ISOLATION AND CHARACTERIZATION OF "8S" RNA IN MURINE SARCOMA VIRUS-INFECTED CELLS.

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SUMMARY : Three new 8S RNA species were identified in rat cells chronically infected with Moloney Murine Sarcoma Virus. Among them, two have identical electrophoretic mobilities, nucleotide compositions and fingerprints with the $8S_A$ and $8S_B$ RNAs recently found in the mouse sarcoma-leukemia virus, M-MSV (MLV) ; the third cellular 8S component, called X, is not incorporated in the virus. Experiments of cellular 8S RNA heat treatment suggest that the $8S_A$ and $8S_B$ RNAs are conformational isomers of each other. The different relative proportions of the two A and B components in the host cell and in the virus and the role of the 8S RNA are discussed.

INTRODUCTION

The presence of"8S" RNA, free (1) or 70S RNA-associated (2) was described in the Mouse Sarcoma Virus, M-MSV (MLV). This small RNA could be analogous to the 7S RNA found in many oncornaviruses (3) (4). Of all known small cellular RNAs, not one hitherto corresponded to the viral 8S RNA. Therefore, we have studied the low-molecular-weight RNAs of the rat cells chronically infected with the MSV (MLV). In this communication, we report the isolation and the characterization of three new cellular 8S RNA components. Two of them, called $8S_A$ and $8S_B$ are similar to the A and B components of the viral 8S RNA. Futhermore, our studies on heat treatment of cellular 8S RNA suggest that the $8S_A$ and $8S_B$ components may be two conformational states of the same molecule.

MATERIALS AND METHODS

Préparation of radioactive cells

78 A_1 is a line of rat cells chronically infected with Moloney Murine Sarcoma Virus (5). Cells were grown as monolayers in a Eagle's Minimal Essential Medium (MEM) supplemented with 10 % heat-inactivated calf serum. Cell cultures were incubated for at least 18 hours with phosphate-free medium containing 10 % dialyzed calf serum and ^{32}P-orthophosphate (20-30 µCi/ml ; CEA) or with MEM containing ^{3}H-uridine (30 µCi/ml-CEA).

Preparation of cellular RNA and cell fractionation

$78A_1$ cell monolayers were trypsinized and washed with phosphate-buffered saline (PBS). Cells were suspended with acetate buffer (0,02 M Na acetate pH 5,1) and lysed with a final concentration of 0.2 % sodium dodecyl sulfate (SDS). The suspension was subjected to three successive phenol extractions at 4°C. RNA was recovered by precipitation in the presence of 0.1 M NaCl with two volumes of ethanol and stored at - 20°C.

Cytoplasm was separated from nuclei in the following manner. The washed cells were resuspended in hypotonic RBS buffer (0.01 M Tris, 0.01 M NaCl, 0.0015 M $MgCl_2$, pH 8.5) and the Nonidet P40 was added at a final concentration of 1 % . After 15 min. at 4°C, the cell suspension was homogenized with 15 strokes of a glass Dounce homogenizer. Nuclei were removed by centrifugation at 3,000 rpm for 10 min. and the supernatant cytoplasm was saved. The nuclear pellet was resuspended with RSB buffer and twice similarly treated.

Low molecular weight cell RNA was separated from 28S and 18S ribosomal RNAs by 5% - 20% sucrose gradient centrifugation at 22,000 rpm for 17 hours at 4°C in a Spinco SW25.1 rotor.

Preparation of viral RNA

M-MSV (MLV) was isolated and purified according to published methods (1). Viral RNA was isolated by the cold phenol-procedure as previously described (6). The high and low molecular weight viral RNAs were separated by 5 % - 20 % sucrose gradient centrifugation for 65 min. at 40,000 rpm in a Spinco SW41 rotor.

Polyacrylamide gel electrophoresis

The low molecular weight cellular and viral RNAs were analyzed by electrophoresis through 10 % polyacrylamide gels according to Tiollais et al (7).

Heat treatment

Low molecular weight cellular RNA was dissolved in acetate buffer. Aliquots of the RNA preparation were heated for four minutes at various temperatures, quickly cooled in ice and finally subjected to polyacrylamide gel electrophoresis.

Determination of nucleotide composition

^{32}P RNA components were eluted from the polyacrylamide gel with acetate buffer. Slices were homogenized with 15strokes of a glass homogenizer. Gel particles were removed by centrifugation and RNA was precipitated with ethanol and carrier RNA. After centrifugation, RNA was hydrolyzed with 10 µl of 0.5 N NaOH at 37°C for 18 hours. Hydrolysate was subjected to electrophoresis on Whatman n°1 paper in pyridine-acetic acid buffer pH 3.5 (3,000 V, 1 hour). Mononucleotides were located by radioautography. Spots were cut out and counted with 10 ml of scintillation fluid.

Oligonucleotide fingerprints

^{32}P RNA components, purified by electrophoresis through and elution from polyacrylamide gel as described above, were hydrolyzed with ribonuclease T_1. Oligonucleotides were analyzed by the fingerprinting procedure reported by Sanger et al (8).

RESULTS

Analysis of low-molecular-weight $78A_1$ cellular RNA on 10 % polyacrylamide gel : isolation of 8S RNA species

The ^{3}H-labeled low-molecular-weight $78A_1$ cell RNA was isolated after sucrose gradient sedimentation as described in Materials and Methods and was analyzed by electrophoresis in a 10 % polyacrylamide gel (Fig. 1 dashed line). ^{32}P-labeled low molecular weight viral RNA was simultaneously migrated through the same gel (Fig. 1 solid line). The cellular RNA profile shows in addition to the cytoplasmic 4S and 5S RNAs and the previously described nuclear RNAs C,D,E,H (9) (10), the presence of three new RNA components having a slower mobility than nuclear C RNA. The first major component migrates with viral $8S_B$ RNA species recently described (1), the second corresponds to viral $8S_A$ component and the third, called X, has a lower electrophoretic migration than the other ones and is not present in the virus. According to their relative proportion to the cellular 5S RNA amount, it has been calculated that these new cellular 8S RNA species represent 0,2 to 0,3 % of the total cellular RNA. We note that the $8S_A$ and $8S_B$ RNA proportions are different in virus and host cells. While $8S_A$ and $8S_B$ RNA amounts are respectively 70 % and 30 % in the MSV (MLV), they are 20 % and 80 % in $78A_1$ cells (these percentages are calculated with RNAs analyzed at room temperature).

Properties of $78A_1$ cell 8S RNA

Molecular weight

The molecular weight of the three new cellular 8S RNA species has been computed from their relative electrophoretic mobility in 10 % polyacrylamide gels using 4S RNA and 5S RNA as reference standards. The estimations of molecular weight by this method are $80,000 \pm 5,000$ for $8S_B$RNA, $85,000 \pm 5,000$ for $8S_A$ RNA and $90,000 \pm 5,000$ for X species. These values could not be determined with accuracy because gel electrophoretic mobility of 8S RNA is subjected to slight variations. This last point is not yet elucidated. Moreover, the calculation did not take into consideration the effect of RNA conformation on relative electrophoretic mobility.

Studies on the 8S RNA conformation by the effect of temperature

Preliminary experiments (not described in this communication) have

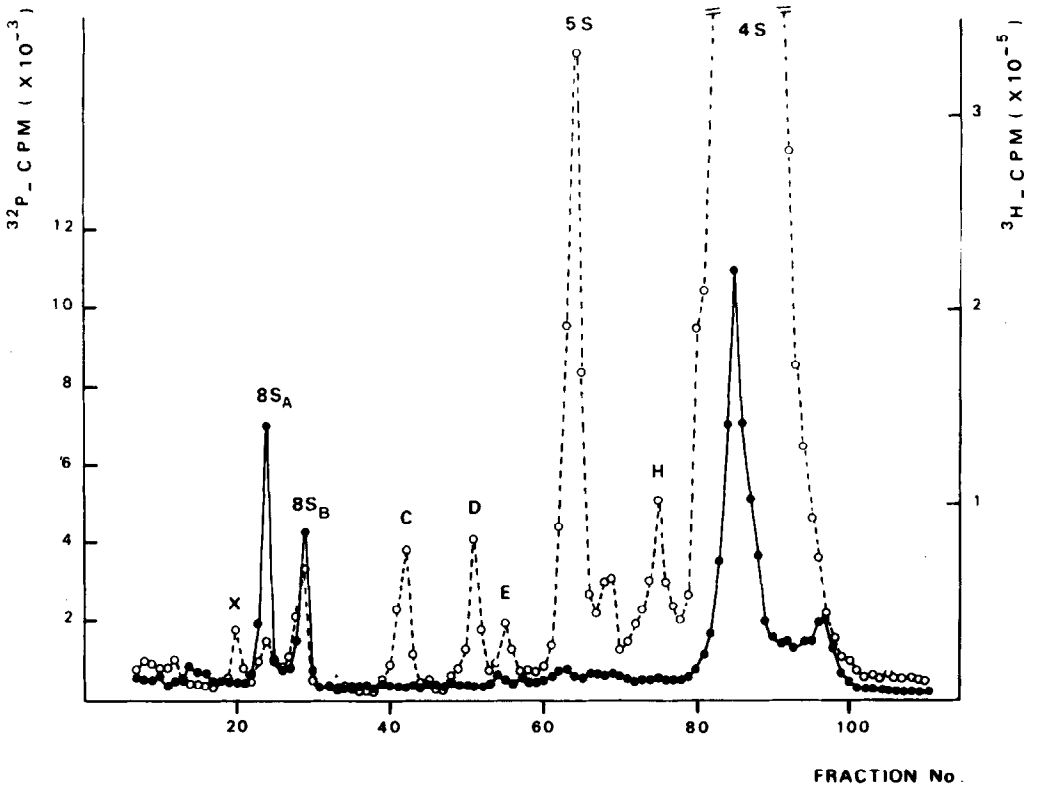

Fig.1 : Coelectrophoresis of 78A$_1$ cellular low-molecular-weight ^{3}H-labeled RNA and M-MSV (MLV) low-molecular-weight ^{32}P-labeled RNA in a 10 % polyacrylamide gel. The cellular total RNA was prepared according to Materials and Methods. The low-molecular-weight cellular RNA (4-10S) was resolved from the high-molecular-weight RNA by sucrose gradient sedimentation and finally subjected to electrophoresis in a gel of 10 % polyacrylamide. The low-molecular-weight viral RNA, prepared as described in Materials and Methods, was electrophoresed in the same gel. Electrophoresis was carried out overnight at room temperature (4 mA/gel). The gel was sliced into 1,5 mm fractions. ^{32}P was located by Cerenkov counting. To measure ^{3}H-radioactivity, gel slices were hydrolyzed in H_2O at 60°C and counted in 10 ml of Bray's solution. o- - - - -o ^{3}H ; ●——● ^{32}P.

shown that an aggregation process can be observed in 8S RNA solutions of high ionic strength. Therefore, 8S RNA was redissolved in a low ionic concentration buffer (acetate buffer) before heat treatment. Fig.2 shows electrophoregrams of cellular ^{32}P-labeled 8S RNA heated at various temperatures between 45°C and 80°C. ^{3}H-labeled unheated cellular 8S RNA components were used as internal standards (arrows-Fig.2) An increase in heating temperature induced conversion of B component into A component. The 80°C gel-profile shows only 8S$_A$ species. On the other hand, the electrophoretic mobility of X component does not change during thermal denaturation. According to the relative proportion of A and B

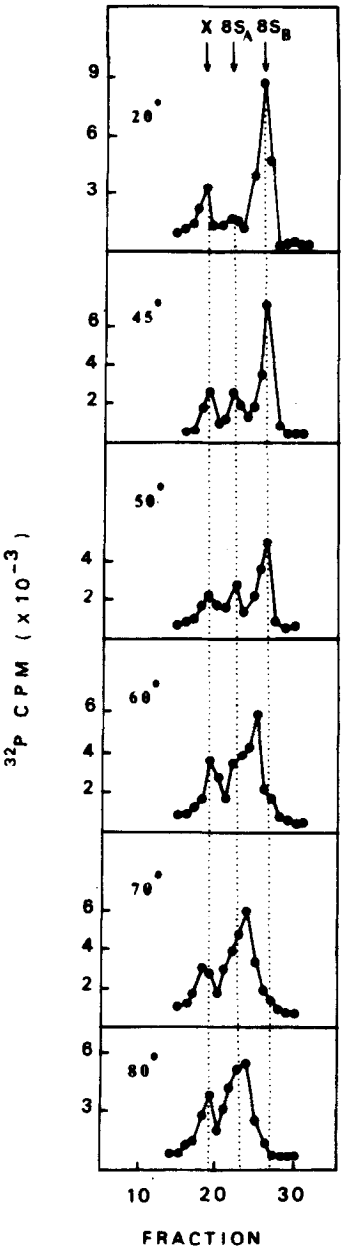

Fig. 2 : Electrophoresis of 78A$_1$ cell heat-treated 8S RNA in gels of 10 % polyacrylamide. Cellular low-molecular weight ^{32}P labeled-RNA was dissolved in acetate buffer. One sample was unheated (20°C) and five samples were heated at the indicated temperature for 4 min. , rapidly cooled and layered directly onto the gel. Only the 8S RNA species are illustrated in the figure. The arrows indicate the position of the three cellular unheated ^{3}H-labeled RNA components used as internal reference markers in the gels.

TABLE 1 : Nucleotide compositions of cellular and viral 8S RNA.

RNA	G	C	A	U	G+C
Cellular 8S (B species)	32,4	27,4	19,8	20,4	59,8
Viral 8S (A + B)	33,7	28,1	20,1	18,1	61,8

Cellular total ^{32}P-labeled RNA was extracted from $78A_1$ cells and M-MSV (MLV) ^{32}P-labeled RNA was extracted from the corresponding supernatant fluids of $78A_1$ cells as described in Materials and Methods. Cellular 8S RNA (B species) and viral 8S RNA (A + B) were isolated by 10 % polyacrylamide gel electrophoresis, eluted from corresponding gel slices as described in Materials and Methods and after precipitation with ethanol, analyzed for nucleotide composition. The results are expressed as mole percentage of nucleotide and represent the average values from two experiments.

components for the different temperatures,half of theB molecules are converted into A component at about 56°C. These results indicate that $8S_A$ and $8S_B$RNA components are conformational isomers of each other. It is likely that these observations are related to our previous results (11) that MSV (MLV) 8S RNA heated at 80°C contained only A species.Such a conformational phenomenon has been described for cellular 5S RNA (12).

Nucleotide composition

The nucleotide composition of purified $8S_B$RNA from $78A_1$ cells is illustrated in Table 1. A high guanosine plus cytosine content (G + C about 60%) is noted which differentiates specially this RNA from nuclear low molecular weight (G + C < 55 %) (9). The nucleotide composition of cellular $8S_B$ RNA is similar to that from viral 8S RNA (Table1). Analysis of small oligonucleotides produced by T_1 and pancreatic ribonuclease digestion has been done on the three cellular purified 8S RNA components (Table 2). There is a identity in oligonucleotide relative yields of $8S_A$ and $8S_B$ RNAs, whereas X component exhibits differences, particularly in the AAG, AG and G proportions.

Fingerprint

The radioautograph obtained after ribonuclease T_1 digestion of cellular $8S_B$RNA is shown in Fig. 3 (A). For comparison, a fingerprint of

TABLE 2 : Oligonucleotide compositions of the three species of cellular 8S RNAs. (a)

Oligonucleotide	$8S_A$RNA	$8S_B$RNA	X RNA
(AAA...N)n	5,41	4,09	6,88
AAAG	1,83	1,96	
AAAU	0,61	0,45	2,28
AAAC	1,01	1,07	0,86
AAG	2,97	2,62	4,10
AAU	2,15	2,13	1,42
AAC	1,32	1,25	1,26
AG	11,61	12,80	9,01
Ax(b)	0,97	0,70	0,64
AU	5,32	5,62	6,67
AC	5,71	5,96	5,23
G + G cyclique	23,73	24,33	20,97
C + C cyclique	22,87	22,54	24,19
U + U cyclique	14,26	14,23	16,32

(a) Cellular ^{32}P-labeled RNA was extracted from $78A_1$ cells. The three components $8S_A$, $8S_B$ and X RNA were isolated by 10 % polyacrylamide gel electrophoresis, eluted from gel slices as described in Materials and Methods and precipitated with ethanol. The RNA was then hydrolyzed with 15 µl of ribonuclease T_1 (2500 U/ml) and 15 µl of ribonuclease A (1 mg/ml) for 60 min. at 37°C.Products of enzyme di gestion were subjected to electrophoresis on DEAE cellulose paper (Whatman DE81) in pyridine-acetic acid buffer pH 3,5 (1500 V, 3hours). Radioactive oligonucleotides were located by radioautography, cut out and counted in 10 ml of scintillation fluid. The results are expressed as mole percentage of oligonucleotide and represent the average values from two experiments.
(b) The dinucleotide Ax has not been characterized.

viral 8S (A + B) RNA is included in the figure 3 (B). The cellular $8S_B$RNA and the viral 8S RNA yield the same fingerprint. In contrast, the X component fingerprint (Fig.3C) reveals differences from the other ones.

Cellular localization of 8S RNA

$78A_1$ cell ^{32}P-labeled 8S RNA species were investigated into cytoplasmic and nuclear fractions. The polyacrylamide gel electrophoregram of the low molecular weight cytoplasmic RNA shows the presence of the three

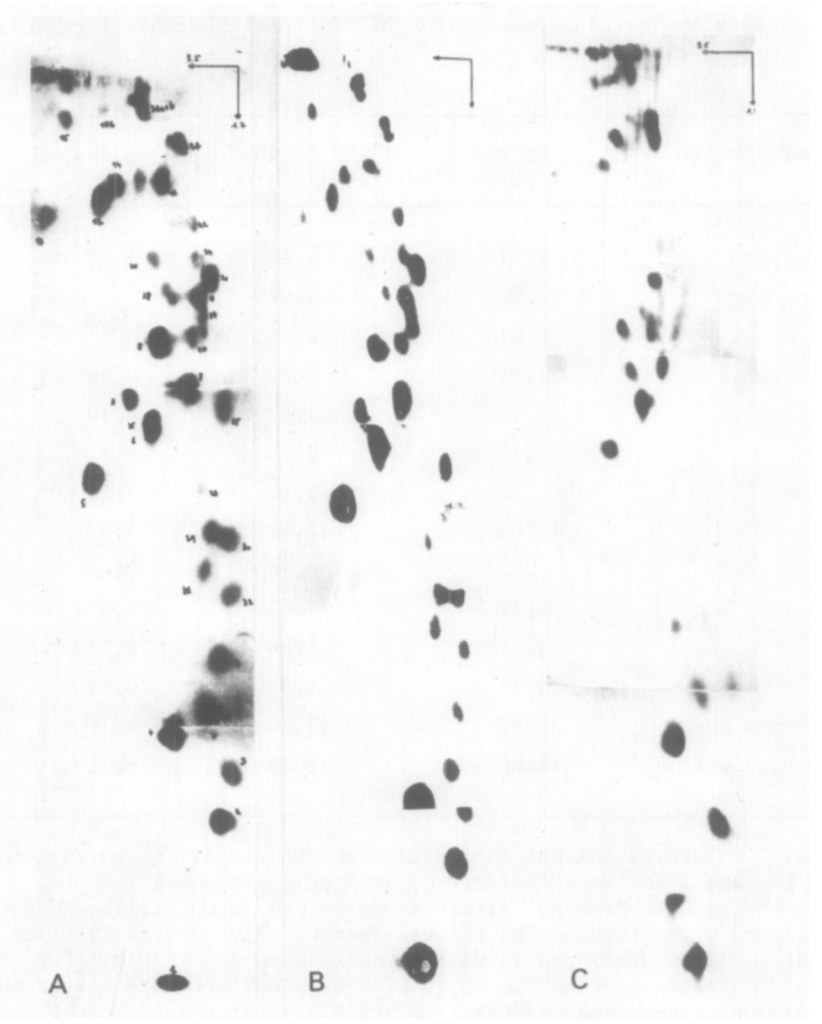

Fig.3 : Radioautographs of the two-dimensional electrophoretic separation of the ribonuclease T_1 digests of three 8S RNAs components. A, $78A_1$ cell $8S_B$ RNA; B, M-MSV (MLV) 8S RNA (A + B) ; C, $78A_1$ cell X RNA component. Each of the 8S RNA components were purified by 10 % polyacrylamide gel electrophoresis, eluted from the corresponding slices as described in Materials and Methods, precipitated with ethanol and digested with ribonuclease T_1 for fingerprinting according to the procedure of Sanger et al (8).

8S RNA components mainly B species (Fig.4A). Fig.4B illustrates gel profile of low molecular weight nuclear RNAs. Characteristic nuclear RNAs and X component are present ; a discrete peak of $8S_B$RNA can also be seen.

DISCUSSION

To understand the origin of the 8S RNA recently found in M-MSV (MLV), low molecular weight RNAs of host cells have been analyzed. In this

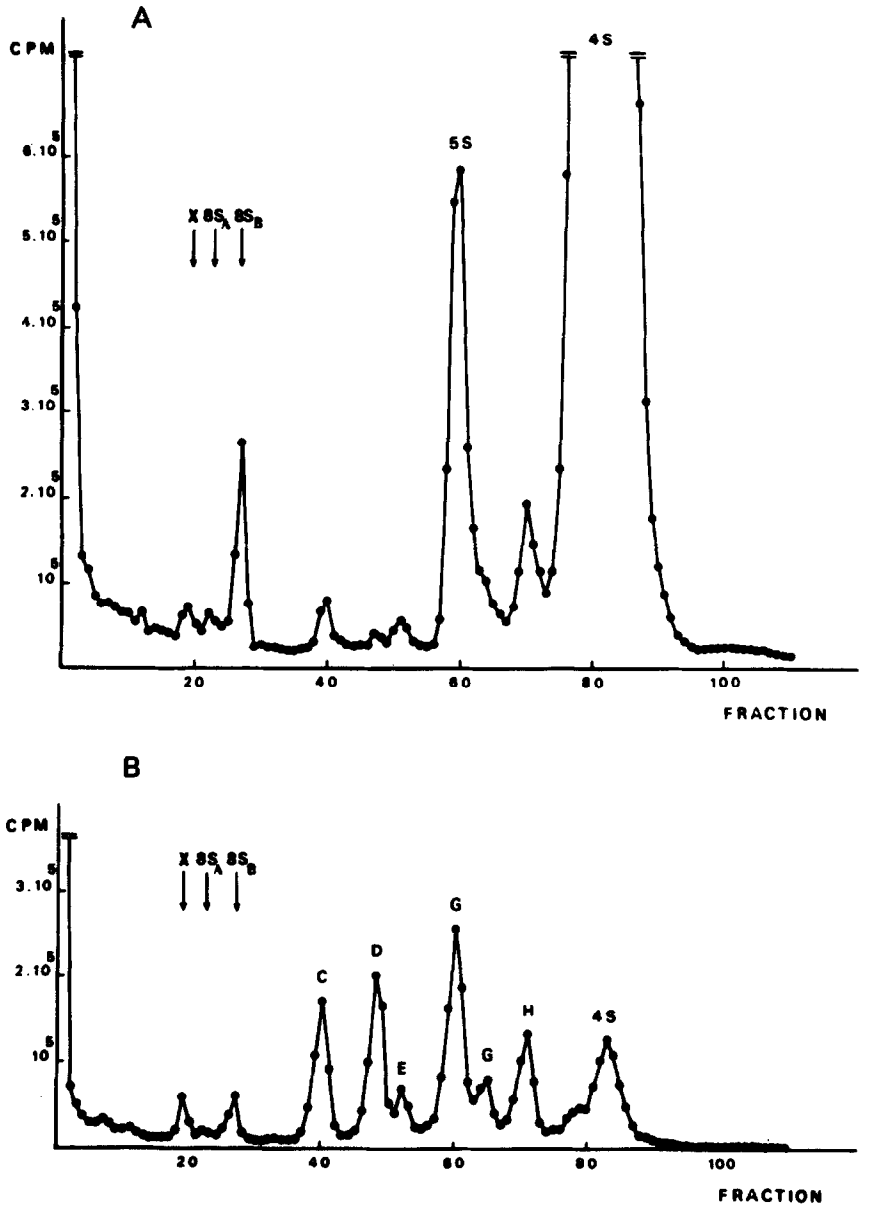

Fig.4 : Cellular localization of 8S RNA. ^{32}P-labeled 78A$_1$ cells were fractionated into nuclear-cytoplasmic fractions. Low-molecular-weight RNAs of each fraction were subjected to electrophoresis in gels of 10 % polyacrylamide. A, Cytoplasmic fraction ; B, Nuclear fraction. The arrows indicate the position of cellular ^{3}H-labeled 8S RNA components used as internal standards.

communication we report the identification of three new 8S RNA species in rat cells chronically infected by the virus. Two among them are essentially located in the cytoplasm and are very similar to viral 8S$_A$ and 8S$_B$ RNA. The third one, called X, not incorporated inside the virus is found in the nuclear and cytoplasmic fractions.

The presence of cellular nucleic acids such as 4S RNA (13), 5S RNA (14) and ribosomal 28S and 18S RNAs (15) has been previously described in oncornaviruses. A similar situation seems to exist with the 8S RNA. However this RNA shows peculiar characteristics. It is formed of two conformational isomers A and B and their relative proportions are not similar in the virus and the host cell : A form is predominant in virus while we found for the most part B form in the cells. Two explanations of this difference may be examined : 1) during the process of budding from the cell surface, virus may trap preferentially A form or 2) since 8S RNA conformation easily changes, B form may be converted into A form when the virus is produced.

No role or specific function of this new 8S RNA is yet known. Its characterization inside the M-MSV (MLV) and the virus-infected cells could have suggested that it was virus-induced. However the presence of a similar RNA in non-infected rat cells (unpublished personal results) appears to indicate that it is a usual cellular RNA incorporated in the virus upon budding. It would also be interesting to compare it with the 7S RNA reported by Erikson et al from uninfected chick fibroblasts (4). Further experiments are in progress to investigate the function of cellular 8S RNA.

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