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Two "Early" mRNA Species in Adenovirus Type 2-Transformed Rat Cells

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mRNA isolated from adenovirus 2-infected HeLa cells at early times during the productive cycle and from two lines of adenovirus 2-transformed rat embryo cells (F17 and T2C4) was fractionated on sucrose gradients after disaggregation. Viral mRNA species were identified by hybridization across such gradients with the separated strands of restriction endonuclease fragments of ^{32}P-labeled DNA known to be complementary to adenovirus 2 "early" and adenovirus 2-transformed cell mRNA. mRNA transcribed from the left-hand 14% of the adenovirus 2 genome was found to comprise two species, 16 to 17*S* and 20 to 21*S*: the same sized mRNA's were present both at early times during productive infection and in the two transformed rat cell lines. Direct comparison of the sequences present in these two mRNA species by additional saturation hybridizations suggests that they are not related to one another. Three additional regions of the adenovirus 2 genome, all of which are located in the right-hand 40% of the adenovirus 2 genome, are complementary to early mRNA sequences: each of these appears to specify one major mRNA species of about 22*S*. Thus, five major species of adenovirus type 2 early mRNA have been identified. Two of these, copied from the left-hand 14% of the viral genome, are also present in adenovirus 2-transformed rat cells.

Before replication of viral DNA begins in adenovirus type 2 (Ad2)-infected HeLa cells, expression of viral information is quite restricted; "early" mRNA is transcribed from only 9 to 13% and 14 to 19% of the l and r stands of Ad2 DNA, respectively (20, 25, 33), and constitutes a small fraction of the infected cells' polysomal mRNA (7, 22, 23). The regions of the viral genome from which Ad2 early mRNA is transcribed have been mapped by saturation hybridization of unlabeled mRNA to the separated strands of restriction endonuclease fragments of ^{32}P-labeled Ad2 DNA (9, 25, 26, 33). These mRNA sequences derive from four widely spaced regions, two on each strand of the Ad2 genome. If the Ad2 genome is considered to be 100 units in length with the zero coordinate at the left end of the DNA (Fig. 1), then early mRNA complementary to the r strand occupies 7 units, lying between 3 and 14 map units and also the region of 81 to 85 map units; mRNA from the l strand has been mapped to about 4 units between positions 58.5 and 70.7 and to a second site at 90 to 94 units (33). A similar map has been derived by Pettersson et al. (25), although the regions of the genome assigned to early mRNA appear slightly larger.

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All lines of Ad2-transformed rat embryo cells that have been examined to date contain a single common set of viral DNA sequences integrated into their chromosomes; these comprise the left-hand 12 to 14% of Ad2 DNA (29, 34). About half of this DNA is expressed as mRNA sequences, which are complementary to the r strand and are indistinguishable from the mRNA sequences copied from this region at early times during productive infection (10).

Several laboratories have described 20*S* Ad2 early and Ad2-transformed cell mRNA species (13, 22, 23, 31, 37, 39, 40). Species of 20*S* early mRNA appear to be complementary to both strands of the viral genome (2, 26), and the more precise localization of different early mRNA species to various restriction endonuclease fragments of Ad2 DNA has been reported (3, 6, 35). However, the exact arrangement of Ad2 early mRNA sequences in RNA chains is not clear. Moreover, the state of the viral DNA from which sequences common to all transformed cells and early mRNA's are transcribed is presumably different in the two cases, autonomous in the infected cell contrasted with integrated in the transformed cell. It is therefore of interest to establish whether or not the species of mRNA present in Ad2-transformed cells are the same as those synthesized early in productive infection.

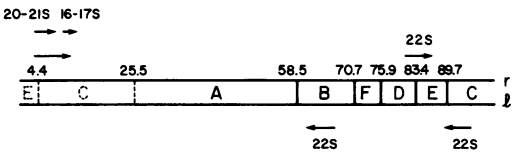

FIG. 1. *Map of Ad2 early mRNA. Ad2 genome is represented by the two horizontal lines. Solid and dashed vertical bars and letters show cleavage sites and fragments generated by EcoRI and HpaI, respectively. Arrows immediately above and below the genome indicate the simplest arrangement of RNA sequences, from which early mRNA is transcribed, consistent with the results of saturation hybridization experiments (9, 10). At the left-hand end, the arrangement of these sequences to form two species, suggested by the studies described here, is also shown. The size of each early Ad2 mRNA species measured here is shown above or below the appropriate arrow.*

This communication describes the fractionation of both unlabeled Ad2 early and Ad2-transformed cell polyadenylic acid [poly(A)]-containing cytoplasmic or polysomal RNA and identification of the species transcribed from different regions of the viral genome by hybridization with the separate strands of restriction endonuclease fragments of ^{32}P-labeled Ad2 DNA. The relationship between two species of mRNA that are complementary to the r strand at the left-hand end of the genome has been explored by saturation hybridization of fractionated mRNA to ^{32}P-labeled r strand DNA derived from the left-hand 24% of Ad2 DNA. Two rather different lines of Ad2-transformed rat embryo cells were chosen for this study: F17, which contains and expresses as little Ad2 information as any characterized cell line, and T2C4, which contains Ad2 DNA sequences from many regions of the viral genome and synthesizes mRNA complementary to all regions of the Ad2 genome from which early mRNA sequences are transcribed (10, 29, 33).

MATERIALS AND METHODS

Cells and virus. The maintenance of HeLa cells in suspension culture and the propagation of Ad2 in them has been described, as has growth of the transformed rat embryo cells F17 and T2C4 (10). All virus stocks were titered on HeLa cell monolayers as described by Williams (43). ^{32}P-labeled virus was prepared as reported previously (10).

Preparation of restriction fragments of Ad2 DNA and separation of their strands. Ad2 DNA was extracted from purified virions as described by Pettersson and Sambrook (24). Fragments of ^{32}P-labeled Ad2 DNA (specific activity, 0.5×10^6 to 1×10^6 cpm/μg) generated by cleavage with *Eco*RI or *Hpa*I were separated by electrophoresis, and the strands of each fragment were separated by electrophoresis of denatured DNA as described in detail elsewhere (9).

Preparation of RNA. All buffers and utensils for RNA preparation were treated with 0.3% diethylpyrocarbonate at 68°C for several hours. Cytoplasmic and nuclear fractions of HeLa cells infected with 10 to 20 PFU of Ad2 per cell for 8 h in the presence of 20 μg of cytosine arabinoside per ml and of F17 and T2C4 cells were separated by treatment with Nonidet P-40 (19). In some experiments, polyribosomes were pelleted from the cytoplasmic fraction by centrifugation through 1.0 M sucrose containing 0.01 M Tris-hydrochloride, pH 7.9, and 1 mM $MgCl_2$ at 23,000 rpm for 6 h in a Spinco SW27 rotor. Further purification of mRNA is described in the legend to Fig. 2.

Sucrose gradient sedimentation. Purified early and transformed cell poly(A)-containing cytoplasmic RNA was heated at 65°C in 95% dimethyl sulfoxide for 5 min and precipitated in 2 volumes of ethanol in 0.10 M sodium acetate at −20°C. Samples of such disaggregated RNA were fractionated in 15 to 30% sucrose gradients containing, in 0.01 M Tris-hydrochloride (pH 7.4): 0.10 M LiCl, 1 mM EDTA, and 0.5% sodium dodecyl sulfate (SDS) previously treated with 0.3% diethylpyrocarbonate. Samples of disaggregated RNA mixed with ^{14}C- or ^{3}H-labeled simian virus 40 (SV40) form I DNA were loaded in volumes of 0.1 ml or less. Centrifugation, in a Spinco SW41 rotor, was at 39,500 rpm for 6.5 h at 25°C. Fractions of 0.15 ml were collected from the bottom of each tube, and 20-μl portions were counted directly in 3 ml of Aquasol II (New England Nuclear Corp.) to locate marker SV40 form I DNA or labeled RNA species. Hybridization of fraction portions to separated strands of restriction endonuclease fragments of ^{32}P-labeled Ad2 DNA is described in the legend to Fig. 2.

RESULTS

Number of Ad2 early mRNA species. Disaggregated, poly(A)-containing cytoplasmic RNA isolated from HeLa cells infected with Ad2 in the presence of cytosine arabinoside to inhibit viral DNA replication was fractionated on 15 to 30% sucrose gradients to separate mRNA's of different size. RNA complementary to different regions of the Ad2 genome was located in the gradient by hybridizing portions of fractions of such gradients to the separated strands of restriction endonuclease fragments of ^{32}P-labeled DNA under conditions in which the extent of hybridization reflected the amount of RNA added. Six probes were used: the r strands of *Hpa*I fragments C and E and *Eco*RI fragments D and E, and the l strands of *Eco*RI fragments B and C. Between them, these contain all the Ad2 early mRNA coding sequences (Fig. 1). In each case, except with the r strand of *Hpa*I fragment C, one discrete peak of RNA hybridized to the probe, and this sedimented slightly faster than SV40 form I DNA (Fig. 2). No hybridization above background (5% of input) was seen to DNA probes not complementary to Ad2 early mRNA, such as the l strand of *Eco*RI

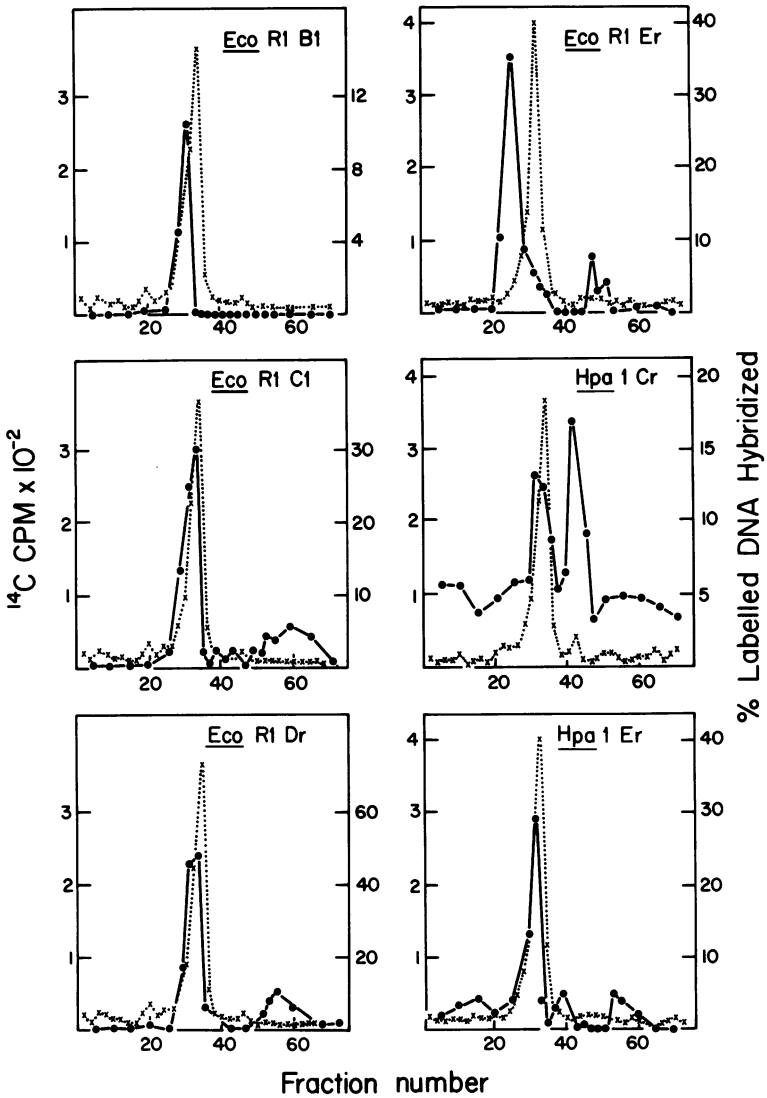

FIG. 2. *Ad2 early mRNA species. Poly(A)-containing cytoplasmic RNA was isolated from HeLa cells infected at a multiplicity of 10 to 20 PFU/cell with Ad2 and maintained at 37°C for 8 h in the presence of 20 μg of cytosine arabinoside per ml, as described in Materials and Methods. After oligodeoxythymidylic acid-cellulose chromatography, poly(A)-containing fractions (0.5 to 2.0 mg) were precipitated with 2 volumes of ethanol in 0.1 M sodium acetate at −20°C overnight. The precipitate was collected, dissolved in 0.01 M Tris-hydrochloride, pH 7.4, containing 0.1 M NaCl, 1 mM EDTA, 0.5% SDS, 0.3% diethylpyrocarbonate, and 95% dimethyl sulfoxide, and heated at 68°C for 5 min. After reprecipitation, the RNA was dissolved in small volumes, 0.1 to 0.5 ml, of the buffer described above without dimethyl sulfoxide. Samples, 0.1 ml, of early RNA prepared in this way were mixed with ^{14}C- or ^{3}H-labeled SV40 form I DNA and applied to 11-ml, 15 to 30% sucrose gradients in 0.01 M Tris-hydrochloride (pH 7.4), 1 mM EDTA, 0.10 M LiCl, 0.5% SDS, and 0.3% diethylpyrocarbonate. Centrifugation was at 39,500 rpm at 25°C in a Beckman SW41 rotor for 6.5 h, and 0.15-ml fractions were then collected from the bottom of each tube. Portions (20 μl) of every other fraction were counted in 3 ml of Aquasol II to determine the position of the internal SV40 form I DNA (●●●). Portions (50 μl) of the fractions shown were also hybridized with ^{32}P-labeled, separated strands of DNA of restriction endonuclease fragments of Ad2 DNA known to be complementary to Ad2 early mRNA (10). Such hybridizations were performed in a final volume of 0.10 to 0.15 ml and contained, besides fractionated RNA, 0.5×10^{-3} to 1×10^{-3} μg of ^{32}P-labeled, single-stranded DNA (specific activity, 0.5×10^{6} to 1×10^{6} cpm/μg), 0.10 M Tris-hydrochloride, pH 7.4, 1.0 M NaCl, and 0.5% SDS. Incubation was at 68°C for 24 to 36 h, and the extent of hybrid formation was analyzed using S_1 nuclease as described previously (11). In calculating the extent of hybridization, correction has been made, where applicable, for spillover of ^{14}C counts into the ^{32}P-channel during counting. The fraction of probe resistant to S_1 nuclease after incubation alone, i.e., background, which ranged from 2 to 6%, has not been substracted. (●—●) Hybridization to each of the six probes complementary to "early" mRNA used here.*

fragment F (data not shown). Thus, the observed hybridization cannot be the result of contaminating viral DNA, but rather represents Ad2 early mRNA species. In some experiments shown in Fig. 2, those with the l strand of *Eco*RI fragment C and the r strand of *Eco*RI fragments D and E, for example, some heterogeneous material near the top of the gradient also appeared to hybridize to a small extent with the ^{32}P-labeled probe DNA. However, this phenomenon was not reproducible with different preparations of early mRNA.

When the r strand of *Hpa*I fragment C was used to assay across the gradient, two discrete peaks of RNA were observed: the larger sedimented just ahead of SV40 form I DNA in a manner very similar to that observed for early mRNA complementary to the r strand of adjacent *Hpa*I fragment E (Fig. 2). The r strand of *Hpa*I fragment E can encode only about 400 nucleotides of early mRNA (10), suggesting that RNA sequences transcribed from the r strands of both *Hpa*I fragments C and E must be present in this 20 to 21*S* species of Ad2 early mRNA. Similar arguments indicate that early mRNA sequences complementary to the r strand of *Eco*RI fragments D and E are also present in the same species.

The pattern of discrete-sized mRNA species shown in Fig. 2 was highly reproducible with different preparations of Ad2 early, poly(A)-containing cytoplasmic RNA, although with some variation in hybridization background dependent on the preparation of ^{32}P-labeled, single-stranded probe DNA; it was also observed when poly(A)-containing, early polyribosomal RNA was analyzed in a similar fashion (data not shown, but included in Table 1). These data demonstrate that Ad2 early mRNA sequences comprise five major species of RNA, which are complementary to discrete regions of the viral genome.

To assign sedimentation coefficients to these Ad2 early mRNA species, the sedimentation of each of the species described above was measured relative to that of SV40 form I DNA. Values from several experiments, with their means, are given in columns 2 and 3, respectively, of Table 1. The sedimentation of several standard RNA species, including 35*S* poliovirus RNA (36), 30*S*, 15*S*, and 12 to 13*S* vesicular stomatitis virus RNA (18), and 28*S* and 18*S* rRNA, relative to that of SV40 form I DNA under these conditions was also determined. The sedimentation coefficients for Ad2 early mRNA shown in column 4 of Table 1 were assigned by comparison with these standard RNA species. The Ad2 early mRNA species observed range in size from 22*S*, early mRNA complementary to the l strand of *Eco*II fragments B and C and the r strand of *Eco*RI fragments D and E, to 16 to 17*S*, the smaller species transcribed from the r strand of *Hpa*I fragment C.

TABLE 1. *Ad2 early and Ad2-transformed cell mRNA species*[a]

Probe	Relative sedimentation	Average relative sedimentation	Sedimentation coefficient (*S*)
Early mRNA			
*Hpa*I Cr I	0.95, 0.97, 0.97	0.96 ± 0.01	21.4
*Hpa*I Cr II	0.85, 0.76, 0.83	0.81 ± 0.05	16.8
*Hpa*I Er	1.05, 1.03	1.04 ± 0.01	22.0
*Eco*RI Bl	1.08, 1.03	1.06 ± 0.03	22.4
*Eco*RI Cl	1.03, 1.03	1.03	21.8
*Eco*RI Dr	1.05, 1.00	1.03 ± 0.03	21.8
*Eco*RI Er	1.03, 1.03	1.03	21.8
F17 mRNA			
*Hpa*I Cr I	0.92, 0.97, 0.83	0.91 ± 0.07	20.0
*Hpa*I Cr II	0.87, 0.76, 0.66	0.76 ± 0.15	16.3
*Hpa*I Er	1.03, 1.03	1.03	21.8
T2C4 mRNA			
*Hpa*I Cr I	0.87, 0.97	0.92 ± 0.07	20.4
*Hpa*I Cr II	0.71, 0.78	0.75 ± 0.04	16.0

[a] Unlabeled Ad2 early or Ad2-transformed cell mRNA was fractionated as described in the legends to Fig. 2 and 3. Species complementary to the probes listed in column 1 were assayed as described in the legend to Fig. 2. mRNA species I and II complementary to the r stand of *Hpa*I fragment C are defined in the legend to Fig. 4. The sedimentation relative to an internal marker, ^{3}H- or ^{14}C-labeled SV40 form I DNA, was measured for each peak of RNA observed. These values and their means are shown in columns 2 and 3, respectively. The sedimentation coefficient (column 4) of each species was determined from the sedimentation of a series of standard RNA species, as described in the text.

It is also possible to estimate the molecular weights of Ad2 early mRNA species from a plot of molecular weight logarithm against relative mobility constructed with the non-rRNA species listed above, whose molecular weights have been determined by electron microscopy or chemical methods as well as by sedimentation and electrophoretic analyses (14, 27, 32, 36). Molecular weights of 7.8×10^5, 7.2×10^5, and 5.1×10^5 were determined in this way for the adenovirus 22*S*, 20 to 21*S*, and 16 to 17*S* species of mRNA, respectively. The 28*S* and 18*S* rRNA species were not included in this molecular weight analysis, as their sedimentation appeared anomalous relative to the non-rRNA species; this presumably reflects the high degree of rRNA secondary structure (42). The limitations inherent in these size estimates are discussed below in relation to the results of saturation hybridization experiments.

Ad2-transformed cell mRNA species tran-

scribed from the left-hand 14% of the genome. Poly(A)-containing cytoplasmic RNA of two Ad2-transformed cell lines, F17 and T2C4, was fractionated on 15 to 50% sucrose gradients, and RNA species complementary to the r strands of *Hpa*I fragments C and E were identified by hybridization as described above. No other viral mRNA sequences are synthesized in F17 cells (10). T2C4 cells express mRNA complementary to other early regions of the Ad2 genome, but they are present at significantly lower concentrations than those from the left end of the genome (11) and are, consequently, more difficult to study by these techniques. Nevertheless, a discrete species of viral RNA complementary to the l strand of *Eco*RI fragment B could be detected (data not shown). However, it is the mRNA from the left-hand "transforming" region that is of greatest interest. The mRNA species synthesized in both F17 and T2C4 cells complementary to the r strand of *Hpa*I fragment C appear very similar, both to one another and to the corresponding Ad2 early mRNA species (Fig. 3). F17 mRNA sedimenting at 20 to 21*S* (Table 1) also hybridized to the r strand of *Hpa*I fragment E and must, therefore, contain RNA sequences complementary to the r strand of *Hpa*I fragments C and E. Presumably, mRNA sequences transcribed from the r strand of *Hpa*I fragment E are similarly arranged in T2C4 cells. The smaller mRNA species arising from the r strand of *Hpa*I fragment C in both F17 and T2C4 cells has a sedimentation coefficient of about 16*S* (Table 1). Thus, these transformed cell mRNA species complementary to the r strand at the left end of the Ad2 genome are identical, by the criteria of size and genome location, to those synthesized during the early phase of productive infection. F17 cells contain viral DNA sequences that are homologous to the region of Ad2 DNA encompassed by 0 to 14 map units (29). Since both the 20 to 21*S* and 16 to 17*S* mRNA species are present in F17 cells, they can be mapped to the left of position 14 on the Ad2 genome.

Saturation hybridization experiments with fractionated Ad2 early and Ad2-transformed cell mRNA. The five Ad2 early mRNA species described here appear to be of sufficient size to account for the early coding capacity of the four regions of the Ad2 genome depicted in Fig. 1. This has been confirmed by saturation hybridization experiments between early RNA species recovered from appropriate regions of sucrose gradients and separated strands of restriction endonuclease fragments of ^{32}P-labeled Ad2 DNA. As shown in Fig. 4, 20 to 24*S* Ad2 early mRNA saturated about 30% of the r strand of *Hpa*I fragment E and the l strands of *Eco*RI B, 40% of the r strand of *Eco*RI fragment D and probably of *Eco*RI fragment E, and about 45% of the l strand of *Eco*RI fragment C. These values are very close to, and in most cases identical with, those previously observed with unfractionated, early cytoplasmic RNA (11, 33).

The relationship between the two species of mRNA complementary to the r strand of *Hpa*I fragment C both at early times during productive infection and in transformed cells has also been investigated by saturation hybridization. Both 20 to 24*S* and 15 to 17.5S early mRNA fractions saturate 10 to 12% of the r strand of *Hpa*I fragment C (Fig. 4D); however, about 25% of this probe enters hybrid when increasing amounts of 20 to 24*S* mRNA are hybridized in the presence of a saturating amount of 15 to 17*S* mRNA. Similar results have been observed with the corresponding species of F17 mRNA (Fig. 4B) and T2C4 mRNA (data not shown) and recently with the r strand of *Sma* fragment

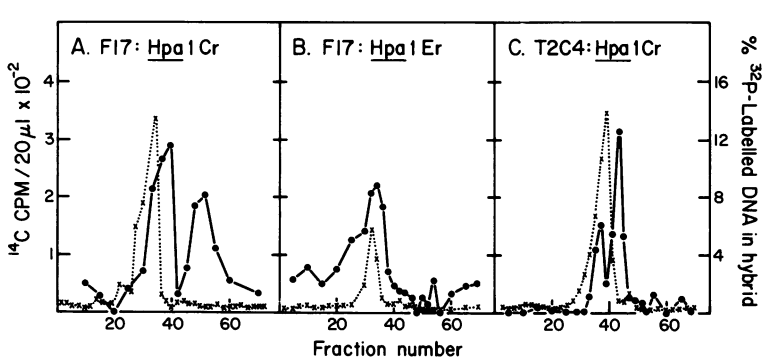

FIG. 3. *Some mRNA species present in two lines of Ad2-transformed rat embryo cells. mRNA species transcribed from the left-hand end of the Ad2 genome in two lines of Ad2-transformed cells, F17 clone 8 and T2C4, were assayed as described for early mRNA in the legend to Fig. 2. Symbols: (●●●) position of internal SV40 form I DNA, and (●—●) hybridization to the single-stranded probes shown.*

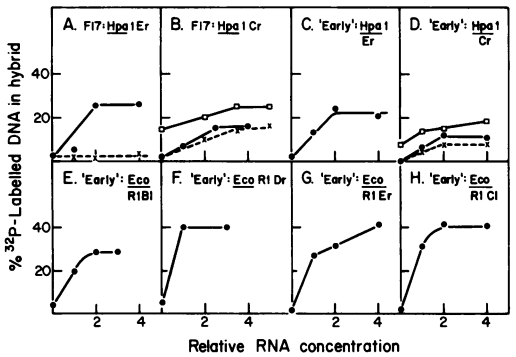

FIG. 4. *Saturation hybridization between fractionated Ad2 early and Ad2-transformed cell RNA and the separated strands of restriction endonuclease fragments of ^{32}P-labeled Ad2 DNA. Early and transformed cell mRNA fractions containing viral sequences were pooled on the basis of hybridization to the r strand of HpaI fragment C, to give two fractions, I and II, which contained RNA sedimenting at 20 to 24S and 15 to 17.5S, respectively. In these experiments, a ^{3}H-labeled SV40 form I DNA internal marker was used. Pool I and II RNA was precipitated with 2 volumes of ethanol overnight at −20°C in the presence of 50 μg of yeast tRNA. The precipitates were dissolved in 0.5 ml of 0.01 M Tris-hydrochloride, pH 7.4, containing 0.15 M NaCl and 1 mM EDTA. Increasing amounts of pool I and pool II RNA were then hybridized with the separated strands of restriction endonuclease fragments of ^{32}P-labeled Ad2 DNA shown in the figure; reactions were performed in 0.10 to 0.15 ml at 68°C for 24 to 36 h and contained pool I or pool II RNA; about 300 cpm of ^{32}P-labeled, single-stranded viral DNA; and 0.10 M phosphate buffer, pH 6.8, containing 1.0 M NaCl and 0.5% SDS. Reactions were analyzed by chromatography on hydroxylapatite. Symbols: (×) and (●) annealing of pool II and pool I early or transformed cell mRNA, respectively, to the probe shown; (□) hybridization of 4 units of pool II RNA in the presence of the increasing concentrations of pool I RNA shown on the abscissa; (□), on the ordinate, hybridization of pool II RNA in the absence of added pool I RNA.*

E as probe (T. Harrison, S. J. Flint, and P. A. Sharp, manuscript in preparation). Although this method cannot eliminate the possibility that some sequence relationship exists between the larger and smaller early and transformed cell mRNA transcribed from *Hpa*I fragment C (the variation observed between experiments is about 5 to 10% of the fraction of the probe saturated), it does establish that the smaller-sized mRNA species does not share the majority of its sequences with the larger.

These data, then, favor the interpretation depicted in Fig. 1: the cytoplasm of Ad2-infected HeLa cells at early times after Ad2 infection and of Ad2-transformed rat embryo cells contains two species of mRNA arising from the left-hand 14% of the viral genome. The larger, 20 to 21*S*, is complementary to both *Hpa*I fragments E and C and can, therefore, be located between 2.5 to 3 and 8 units. The smaller, about 3 to 4 units long, is distinct from the larger and would, therefore, map between 8 and 14 units, but it cannot be placed more precisely within this region on the basis of available data.

DISCUSSION

This report describes the size distribution and genome localization of viral mRNA species synthesized during the early phase of productive Ad2 infection and in two lines of Ad2-transformed rat embryo cells. Ad2 mRNA species were identified by hybridization of disaggregated mRNA, following fractionation on 15 to 30% sucrose gradients to the separated strands of appropriate restriction endonuclease fragments of ^{32}P-labeled DNA, under conditions likely to approach DNA excess (11). By this method, five discrete species of Ad2 early mRNA, two of which are also present in the F17- and T2C4-transformed cell lines, have been identified: their size properties and localization on the Ad2 genome are summarized in Table 1 and Fig. 1, respectively.

In this study, early mRNA was heated in 95% dimethyl sulfoxide before its size was analyzed, a method that has been shown to achieve efficient disaggregation of viral RNA synthesized in the nucleus of cells producing murine leukemia virus and to denature poliovirus RNA (15). Moreover, when this treatment was omitted, early viral RNA was also observed in the region of the gradients to which 28*S* rRNA sedimented (data not shown). It therefore seems unlikely that the mRNA species described in this report represent aggregates of smaller RNA chains, although this procedure probably does not eliminate secondary structure inherent in the single mRNA molecules themselves.

After disaggregation, RNA sequences complementary to each of the early regions of the Ad2 genome, except that ubiquitously expressed in adenovirus-transformed rodent cells, sedimented at about 22*S* (Fig. 2 and Table 1). Estimates of the size of these three mRNA species calculated from the fraction of the Ad2 genome saturated by each of them range from 1,500 to 1,950 bases. This is about 25% lower than the length of 2,300 bases determined from their sedimentation behavior alone. The errors associated with these two different methods of size estimation are: (i) some underestimation in size, calculated from the saturation values,

arising from the fact that the maximum fraction of input probe DNA that can be scored as hybrid is 95% rather than 100%, and (ii) the effect secondary structure of RNA can have on its sedimentation rate. Given these considerations, the lengths of 22S adenovirus early mRNA species calculated from sedimentation and saturation data are in reasonable agreement.

Early mRNA species of similar size, 19 to 20.5S, complementary to *Eco*RI fragments B, D, E, and C have been observed by Tal et al. (35) and Büttner et al. (3), but the former authors also describe an 11S species transcribed from *Eco*RI fragments D and E. Such smaller RNA species were not detected in this study. The reasons for these differences are not obvious, although they might in part reflect the different analytical methods employed in the two studies; the hybridization of unlabeled RNA to labeled DNA in the conditions used here might fail to detect viral RNA present at low concentrations. The results of saturation hybridization experiments, depicted in Fig. 4, indicate that the 22S mRNA species described here are complementary to the total early coding capacity of these regions, i.e., the three right-hand early regions (Fig. 1), as measured by saturation hybridization experiments with unfractionated Ad2 early mRNA (10). Thus, the status of smaller RNA species complementary to these same regions, which could then only be derived from the larger molecules, is not clear: they could, for example, represent the products of either meaningful cleavage(s) of larger mRNA species or merely nonspecific degradation.

Two species of early and transformed cell mRNA, 20 to 21S and 16 to 17S, complementary to the r strand between 0 and 14 units on the Ad2 genome are identified here. Recently, Bachenheimer and Darnell (1) have described two very similar species of viral mRNA, 20S and 15S, complementary to the region of the Ad2 genome between 2.9 and 11.1 map units (*Sma* fragment E) present in the 8617 line (12) of the Ad2-transformed rat cells. Viral mRNA species of 22 to 24S and 14S transcribed from this same region both early during productive infection (3) and in 8617 cells (5) have also been reported. There is, therefore, general agreement among these studies and the results reported here on the size of viral mRNA species complementary to the r strand at the left-hand end of the Ad2 genome.

However, the sequence relationship between the larger and smaller early and transformed cell mRNA species remains controversial. Büttner et al. (3) and Chinnadurai et al. (5) were able to select both species of viral mRNA by hybridization to fragments of Ad2 DNA derived from the left-hand of the viral genome, such as *Hpa*I fragment E, that are far too small to encode the two species. These authors therefore postulate that the smaller mRNA, which in their hands hybridizes to both *Hpa*I fragment E and *Hpa*I fragment C, probably contains two species of mRNA, both of which may be derived from the larger. On the other hand, the results reported here are most consistent with a different interpretation, that depicted in Fig. 1; the observations that the 16 to 17S early and transformed cell mRNA species do not hybridize to *Hpa*I fragment E (Fig. 2–4) and contain a set of sequences complementary to the r strand of *Hpa*I fragment C, distinct from those comprising the 20 to 21S mRNA (Fig. 4B and D), suggest that little or no sequence relationship exists between these mRNA species. Sedimentation analysis indicates that the 20 to 21S and 16 to 17S species comprise 2,000 and 1,400 bases, respectively, whereas the estimates based on saturation hybridization data are 1,500 and 1,110 bases, respectively. Thus *Sma* fragment E (2.9 to 11.1 map units, 2,900 bases), to which both the larger and smaller early and transformed cell mRNA's hybridize (1, 3, 5), appears to contain sufficient information to encode both the 20 to 21S and the 16 to 17S Ad2 mRNA species with little or no sequence overlap. Unfortunately, however, we have no simple explanation for the apparent discrepancy between the data reported here and the previous observations described above (3, 5). In this context, it should be noted that Craig and her colleagues detect 22S early RNA in nuclear preparations (8), but only 11S and 13S mRNA species (6) in cytoplasmic RNA preparations.

Taking the sizes of the 22S, 20 to 21S, and 16 to 17S viral mRNA species, estimated from their sedimentation behavior and from saturation hybridization experiments as upper and lower limits, respectively, of their coding capacities, we estimate that these three species encode 59,000 to 78,000, 50,000 to 65,000, and 35,0000 to 48,000 daltons of protein, respectively. The 22S mRNA species transcribed from the l strand of *Eco*RI fragment B is not too dissimilar in calculated coding capacity from the 72,000-dalton, single-stranded, DNA binding protein species by this region (21, 38). However, all other species appear to have the ability to code for a considerably larger amount of protein than has actually been ascribed to the corresponding regions of the viral genome; early mRNA transcribed from the r strand of *Hpa*I fragments C and E, for example, apparently could specify a total of some 85,000 to

113,000 daltons of protein, but only a 15,000-dalton and a 45,000- to 50,000-dalton polypeptide have been assigned to this region (21). Six infected, cell-specific early polypeptides have been shown to be viral in origin (21), but several others have been described (4, 16, 28). Thus, it is not obvious at the present time whether each of the early mRNA species described here encodes more than one viral polypeptide, or whether much of their information is not expressed. We can, however, anticipate that at least two early viral polypeptides, one or both of which may be necessary and sufficient for initiation and maintenance of the transformed phenotype, are synthesized in all lines of Ad2-transformed rat embryo cells that contain the left-hand 14% of the viral genome.

Finally, it is clear from the observations reported here that the same species of viral mRNA are synthesized both at early times during productive infection and in Ad2-transformed cells. These data, together with the preferential synthesis of early mRNA sequences in transformed cells (10, 15), suggest that it is unnecessary to postulate the existence of different transcriptional modes of adenovirus DNA sequences present in the various environments of productively infected and transformed cells.

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