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Mappings of Adenovirus Type 7 Cytoplasmic RNA Species Synthesized Early in Lytically Infected Cells and Synthesized in Transformed Cells

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Early virus-specific RNA synthesized in KB cells infected with adenovirus type 7 and virus-specific RNA synthesized in rat embryo cells (7IJY1-2) transformed by the adenovirus type 7 *Hin*dIII-I·J fragment (left-hand 8.1% of the viral genome) have been mapped on the viral genome. About 25% of the viral genome, four discrete regions, two on each strand of the viral genome, are expressed as "early" mRNA. Almost similar regions in the left-hand 8.1% of the viral genome are transcribed both in KB cells at early times after infection and in 7IJY1-2 cells.

In human adenovirus subgroup C (adenovirus type 2 [Ad2], Ad5) and subgroup A (Ad12), "early" mRNA is transcribed from four separated regions of the viral genome, two on each strand (2-5, 10, 14, 15). Almost similar DNA sequences constituting the left-hand 12% of the adenovirus genome were expressed as mRNA both in lytically infected early cells and in transformed cells (6, 10, 14). In subgroup B, Tibbetts (17) showed that Ad7 mRNA isolated 16 h after infection was transcribed from nearly corresponding regions to the gene clusters expressed as early and late mRNA in the Ad2-infected cells. However, no mapping experiments have been reported with mRNA species synthesized during the early phase after infection with Ad7 and virus-specific RNA species in Ad7-transformed cells. In this paper, we report transcriptional maps of Ad7 cytoplasmic mRNA isolated from KB cells during the early phase of lytic infection by Ad7 and cytoplasmic RNA isolated from the rat embryo cells transformed by a DNA fragment comprising the left-hand 8.1% of the Ad7 genome.

Grider strain of Ad7 was grown in KB cells, and viral DNA was purified from virions by the method of Green and Pina (7, 8). ^{32}P-labeled Ad7 was prepared from infected cells labeled with 100 μCi/ml as described previously (12). Viral DNA was cleaved by restriction endonuclease, and each DNA fragment was separated by 1.4% agarose gel electrophoresis as described elsewhere (13).

Single-stranded DNA probes are a prerequisite to map viral RNA sequences by hybridization with ^{32}P-labeled DNA representing discrete regions of the viral genome. Gel electrophoresis was used to isolate complementary strands of Ad7 DNA fragments as described by Hayward (9). Each of the single-stranded DNAs was annealed to heavy (H) and light (L) strands of whole Ad7 DNA which were prepared by equilibrium centrifugation in alkaline CsCl as described in the footnote for Table 1. Each fast and slow strand of the nine *Hin*dIII fragments hybridized significantly with only one of the two strands of whole Ad7 DNAs as shown in Table 1.

To determine the polarity of the H and L strands of Ad7 DNA, 3′ termini of intact Ad7 DNA were labeled in vitro with α-[^{32}P]dATP by phage T4 DNA polymerase. Labeled Ad7 DNA was cleaved with *Hin*dIII and subjected to electrophoresis in a 1.4% agarose slab gel to separate the strands. Only the slowly migrating strands of each terminal *Hin*dIII fragment B and I of Ad7 DNA were labeled at 3′ termini (data not shown). From the results in Table 1, 3′ termini of intact Ad7 DNA are present in the H strand of *Hin*dIII fragment I and in the L strand of *Hin*dIII fragment B. Therefore, when the transforming region of Ad7 DNA was positioned to the left, the H strand of Ad7 DNA is transcribed to the right, being designated as the r strand, and its complement is designated as the l strand.

Increasing amounts of early mRNA extracted from the cytoplasm of KB cells infected with Ad7 were annealed to each labeled *Hin*dIII fragment as shown in Fig. 1. Ad7 early mRNA saturates 20% of the r strand and 35% of the l

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TABLE 1. *Hybridization of the complementary strands of HindIII fragments of Ad7 DNA with H and L strands of whole Ad7 DNA*[a]

^{32}P-labeled *Hin*dIII		% ^{32}P-labeled DNA in hybrid		Strand assignment
Fragment	Strand	H	L	
B	Fast	6.3	77.8	r
	Slow	86.6	5.3	l
C	Fast	12.6	63.2	r
	Slow	81.5	20.5	l
D	Fast	64.5	15.5	l
	Slow	8.4	30.2	r
E	Fast	71.0	21.6	l
	Slow	15.0	60.0	r
F	Fast	10.0	63.7	r
	Slow	80.8	14.4	l
G	Fast	8.5	48.5	r
	Slow	80.9	12.2	l
H	Fast	6.2	45.3	r
	Slow	76.3	8.3	l
I	Fast	64.0	7.2	l
	Slow	6.0	58.5	r
J	Fast	74.1	5.4	l
	Slow	8.4	74.3	r

[a] ^{32}P-labeled single strands (10^6 to 2×10^6 cpm/μg) were prepared from in vivo-labeled Ad7 *Hin*dIII fragments by gel electrophoresis as described by Hayward (9) and purified by self-annealing at 68°C for a time equivalent to about 50× $C_0t_{1/2}$ of Ad7 DNA to remove any contamination of complementary strands. The H and L strands of whole Ad7 DNA were prepared as described by Sussenbach et al. (16). Ad7 DNA in 0.1 M Tris-hydrochloride (pH 8.1) containing 5 mM EDTA was alkaline denatured and adjusted to the density of 1.77 g/ml by adding CsCl. The samples were centrifuged in polycarbonate tubes for 72 h in a type 40 spinco rotor at 30,000 rpm at 10°C. The H and L fractions of the single peak observed were collected, ethanol-precipitated, and self-annealed for 12 h at 60°C. Any duplex DNA were removed by chromatography on hydroxyapatite. Hybridizations were carried out as follows. About 400 to 500 cpm (0.4×10^{-4} to 0.6×10^{-4} μg) of purified fast- and slow-strand DNA of the nine *Hin*dIII fragments was added to the reaction mixtures (50 μl) containing 0.2 μg of H or L strands of Ad7 DNA and 0.4 M sodium phosphate buffer (pH 6.8). Hybridization mixtures were incubated at 68°C for 15 h. Hybrid formation was analyzed by batch absorption on hydroxyapatite as described elsewhere (18).

strand of *Hin*dIII fragment B. Ad7 early mRNA saturates 35, 20, 45, and 55% of the r strand of *Hin*dIII fragments E, F, I, and J, respectively. Ad7 early mRNA also saturated 20 and 25% of the l strand of *Hin*dIII fragment C and G, respectively. The r strand of *Hin*dIII fragment H was not saturated at the concentration of 60 μg of early mRNA per ml. Similar results were also observed in two independent experiments using early mRNA and ^{32}P-labeled *Hin*dIII fragment H prepared at different times. Early mRNA complementary to the *Hin*dIII fragment H appears to be present in the cytoplasm at a relatively low concentration. However, we do not

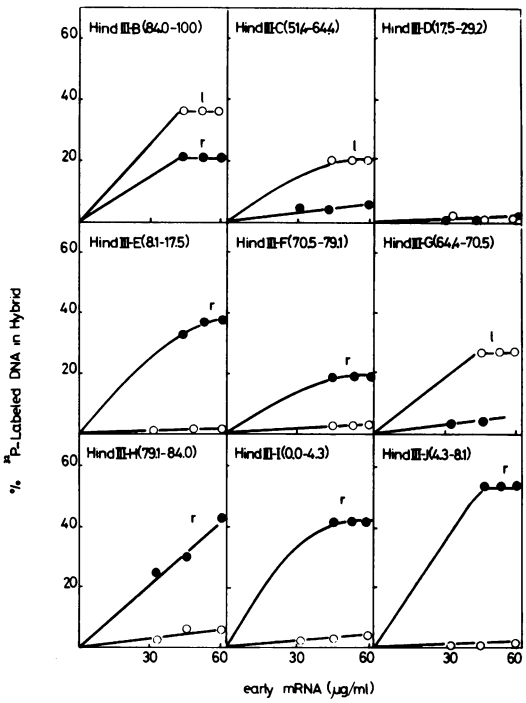

FIG. 1. *Hybridization of ^{32}P-labeled strands of HindIII fragments to early mRNA from KB cells infected by Ad7. Cytoplasmic RNA was extracted from KB cells infected with 20 PFU of Ad7 per cell for 5.5 h at 37°C by the method of Wall et al. (19). Polyadenylic acid-containing RNA was selected by oligodeoxythymidylic acid-cellulose chromatography and subjected to hybridization. ^{32}P-labeled strands of HindIII fragments of Ad7 DNA were prepared as described in the footnote to Table 1. Hybridization was carried out at 68°C for 24 h in 50 μl of 0.1 M sodium phosphate buffer (pH 6.8) containing 1.0 M NaCl, 0.5% sodium dodecyl sulfate, 600 μg of E. coli tRNA, 300 to 400 cpm (4×10^{-4} to 6×10^{-4} μg) of ^{32}P-labeled DNA, and increasing amounts of Ad7 early mRNA. The fraction of DNA entering into hybrid after annealing with the l strand (○) and the r strand (●) was determined by batch absorption on hydroxyapatite. Background of 4 to 6% of the input was subtracted, and maximum hybridization of 80 to 85% in the presence of 100 μg of Ad7 DNA per ml was normalized to 100%. Numbers in parentheses at the top of each panel are map positions of each viral DNA fragment.*

exclude the possibility that only a part of the *Hin*dIII fragment H is transcribed. Because the strands of *Hin*dIII fragment A were not separated by gel electrophoresis, we used nick-translated *Hin*dIII fragment A with a higher specific activity (10^8 to 2×10^8 cpm/μg) as a probe for hybridization. Before hybridization, snap-back sequences were removed by chromatography on hydroxyapatite, corresponding to about 10% of total counts of ^{32}P-labeled *Hin*dIII fragment A. The nick-translated DNA (3.4×10^{-3} ng) prepared as described (Y. Sawada et al., submitted for publication) was annealed to increasing amounts of Ad7 early mRNA as described in the legend of Fig. 1. Only 5% or less entered into hybrid fraction in the presence of early mRNA (data not shown).

We further studied the right- and left-hand end of the viral genome in more detail with the separated strands of *Bam*HI fragments as shown in Fig. 2. Ad7 early mRNA saturates about 70% of the l strand of *Bam*HI fragment E (map position 92.3–100), whereas its r strand forms no hybrid. Ad7 early mRNA saturates 20, 60, and 60% of the r strand of *Bam*HI fragments H (0–2.0), F (2.0–5.3), and G (5.3–8.1), respectively. No hybrid formation with their l strands was detected, as predicted from the results indicated in Fig. 1.

The transformed cell line 7IJY1-2 induced by the Ad7 *Hin*dIII-I·J contains the DNA sequences of the Ad7 *Hin*dIII fragments I and J, comprising the left-hand 8.1% of the Ad7 genome (13). Viral RNA in the cytoplasm of 7IJY1-2 cells derived from this region was studied by the similar hybridization method. RNA extracted from the cytoplasm of 7IJY1-2 cells was annealed to ^{32}P-labeled, separated strands of the Ad7 *Hin*dIII fragments I and J and *Bam*HI fragments F, G, and H, as shown in Fig. 3. Cytoplasmic RNA in 7IJY1-2 cells saturates 55% of the r strands of both Ad7 *Hin*dIII fragments I and J, and no hybridizations with l strands of both fragments were detected. Cytoplasmic RNA also saturates 50, 55, and 30% of the r strands of *Bam*HI fragments F, G, and H, respectively. No hybrid formations with the l strands of these fragments were observed.

Table 2 summarizes the saturation values observed in hybridization experiments between *Hin*dIII and *Bam*HI fragments of Ad7 DNA and Ad7 early mRNA or cytoplasmic RNA from 7IJY1-2 cells. In a right-end column, the fraction of the total genome complementary to Ad7 early mRNA was given, which is calculated assuming that one strand equivalent of Ad7 DNA is informational and all the DNA sequences of *Hin*dIII fragment H are complementary to early mRNA. At early times after infection, about 25% of the genome is expressed as mRNA. This value is similar to that obtained at early times after infection of Ad5 (5), but lower than those of Ad2 (1, 2, 11). From the results shown in Table 2, we can construct tentative maps of Ad7 early mRNA and cytoplasmic RNA of 7IJY1-2 cells, if we assume the following. When RNA sequences are complementary to the same strand of adjacent restriction endonuclease fragments, they form one chain (6, 14). When the whole genome is represented as 100 units, early mRNA encoded in the left-hand region is complementary to 0.4, 2.0, 1.6, 1.9, 2.1, and 3.3 units of the

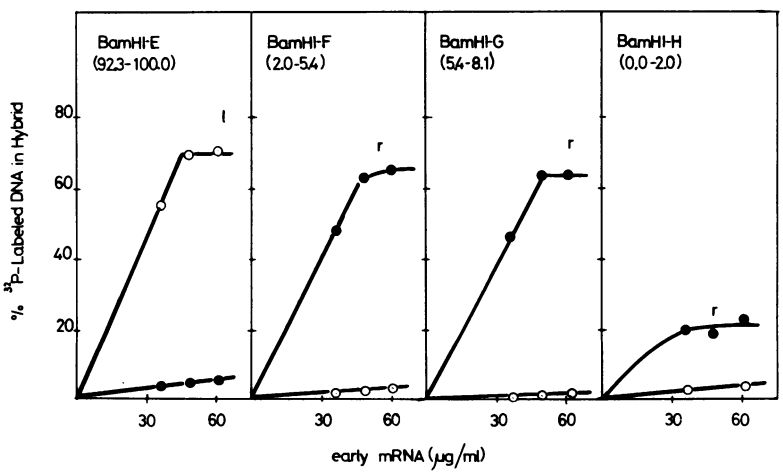

FIG. 2. *Hybridization of ^{32}P-labeled strands of BamHI fragments E, F, G, and H of Ad7 DNA to early mRNA from KB cells infected with Ad7. Isolation of early mRNA and hybridization reactions were performed as described in the legend to Fig. 1. The r strand (●) or the l strand (○) of each DNA fragment was hybridized with early mRNA.*

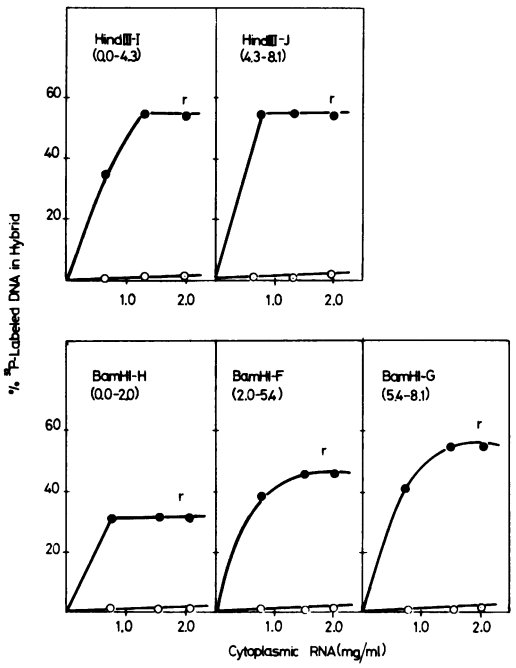

FIG. 3. *Hybridization of ^{32}P-labeled strands of HindIII and BamHI fragments of Ad7 DNA to cytoplasmic RNA from 7IJY1-2 cells. RNA was purified from the cytoplasmic fraction of 7IJY1-2 cells as described by Zieve and Penman (20). Hybridizations were carried out as described in the legend to Fig. 1. The r strand (●) or l strand (○) of each DNA fragment was hybridized with cytoplasmic RNA isolated from 7IJY1-2 cells.*

r strands of *Bam*HI fragments H (0–2.0), F (2.0–5.3), G (5.3–8.1), *Hin*dIII fragments I (0–4.3), J (4.3–8.1), and E (8.1–17.5), respectively. The coding region in the adjacent *Hin*dIII fragment J and E is considered to be located from 2.1 units to the left of *Hin*dIII fragment J·E junction to 3.3 units to its right, that is, from 6.0 to 11.4. Early mRNA complementary to the adjacent *Bam*HI fragments H and F will be mapped from 1.6 to 4.0. The length of this region roughly corresponds to the 1.9 unit length estimated from the hybridization value of *Hin*dIII fragment I. Viral RNA in 7IJY1-2 cells complementary to the r strands of adjacent *Bam*HI fragments H and F will be mapped from position 1.4 to 3.7. This 2.3 unit length coincides with the length calculated from the saturation value of *Hin*dIII fragment I. An another region coding for viral RNA in 7IJY1-2 cells must lie within the r strand of the *Hin*dIII fragment J (4.3–8.1), but cannot be determined further from these data. Ad7 early mRNA complementary to their strands of the adjacent *Hin*dIII fragments B, F, and H could be mapped from position 77.4 to 87.2, that is, 9.8 unit lengths, assuming that all the DNA sequences of *Hin*dIII fragment H are complementary to early mRNA. Ad7 early mRNA is also complementary to 35 and 70% of the l strand of the *Hin*dIII fragment B and the *Bam*HI fragment E, corresponding to 5.6 and 5.4 unit lengths, respectively. Because the map coordinate from 92.3 to 100 is common in both fragments, the region coding for early mRNA will be located from 0.2 units to the left of position 92.3 to 5.4 units to its right, that is, from position 97.7 to 92.1. The final region coding for early mRNA will be mapped from position 65.9 to 61.8 on the l strand of the adjacent *Hin*dIII

TABLE 2. *Ad7 DNA sequences complementary to early mRNA and cytoplasmic RNA*[a]

Fragment	Strand	% ^{32}P-labeled DNA in hybrid		% Total genome expressed			
		Ad7 early mRNA	7IJY1-2 cell cytoplasmic RNA	Ad7 early mRNA		7IJY1-2 cell cytoplasmic RNA	
				r	l	r	l
*Hin*dIII				15.4	9.7	4.4	0
B	r	20					
	l	35					
C	r	0					
	l	20					
D	r	0					
	l	0					
E	r	35					
	l	0					
F	r	20					
	l	0					
G	r	0					
	l	25					
H	r	100					
	l	0					
I	r	45	55				
	l	0	0				
J	r	55	55				
	l	0	0				
Bam HI						3.8	0
E	r	0					
	l	70					
F	r	60	50				
	l	0	0				
G	r	60	55				
	l	0	0				
H	r	20	30				
	l	0	0				

[a] The fraction of ^{32}P-labeled DNA entering into hybrids with Ad7 early mRNA or cytoplasmic RNA of 7IJY1-2 cells is summarized for the strands of each *Hin*dIII and *Bam*HI fragment. The saturation values for early mRNA are taken from Fig. 1 and 2, and those for cytoplasmic RNA of 7IJY1-2 cells are from Fig. 3. The percentage of total genome expressed as mRNA was calculated by summing the products of the fractions of fragment strands annealing to RNA and the fractional lengths of those fragments. The fractional lengths of *Hin*dIII fragments B, C, D, E, F, G, H, I, and J of Ad7 DNA are 16.0, 13.0, 11.7, 9.4, 8.6, 6.1, 4.9, 4.3, and 3.8, respectively (12). *Bam*HI fragments E, F, G, and H comprise 7.7, 3.4, 2.7, and 2.0 respectively (K. Yoshida, K. Sekikawa, and K. Fujinaga, Tumor Res., in press).

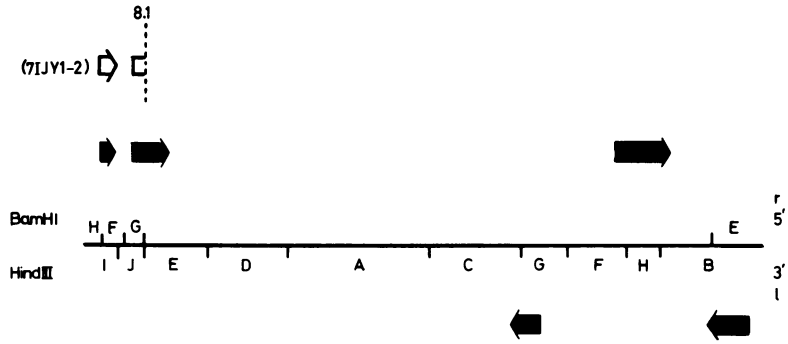

FIG. 4. *Maps of Ad7 DNA sequences expressed in KB cells early after infection and in transformed cells, 7IJY1-2. The Ad7 DNA was represented by the solid horizontal line in the center, and the cleavage site on Ad7 DNA was noted by vertical lines. The capital letters above and below the horizontal line give the nomenclatures of each cleavage fragment of BamHI and HindIII, respectively. The closed thick bars indicate the regions of the genome complementary to Ad7 early mRNA, whereas the open thick bars show the DNA sequences expressed in the cytoplasm of 7IJY1-2 cells. Arrows show the direction of transcription.*

fragments C and G. Figure 4 shows the most conceivable transcriptional maps of Ad7 early mRNA and Ad7-specific RNA of 7IJY1-2 cells. There are four regions coding for early mRNA, two on each strand of Ad7 DNA. Similar DNA sequences in the left-hand 8.1% of the viral genome are transcribed both in 7IJY1-2 cells and in KB cells early after infection. Except for the left-hand region, this map of early mRNA is compatible with those of Ad2, Ad5, and Ad12 (1, 2, 3, 5, 10, 11, 14, 15). A mapping study of early mRNA of Ad2, Ad5, and Ad12 by saturation hybridization showed that most of the sequences in the left-hand 12% of the viral genome were expressed as early mRNA (5, 10, 11, 14). Ad7 early mRNA appears to be transcribed from two separate regions, mapped at 1.6–4.0 and 6.0–11.4. The gap (4.0–6.0) may be explainable by lower estimation of hybridization values, because we have observed by the S1 nuclease technique that most of the DNA sequences mapped at 1.5–11.5 are complementary to Ad7 early mRNA (K. Yoshida and K. Fujinaga, unpublished data). More precise mappings of Ad7 early mRNA and virus-specific RNA in transformed cells are now under investigation by the S1 nuclease technique.

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