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Identification of Early Adenovirus Type 2 RNA Species Transcribed from the Left-Hand End of the Genome

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Unique fragments of adenovirus type 2 DNA generated by cleavage with endonuclease R·*Eco* RI or endonuclease R·*Hsu* I (*Hin* dIII) were used to map cytoplasmic viral RNAs transcribed early in productive infection. Radioactive early viral RNA was first fractionated by polyacrylamide gel electrophoresis. Eluted viral RNAs were then tested for hybrid formation with DNA fragments. The *Eco* RI DNA fragment (*Eco* RI-A) which contains the left-hand 58% of the genome hybridized 13*S* and 11*S* RNAs. More detailed mapping of these RNAs was achieved by hybridization to the seven *Hsu* I fragments of *Eco* RI-A. The early RNA annealed only to *Hsu* I-G and C, two fragments which comprise the extreme left-hand 17% of the genome. Viral RNA migrating as 13*S* and 11*S* annealed to *Hsu* I-G, and 13*S* RNA annealed to *Hsu* I-C. A 13*S* RNA is transcribed from *Eco* RI-A late in infection (18 h). Hybridization-inhibition studies with *Eco* RI-A DNA, early cytoplasmic RNA, and ^{3}H-labeled 13*S* late RNA demonstrated that this RNA synthesized at late times is an early RNA species which continues to be synthesized in large amounts at 18 h. This 13*S* RNA synthesized at 18 h hybridized to *Hsu* I-C but not to *Hsu* I-G DNA. These results establish that the 13*S* RNAs transcribed from *Hsu* I-G and C at early times must be different species.

Lytic infection of adenovirus type 2 in cultured human cells consists of a series of sequential events that can be divided into early and late processes (9). The early period terminates at the beginning of viral DNA synthesis, approximately 6 h after infection (26). In this early period only a limited portion of the coding capacity of the viral genome, approximately 25%, is transcribed into cytoplasmic viral RNA (4, 7, 21, 27). Polyacrylamide gel electrophoresis of the early cytoplasmic viral RNA revealed two major size classes, 19 to 20*S* and 11 to 15*S* RNAs (4, 10, 15).

The use of restriction endonucleases to produce specific fragments of adenovirus 2 DNA permits further analysis of virus-specified transcription in lytic infection and in cells transformed by the virus. For example, endonuclease R·*Eco* RI cleaves adenovirus 2 DNA into six fragments (16). Hybridization of size-fractionated early cytoplasmic RNA to each of the six *Eco* RI DNA fragments has allowed the localization of individual viral RNA species to regions along the genome (25). These studies established that the two major size classes were composed of at least six different RNA species.

One of the six *Eco* RI fragments (RI-A) comprises 58% of the adenovirus 2 genome (16). In a previous study (25), this portion of the genome was shown to code for two different classes of early cytoplasmic RNA, molecules migrating as 13*S* and 11*S* RNA. To map in greater detail the genome location of these viral RNAs transcribed from RI-A, we have utilized endonuclease R·*Hsu* I which cleaves RI-A DNA into seven unique fragments (R. J. Roberts, personal communication). Using these seven DNA fragments we have found that (i) the RI-A fragment specifies early viral mRNA's that are transcribed from regions very close to the left-hand end of the genome; (ii) these regions code for at least two and possibly three different viral RNAs; and (iii) only one of these viral RNA species is still synthesized late in infection. A comparison of the results obtained here and recent analyses of cell transformation by adenoviruses (8, 8a, 22) indicates that some of the early viral RNA species coded for by RI-A are transcribed from the small portion of the viral genome which is required for the establishment and maintenance of cell transformation.

MATERIALS AND METHODS

Suspension cultures of exponentially growing KB cells were infected with CsCl-purified adenovirus 2 and harvested as previously described (3, 5, 20). For radioactive early RNA, [^{3}H]uridine was added 2 to 6 h after infection (3); for late RNA, cultures were labeled with [^{3}H]uridine 18 to 21 h after infection (24). All cultures harvested at 6 h after infection were treated with cycloheximide (25 μg/ml) to prevent the beginning of late mRNA synthesis (3). RNA was purified by phenol and chloroform-isoamyl alcohol extraction as previously described (3), and molecules containing poly(A) were separated from molecules lacking poly(A) by selective retention on oligo-dT cellulose (1, 3). RNA was fractionated on 11-cm 3.2% polyacrylamide gels cross-linked with ethylene diacrylate; the RNA in each gel slice was solubilized by incubating at 60 C in 6× Sal/Cit (1× Sal/Cit is 0.15 M NaCl-0.015 M sodium citrate) containing 0.1% sodium dodecyl sulfate ($NaDodSO_4$) (4). The approximate sedimentation values assigned to viral RNA size classes were obtained by comparing the migration of viral RNAs with 28*S* and 18*S* rRNA.

Adenovirus 2 DNA was purified as previously described (5, 16). Endonuclease R·*Eco* RI was purified and used according to the procedure of Mulder and Delius (13); endonuclease R·*Hsu* I, an isoschizomer of endonuclease R·*Hin* dIII (R. J. Roberts and P. A. Myers, personal communication), was prepared and used as described by Graham et al. (8a). The RI-A fragment to be used for subcleavage by endonuclease R·*Hsu* I was purified free from the other RI fragments by sucrose gradient centrifugation as described by Graham et al. (8a). After enzyme digestion, DNA fragments were resolved by electrophoresis in 1.0% agarose gels containing ethidium bromide, and the bands containing DNA were visualized by UV light (23). The DNA was either extracted from gel slices as described before (8a) or eluted from gel slices by high-voltage electrophoresis (16, 25); recovery was monitored with [^{32}P]DNA.

Hybridizations with 6.5-mm membranes were performed in 6× Sal/Cit-0.1% $NaDodSO_4$ as described previously (3, 19). For all hybridizations to *Hsu* I fragments, six or seven membranes, each containing DNA from a unique fragment, were incubated simultaneously with [^{3}H]RNA in 200 μl. Hybridization-inhibition experiments were carried out as previously described (3, 5).

RESULTS

The six adenovirus 2 DNA fragments produced by cleavage with endonuclease R·*Eco* RI are shown in Fig. 1. In a previous study, hybridization of ^{3}H-labeled early cytoplasmic viral RNA to these six fragments demonstrated the presence of transcripts from all of the *Eco* RI fragments except for *Eco* RI-F (25). When ^{3}H-labeled early cytoplasmic RNA was resolved by polyacrylamide gel electrophoresis, and the fractionated RNA was hybridized to *Eco* RI-A DNA, 13*S* and 11*S* viral RNAs were identified (25). The DNA required as template for one 11*S* and one 13*S* mRNA is less than 10% of the RI-A fragment; to allow the assignment of these RNAs to a more precise region within RI-A, further analysis was performed with DNA fragments obtained by cleaving *Eco* RI-A with endonuclease R·*Hsu* I. (This endonuclease cleaves the same DNA sequences recognized by endonuclease R·*Hin* dIII [R. J. Roberts, personal communication].) The fragments produced by cleavage of adenovirus 2 DNA with endonuclease R·*Hsu* I are illustrated in Fig. 1. The genome order of the *Hsu* I fragments produced by cleavage of *Eco* RI-A is G, C, B, I, J, D, and A-A; the latter notation (*Hsu* I A-A) refers to the DNA segment bounded on one side by the sixth endonuclease R·*Hsu* I cleavage site and on the other side by the first endonuclease R·*Eco* RI scission, between fragments *Eco* RI-A and *Eco* RI-B.

Hybridization of total early and late cytoplasmic RNA to DNA fragments generated from Eco RI-A. When ^{3}H-labeled early cytoplasmic RNA was annealed simultaneously to the seven *Hsu* I DNA fragments generated from RI-A, significant hybridization occurred only with fragments *Hsu* I-G and a mixture of *Hsu* I-C and *Hsu* I-D (Table 1). For this initial experiment *Hsu* I fragments C and D, which are very similar in size, were not separated. For comparison, hybridization with ^{3}H-labeled late RNA purified from cells harvested 18 h after infection revealed transcripts from *Hsu* I fragments B, C plus D, J and A-A. Hybridization of ^{3}H-labeled late RNA to *Hsu* I fragments G or I was not detected.

Hybridization of size-fractionated early RNA to DNA fragments. As noted above, analysis of early cytoplasmic RNA fractionated by size indicated that molecules migrating as 11*S* and 13*S* are transcribed from RI-A (25). The size distribution of early cytoplasmic transcripts from RI-A is shown in the top panel of Fig. 2. To determine more exactly the location of these RNA molecules on the adenovirus genome, poly(A)-containing ^{3}H-labeled early RNA was hybridized to the seven fragments generated by cleavage of RI-A with endonuclease R·*Hsu* I (Fig. 2). The 11*S* to 13*S* RNA annealed to *Hsu* I fragments C and G. The material that hybridized to *Hsu* I-G DNA migrated as a peak with a shoulder. Hybridization of RNA from a gel which had been run for a longer time revealed two peaks hybridizing to *Hsu* I-G (inset, Fig. 2). The transcripts from *Hsu* I-C and the larger transcripts from *Hsu* I-G appear to comigrate with the 13*S* RNA detected by hybridization to RI-A. No hybridization was

FIG. 1. *The genome location of Eco RI and Hsu I (Hin dIII) adenovirus 2 DNA fragments. The cleavage sites of endonuclease R-Eco RI are based on the study of Mulder et al. (12). The cleavage sites of endonuclease R·Hin dIII and endonuclease R·Hsu I are identical and were determined by R. J. Roberts and collaborators (personal communication). The space between each pair of small vertical bars represents 10% of the genome. When Eco RI-A is cleaved by endonuclease R·Hsu I, a new fragment is generated which contains the overlapping regions of Eco RI-A and Hsu I-A. In the text this fragment is designated as Hsu I A-A.*

TABLE 1. *Hybridization of early and late [^{3}H]RNA to DNA fragments generated by cleavage of Eco RI-A DNA with endonuclease R-Hsu I*[a]

Hsu I fragment	^{3}H-labeled early RNA hybridization (counts/min)	^{3}H-labeled late RNA hybridization (counts/min)
B	9	287
C plus D	121	451
A-A	9	476
G	352	19
I	2	11
J	14	98

[a] ^{3}H-labeled early RNA synthesized in the presence of cycloheximide and ^{3}H-labeled late RNA were separated into poly(A)-containing and non-poly(A)-containing RNA by chromatography on oligo-dT cellulose. Poly(A)-containing early RNA (25,000 counts/min) and late RNA (12,000 counts/min) were each hybridized simultaneously to six filters each containing the amount of *Hsu* I fragment DNA derived from 0.57 μg of *Eco* RI-A DNA. The genome location of the *Hsu* I fragments is shown in Fig. 1. *Hsu* I fragments C and D were not separated in the preparation used for this experiment. The hybridizations were performed in 200 μl of 6× Sal/Cit containing 0.1% NaDodSO$_4$.

detected to *Hsu* I fragments B, I, J, D, and A-A.

Identification of an early RNA species from Eco RI-A which is transcribed at late times. Among the cytoplasmic viral RNAs synthesized at late times after infection are 11*S* to 13*S* molecules which include some sequences transcribed at early times (24). This 11*S* to 13*S* size class includes at least one RNA species transcribed from RI-A (25). The possible relationship between this RNA species and the 13*S* RNAs transcribed from RI-A at early times was analyzed by a hybridization-inhibition experiment. Poly(A)-containing ^{3}H-labeled late cytoplasmic RNA was fractionated by polyacrylamide gel electrophoresis. The profile of total radioactivity is plotted in the upper panel of Fig. 3. At 18 h after infection 80% or more of the newly synthesized poly(A)-containing cytoplasmic RNA is virus specific (2, 10, 24); therefore the major viral RNA size classes (27*S*, 24*S*, and 19*S*) synthesized at 18 h can be distinguished even in the unhybridized size fractionated RNA (2, 10, 14, 18).

The hybridization-inhibition experiment was performed with the RNA migrating faster than 18*S* molecules. The RNA in individual fractions of the gel was divided into three aliquots. Each aliquot was then tested for annealing to a filter containing *Eco* RI-A DNA which had been preincubated with one of the following unlabeled RNA preparations: RNA from uninfected KB cells, RNA purified from cells harvested at 6 h after infection, or RNA purified from cells harvested at 18 h after infection (Fig. 3b). The hybrids formed with the filters preincubated with control RNA from uninfected cells revealed the 13*S* RNA transcribed from RI-A. Homologous late RNA inhibited hybridization of all size classes of RNA more than 90%. Early RNA inhibited the hybridization of 13*S* RNA but apparently did not inhibit the hybridization of larger RNA molecules transcribed from the A fragment. Thus the 13*S* RNA transcribed from RI-A at late times contains early sequences.

Hybridization of the late Eco RI-A 13S RNA to Hsu I DNA fragments. Since the 13*S* RNA transcribed from RI-A at 18 h is also present in the cytoplasm of cells at 6 h after infection (Fig. 3) and all early cytoplasmic RNA transcribed from RI-A DNA anneals to *Hsu* I-C and G fragments (Fig. 2), this 13*S* RNA must be transcribed from either *Hsu* I-C or G. To determine which portion of RI-A codes for the late 13*S* RNA, late RNA was fractionated by size and the 10*S* to 18*S* molecules were annealed to the *Hsu* I fragments derived from RI-A (Fig. 4). No hybridization to fragment G could be detected; a 13*S* peak hybridized to *Hsu* I fragments C plus D (which were not separated for this experiment). No peak of hybridization to any other fragment was detected.

DISCUSSION

Using the seven DNA fragments generated by digestion of adenovirus 2 *Eco* RI-A with endonuclease R·*Hsu* I, the early cytoplasmic RNA

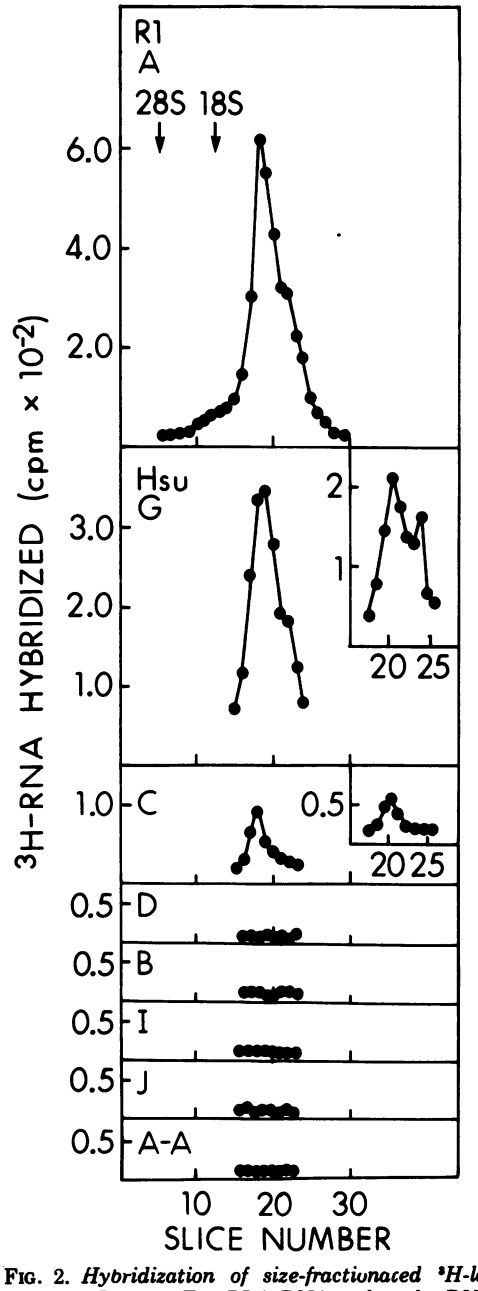

FIG. 2. *Hybridization of size-fractionated ^{3}H-labeled early RNA to Eco RI-A DNA and to the DNA fragments resulting from cleavage of Eco RI-A DNA with endonuclease R·Hsu I. Early RNA was labeled with [^{3}H]uridine from 2 to 6 h after an infection performed in the presence of cycloheximide. Poly(A)-containing RNA (4 × 10^5 counts/min) was layered on each of two 3.2% polyacrylamide gels; electrophoresis was performed at 5 mA/tube for 4 h. Gel slices (2 mm) were dissolved by incubation in 6× Sal/Cit containing 0.1% NaDodSO$_4$ for 16 h at 60 C. The RNA from one gel was hybridized to the amount of Eco RI-A DNA derived from 0.5 μg of adenovirus 2 DNA (top panel). The RNA in slices 15 to 24 of the parallel gel, those slices containing RNA transcribed from Eco RI-A DNA, were hybridized to the seven Hsu I fragments derived from Eco RI-A DNA. The results of the hybridizations are presented in the linear order of the fragments, G, C, B, I, J, D, and A-A. These hybridizations were performed simultaneously with seven filters, each containing the amount of DNA derived from 0.28 μg of Eco RI-A DNA. ^{14}C-labeled rRNA was applied to a third gel; the arrows indicate the positions of 28S and 18S rRNA. The insets in the panels for Hsu I-G and C show the result of another experiment in which the electrophoresis was for 5.5 h and the RNA was then annealed to Hsu I-G and C DNA.*

transcribed from *Eco* RI-A was shown to originate from the extreme left-hand 17% of the viral genome; ^{3}H-labeled early RNA annealed exclusively to *Hsu* I fragments G and C. This result is in agreement with recent liquid hybridization studies using nonradioactive early cytoplasmic RNA and fragments of adenovirus 2 DNA derived by cleavage with endonuclease R·*Eco* RI (5, 17, 21) and endonuclease R·*Hpa* I (17, 21).

Early RNA migrating as 13*S* in polyacrylamide gel electrophoresis annealed to both *Hsu* I-G and C DNA (Fig. 2). From the data of two experiments, we infer that a 13*S* RNA transcribed at 18 h is the same as the early *Hsu* I-C message. (i) Hybridization-inhibition experiments demonstrated that the 13*S* RNA transcribed from RI-A at late times was composed in large part if not entirely of early sequences (Fig. 3); and (ii) this late 13*S* RNA annealed only to membranes containing the combined *Hsu* I-C and D fragment DNAs, not to those containing *Hsu* I fragments G, B, I, J, and A-A (Fig. 4). Since there is no early viral RNA species transcribed from *Hsu* I-D (Fig. 2), this 13*S* RNA transcribed at 18 h must be the early species from *Hsu* I-C. This result demonstrates that the 13*S* RNAs transcribed from *Hsu* I-G and C must be different species, not a single molecule which overlaps the cleavage site between *Hsu* I-G and C, for the *Hsu* I-C 13*S* RNA is transcribed at late times, whereas 13*S* transcripts of *Hsu* I-G were not detected at 18 h (Fig. 4). Recent experiments with adenovirus 2 fragments generated by digestion with endonuclease R·*Sma* I have confirmed that the 13*S* RNA synthesized at late times is transcribed from *Hsu* I-C.

From the data presented here and those previously reported by Tal et al. (25), we conclude that there are seven and possibly eight early viral mRNAs. We had previously reported the detection of 13*S* and 11*S* RNAs transcribed from *Eco* RI-A, 19*S* and 11*S* RNAs from *Eco*

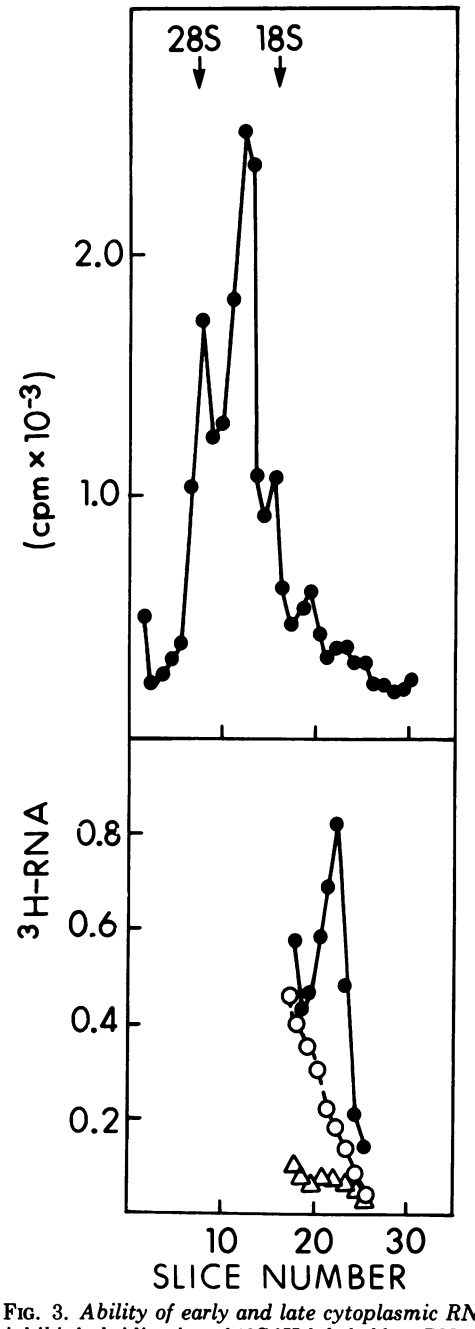

FIG. 3. *Ability of early and late cytoplasmic RNA to inhibit hybridization of 13S ^{3}H-labeled late RNA to Eco RI-A DNA. Poly(A)-containing RNA (5 × 10^6 counts/min) purified from cells labeled 18 to 21 h after infection was applied to a 3.2% acrylamide gel. Electrophoresis was performed at 5 mA/tube for 5 h. The gels were cut into 2-mm slices, and the slices were solubilized in 200 μl of 6× Sal/Cit containing 0.1% NaDodSO$_4$; 5-μl aliquots were removed and counted (upper panel). The 9S to 17S region of the gel (slices 18 to 26) was used for the hybridization-inhibition experiments. These fractions were divided into three aliquots. Each aliquot was hybridized simultaneously to three filters preincubated with a different RNA preparation. One set of filters (●) was preincubated with 3 mg of RNA per ml purified from uninfected KB cells; a second set was incubated with 2 mg of cytoplasmic RNA per ml harvested from cells 20 h after infection (△); a third set of filters was incubated with 3 mg of cytoplasmic RNA per ml purified from cells harvested at 6 h after infection (○). ^{14}C-labeled rRNA was applied to a second gel; the arrows indicate the positions of 23S and 18S rRNA.*

RI-B, 20*S* and 13*S* RNAs from *Eco* RI-D, 13*S* RNA from *Eco* RI-E, and heterogenous transcripts from *Eco* RI-C (25). Several observations suggest that the 13*S* RNA from RI-D and RI-E represents a single species which overlaps the *Eco* RI cleavage site between RI-E and D (25). From the studies presented here it is now clear that the 11*S* to 13*S* RNA transcribed from *Eco* RI-A is not related to the 11*S* RNA transcribed from *Eco* RI-B, for the early RNAs transcribed from *Eco* RI-A are derived from the portion of *Eco* RI-A which is farthest from the *Eco* RI-B segment. The early RNAs transcribed from *Eco* RI-A include two species we have designated 13*S*, one from *Hsu* I-G and one from *Hsu* I-C, and possibly an 11*S* RNA also from *Hsu* I-G. All preparations show either a shoulder or a distinct peak of 11*S* RNA that anneals to *Hsu* I-G DNA; experiments in progress with other sets of DNA fragments should determine if this 11*S* RNA is unique compared to the others. In comparison, genetic studies with adenovirus 5 temperature-sensitive mutants have identified only two complementation groups whose defects can be classified as early functions (6, 31).

At early times, all cytoplasmic transcripts from RI-A are from the l strand of the genome (17, 21). Liquid hybridization studies (17, 21) have shown that the early *Eco* RI-A transcripts are derived exclusively from two *Hpa* I DNA fragments, E and C, which encompass the left-hand 24% of adenovirus 2 DNA. The early cytoplasmic RNA contains transcripts of 42% of *Hpa* I-E and 30% of *Hpa* I-C (17, 21). Since the molecular weight of *Hpa* I-E DNA is 0.9 × 10^6 and that of *Hpa* I-C is 4.6 × 10^6 (12), the molecular weight of the cytoplasmic transcripts from these segments would be 180,000 from *Hpa* I-E and 700,000 from *Hpa* I-C. Because of the considerable uncertainty in the precise sedimentation values and hence the corresponding molecular weights assigned to the early RNAs transcribed from *Eco* RI-A, the total transcripts from *Hpa* I-E and *Hpa* I-C (880,000) might be able to accommodate the two RNAs we have designated 13*S* (380,000 each) and one 11*S* RNA

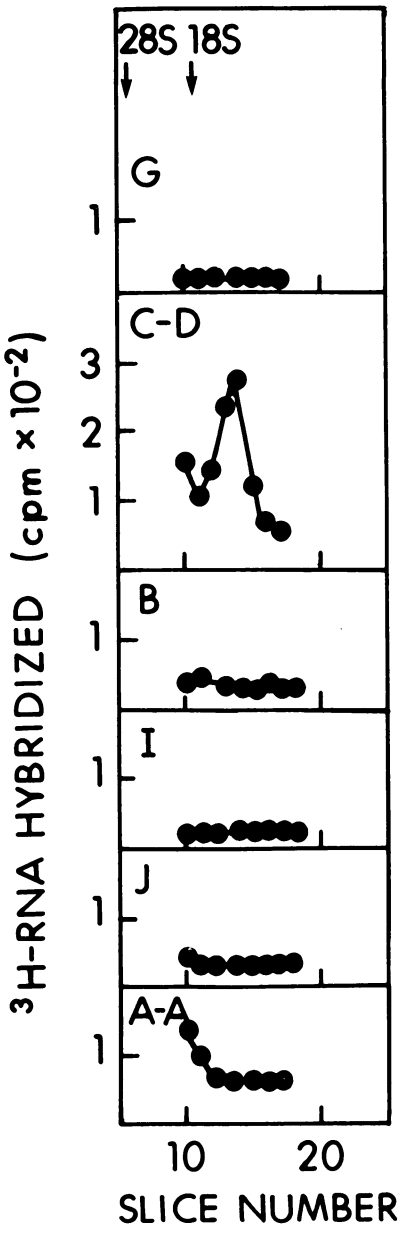

FIG. 4. *Hybridization of size-fractionated ^{3}H-labeled RNA to the Hsu I fragments resulting from cleavage of RI-A DNA. Poly(A)-containing ^{3}H-labeled late RNA (4 × 10^6 counts/min) was fractionated on 3.2% polyacrylamide gels. Electrophoresis was carried out for 3 h at 5 mA/tube. Gel slices were dissolved by incubation for 16 h at 60 C in 6× Sal/Cit containing 0.1% NaDodSO$_4$. The RNA from gel slices in the 10S to 18S region (slices 10 to 19) was hybridized simultaneously to six filters. Each filter contained one (or two) of the fragments obtained by digestion of 0.28 μg of RI-A by endonuclease R-Hsu I. ^{14}C-labeled rRNA was applied to a third gel; the arrows indicate the positions of 28S and 18S rRNA.*

of 300,000. Figure 5 shows two possible maps assigning the early viral RNA species transcribed from *Eco* RI-A to regions of the genome defined by the *Hpa* I and *Hsu* I cleavage sites. Since the 3′ end of the l strand is at the *Hsu* I-G end of the molecule (21), the early mRNA's transcribed from RI-A must have the polarity shown in Fig. 5. In addition to the 13*S* RNAs transcribed from *Hsu* I-G and *Hsu* I-C DNAs, the 11*S* RNA which may be transcribed from *Hsu* I-G DNA is also shown.

The early cytoplasmic RNA is composed of two sequence classes: class I, which is present in greatly diminished concentrations at late times (18 h), and class II, which remains in high concentrations at 18 h (4, 11). Hybridization-inhibition studies with total ^{3}H-labeled early cytoplasmic RNA and RI-A DNA have shown that the early transcripts from RI-A contain both class I and class II sequences (5). Since large amounts of 13*S* RNA are transcribed from *Hsu* I-C at late times, and this RNA is composed largely if not entirely of early sequences, this 13*S* species is likely to be a class II molecule. Thus the left-hand 17% of the l strand appears to code for both class I and class II species.

The extreme left-hand end of the viral DNA is of particular interest, for this region of the genome has been found in all adenovirus 2-transformed rat cells examined to date (8, 22). Although additional portions of the genome are present in some transformants, all lines examined contain at least 14% of the left-hand end of the genome (8), corresponding to all of *Hpa* I-E and approximately half of *Hpa* I-C (Fig. 5). In addition Graham et al. (8a) have shown that *Hsu* I-G DNA alone can transform rat embryo cells and that efficiency of transformation with this unique DNA fragment approaches the efficiency of transformation with total viral DNA.

Sharp et al. (21) have reported that the cytoplasmic RNA of transformed cell lines containing 14% of the genome is complementary to 30% of *Hpa* I-C, as in early lytic infection, but only 30% of *Hpa* I-E as compared to 42% in early lytic infection (21); all these sequences are found in the cytoplasm of productively infected cells at early times (7, 21). Thus, nearly all the sequences in the 11*S* to 13*S* mRNA's transcribed from *Hsu* I, G, and C are also transcribed in these transformed cells. Although the translation products of the 11*S* to 13*S* RNAs synthesized in productive infection have not been identified, they could code for peptides having a molecular weight of 34,000 to 40,000.

The size of cytoplasmic viral RNA in trans-

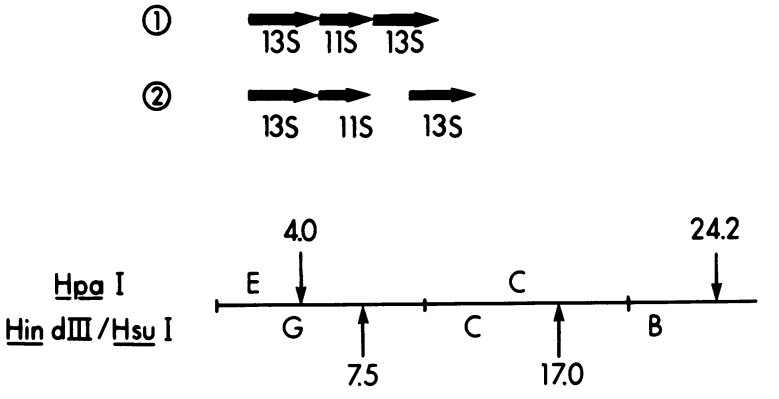

FIG. 5. *Possible genome locations of early viral RNA species transcribed from Hsu I-G and C DNA. The data presented in this report and the liquid hybridization studies with Hpa I fragments E and C (17, 21) were used to assign early viral RNA species to segments of Eco RI-A defined by the Hsu I and Hpa I cleavage sites. The drawing represents the extreme left-hand end of the genome; the space between each pair of small vertical lines represents 10% of the genome. The Hpa I cleavage sites have been identified by Mulder et al. (12), and the Hsu I fragments are shown in Fig. 1. The cleavage sites for endonuclease R·Hpa I are shown above the horizontal line, and the sites for endonuclease R·Hsu I are given below the line. The length of the RNAs is proportional to their molecular weights as calculated from electrophoretic mobility. Although the existence of a unique 11S RNA from Hsu I-G is not certain, such a species is shown because of observations presented in this report (Fig. 2). Within Hsu I-G DNA the relative order of 13S and 11S RNAs is not known. The 13S RNA transcribed from Hsu I-C could be located anywhere within the portion of Hpa I-C that extends from 7.5 to 17.0% of the genome (models 1 and 2). The early RNAs from RI-A are transcribed from the l stand of the genome and therefore (21) have the polarity shown here, with the head of the arrow representing the 3' end of the RNA molecule.*

formed cells has been examined in only one cell line, 8617 (15, 30). In this particular line the majority of viral RNA sedimented as 20*S*, molecules twice as large as the early 13*S* to 11*S* molecules transcribed from the left-hand end of the genome. Since 8617 contains 50% of the viral genome (22), considerably more viral DNA sequences than many transformants (8), the predominant viral RNA in this line may not represent transcripts of the left-hand end of the genome. Alternatively, the viral mRNA's synthesized in transformed cells may differ from their counterparts in lytically infected cells. For example, two viral mRNA's may be fused together or cell sequences may be covalently linked to viral sequences (28, 29).

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