

In Vitro Translation Products Specified by the Transforming Region of Adenovirus Type 2

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Region 1 DNA sequences (map positions 0 to 11% on the linear adenovirus 2 genome) are expressed both early and late in lytic infection and are required for transformation by the virus. During productive infection six distinct cytoplasmic RNAs are synthesized from this region. These RNAs comprise two families, each consisting of three size classes that share 3' sequences. Region 1 RNAs were purified by hybridization selection, using restriction fragments bound to nitrocellulose membranes, and by size fractionation. The isolated RNAs were then translated in cell-free systems derived from wheat germ and rabbit reticulocytes. The family of RNAs specified by 0 to 4.4 sequences includes two RNAs, which are 12S and 13S in size. These RNAs were partially separated by molecular weight and translated. The 13S RNA produced 53,000-dalton (53K) and 41K peptides, and the 12S RNA synthesized 47K and 35K products. The family of RNAs mapping from 4.4 to 11.0 encodes three separate polypeptides, each of which can be assigned to a specific RNA. A 12K product that comigrates with structural polypeptide IX is synthesized from the 9S RNA as previously reported (U. Pettersson and M. B. Mathews, *Cell* 12:741-750, 1977). The 13S RNA encodes a 15K polypeptide that corresponds to a 15K polypeptide in infected cell extracts. The 22S RNA encodes a 52K protein distinct from the 0 to 4.4 polypeptides.

Only a small part of the viral genome is required for cell transformation by adenoviruses. These viral sequences are part of region 1, a DNA segment that is expressed at early times in productive infection. Early region 1 is located at the left end of the linear adenovirus genome. For adenovirus 2 this region includes the sequences in map positions 0 to 11 when the entire 23×10^6 -dalton viral DNA is defined as 100 map units. Two types of evidence define the correlation of early region 1 with transformation: these sequences are present in all transformed cell lines (13, 14, 41, 42), and transfection with specific restriction fragments from this region can establish and maintain the transformed state (17, 18).

The mRNA's and proteins specified by early region 1 have been studied in both transformed and lytically infected cells. In vitro translation of adenovirus-specific mRNA's isolated from transformed cells revealed polypeptides having molecular weights of 42,000 (42K) to 50K and 15K that are common to all transformed lines studied (28). Polypeptides of corresponding molecular weights were translated in vitro when early RNA was isolated from lytically infected cells (19, 28, 40). Transformation-specific pro-

teins have also been studied immunologically. Antisera were prepared from animals inoculated with adenovirus-transformed cells or from animals bearing adenovirus-induced tumors. Antisera elicited by different transformed lines were reacted with extracts of early infected cells. The only precipitated peptides common to all antisera tested were proteins of molecular weights 53K to 58K and 10K to 15K (16, 25, 26, 39). The relationship between the latter proteins and those identified by in vitro translation experiments has not yet been established.

Adenovirus mRNA's, including those specified by region 1, comprise families which share sequences (7, 30, 31, 33). In most cases the individual mRNA's are mosaic molecules containing transcripts from noncontiguous DNA sequences (2, 7-9, 21, 22). These properties of the mRNA's have made the relationship between a DNA sequence, its mRNA, and the protein product of the gene less straightforward than previously assumed. We have reported that two families of cytoplasmic RNAs are specified by early region 1 of the adenovirus 2 genome (43). In this communication, we extend these studies to a description of the proteins made in vitro from purified region 1 RNAs. The results show a

direct correlation between the individual RNA species within each family and their translated products.

MATERIALS AND METHODS

Virus infection and RNA isolation. Maintenance of KB suspension cell cultures and infections with adenovirus type 2 were performed as described previously (11, 43). For the preparation of [³⁵S]methionine-labeled virions, cells at 16 h after infection were concentrated twofold in Joklik minimal essential medium containing 1/50 the normal concentration of methionine and labeled for 4 h with 10 μ Ci of [³⁵S]-methionine (700 to 1,000 Ci/mmol; Amersham Corp., Chicago, Ill.) per ml. Cells were diluted to the original volume at 20 h and harvested as previously described. Labeled virus was purified as described previously (11). Early cytoplasmic RNA was isolated from cells infected for 9 h in the presence of 20 μ g of cytosine arabinoside (Sigma Chemical Co., St. Louis, Mo.) per ml to block viral DNA synthesis and prevent the onset of late gene expression. Late RNA was isolated from cells harvested at 22 h after infection. Cultures were labeled with 5 to 10 μ Ci of [5,6-³H]uridine (50 Ci/mmol; New England Nuclear Corp., Boston, Mass.) per ml for 6 to 10 h before harvesting. This level of radioactivity was adequate to monitor the hybridization selection and elution (see below). Cytoplasmic RNA was purified as previously described (43), and polyadenylic acid-containing RNA was selected by oligodeoxythymidylic acid-cellulose chromatography (1).

Preparation of DNA fragments. Adenovirus 2 DNA was prepared by the procedure of Tibbetts et al. (44). Viral DNA was digested with endo R-*Sma*I (10) or endo R-*Xba*I (Bethesda Research Laboratories, Rockville, Md.). Fragments were eluted and repurified for subcleavage as previously described (30). The fragment *Sma*I-E (coordinates, 2.9 to 10.7) was subcleaved with endo R-*Ba*II (15, 43). When viral DNA was digested with endo R-*Xba*I, five fragments were produced (M. Zabeau, personal communication). The two smallest fragments, *Xba*I-D (84.8 to 88.8) and *Xba*I-E (0 to 3.8), ran as a single band in 1% agarose gels. The fragments were eluted from the gels and purified on hydroxyapatite. Endo R-*Alu*I subcleavage (38) of the mixture gave a series of small fragments. The largest fragment was well resolved in 1.6% agarose gels and has the map position 1.8 to 3.8 (D. Spector, unpublished data).

Hybridization selection and fractionation of RNAs used to prime in vitro translation. Preparative hybridization of RNAs to DNA fragments bound to nitrocellulose membranes and thermal elution of specific RNAs have been described (32). For in vitro translation, the eluted RNA solution was adjusted to 0.5 M KCl and 10 mM Tris-hydrochloride, pH 7.5, and was rebound to oligodeoxythymidylic acid-cellulose. Polyadenylic acid-containing RNA was eluted with 10 mM Tris-hydrochloride, pH 7.5. After the addition of 0.1 M potassium acetate and 3 μ g of calf thymus tRNA per ml, RNA was precipitated overnight with 2 volumes of ethanol at -20°C.

In some experiments selected RNAs were fractionated by size before translation. The RNA was sub-

jected to electrophoresis for 6 h at 2.5 mA/gel in a 3.5% polyacrylamide gel containing 98% formamide (12). A parallel marker gel contained a sample of the same RNA together with ¹⁴C-labeled rRNA from KB cells. The marker gel was cut into 2-mm slices, and the RNA was eluted from each slice with NH₄OH and assayed for radioactivity. Based on the marker gel, appropriate fractions were then pooled from the sliced sample gel. The RNA was extracted by using the following procedure. Pooled gel slices were placed in a 3-ml syringe fitted with a 20-gauge needle; 0.3 ml of TES (50 mM Tris-hydrochloride, pH 7.5, 20 mM EDTA, 0.2% sodium dodecyl sulfate) was added to the syringe, and the slices were forced through the needle. The syringe and needle were washed with 0.2 ml of TES, and the wash was added to the gel suspension. The suspension was thoroughly mixed. Large gel particles were removed by passing the suspension through a 1-ml syringe fitted with a 20-gauge needle and plugged with glass wool. The syringe was then washed two times with 0.5 ml of TES, and these washes were added to the suspension. RNA was extracted two times with an equal volume of water-saturated phenol and precipitated with 2 volumes of ethanol at -20°C. The precipitate was centrifuged immediately at 12,000 rpm for 10 min. The pellet was resuspended in 10 mM Tris-hydrochloride (pH 7.5)-0.5 M KCl, reselected by oligodeoxythymidylic acid-cellulose chromatography, and prepared for translation as described above.

Rabbit reticulocyte cell-free translation system. For translation, RNA was pelleted at 25,000 rpm, dried with nitrogen, and resuspended in 5 to 10 μ l of sterile distilled water. Nuclease-treated reticulocyte lysates were prepared by published procedures (34). Translation assays were carried out in a total volume of 25 μ l. The reaction mixture contained 10 μ l of lysate, 5 μ l of [³⁵S]methionine (700 to 1,000 Ci/mmol; Amersham Corp.), 1 to 2 μ l of selected RNA, 1 mM magnesium acetate, 80 mM potassium acetate, 20 μ g of calf thymus tRNA per ml, 0.5 mM spermidine, 20 mM HEPES (*N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid), pH 7.6, 1 mM ATP, 0.2 mM GTP, 1 mM dithiothreitol, 8 mM creatine phosphate, 35 μ g of creatine phosphokinase per ml, and 60 μ M concentrations of 19 unlabeled amino acids. Incubation was at 30°C for 1 h. A sample was removed for trichloroacetic acid precipitation, and the remainder was processed for gel electrophoresis as described below.

Wheat germ cell-free translation system. The wheat germ cell-free system was used to assay translation of small polypeptides. The large amount of globin present in the reticulocyte lysate interferes with migration and identification of globin-sized translation products (unpublished data).

Translation systems were prepared by established procedures (29). Translation reactions were carried out in a total volume of 25 μ l containing 12.5 μ l of wheat germ S-30 extract, 2.5 μ l of [³⁵S]methionine, 1 to 2 μ l of selected RNA, 0.35 mM spermidine, 1.5 mM magnesium acetate, 110 mM potassium acetate, 20 μ g of wheat germ tRNA per ml, 20 mM HEPES, pH 7.6, 37.5 μ M concentrations of 19 unlabeled amino acids, 1 mM ATP, 0.2 mM GTP, 1 mM dithiothreitol, 8 mM creatine phosphate, and 35 μ g of creatine phosphokinase per ml. Translation reactions were incubated at 30°C for 1 h.

Gel electrophoresis. Samples from reticulocyte translations were prepared for electrophoresis in the following manner. EDTA (5 mM) and RNase A (100 μ g/ml) were added, and the extracts were incubated for 15 min at 30°C. Three volumes of sample buffer (100 mM Tris, pH 6.8, 2.5% sodium dodecyl sulfate, 2.5% glycerol, 0.7 M mercaptoethanol, and 0.0125% bromophenol blue) was added, and then the samples were heated to 100°C for 3 min. Samples from wheat germ translations were treated with EDTA and RNase A as described above and then precipitated with 10% trichloroacetic acid. Precipitates were washed with 10% trichloroacetic acid-acetone (4°C) and air dried. They were then suspended in sample buffer and heated at 100°C for 3 min. Samples of either reticulocyte or wheat germ assays were run on a modified Laemmli polyacrylamide gel system (23) with a ratio of acrylamide to bisacrylamide of 30:0.174. Slab gels were cast 1.5 mm thick with a 1.0-cm 5% stacking gel above an 8-cm resolution gel of 15 or 17.5%. Electrophoresis was at 22.5 mA (constant current) for 3 to 4 h. Gels were prepared for fluorography by published procedures (6, 24). Molecular weight determinations were based on published values for virion polypeptides (27).

RESULTS

The two families of region 1 mRNA's are illustrated in Fig. 1. The 13S and 12S molecules from the 0 to 4.4 region (region 1A) have common 3' and 5' termini but differ in the size of an internal splice (5). Both of the cytoplasmic 9S RNAs accumulate label *in vivo* only at late times, whereas the other species are labeled throughout infection. At late times the rate of appearance of cytoplasmic 13S and 9S RNA from the 4.4 to 10.7 region (region 1B) is at least 10-fold greater than that of 22S RNA (43).

The experiments presented below rely on a variation of deletion mapping. Highly purified viral RNAs can be isolated by preparative hybridization-elution using specific viral DNA fragments (32). In performing these selections, we

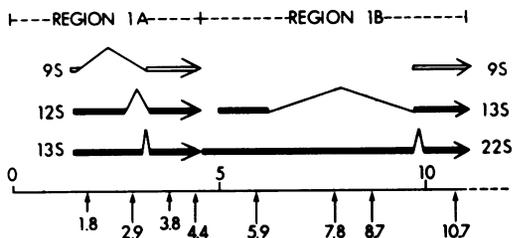


FIG. 1. Region 1 mRNA's. The 5' and 3' termini of RNA molecules and splice positions are based on filter hybridization (43), nuclease S1 (5), and electron microscopy (9, 46) data. RNAs which accumulate predominantly at late times (43) are indicated by open arrows. Restriction endonuclease cleavage sites have been reported previously (43), except for the *Alu* site at 1.8 and the *Xba*I site at 3.8 (see text).

took advantage of two features of region 1 RNAs: (i) the differential expression of these RNAs during productive infection and (ii) the sequences unique to each member of a family of overlapping RNAs. For example, 7.8 to 8.7 DNA contains sequences present only in the 22S RNA and not in its overlapping 13S RNA. In one case it was necessary to partially separate by size two RNAs closely related in sequence. The RNAs purified by these methods were then translated *in vitro* and assigned to specific protein products.

Proteins specified by 0 to 4.4 mRNA's. As noted above, transcripts from region 1A consist of a 9S species detected only at late times after infection and a 12S to 13S size class detected at both early and late times after infection (Fig. 1). The larger size class consists of two mRNA species which differ only in the size of an internal splice. This observation has been confirmed in our laboratory by using the S1 nuclease assay (3) with early RNA selected by hybridization to 2.9 to 5.9 DNA (data not shown).

To obtain purified early viral RNA for *in vitro* translation, KB cells were infected for 9 h in the presence of cytosine arabinoside in order to block the onset of viral DNA synthesis. Polyadenylic acid-containing cytoplasmic RNA was isolated and hybridized to nitrocellulose membranes containing 0 to 2.9 DNA. Nonspecific RNAs were removed by a thermal elution step as previously described in detail (32), and RNAs specific for 0 to 2.9 DNA were eluted at 85°C. The eluted RNAs were then further purified by oligodeoxythymidylic acid-cellulose chromatography. This RNA was used to program nuclease-treated rabbit reticulocyte or wheat germ cell-free translation systems. In the reticulocyte extract the RNA purified from 0 to 2.9 DNA directed the *in vitro* translation of four polypeptides with molecular weights of 35K to 53K (Fig. 2, lane 3). One of these products comigrated with the background band synthesized in the absence of exogenous mRNA (Fig. 2, lane 2). The presence of a viral product of this size was confirmed by translation in a wheat germ cell-free system which does not produce any background in this molecular weight range (see below). Polypeptides of identical mobilities were translated, although there were differences in the relative proportions of the four polypeptides.

Since two known RNA species were selected with the 0 to 2.9 fragment, we performed an experiment in order to correlate polypeptide products with each RNA. For these experiments RNAs were partially separated by size before translation *in vitro*. RNA was selected by hybridization to 0 to 2.9 DNA. The specific RNA was eluted and then subjected to electrophoresis in a 3.5% formamide-polyacrylamide gel. As de-

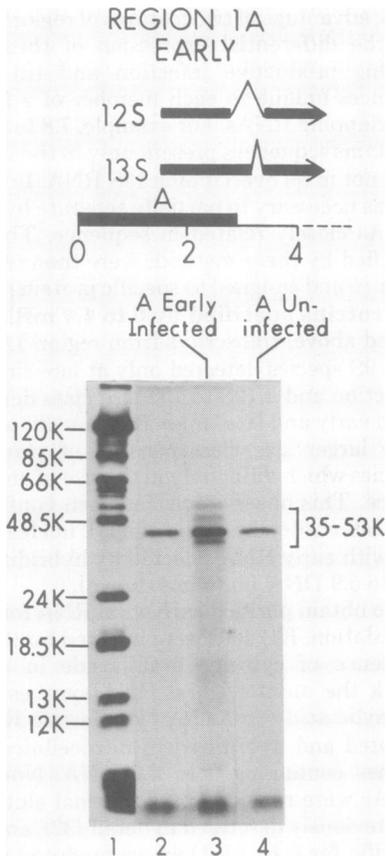


FIG. 2. *In vitro* translation of RNAs selected by 0 to 2.9 DNA. Cytoplasmic polyadenylic acid-containing RNA from 4.5×10^8 early infected or uninfected KB cells was selected by preparative hybridization to 0 to 2.9 DNA and elution. A sample of RNA was translated in reticulocyte lysates. The map of early region 1A RNA is provided for reference. Lane 1, ^{35}S -labeled virion markers; lane 2, no added RNA; lane 3, 0 to 2.9 specific RNA from early infected cells; lane 4, 0 to 2.9 specific RNA from uninfected KB cells.

scribed above, a parallel gel containing selected RNA was used to locate the 12S to 13S peak (Fig. 3). The larger of the two RNAs in this peak is present in much greater abundance (5). We predicted that by splitting the peak we could obtain a sample of the larger RNA free of contamination by the smaller. The sample gel was then sliced, and fractions 12 to 14 (pool I) and 15 to 17 (pool II) were eluted for translation in the wheat germ *in vitro* system (Fig. 4). Pool I RNA (Fig. 4, lane 2) encoded only 53K and 41K proteins, whereas pool II (lane 3) specified, in addition, the 47K and 35K proteins. Nuclease S1 assays of the RNA samples from a repeat experiment confirmed that pool I contained only the larger and predominant species of RNA, whereas pool II contained both RNAs (data not

shown). On this basis, we assigned the 53K and 41K proteins to the 13S RNA and the 47K and 35K proteins to the 12S RNA.

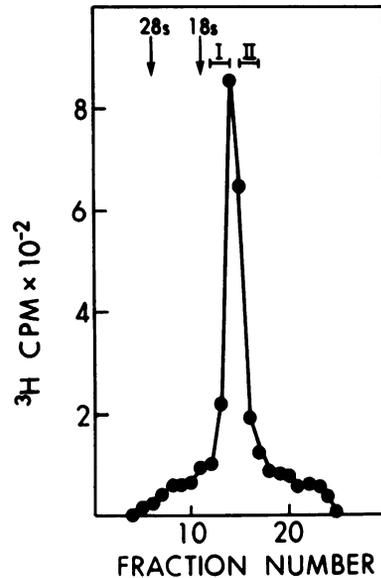


FIG. 3. Separation of region 1A early RNA by size before *in vitro* translation. Early RNA was selected by hybridization to 0 to 2.9 DNA; 3.9×10^4 cpm of selected RNA (from 9×10^8 infected cells) was subjected to electrophoresis on a formamide-polyacrylamide gel for 6 h at 2.5 mA/gel. The marker gel shown here contained 1.5×10^4 cpm of high-specific activity RNA selected by the same fragment and ^{14}C -labeled rRNA from KB cells. After elution of RNA from the sample gel, 5.3×10^3 cpm was recovered in pool I and 7.9×10^3 cpm was recovered in pool II.

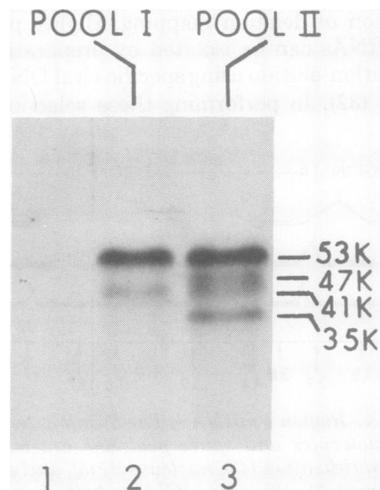


FIG. 4. Translation of two size classes of early region 1A RNA. Samples of RNA were translated in wheat germ lysate. Lane 1, No added RNA; lane 2, pool I RNA specific for 0 to 2.9 DNA (see Fig. 3); lane 3, pool II RNA specific for 0 to 2.9 DNA (see Fig. 3).

We considered the possibility that one or more of these products were host coded. Such mRNA's might have been selected because of a limited sequence homology with the viral sequences in region 1A. To examine this question, the following experiment was performed. Polyadenylated cytoplasmic RNA was isolated from uninfected KB cells and then hybridized to 0 to 2.9 DNA. Bound RNA was eluted and translated as described above. No prominent *in vitro*-synthesized proteins were detected (Fig. 2, lane 4).

Cytoplasmic RNA was also isolated from cells at 22 h after infection and selected by hybridization to 1.8 to 3.8 DNA. When late RNA was selected with this fragment and translated, the four proteins found in early samples were synthesized (data not shown). The sequences in this fragment also specify a 9S RNA which can be detected only at late times (43) (Fig. 1). Although samples labeled with [³H]Juridine at 12 to 22 h postinfection revealed the 9S sequence (data not shown), no evidence was found for a prominent polypeptide that might be assigned to the region 1A 9S RNA.

Proteins specified by 4.4 to 10.7 mRNA's.

Early RNA was selected by hybridization to two different restriction fragments which discriminate between the two early RNAs from region 1B (Fig. 1). The 8.7 to 10.7 fragment contains sequences present in both the 13S and 22S species, whereas the 7.8 to 8.7 DNA fragment contains only 22S sequences. When translated *in vitro*, RNA selected by 8.7 to 10.7 DNA produced two peptides with molecular weights of 52K and 15K (Fig. 5, lane 2). RNA specific for the 7.8 to 8.7 fragment produced only a 52K polypeptide (Fig. 5, lane 9). On the basis of these results, we assigned the 15K protein to the 13S mRNA and the 52K protein to the 22S mRNA. In our gel system, this 22S RNA-specified 52K protein did not comigrate with any of the four polypeptides made from RNA selected by the 0 to 4.4 region (Fig. 5, lanes 5 and 6). It should also be noted that none of these peptides comigrated with the protein described by Rekosh et al. (37), which is covalently linked to the 5' termini of adenovirus DNA (S. Straus and D. Halbert, unpublished data).

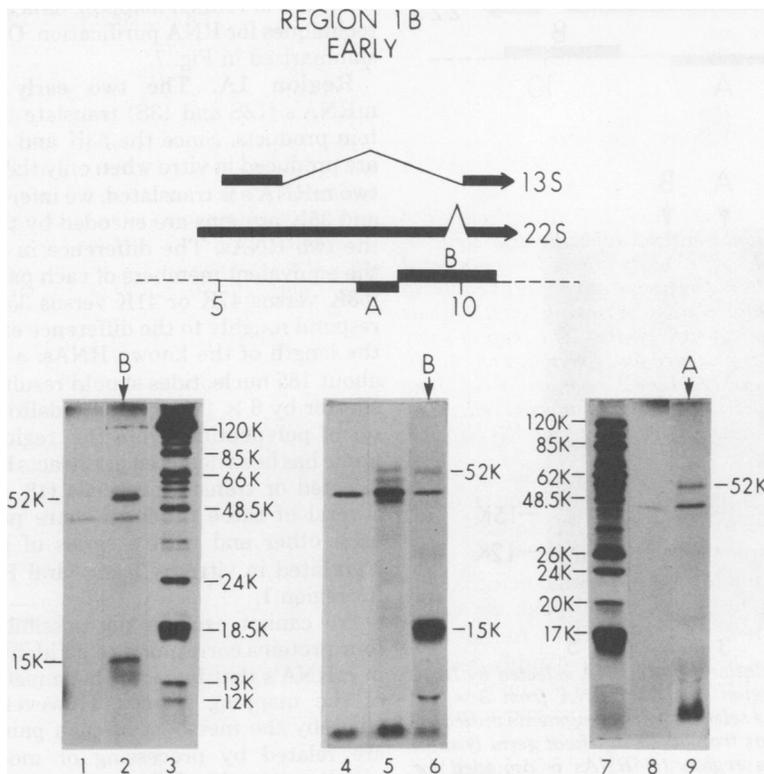


FIG. 5. Translation of early RNA selected by DNA fragments in region 1B. Early RNA from 4.5×10^8 infected cells was selected by the DNA fragments indicated and samples were translated in reticulocyte lysates as described in the text. The map of early region 1B RNA is provided for reference. Lane 1, No RNA added; lane 2, RNA selected by 8.7 to 10.7 DNA (B); lane 3, ³⁵S-labeled virion marker; lane 4, no RNA added; lane 5, RNA selected by 0 to 2.9 DNA (see text); lane 6, RNA selected by 8.7 to 10.7 DNA (B); lane 7, unselected late infected cell RNA; lane 8, no RNA added; lane 9, RNA selected by 7.8 to 8.7 DNA (A).

At late times region 1B specifies three RNAs (Fig. 1). Two of these RNAs are indistinguishable from the 13S and 22S species synthesized early. The third RNA migrates as a 9S molecule. We have previously observed that late region 1B RNA labeled for 2 h contains much more 13S and 9S RNA than 22S RNA (43). The same result was obtained in RNA preparations labeled for 10 h (data not shown), indicating a substantially higher steady-state concentration of the smaller messages with respect to the 22S RNA. Therefore, late RNAs selected by hybridization to 8.7 to 10.7 DNA consist of mostly 13S and 9S RNA. Peptides of molecular weight 15K and 12K were synthesized from this RNA (Fig. 6,

lane 4). Late RNA selected by hybridization to 5.9 to 7.8 DNA consists of mostly 13S RNA. When translated, only a 15K protein is discerned (Fig. 6, lane 3). As expected, this product comigrated with the early polypeptide from this region (data not shown). These results support the assignment of the 15K protein to the 13S RNA and agree with previous results (36) that a 9S RNA from this region codes for viral structural polypeptide IX, a 12K polypeptide.

DISCUSSION

Several earlier attempts to identify adenovirus polypeptides related to cell transformation have relied on immunological methods. With this approach it is difficult to distinguish between virus-specified proteins and cellular proteins that may be induced as a result of transformation. This ambiguity can be overcome by purifying the viral mRNA's on the basis of nucleic acid sequence and then translating these RNAs in vitro. Previous efforts toward such goals utilized RNA preparations whose content was less rigorously defined (18, 28, 40). In the present study, we made use of refined mapping data and improved techniques for RNA purification. Our results are summarized in Fig. 7.

Region 1A. The two early overlapping mRNA's (12S and 13S) translate into four protein products. Since the 53K and 41K proteins are produced in vitro when only the larger of the two mRNA's is translated, we infer that the 47K and 35K proteins are encoded by the smaller of the two RNAs. The difference in size between the equivalent members of each pair of proteins (53K versus 47K or 41K versus 35K) does correspond roughly to the difference expected from the length of the known RNAs: a difference of about 185 nucleotides should result in a protein shorter by 6×10^3 to 7×10^3 daltons. A similar set of polypeptides from this region of the genome has been detected in extracts from lytically infected or transformed cells (18, 20). At least several of these products share peptides with each other and with a series of polypeptides translated in vitro by using viral RNA specific for region 1.

We cannot exclude the possibility that the four proteins correspond to an identical number of mRNA's that have not been resolved by any of the mapping studies. However, a scheme whereby the members of each pair of proteins are related by processing or modification is equally attractive. In our gel system each member differs in size from its partner by the same amount, approximately 12,000 daltons. This difference could be due to either similar proteolytic cleavage of the larger proteins or modifications that alter the mobility of the primary product.

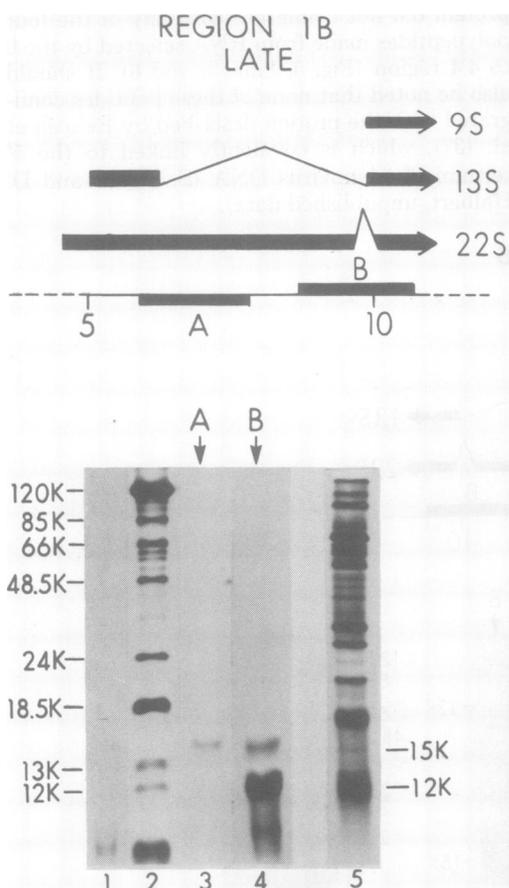


FIG. 6. Translation of late RNA selected by DNA fragments in region 1B. Late RNA from 3×10^8 infected cells was selected by the fragments indicated and a sample was translated in wheat germ lysates. The map of late region 1B RNAs is provided for reference. The 22S RNA was present in very low concentrations (see text). Lane 1, No RNA added; lane 2, ^{35}S -labeled virion marker; lane 3, late RNA selected by 5.9 to 7.8 DNA (A); lane 4, late RNA selected by 8.7 to 10.7 DNA (B); lane 5, unselected late RNA.

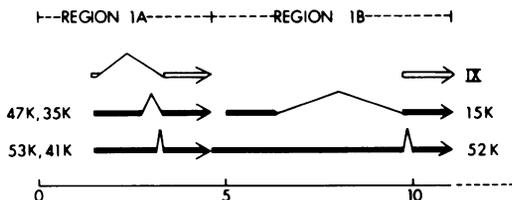


FIG. 7. Transcription and translation map of region 1. Assignments of polypeptides to RNAs are discussed in the text.

A 10K polypeptide from transformed cells has been mapped to region 1 by immunological methods (16, 25, 26, 39). Although we do not detect this peptide in our *in vitro* system, such a cleavage product could have a function in infected or transformed cells and thus be conserved. It has been suggested by analogy to simian virus 40 early RNA (4) that the different splice patterns in the 12S and 13S RNAs result in the exclusion of a translation terminator. Because the large 13S region 1A mRNA codes for the larger proteins, we consider it unlikely that a functional terminator is removed by the increased splice. An analysis of recently published sequence data for adenovirus 5 region 1 (45) is also consistent with this conclusion.

We have been unable to assign the late 9S RNA species from region 1A to a distinct polypeptide. Cells infected in the presence of 20 μ g of cycloheximide per ml synthesize an early protein of about 26K (35). Treatment of cells with cycloheximide results in elevated levels of RNA synthesis from all early regions of the genome and the appearance of 9S RNA from region 1. We have recently mapped this RNA to region 1A and have detected a 28K polypeptide from *in vitro* translation systems programmed with the cycloheximide 9S RNA (D. Spector, L. Crossland, D. Halbert, and H. J. Raskas, manuscript in preparation). The 28K polypeptide is a candidate for the 9S RNA product in non-drug-treated cells.

All of the proteins encoded by region 1A ran anomalously large in our gel system. An mRNA of 1,100 nucleotides (13S) does not contain enough coding capacity for 53,000 daltons of protein. Sequence data for region 1 indicate a large number of proline and glutamic acid codons in the only open reading frame to the right of map position 3.3 (45). High proline content may increase the apparent molecular weight of proteins in gels. The fact that all four proteins coded for by the 12S and 13S RNAs show this apparent increase in molecular weight is consistent with the contention that both mRNA's are translated past the splice point at map position 3.3. They would then be translated in the same reading frame to the right of map position 3.3.

One might speculate that any processing of the proteins would take place at the amino-terminal end.

Region 1B. Region 1B codes for three mRNA's and three proteins. Persson et al. (35) have reported that polypeptide IX can be detected in early infected cell extracts and by translation of mRNA isolated from cells infected in the presence of either cycloheximide or cytosine arabinoside. We do not detect this polypeptide when early RNA from this region is translated. A late 15K protein has been mapped previously to region 1 by *in vitro* translation (27, 28). Our data demonstrate that a 15K protein can be translated from early and late mRNA. However, late RNA is a much more efficient template for synthesis of this protein. This result correlates with our previous observation of an increased accumulation rate of 13S mRNA from region 1B after the onset of viral DNA synthesis (43).

The genome site specifying the 52K protein has not been determined previously. The 52K protein is distinct from the four proteins identified in region 1A by the following criteria: (i) it does not comigrate with any of the four region 1A proteins; and (ii) translation of size-fractionated region 1 mRNA's demonstrates distinct size classes of approximately 13S for region 1A proteins and approximately 22S for the 52K protein. Also, this 52K protein does not comigrate with the 50K to 60K protein isolated from the termini of adenovirus 2 DNA (data not shown). Proteins in the 50K to 60K range have been identified by immunological methods in infected and transformed cells (16, 20, 25, 26, 39). A 58K protein has been mapped to region 1B (S. Ross and A. J. Levine, personal communication).

The region 1B 22S mRNA (approximately 2,300 nucleotides) is capable of encoding more than 70,000 daltons of protein, whereas the 13S mRNA (approximately 1,100 nucleotides) can encode more than 35,000 daltons of protein. Thus, there must be some restriction as to which sequences are translated in these overlapping mRNA's, and this restriction may be governed by splicing patterns. Comparative peptide mapping of 53K and 15K polypeptides immunoprecipitated from lytically infected cells (M. Green, W. S. M. Wold, K. Brackmann, and M. A. Cartas, personal communication) has revealed that most if not all of the methionine-labeled peptides of 15K polypeptide are shared by 53K polypeptide. Similar comparisons of the *in vitro* products are in progress. This information, in conjunction with mRNA mapping studies and DNA sequencing, should provide a more complete understanding of the transforming region of adenovirus.

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