Identity of the two 8S RNA components of the mouse sarcoma virus (Moloney)

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ABSTRACT

The two 8S A and B RNAs of the Mouse Sarcoma Virus (Moloney) can be converted by heating into a homogeneous population. After digestion with T₁ RNase, they give identical fingerprints. It is concluded that these two molecules represent conformational isomers of the same molecular species.

INTRODUCTION

The major Ribonucleic acid component of Oncornaviruses is a 60-70S complex which constitutes the viral genome (1). Among the smaller molecular species which are present in the virions, a 7-8S RNA has been reported in the avian as well as in the murine Oncornaviruses (2, 3, 4, 5). We demonstrated previously that the 8S RNA of the Mouse Sarcoma Virus M-MSV(MLV) is resolved in a 10 % polyacrylamide gel into two bands which have been called A and B (3). These two bands could represent two different RNA species or two conformational states of the same molecule. In this report, we present evidence that the 8S_A and 8S_B RNAs are conformational isomers of each other : after heating, they have the same electrophoretic mobility and they possess the same primary structure as judged by fingerprinting analysis.

MATERIALS AND METHODS

Methods for growing and labelling the virus-producing cell line have been previously described (3). Virus stocks were obtained from 24 hours culture supernatants and were purified by isopycnic centrifugation in a sucrose gradient (10-70 %, w/w ; 0.01 M Tris-HCl, pH 7.4, 0.1 M NaCl, 0.001 M EDTA). Nucleic acids were extracted by the cold phenol Sodium Dodecylsulfate procedure (6). Heavy (70S) and light (4 to 8S) RNAs were separated by velocity centrifugation through a 5-30 % sucrose gradient. Light RNA was further analyzed by electrophoresis in 10 % polyacrylamide gels (7). When 8S RNAs were to be fingerprinted, purification was achieved by chromatography of the light RNA on a methylated albumin (MAK) column, followed by filtration on G-100 Sephadex co-

lumn (90 x 1.8 cm, 0.1 M NaCl, 0.02 M Sodium Acetate, pH 5.0). Purified RNA was digested with T_1 RNase (Sankyo) and subjected to the two dimensional analysis as described by Sanger et al (8).

RESULTS AND DISCUSSION

When light RNA previously separated from the 70S RNA by sucrose gradient centrifugation was subjected to electrophoresis in a 10 % polyacrylamide gel, a pattern presented on fig. 1A was obtained. As previously reported, $8S_A$ and $8S_B$ RNAs were clearly resolved from each other (3). An aliquot of the same (original) preparation was heated for 3 mn at 80°C before being applied at the top of the gel (fig. 1B). A single peak moving at the position of the $8S_A$ species was still present but there was no more $8S_B$ peak. To decide whether the $8S_B$ RNA had been converted into the $8S_A$ species by the hot treatment, pure $8S_B$ RNA was prepared, heated in the same conditions as above, then electrophoresed again in a 10 % gel. The heated RNA moved at the position of the $8S_A$ species.

From the foregoing results, we concluded that $8S_A$ and $8S_B$ RNAs could be two conformational forms of the same molecule. Fingerprinting studies were undertaken to test this hypothesis. Total 8S RNA was digested by T_1 RNase and analyzed by the two-dimensional electrophoresis procedures (fig. 2). Table I gives the list of the products and their relative yield. Separate fingerprints of the $8S_A$ and $8S_B$ were obtained and compared to the one of the total 8S RNA. No difference was noted between the three radioautographs.

In this report, we have shown that the two 8S RNA of the MSV (MLV) virions are the two forms of the same molecular species. The B form is converted into the A form by thermal denaturation. We assume that this is due to a conformational transition analogous to that described for 5S RNA (9). As for this species, no correlation has been established between this change and any biological function. In the case of the 8S RNA, it may be significant to note that it is present in infected cells mostly under the B form (10). It is plausible that the conformational transition takes place during the maturation of the virions or in the initial moments of the extracellular life.

Examination of the T_1 fingerprints of the $8S_A$ and $8S_B$ RNAs of the MSV (MLV) confirmed that they are two conformational isomers. As it was difficult to get sufficient amounts of radio-active products needed for a detailed analysis of each form, fingerprints of total 8S RNA were obtained and compared to the autoradiographs of $8S_A$ and $8S_B$ RNA respectively. All the spots observed on the pattern of the total 8S RNA were found in the same relative intensities on the patterns of the A and the B forms.

Erikson et al recently analyzed the 7-8S RNAs of several avian or

murine oncornaviruses (5) by fingerprint analysis and have found differences clearly related to the cellular origin of the viruses. The Mouse Sarcoma Virus which they used was grown on hamster cells, while the MSV(MLV) employed in our

Figure I : Polyacrylamide gel electrophoresis of light MSV (MLV) RNA. ^{32}P RNA was analyzed in 10 % polyacrylamide gel. Migration was performed overnight at 120 V and 3mA per gel. 1.5 mm slices were cut out and ^{32}P RNA was located by Cerenkov counting in a Packard scintillation counter. a) Control RNA. b) Heated RNA (3' at 80°C in NTE buffer pH 7.4).

laboratory came from rat cells. In spite of this difference, the fingerprints of the 7-8S MSV RNAs are almost identical with the exception of the spot n°25 which is not present on the radioautograph presented by Erikson et al. Some slight differences also appear in the relative yields of a few nucleotides, but they most probably result from uncertainties of the measurements.

Presence of 7-8S RNAs in Oncornaviruses seems to be a general feature as their existence has been reported in several viruses of avian or murine origin (2, 3, 4, 5). However, the cellular origin of these RNAs is suggested by two lines of facts : i) they are located in the viral envelope which comes from the cell membrane (3) ; ii) they have been found in uninfected cells (5, 10). Therefore the minor but consistent differences observed at the level of their primary structure most likely reflect evolutionary divergences of the animals from which the cells were derived.

The function of these molecules in the cell is totally unknown. At present one cannot decide if they are accidentally acquired by the virions at the time of the budding process and/or have specific function when a virus infects a new cell.

Figure II : Oligonucleotide pattern of the two-dimensional fractionation of ribonuclease T₁-digested 8S_{A+B} RNA. Spots numbers refer to those of the Table I.

TABLE 1

Spot number	Relative molar yield (a)	PRODUCTS OF DIGESTION WITH T ₁ RNase					Deduced sequence
		C	Alkaline hydrolysis(b)			Pancreatic RNase analysis (c)	
			A	G	U		
1	29.6	0	0	+	0	G	G
2	6.4	1	-	1	0	C,G	CG
3	3.2	2	-	1	0	C(2),G(1)	CCG
4	10.0	0	1	1	0	AG	AG
5	8.0	0	0	1	1	U(1),G(1)	UG
6	4.0	1	0	1	1	C(1),U(1),G	(C,U)G
7	3.7	2	0	1	1	C(2),U(1),G	(C,C,U)G
8	2.1	1	1	1	1	AU(1),C(1),G	(AU,C)G
9	3.1	0	1	1	1	U(1),AG(1)	UAG
10	0.7	3	1	1	1	U(1),C(3),AG	(U,C ₃)AG
11	1.0	2	2	1	1	U(1),C(2),AAG	(U,C ₂)AAG
12	2.1	1	0	1	2	U(2),C(1),G	(U,U ₂)C)G
13	1.1	0	0	1	2	U(2),G	UUG
14	1.1	1	1	1	2	AU(1),C(1),U(1),G	(AU,C,U)G
15	1.5	1	0	1	3	U(3),C(1),G	(U ₃ ,C)G
16	0.9	3	1	1	2	AC(1),U(2),C(2),G	(AC,U ₂ ,C ₂)G
17	0.8	2	2	1	1	U(1),C(1),AC(1),AG	(U,C,AC)AG
18	1.1	1	2	1	1	AU(1),C(1),AG	(AU,C)AG
19	1.1	2	1	1	1	U(1),C(2),AG	(U,C ₂)AG
20	1.0	0	3	1	1	AAU(1),AG	AAUAG
21(d)		6-7	4	2	2	U(2),C(4),AC(3) AG(1),G(1)	
22	0.8	2	4	1	1	C(1),AAC(1),AU(1)	(C,AAC,AU)AG
23-A)	(g) 1.1	2	4	1	3	AAU(1),AU(2),C(2),G	(AAU,AU,AU,C,C)G
23-B)							
24	0.7	2	3	1	1	U(1),C(1),AC(1),AAG	(U,C,AC)AAG
25	0.7	4	3	1	0	AC(2),C(2),AG	(AC,AC,C,C)AG
26	1.1	1	3	1	0	AAAC(1),G	AAAC G
27	0.6	5	4	1	1	AAAAC(1),C(4),U(2), G	(AAAAC,C ₄ ,U ₂)G
29	0.9	2	2	1	0	C(1),AAC(1)G	(C,AAC)G
30	0.8	4	1	1	0	C(3),AC(1),G	(C ₃ ,AC)G
31	0.9	1	2	1	0	AC(1)AG	ACAG
32	0.7	3	1	1	0	C(3),AG	CCCAG
33	1.4	2	1	1	0	C(1),AC(1)G	(C,AC)G
34	1.7	1	1	1	0	C(1),AG	CAG
15b(e)	0.4	+			+	U(4),C(2)	(U ₄ ,C ₂)X _{OH}

Table I : a) Relative yield of the oligonucleotides. Each oligonucleotide was located by radioautograph cut out from the DEAE paper and counted in scintillation vials. Indicated values are the average of 5 determinations. The spot n°22 was taken as reference for these calculations.

b) Base composition was determined by hydrolysis for 18 hours at 37°C with 0.5 N KOH. Mononucleotides were separated by electrophoresis at 3,000 volts in the Pyridine-acetic acid pH 3.5 system.

c) Pancreatic RNase was used at the concentration of 400 µg/ml for 1 hour at 37°C. (Continued on the next page).

d) Spot 21 is a mixture of two oligonucleotides. The global composition gives U₂, C₄, AC₃, G₁, AG₁. Each oligonucleotide is represented once in the molecule.

e) Spot 15b most likely represents the 3' end of the molecule. It is only one lacking G.

f) The nature of the spot X is undetermined. Such material was also mentioned by Erikson et al (5).

g) The spot 23 can be dedoubled during the second dimension.

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