Early RNA of Adenovirus Type 3 in Permissive and Abortive Infections

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Early adenovirus type 3 cytoplasmic polyadenylated RNAs from HeLa and BHK-21 cells were detected and mapped on the viral genome by gel blotting and hybridization techniques. The sizes and locations of the 16 adenovirus type 3 RNAs were identical in the two cell types, although relative molarities of the various RNA species differed. Each of the early adenovirus type 3 RNAs was associated with polysomes in both cell types, suggesting that the abortive infection of hamster cells does not result from a defect in early adenovirus type 3 mRNA biosynthesis. No RNAs from regions transcribed late in infection of permissive cells were detected in BHK-21 cells.

The infection of rodent cells with the oncogenic class A or class B adenoviruses is abortive, resulting in no viral DNA synthesis and yielding no infectious virus (19). Adenovirus type 12 (Ad12), a class A adenovirus, adsorbs to and penetrates hamster cells but fails to synthesize viral DNA (20). These observations indicate that adenovirus multiplication is blocked either in early gene expression or directly at the level of DNA replication, and it has been suggested that nonpermissive cells lack a function essential for these processes. Ad12 DNA synthesis has been detected in hamster nuclei after infection of heterokaryons produced by fusion of hamster and human cells (25) and in mouse-human hybrid cells (4). This system, in which the nature of a possible missing function in nonpermissive cells can be studied, is made more intriguing by the fact that class C adenoviruses, such as adenoviruses types 2 and 5, can initiate viral DNA synthesis and produce infectious virus in hamster cells (19, 26).

Several reports have suggested restriction of early adenovirus mRNA biosynthesis in hamster cells. By competition hybridization, Raska and Strohl (17) found that about 60% of the RNA sequences transcribed in the lytic system are present in Ad12-infected BHK-21 cells. Hybridization of labeled restriction fragments of Ad12 to mRNA indicated that some sequences from each of four early gene regions were missing from stable mRNA populations in BHK-21 cells (16).

In this report, early cytoplasmic RNA produced in human and hamster cells by the class B adenovirus type 3 (Ad3) was identified and mapped by gel blotting and hybridization procedures to determine whether any specific early mRNA fails to be synthesized in hamster cells. Our approach was to purify cytoplasmic RNA from Ad3-infected cells, select polyadenylated RNA, denature the RNA with glyoxal, subject the RNA to electrophoresis in 1.2% agarose gels, and transfer the RNA to diazobenzyloxymethyl paper (1, 13, 15, 23). Ad3 restriction fragments were purified from agarose gels by electroelution, labeled with ³²P by nick translation, and hybridized to the RNA-paper (7, 14, 18, 22, 24). Hybridization was detected by autoradiography. No hybridization to RNA from uninfected cells was detected.

For identifying early Ad3 RNA in human cells, HeLa cells were infected with Ad3 (10 PFU per cell) and incubated in medium containing 20 µg of cytosine arabinoside per ml for 8 h after infection, and RNA was extracted and selected for polyadenylation. Hybridization with the *Sma*I restriction fragments between 0 and 77.9 map units, *Xba*I-C', and *Xba*I-D revealed at least 16 distinct Ad3 RNAs early after infection (Fig. 1). Using additional Ad3 restriction fragments, we mapped the coding sequences of these early Ad3 RNAs. The results of these hybridizations were consistent with the existence of four early gene regions, as has been observed with other adenoviruses (Fig. 2) (2, 3, 6, 12, 16).

Early region 1 (E1) contained 2.4- and 1.1-kilobase (kb) RNAs between 0 and 13.5 map units. In some analyses, the 1.1-kb RNA ap-

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FIG. 1. Early Ad3 RNA in HeLa cells. HeLa cells were infected in suspension with Ad3 (10 PFU per cell) and incubated with 20 μ g of cytosine arabinoside per ml. Cytoplasmic RNA was isolated 8 h after infection. Polyadenylated RNA was denatured with glyoxal, fractionated by electrophoresis in 1.2% agarose gels, and transferred to diazobenzyloxymethyl paper. 32 P-labeled Ad3 DNA restriction fragments were hybridized separately to each lane for 16 h. Filters were exposed to hypersensitized X-omat R film with intensifying screens at -80°C for 72 h. The positions of the 28S (5.2-kb) and 18S (1.8-kb) ribosomal RNAs are indicated. Lanes a through e contained 7 μ g of RNA per lane and were subjected to electrophoresis at 40 V for 17 h. Lanes f through h contained 5 μ g of RNA per lane and were subjected to electrophoresis at 30 V for 16 h. (a) SmaI-D, 0 to 13.5 map units; (b) SmaI-G, 13.5 to 19.0 map units; (c) SmaI-A, 19.0 to 46.7 map units; (d) SmaI-C, 46.7 to 61.0 map units; (e) SmaI-F, 16.0 to 67.6 map units; (f) SmaI-E, 67.6 to 77.9 map units; (g) XbaI-C', 76.5 to 90.7 map units; (h) XbaI-D, 90.7 to 100 map units.

peared as a doublet. Early region 2 (E2) contained 2.1- and 1.8-kb RNAs between 61.0 and 76.5 map units. Early region 3 (E3) contained four abundant RNAs of 4.2, 3.1, 2.1, and 1.5 kb and four rare RNAs of 5.3, 3.5, 2.6, and 0.9 kb between 75.3 and 90.7 map units. Early region 4 (E4) contained three abundant RNAs of 2.5, 2.0, and 1.6 kb and one rare RNA of 2.9 kb between 90.7 and 100 map units. No early Ad3 RNA was detected between 13.5 and 61.0 map units.

Ad3 E1 appears to contain two adjacent intervals. BamHI-I (0 to 2.0 map units) hybridized only the 1.1-kb RNA. BamHI-G (5.0 to 9.6 map units) hybridized only the 2.4-kb RNA (data not shown). Therefore, E1A 1.1-kb RNA maps between 0 and 5.0 map units, and E1B 2.4-kb RNA maps between 2.0 and 13.5 map units.

The direction of transcription of each early region was deduced by comparing the hybridization of two specific cDNA's to restriction fragments subdividing each early region (Table 1) and determining the 5' or 3' sequences of each region. cDNA was synthesized to polyadenylated RNA purified from cytosine arabinoside-treated HeLa cells 8 h after infection (8, 9). Oligodeoxythymidylic acid-primed cDNA contained primarily 3' sequences. Calf thymus oligodeoxynucleotide-primed cDNA contained sequences representative of the entire RNA molecule (21). This analysis demonstrated that E2 and E4 RNAs were transcribed leftward. E1 and E3 RNAs were transcribed rightward.

For determining the Ad3 RNAs synthesized in hamster cells, polyadenylated RNA was purified from serum-arrested BHK-21 cells 16 h after infection (50 PFU per cell) and analyzed as described above (Fig. 3). These hybridizations initially distinguished 15 Ad3 RNAs corresponding to early Ad3 RNAs from HeLa cells. In later experiments, we were able to detect an additional RNA of 1.1-kb corresponding to E1A RNA. Mapping these RNAs demonstrated the following: (i) RNA synthesized in BHK-21 cells was encoded in four regions of the Ad3 genome; (ii) the limits of the coding sequences were identical to the limits of the Ad3 early regions observed in HeLa cells; (iii) the number and apparent sizes of the Ad3 RNAs from each region were identical in each cell type; and (iv) only early Ad3 RNAs were detected in BHK-21 cells.

The only difference between the Ad3 RNAs in BHK-21 cells and the early Ad3 RNAs in HeLa cells was in the relative molarities of the RNAs derived from each region. Because the RNA was purified from the two cell types at different times after infection, it was necessary to determine whether the relative molarities changed during the course of infection. Therefore, polyadenylated cytoplasmic RNA was pur-

FIG. 2. Ad3 early gene regions. The sequences expressed as RNA early after infection and directions of transcription are represented by arrows. The sizes of the cytoplasmic polyadenylated RNAs (in kb) are listed. For E3, the major RNAs are listed in the left column, and the minor RNAs are listed in the right column.

TABLE 1. *cDNA hybridization to Ad3 restriction fragments*

Enzyme	Frag- ment	Location ^a	Hybridization ^b of:	
			cDNA _{3'}	cDNA _{rep}
<i>Sma</i> I	D	0.0-13.5	51.1	20.4
	F	61.0-67.6	30.4	53.6
	E	67.6-77.9	ND ^c	4.5
	B	77.9-97.4	18.5	21.1
	H	97.4-100	ND	0.4
<i>Bam</i> HI	I	0.0-2.0	ND	0.7
	H	2.0-5.0	14.7	2.8
	G	5.0-9.6	20.6	9.3
	A	9.6-36.7	19.2	2.8
	E	60.2-70.0	22.4	35.0
	C	70.0-83.1	ND	26.0
	F	83.1-89.7	20.6	6.9
	D	89.7-100	2.5	16.5
<i>Hind</i> III	I+J	0.0-7.1	20.3	6.2
	E	7.1-17.0	45.6	8.4
	C	50.6-64.3	9.8	10.0
	G	64.3-70.4	8.9	26.0
	F	70.4-79.6	1.6	22.7
	B	84.1-100	13.7	26.7
<i>Xho</i> I	C	0.0-10.8	58.7	14.0
	E	10.8-14.1	1.3	ND
	A	22.9-75.3	23.2	63.3
	B	76.5-100	16.8	27.7

^a Intervals which are not listed are those in which there was no detectable hybridization to restriction fragments with either probe.

^b Expressed as a percentage of total hybridization to restriction fragments produced by each endonuclease. cDNA_{3'}, oligodeoxythymidylc acid-primed cDNA containing primarily 3' sequences; cDNA_{rep}, calf thymus oligodeoxynucleotide-primed cDNA containing sequences representative of the entire RNA molecule.

^c ND, None detected.

ified from Ad3-infected BHK-21 cells and from cytosine arabinoside-treated HeLa cells at various times after infection and analyzed as described above (Fig. 4). Each of the 16 Ad3 RNAs in BHK-21 cells comigrated with the corresponding early Ad3 RNA purified 8 h after infection, confirming the identity of sizes of Ad3 RNAs in both cell types. With the exception of E2 RNAs, the relative molarities of the Ad3 RNAs did not change during the course of infection, indicating that the observed differences in abundances were determined by the cell type.

The following two differences were observed between Ad3 RNAs purified 8 and 16 h after infection of cytosine arabinoside-treated HeLa cells: (i) a 3.4-kb RNA, containing sequences from both E1A and E1B, was detected only at 16 h after infection; and (ii) the 1.8-kb E2 RNA observed in HeLa cells at 16 h after infection

migrated slightly faster than the 1.8-kb RNA observed at 8 h after infection.

For determining if the early Ad3 RNAs function as mRNA's, polysomal RNA was purified from Ad3-infected HeLa and BHK-21 cells (5) and analyzed as described above. All of the early Ad3 RNAs were polysome associated, suggesting that all of the early cytoplasmic polyadenylated Ad3 RNAs can function as mRNA's in both HeLa and BHK-21 cells.

We found that all early Ad3 RNAs detected in HeLa cells were also detected in BHK-21 cells and associated with polysomes. Although this analysis is limited by the resolution of mRNA in the gel system (for example, we could not resolve two E1A species), our findings suggest that the abortive infection of BHK-21 cells by Ad3 did not result from a lesion in biosynthesis of the predominant early mRNA's.

The complete lack of Ad3 DNA synthesis in hamster cells cannot be adequately explained by the observed differences in mRNA molarities. The largest difference was only threefold, observed in E3, and we know that much of the Ad3

FIG. 3. *Ad3 RNA in BHK-21 cells.* Serum-arrested BHK-21 cells were infected with Ad3 (50 PFU per cell). Cytoplasmic RNA was isolated 16 h after infection. Polyadenylated RNA was selected, transferred to diazobenzylxymethyl paper, and hybridized to DNA as described in the legend to Fig. 1. Lanes a through e contained 8 µg of RNA per lane and were subjected to electrophoresis at 30 V for 20 h. Lanes f through h contained 5 µg of RNA per lane and were subjected to electrophoresis at 30 V for 16 h. The positions of the 28S (4.8-kb) and 18S (1.8-kb) ribosomal RNAs are indicated. (a) *Sma*I-D; (b) *Sma*I-G; (c) *Sma*I-A; (d) *Sma*I-C; (e) *Sma*I-F; (f) *Sma*I-E; (g) *Xba*I-C'; (h) *Xba*I-D.

FIG. 4. Time course of Ad3 RNA synthesis in BHK-21 and HeLa cells. Cytoplasmic polyadenylated RNA was purified from Ad3-infected, serum-arrested BHK-21 cells at 8, 12, and 16 h after infection and from Ad3-infected HeLa cells incubated with 20 μ g of cytosine arabinoside per ml at 8 and 16 h after infection. RNA from 3×10^7 cells at each time point was subjected to electrophoresis in 1.2% agarose gels for 16 h at 30 V and transferred to diazobenzylxymethyl paper. Filters were hybridized with Ad3 restriction fragments and exposed for 20 h, except in panel D, where the HeLa lanes were exposed for 9 h. The positions of the 28 and 18S ribosomal RNAs are indicated. (A) E1, XbaI-A, 0 to 44.0 map units; (B) E2, XbaI-B, 44.0 to 68.5 map units; (C) E3, XbaI-C', 76.5 to 90.7 map units; (D) E4, XbaI-D, 90.7 to 100 map units.

E3 can be deleted without altering the viability of the virus (11). Furthermore, preliminary data indicate that those E3 RNAs produced by the Ad3 deletion mutant, H3dl102, are expressed at the same relative levels in HeLa and BHK-21 cells.

Ad12 infection of hamster cells is also abortive, resulting in no detectable viral DNA replication. Studies of expression of Ad12 early genes have suggested that only a subset of the se-

quences expressed early in permissive infection are present in the cytoplasm of infected hamster cells (16, 20). Cell-free translation of Ad12 mRNA from hamster cells produced some, but not all, of the early gene products (10). These observations may represent real differences between Ad12 and Ad3 abortive infections but more likely reflect differences in the methods used.

Two alternative hypotheses about the cause

of the abortive infection of hamster cells by the oncogenic adenoviruses should be considered. (i) One or more of the essential early gene products are not functional in hamster cells. (ii) DNA synthesis of the oncogenic adenoviruses cannot proceed in hamster cells. The use of *in vitro* replication systems will help define human cell factors that can support DNA synthesis of the oncogenic adenoviruses in hamster cells. Such an approach may ultimately reveal the mechanism of the abortive infection of hamster cells by adenoviruses.

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