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Series of 4.5S RNAs associated with poly(A)-containing RNAs of rodent cells

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ABSTRACT

Uninfected mouse kidney cells and mouse leukemia cells L1210 in culture contained a series of 4.5S RNAs which was structurally identical to the series of 4.5S RNAs assoicated with genomic RNAs of murine retroviruses and poly(A)-containing RNAs from virus infected cells. Normal rat kidney cells and baby hamster kidney cells in culture also contained a series of 4.5S RNAs. The structures of the 4.5S RNAs from mouse, rat and hamster cells were very similar, but not identical. These 4.5S RNAs were not found in cultured cells of other vertebrates, such as human, monkey, cat, mink, rabbit and chicken cells.

INTRODUCTION

Series of low molecular weight RNAs (4.5 to 5.5S) have been isolated from Moloney murine leukemia virus (Mo-MuLV) and Friend spleen focus forming virus (SFFV)(1,2). These RNAs are specifically associated with the genome in the virions and are released by denaturing the 70S genomic RNA complex. In Mo-MuLV, this series of RNAs is composed of five 4.5S RNAs of different lengths (1), whereas in SFFV, the series of RNAs is composed of more than thirty RNAs of 4.5 to 5.5S (2). The same series of RNAs is associated with nulcear and cytoplasmic poly(A)-containing RNAs from SFFV-infected cells (2). The nucleotide sequences of these molecules are identical except for their 3'-termini : larger RNAs have progressively more uridylic acid residues at their 3'-end, and thus larger molecules contain poly(U) sequences at their 3'-termini(2).

To determine whether these molecules are coded by the host or viral genome, we analyzed the small RNAs associated with poly-(A)-containing RNAs from several mammalian and avian cells as well as those from uninfected mouse cells. Uninfected mouse, rat

and hamster cells contained the same series of 4.5S RNAs, but this series was not found in human, monkey, cat, mink, rabbit and chicken cells.

MATERIALS AND METHODS

Materials. The sources of the materials used in this study have been described previously (2).

Preparation of ^{32}P-labeled RNAs. Various cell lines and primary or secondary cultures of cells were obtained from the following sources : Mouse kidney cells (C3H2K), normal rat kidney cells (NRK), mink lung cells (American Type Culture Collection, cat. No, CCL64), rabbit corneal cells (SIRC), and a transformed subclone of cat cells infected by Moloney sarcoma virus (Cat S^+L^- 8c) from H. Yoshikura of the Institute of Medical Science, University of Tokyo ; African green monkey kidney cells (CV-1) and a secondary culture of human embryo kidney cells (HEK) from K. Shiroki of the Institute of Medical Science ; another line of African green monkey kidney cells (Vero) from H. Shibuta of the Institute of Medical Science ; a human lymphoblastoid cell line transformed by Epstein Barr virus (NL-3) from A. Oikawa of Tohoku University ; HeLa cells and a cell line of mouse lymphoma (L1210/C) from H. Okada and H. Tanaka of this institute ; a primary culture of chicken embryo fibroblast cells (CEF) from Y. Yaoi and K. Motohashi of this institute ; baby hamster kidney cells (BHK-21) from Flow Laboratory.

Cells were labeled for 4 hours with ^{32}P as described previously (2). Then they were washed with TSE buffer (0.02M Tris-HCl, pH 7.5 ; 0.1M NaCl ; 0.001M EDTA), and lysed directly with TSE buffer containing 0.5% SDS for 15 minutes at room temperature. The suspension was extracted with the same volume of phenol-chloroform mixture (1:1, v/v), and the resulting phenol layer was re-extracted with the same volume of 0.1M Tris-HCl (pH 9.0), 0.1M NaCl, 1mM EDTA, and 0.5% SDS(3). The two upper layers were then combined and extracted with chloroform-isoamyl alcohol (24:1, v/v). The aqueous phase was adjusted to 0.3M NaCl and nucleic acids were precipitated with two volumes of ethanol. The fibrous precipitate of DNA was removed with a glass rod before centrifugation.

Purification of poly(A)-containing RNA by poly(U)-Sepharose column chromatography, fractionation of low molecular weight RNAs by two dimensional polyacrylamide gel (2-D gel) electrophoresis (4,5) and fingerprint analysis (1,6) were carried out as described previously (2).

RESULTS

Analysis of small RNAs associated with poly(A)-containing RNAs. Since both nuclear and cytoplasmic poly(A)-containing RNAs from SFFV-infected cells contained the series of RNAs(2), the total cellular poly(A)-containing RNA of cells was analyzed by 2-D gel electrophoresis. Figure 1 shows the 2-D gel patterns of small RNAs released form poly(A)-containing RNA of these cells. Very characteristic spots of the series of RNAs in the 4.5S region of the gels were obtained from C3H2K, L1210, NRK and BHK cells (Fig. 1a through d). In a separate experiment, cytoplasmic and nuclear poly(A)-containing RNAs were purified from L1210 cells and analyzed in the same way. The series of RNAs was obtained from both samples. On the other hand, these spots were not seen on gels of materials from HeLa, Vero, SIRC and CEF cells (Fig. 1e through h). Moreover this series of RNAs was not found in poly(A)-containing RNAs of the other mink, cat, monkey and human cells listed in the Materials and Methods (data not shown). Although some of these cells gave spots that moved to the same area as the series of RNAs, their RNase T1 fingerprints were different from that of the series of RNAs.

Analysis of flow-through fractions from a poly(U)-Sepharose column. Previously, we showed that the flow-through fractions obtained on poly(U)-Sepharose column chromatography of nuclear and cytoplasmic RNAs from SFFV-infected cells contained considerable amounts of the series of RNAs(2). The flow-through fractions of L1210, NRK (Fig. 2a and b), C3H2K and BHK (not shown) cells also contained the same series of 4.5S RNAs, and their RNase T1 fingerprints were identical with those of the RNAs released from poly(A)-containing RNAs. On the other hand, the flow-through fractions of Vero, SIRC, cat, mink (Fig. 2c through f) and the other monkey, human and chicken cells listed in the

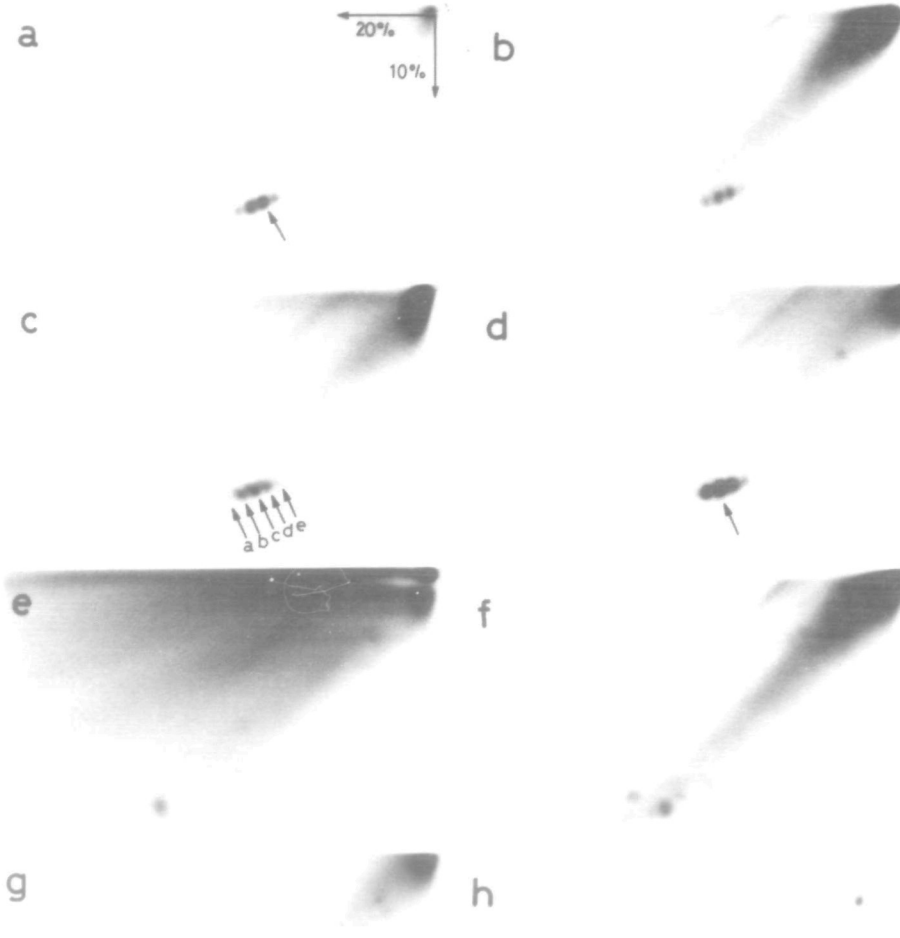

Figure 1. 2-D Gel electrophoretic patterns of small RNAs released from poly(A)-containing RNAs of various cells. (a) C3H2K cells; (b) L1210 cells;(c) NRK cells;(d) BHK cells;(e) HeLa cells ;(f) Vero cells;(g) SIRC cells;(h) CEF cells. Arrows indicate the spots used for RNase T1 fingerprints in Figure 3.

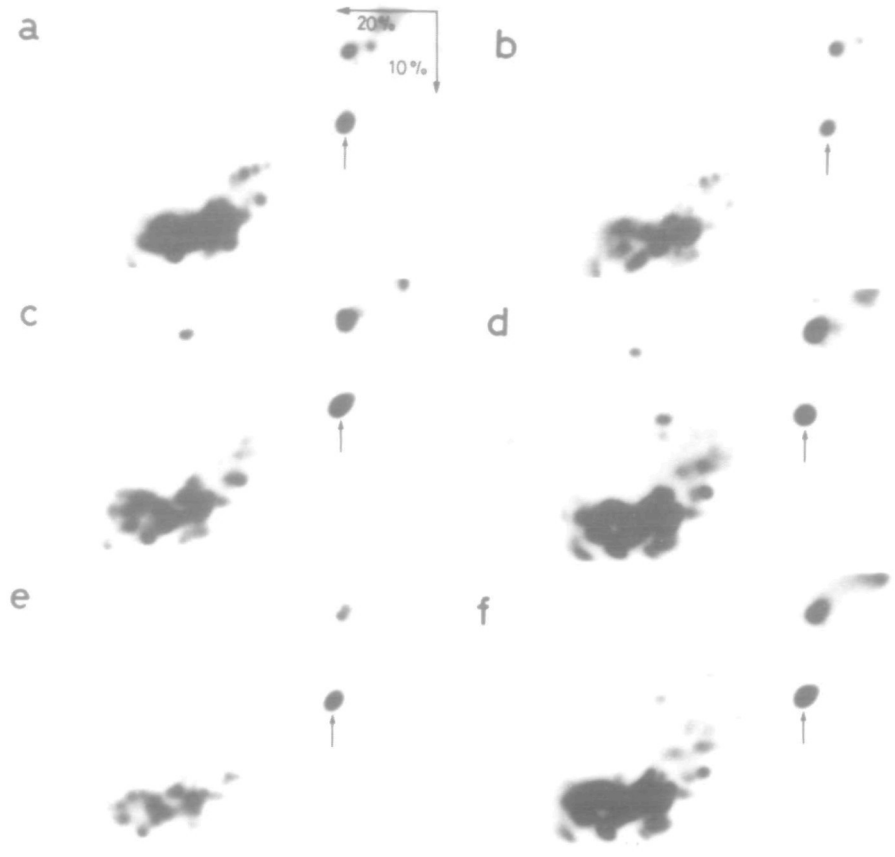

Figure 2. 2-D Gel electrophoretic patterns of small RNAs of flow-through fractions on poly(U)-Sepharose column chromatography. (a) L1210 cells;(b) NRK cells;(c) Vero cells;(d) SIRC cells;(e) cat cells;(f) mink cells. Upward arrows indicate the position of ribosomal 5S RNA.

Materials and Methods did not contain this series of RNAs. The RNase T1 fingerprints of the spots from these cells that moved to the position of the series of RNAs were different from the fingerprints of the series of RNAs of rodent cells.

Fingerprint analysis of the 4.5S RNAs from mouse, hamster and rat cells. Figure 3 shows the RNase T1 fingerprints and a schematic drawing of the 4.5S RNAs from C3H2K, BHK and NRK cells.

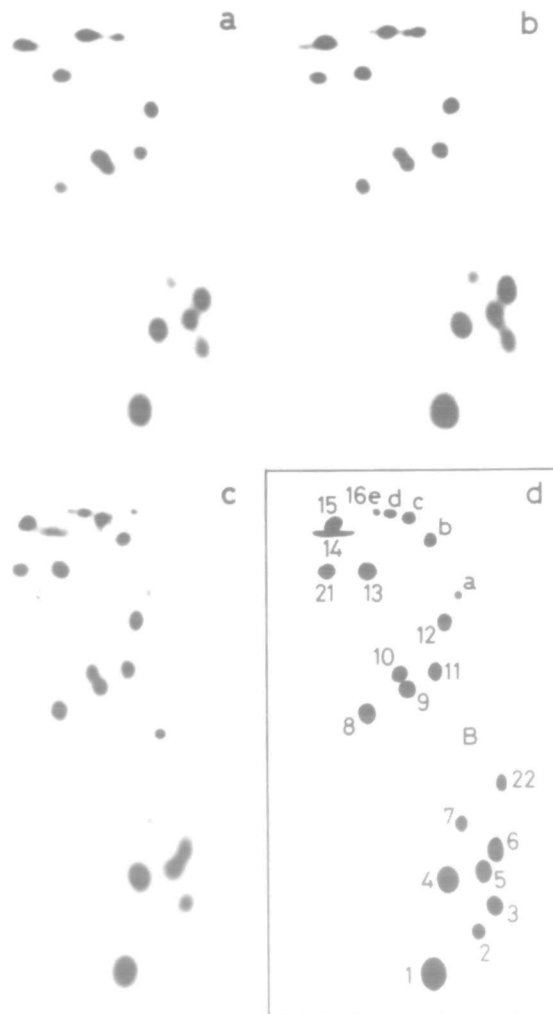

Figure 3. RNase T1 fingerprints and schematic drawing of the 4.5S RNAs from various rodent cells. The RNAs examined were those indicated by arrows in Figure 1. First dimension (right to left) : electrophoresis on cellulose acetate in pyridine acetate (pH 3.5)-7M urea. Second dimension (top to bottom) : electrophoresis on DEAE-cellulose in 7% formic acid. (a) C3H2K cells ;(B) BHK cells;(c) mixture of five different spots from NRK cells;(d) schematic presentation of the electropherograms and the numbering system. 16a through e refer to the 3'-terminal oligonucleotides from corresponding RNAs a through e in Figure 1c. B denotes the position of the blue dye marker (xylene cyanol FF).

The 4.5S RNAs from L1210 cells gave an identical fingerprint to those of C3H2K cells. In Figure 3c, oligonucleotide 14 is shifted to the right of oligonucleotide 15. This difference resulted from use of a different batch of cellulose acetate. Oligonucleotides 16a through e in Fig. 3c were the 3'-terminal oligonucleotides derived from corresponding RNAs(a through e of Fig. 1c). These oligonucleotides differed in that the larger RNAs had progressively more uridylic acid residues at their 3'-termini (1,2). Therefore, oligonucleotide 16e contains four more uridylic acid residues than oligonucleotide 16a at its 3'-terminus. In the fingerprint of the spot from C3H2K or BHK cells, 16d was the major 3'-terminus and a small amout of 16c was also obtained as a contaminant of smaller RNA, which was located to the left of the sample spot (Fig. 1a and d). Otherwise, these three fingerprints were very similar to one another except for the following differences. (i)Oligonucleotide 21 was absent from mouse 4.5S RNAs. Preliminary experiments showed that the sequences of oligonucleotides 21 and 10 are U-U-Gp and U-A-Gp, respectively. There are two moles of U-A-Gp in mouse 4.5S RNAs, but one mole each of U-U-Gp and U-A-Gp in BHK and NRK 4.5S RNAs. These differences must result from substitution between T and A at the same position of the genes for these RNAs. (ii) There was a submolar amount of oligonucleotide 22 in the fingerprint of NRK 4.5S RNAs. Since this oligonucleotide was consistently found in different preparations of NRK 4.5S RNAs, partial base substitution must have occurred on the genes for the 4.5S RNAs of NRK cells.

DISCUSSION

In this work we found that the series of 4.5S RNAs that has been obtained from Mo-MuLV (1), SFFV and poly(A)-containing RNAs of SFFV-infected mouse spleen cells(2), can also be isolated from uninfected mouse, rat and hamster cells. Thus, we conclude that this series of 4.5S RNAs is probably cellular in origin. Although the function of this series of RNAs is not known at the present time, the specific association of these molecules to nuclear and cytoplasmic poly(A)-containing RNAs suggests that it could have important cellular function(s) such as participation

in splicing and joining of nuclear poly(A)-containing RNA, transportation of mature mRNA from the nucleus to the cytoplasm, translational control of mRNA, and protection of poly(A)-containing RNA from nuclease attack. The RNase T1 fingerprints of the 4.5S RNAs from mouse, rat and hamster cells were very similar, but not identical. Probably during the course of evolution, some base substitution has occurred on the genes for these 4.5S RNAs. We could not find these molecules in human, monkey, cat, mink, rabbit and chicken cells, but, since several other small RNAs were released from the poly(A)-containing RNAs of these cells, some of these small RNAs could have the same function(s) as the series of 4.5S RNAs of rodent cells.

Jelinek and Leinwand isolated a group of small RNAs associated with poly(A)-containing RNAs from cultured Chinese hamster ovary cells(7). Since they used a different fingerprint system their small RNAs can not be directly compared with the 4.5S RNAs from BHK cells demonstrated in this work. However, their small RNAs may well be the same as those of BHK cells, judging from their size and ability to associate with poly(A)-containing RNA.

Ro-Choi et al. isolated three distinct species of 4.5S RNAs(4.5S RNAI, II and III) from the nuclei of Novikoff hepatoma cells(8). The primary structure of one of these molecules (4.5S RNA_I) has been determined(9). Although, on the gel, this molecule partly overlapped the series of 4.5S RNAs, 4.5S RNA_I is metabolically stable, whereas the series of 4.5S RNAs is rapidly metabolyzed (1,F. Harada, unpublished data). Moreover, the fingerprint of 4.5S RNA_I differs from that of the series of 4.5S RNAs. 4.5S RNA_{III} contains many modified nucleosides (10,11), but the series of 4.5S RNAs does not contain any minor nucleoside (1, our unpublished data). Therefore, neither 4.5S RNA_I nor 4.5S RNA_{III} are identical to the series of 4.5S RNAs. Since 4.5S RNA_{II} has not been analyzed in detail, it is uncertain whether it is related to the series of 4.5S RNAs.

Sequence studies of these series of 4.5S RNAs are now in progress.

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REFERENCES

1. Peters, G.G., Harada, F., Dahlberg, J.E., Panet, A., Haseltine, W.A. and Baltimore, D. (1977) J. Virol. 21, 1031-1041
2. Harada, F. and Ikawa, Y. (submitted to Nucleic Acids Res.)
3. Brawerman, G., Mendecki, J. and Lee, S.Y. (1972) Biochemistry 11, 637-641
4. Ikemura, T. and Dahlberg, J.E. (1973) J. Biol. Chem. 248, 5024-5032
5. Ikemura, T., Shimura, Y., Sakano, H. and Ozeki, H. (1975) J. Mol. Biol. 96, 69-86
6. Sanger, F., Brownlee, G.G. and Barrell, B.G. (1965) J. Mol. Biol. 13, 373-398
7. Jelinek, W. and Leinwand, L. (1978) Cell 15, 205-214
8. Ro-Choi, T.S., Moriyama, Y., Choi, Y.C. and Busch, H. (1970) J. Biol. Chem. 245, 1970-1977
9. Ro-Choi, T.S., Reddy, R., Henning, D., Takano, T., Taylor, C.W. and Busch, H. (1972) J. Biol. Chem. 247, 3205-3222
10. El-Khatib, S.M., Ro-Choi, T.S., Choi, Y.C. and Busch, H. (1970) J. Biol. Chem. 245, 3416-3421
11 Reddy, R., Ro-Choi, T.S., Henning, D., Shibata, H., Choi, Y.C. and Busch, H. (1972) J. Biol. Chem. 247, 7245-7250