THREE NEW VIRUS-SPECIFIC LOW MOLECULAR WEIGHT RNAs IN ADENOVIRUS TYPE 2 INFECTED KB CELLS

Karel Raska, Jr., Lynne M. Sehulster, and Frederick Varricchio*
Department of Microbiology, College of Medicine
and Dentistry of New Jersey, Rutgers Medical School
Piscataway, New Jersey 08854 U.S.A.
and
Sloan-Kettering Cancer Center*, New York, New York 10021 U.S.A.

Received December 29, 1975

SUMMARY

Low molecular weight RNA synthesized at 16-24 hours after infection (h.p.i.) in adenovirus type 2 (Ad2) infected KB cells was analyzed by two-dimensional polyacrylamide gel electrophoresis. Three discrete species of RNA moving between the cellular 5S RNA and viral VA RNA were detected. DNA-RNA hybridization indicates that they are coded by the Ad2 genome.

INTRODUCTION

During productive infection with adenoviruses, large amounts of RNA sedimenting at 5.5S are synthesized in infected cells (1). This so-called VA RNA, coded by the virus genome, was completely sequenced, but its function remains unknown (2). We have previously reported that 4S RNA from Ad2-infected KB cells, from which VA RNA was separated by methylated albumin kieselguhr (MAK) column chromatography contained material complementary to the Ad2 genome (3). As no amino acid acceptor activity was associated with this RNA, it was concluded that it represented fragments of Ad2 mRNA.

With the refinement of methodology for RNA separation, we decided to reexamine the synthesis of virus-specific low molecular weight RNA in Ad2-infection. Here we report, that in addition to VA RNA, 3 other discrete species of Ad2-specific low molecular weight RNA can be detected in Ad2-infected KB cells.

MATERIALS AND METHODS

Cells and virus. A mycoplasma-free line of KB cells grown in "spinner" flasks was infected with plaque-purified adenovirus type 2 at input multiplicity of infection of 20-60 plaque forming units as described previously (4).

Radioactive isotope labeling and isolation of low molecular weight RNA. The cells were labeled with $^{32}P_i$ (25 μCi/ml) in phosphate-free medium from 16 to 24 hours after infection. The low molecular weight RNA was isolated by phenol extraction and differential precipitation in 1M sodium chloride (5). The RNA was treated with ribonuclease-free deoxyribonuclease (10 μg/ml) and reextracted with phenol.

Polyacrylamide gel electrophoresis. Low molecular weight RNA was separated by two-dimensional polyacrylamide gel electrophoresis (6). The first dimension was in 15% gel, pH 5.8, containing 6M urea; the second dimension was in 16% gel, pH 8.3. The second dimension slab gels were stained with methylene blue and autoradiographed using Chronex-4 X-ray film. The RNA was extracted by phenol from the respective areas of the gel.

DNA-RNA hybridization. Adenovirus type 2 was purified (4) and viral DNA was isolated by the method of Doerfler (7). The 5S RNA, the VA RNA, and the RNA species moving between the 5S RNA and VA RNA were hybridized to denatured Ad2 DNA under standard conditions on nitrocellulose filters (8). Filters were then processed as previously described (4). Blank filters and filters containing *E. coli* DNA were used as controls.

RESULTS

By two-dimensional gel electrophoresis the low molecular weight RNA from KB cells is resolved into 36 spots of tRNA and 2 spots corresponding to cellular 5S RNA (9). In Ad2-infected cells, the more slowly moving intense spot is VA RNA (Fig. 1A). In between the 5S RNA and the VA RNA, three other spots can be detected on the autoradiograms (Fig. 1B). Table I indicates the incorporation of radioactivity into the individual RNA species in the 5S RNA-VA RNA area of the gel. The radioactivity in these three spots is sensitive to ribonuclease digestion. It is apparent that from 16 through 24 h.p.i. the rate of synthesis of the RNA in the spot C corresponds to about one-half of that in the major 5S RNA spot. Incorporation into the other 2 spots is considerably lower, but the relative amounts of radioactivity in the 3 RNA spots (A, B, and C) are similar in different experiments. From the known size of cellular 5S RNA and VA RNA, the size of these 3 RNA species can be estimated between 120 and 156 base residues. The VA RNA, the 5S RNA, and material present in the spots A, B, and C

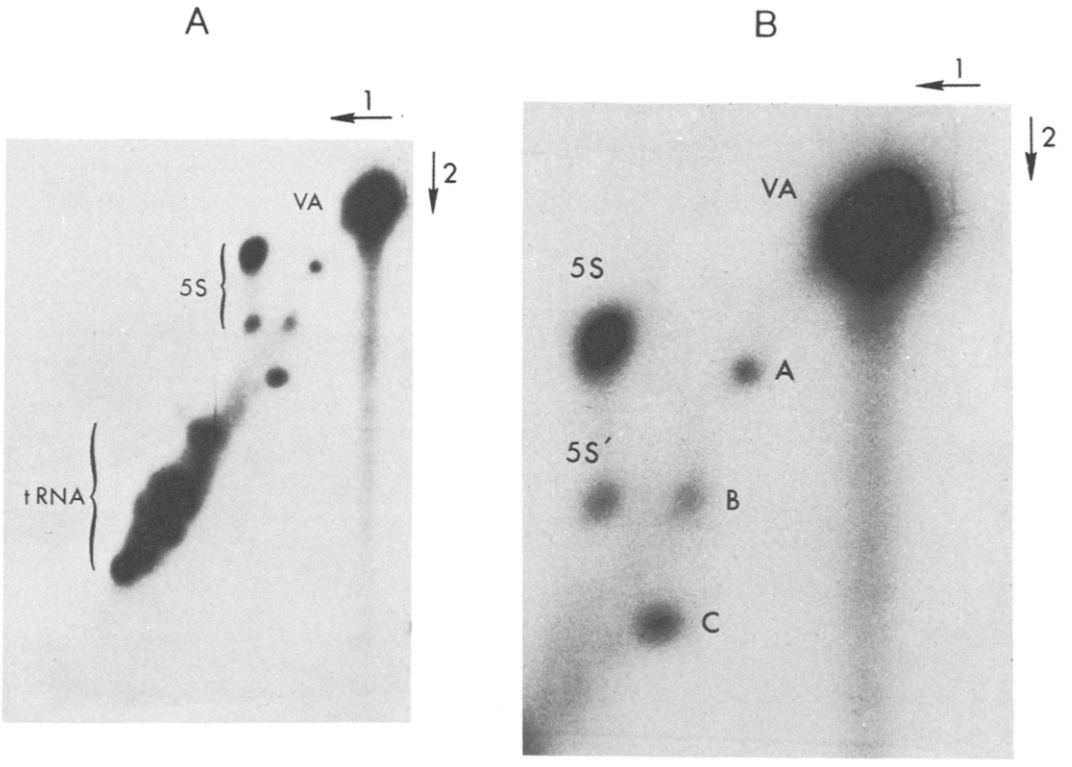

Figure 1. Two-dimensional gel electrophoresis of the low molecular weight RNA from Ad2-infected KB cells.

The RNA was isolated from cells labeled at 16-24 h.p.i. A-autoradiogram of the second dimension gel slab (exposure 72 hours). B-enlarged 5S-7S area of the gel. 5S' RNA corresponds to the component of cellular 5S RNA moving faster on the second dimension gel.

(Fig. 1B) were recovered from the gel and hybridized to 10 μg of purified denatured Ad2 DNA. Results summarized in Table II indicate that RNA material present in all 3 spots A, B, and C contains RNA complementary to Ad2 DNA. The specificity of the reaction was determined by lack of complementarity of 5S RNA and by hybridization of VA RNA, which is virus coded. No significant hybridization of this material was observed with E. coli DNA. This result indicates that in Ad2 infected cells discrete small RNA species, other than VA RNA, are complementary to viral DNA.

Table I

Incorporation of $^{32}P_i$ into Low Molecular Weight RNA Late After Ad2 Infection[1]

RNA Species[2]	Incorporation (c.p.m.) Exp. 1	Exp. II
5S RNA	6,005	3,800
5S' RNA	1,608	968
A	599	348
B	817	641
C	3,111	2,062
VA RNA	60,693	42,300

[1]The cells were labeled at 16-24 h.p.i. with $^{32}P_i$. 30 µg of low molecular weight RNA were separated on the second dimension gel slab.

[2]Corresponds to RNA in spots labeled in Fig. 1B. The spots detected on autoradiograms were recovered and their radioactivity was determined after digesting in Protosol by counting in Aquasol.

DISCUSSION

The finding of 3 discrete species of virus-specific RNA moving between 5S RNA and VA RNA is of considerable interest. They most probably represent RNA responsible for previously observed complementarity to Ad2 DNA of 4S RNA in infected KB cells (3). The present study does not exclude the possibility that these low molecular weight RNAs (other than VA RNA) represent fragments of viral mRNA, although the results are extremely reproducible. It was recently observed that an additional species of VA RNA, smaller than that originally described, is detected in Ad2-infected KB cells (10). This minor RNA is coded for by a gene that is located immediately to the right of the one coding for the originally described VA RNA. One of the RNA spots detected in our two-dimensional gels

Table II

Hybridization of Low Molecular Weight RNA to Ad2 DNA[1]

RNA Species[2]	Total Radioactivity Added (c.p.m.)	Radioactivity Bound to Filters Containing Ad2 DNA	
		c.p.m.	Fraction of total (%)
5S RNA	8,200	7	0.08
A	869	165	18.9
B	1,178	160	13.6
C	589	144	24.4
VA RNA	28,400	248	0.87

[1]RNA was isolated from cells labeled with $^{32}P_i$ at 16-24 h.p.i. and separated by two-dimensional electrophoresis.[1] RNA was recovered and hybridized to filters containing 10 µg of Ad2 DNA. The same amounts of radioactivity were incubated with filters containing 10 µg of E. coli DNA or blank filters. Radioactivity on control filters not exceeding 9 c.p.m. was subtracted as background.

[2]Corresponds to spots labeled in Fig. 1B.

may correspond to this minor RNA species. Synthesis of discrete RNA species similar to those reported here was also observed in isolated nuclei of adenovirus-infected cells on one-dimensional gel electrophoresis. Results from this system indicate the presence of at least 2 species of virus-specific RNA that are smaller than VA RNA (11). Their results from isolated nuclei suggest that these discrete RNA species are not fragments of viral mRNA, as they appear to be synthesized by RNA polymerase III (11), while the adenovirus mRNA is a product of RNA polymerase II (12).

ACKNOWLEDGEMENTS

This work was supported by Grants CA-08748 and CA-08851 from the National Cancer Institute and by Grant DRG-1289 from the Damon Runyon-

Walter Winchell Cancer Fund. LMS is supported by National Cancer Institute Training Grant CA-09069.

REFERENCES

1. Reich, P.R., Forget, B.G., Weissman, S.M., and Rose, J.A. (1966). J. Mol. Biol. 17, 428-439.

2. Ohe, K., and Weissman, S.M. (1971). J. Biol. Chem. 246, 6991-7009.

3. Raska, Jr., K., Frohwirth, D., and Schlesinger, R.W. (1970). J. Virol. 5, 464-469.

4. Raska, Jr., K., Prage, L., and Schlesinger, R.W. (1972). Virology 48, 472-484.

5. Varricchio, F. (1971). In Methods in Molecular Biology, (Last, J., and Laskin, A., eds.), vol. 1, p. 297, Marcal Dekker, Inc., New York.

6. Stein, M., and Varricchio, F. (1974). Anal. Biochem. 61, 112-119.

7. Doerfler, W. (1969). Virology 38, 587-606.

8. Gillespie, D., and Spiegelman, S. (1965). J. Mol. Biol. 12, 829-842.

9. Sehulster, L.M., Varricchio, F., and Raska, Jr., K. (1975). Proc. Amer. Assoc. Cancer Res. 16, 71.

10. Mathews, M.B. (1975). Cell 6, 223-229.

11. Raskas, H.J., Weinman, R., Jaehning, J.A., and Roeder, R.G., personal communication.

12. Price, R., and Penman, S. (1972). J. Virol. 13, 621-626.