE1A-WT DNA as probe. *sub*360-3′-INV carries an inverted copy of the tripartite leader segment inserted at the same site as that in *sub*360-3′-L1,2,3. (*C*) Polypeptides. Cultures were labeled for 20 min with [^{35}S]methionine 24 hr after infection. Immunoprecipitations were carried out as in Fig. 3.

uously identify the sequences responsible for this effect. Their results taken together with our experiments indicate that the tripartite leader segment carries an important *cis*-acting regulator of translation in adenovirus-infected cells.

Solnick (20) has described a recombinant adenovirus carrying the E1A coding region fused to the major late transcriptional control region plus a portion of the first leader segment. This virus produced substantial amounts of E1A-specific mRNA but little polypeptide. This observation is consistent with our results. Portions of the tripartite leader sequence enhance translation to a modest extent, at best (Fig. 3 and Table 1).

How efficiently is the tripartite leader-carrying E1A mRNA translated as compared to a normal late message that carries this sequence? We have compared the efficiency with which the *sub*360-L1,2,3 E1A and hexon (L3) mRNAs are translated. We determined relative steady-state levels of the two mRNAs, assayed incorporation of [^{35}S]methionine during a short labeling period (15 min, since E1A polypeptides have a shorter half-life than the hexon species), and corrected for the different methionine content of the two polypeptides. This calculation indicates that the hexon mRNA is translated 5- to 10-fold more efficiently than the E1A species. This difference could be due to the fact that the E1A message contains only 172 out of the total of 200 nucleotides that make up the tripartite leader (see Fig. 1*A*). Perhaps a fully intact leader would be more efficient. Alternatively, factors such as codon usage, nucleotides surrounding the AUG, or other unknown effects could cause this difference.

The action of the tripartite leader is not due to a structural or sequence effect that alters the intrinsic efficiency with which a mRNA is translated. If the intrinsic efficiency of translation were altered, mRNAs that carry these sequences would be more efficiently translated under all conditions. This is not the case. The tripartite leader exerts an effect within adenovirus-infected cells late after infection (Fig. 3) but not early after infection (Fig. 5*A*) or in reticulocyte lysates. Something is different about cells late after adenovirus infection, and this difference enables the tripartite leader sequence to mediate enhanced translation.

The changes that occur from early to late times during the adenovirus infectious cycle that could affect translation are of two types. First, the environment within the infected cell can change. For example, infection can alter permeability of the cell membrane, causing an imbalance in small compounds such as ions, thiol compounds, and ATP (e.g., see refs. 21–23). Such changes could conceivably alter sequence and structural requirements for efficient translation of a mRNA. The second difference within infected cells at late as compared to early times is the presence of new viral gene products. A new gene product could be synthesized late after infection that could interact with the tripartite leader to facilitate translation of the mRNA. VAI RNA could be this new gene product. This small, RNA polymerase III-transcribed RNA is required for efficient translation of viral mRNAs late after infection (24). Mathews (25) showed that VAI RNA will hybridize to the intact tripartite leader, and we find by computer search that there are several regions of complementarity between VAI RNA and the tripartite leader. Thus, it is tempting to speculate that VAI RNA interacts with the tripartite leader sequence to somehow facilitate translation. There is a problem with this hypothesis. Namely, VAI RNA is required for efficient translation of all viral mRNAs late after infection, not just those that carry the tripartite leader sequences (24). Further experiments are necessary to determine whether the translational effects mediated by the tripartite leader and VAI RNA are mechanistically related.

As yet, it is not clear at which point during translation the tripartite leader sequences act to enhance the process. Given the natural 5′ location of the sequences and the fact that they don't function when inserted into the 3′ noncoding region of a mRNA, it seems likely that they play a role during initiation of translation.

Do sequences analogous to the adenovirus tripartite leader sequence modulate the efficiency with which cellular RNAs are translated? So far, such sequences have not been identified. The heat-shock response could, however, be an analogous situation. After heat shock, particular cellular mRNAs are preferentially translated (26–28). These RNAs apparently carry sequences that allow them to be recognized and their translation enhanced under certain conditions.

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1. Berget, S. M., Moore, C. & Sharp, P. A. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3171–3175.
2. Broker, T. R., Chow, L. T., Dunn, A. R., Gelinas, R. E., Hassell, J. A., Klessig, D. G., Lewis, J. B., Roberts, R. J. and Zain, B. S. (1977) *Cold Spring Harbor Symp. Quant. Biol.* **42,** 531–553.
3. Zain, S., Sambrook, J., Roberts, R. J., Keller, W., Fried, M. & Dunn, A. R. (1979) *Cell* **16,** 851–861.
4. Weil, P. A., Luse, D. A., Segall, J. & Roeder, R. G. (1979) *Cell* **18,** 469–484.
5. Hearing, P. & Shenk, T. (1983) *Cell* **33,** 695–703.
6. Jones, N. & Shenk, T. (1979) *Cell* **17,** 683–689.
7. Stow, N. D. (1981) *J. Virol.* **37,** 171–180.
8. Chinnadurai, G., Chinnadurai, S. & Brusca, J. (1979) *J. Virol.* **32,** 623–628.
9. Graham, F. L., Smiley, J., Russell, W. C. & Nairn, R. (1977) *J. Gen. Virol.* **36,** 59–72.
10. Gaynor, R. B., Tsukamoto, A., Montell, C. & Berk, A. J. (1982) *J. Virol.* **44,** 276–285.
11. Hearing, P. & Shenk, T. (1983) *J. Mol. Biol.* **167,** 809–822.
12. McKnight, S. L., Gavis, E. R., Kingsbury, R. & Axel, R. (1981) *Cell* **25,** 385–398.
13. Lenk, R., Ransom, L., Kaufman, Y. & Penman, S. (1977) *Cell* **10,** 67–78.
14. Sarnow, P., Ho, Y.-S., Williams, J. & Levine, A. J. (1982) *Cell* **28,** 387–394.
15. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250,** 4007–4021.
16. Gingeras, T. R., Sciaky, D., Gelinas, R. E., Bing-Dong, J., Yen, C. E., Kelly, M. M., Bullock, P. A., Parsons, B. L., O'Neill, K. E. & Roberts, R. J. (1982) *J. Biol. Chem.* **257,** 13475–13491.
17. Van Ormondt, H., Maat, J., De Waard, A. & Van der Eb, A. J. (1978) *Gene* **4,** 309–328.
18. Kozak, M. (1983) *Cell* **34,** 971–978.
19. Thummel, C., Tjian, R., Hu, S.-L. & Grodzicker, T. (1983) *Cell* **33,** 455–464.
20. Solnick, D. (1983) *EMBO J.* **2,** 845–851.
21. Carrasco, L. (1978) *Nature (London)* **272,** 694–699.
22. Fernandez-Puentes, C. & Carrasco, L. (1980) *Cell* **20,** 769–775.
23. Ramabhadran, T. V. & Thach, R. E. (1981) *J. Virol.* **39,** 573–583.
24. Thimmappaya, B., Weinberger, C., Schneider, R. J. & Shenk, T. (1982) *Cell* **31,** 543–551.
25. Mathews, M. B. (1980) *Nature (London)* **285,** 575–577.
26. Ashburner, M. & Bonner, J. J. (1979) *Cell* **17,** 241–254.
27. Bienz, M. & Gurdon, B. (1982) *Cell* **29,** 811–819.
28. Scott, M. P. & Pardue, M. L. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3353–3357.