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NUCLEOTIDE SEQUENCE OF 4.5S RNA (C8 OR hY5) FROM HeLa CELLS

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SUMMARY : The nucleotide sequence of C8 RNA, one of the 4.5S RNAs of HeLa cells, was determined. C8 RNA consists of 83 or 84 nucleotide residues containing pppA at its 5'-terminus and oligo U at its 3'-terminus. This RNA is rich in uridylate residues (about 38 %) and does not have any modified nucleosides. C8 RNA is present mainly in the cytoplasm, with some in the nucleus and the C8 RNAs from the nucleus and cytoplasm have the same sequence.

INTRODUCTION

In mammalian cells, several low molecular weight RNAs that differ from tRNA, 5S rRNA and 5.8S rRNA, have been reported (for review, see 1 and 2). Previously, we and others reported 4.5S RNA associated with nuclear and cytoplasmic poly(A) containing RNA in rodent cells (4.5S RNA_H) (3-5). The nucleotide sequence of this RNA, which shows homology with the rodent Alu-equivalent consensus sequence, has been determined (6-8). This RNA is specific to cells of small rodents, such as mice, rats and hamsters, and is not found in other vertebrates, such as humans, monkeys, cats, minks, rabbits, guinea pigs or chickens (4). To determine whether human cells contain RNA possessing analogous characters to 4.5S RNA_H, we analyzed low molecular weight RNAs of HeLa cells.

MATERIALS AND METHODS

Materials. The sources of the materials used in this study have been described previously (9).

Purification of low molecular weight RNAs. HeLa cells were grown in phosphate-free RPMI 1640 medium containing 0.67 mCi of $^{32}PO_4^{3-}$ per ml and 10 % dialyzed fetal calf serum. After 20 hours of labeling, cells were washed twice with TSE buffer (20 mM Tris-HCl, pH 7.5; 0,1 M NaCl; 1 mM EDTA), and the cytoplasmic fraction was prepared by a published method (10) without using detergent. Mitochondria were precipitated by centrifugation at 10,000g for 10 min. Cytoplasmic RNA was purified by phenol-sodium dodecyl sulfate extraction and ethanol precipitation from the post-mitochondrial supernatant (3). Nuclear RNA was purified as described previously (3). Nuclear and cytoplasmic low molecular weight RNAs were fractionated by two dimensional polyacrylamide gel (2-D gel) electrophoresis (3).

Unlabeled C8 RNA was isolated from a large amount of HeLa cells (~10^9 cells) as follows. The cytoplasmic RNA, prepared as described above, was loaded on a column of DEAE-Sephadex A-50 (1.2 cm x 4.5 cm). The column was washed with 0.2 M NaCl-10 mM $MgCl_2$-10 mM Tris-HCl (pH 7.5), and low molecular weight RNA was eluted from the column with 1 M NaCl-10 mM $MgCl_2$-

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10 mM Tris-HCl (pH 7.5). This low molecular weight RNA was mixed with uniformly ^{32}P-labeled cytoplasmic RNA as marker and fractionated by 2-D gel electrophoresis. The C8 RNA fraction was eluted, labeled with [5'-^{32}P]pCp at the 3'-end (11) and purified by 2-D gel electrophoresis. Since the 3'-terminus of C8 RNA is heterogeneous with respect to the number of uridylate residues (see text), the 3'-end-labeled C8 RNA was subjected to further electrophoresis in 20 % polyacrylamide gel containing 7 M urea and separated into two bands. Major species (fast moving band) was designated as C8 RNA$_{\text{I}}$ and minor species (slowly moving band) was designated as C8 RNA$_{\text{II}}$.

Sequence analysis. Standard procedures (6,12) were used for enzymatic digestion of uniformly ^{32}P-labeled C8 RNA, fingerprinting and identification of oligonucleotides from fingerprints. Modified nucleotides were analyzed by two dimensional thin layer chromatography (13). Large fragments of uniformly ^{32}P-labeled C8 RNA were obtained by nuclease S1 digestion (9). The total sequence of the C8 RNA was obtained by the chemical sequence method (14) using 3'-end-labeled C8 RNAs.

RESULTS

Purification of 4.5S RNAs. ^{32}P-labeled cytoplasmic RNA was prepared from the post-mitochondrial supernatant of HeLa cells and low molecular weight RNA was separated by 2-D gel electrophoresis (Figure 1a). Nine discrete "4.5S" RNAs (C1-C9) were obtained between tRNAs and 5S rRNA. On the other hand, the nuclear fraction of HeLa cells contained only C1 and C8 RNAs (Figure 1b). It is noteworthy that neither 4.5S RNA$_{\text{H}}$ (4) nor 4.5S RNA$_{\text{I}}$ (15,16) of rodent cells were present in HeLa cells.

Since C1 RNA gave the same RNase T1 fingerprint and the same modified nucleotides as U6 RNA of mouse or rat cells (16,17)(data not shown), it must be U6 RNA of HeLa cells. Because the cellular localization of C8 RNA in HeLa cells is similar to that of 4.5S RNA$_{\text{H}}$ in rodent cells, we tried to determine the primary structure of C8 RNA. The amount of nuclear C8 RNA was half to one third as much as that of cytoplasmic C8 RNA.

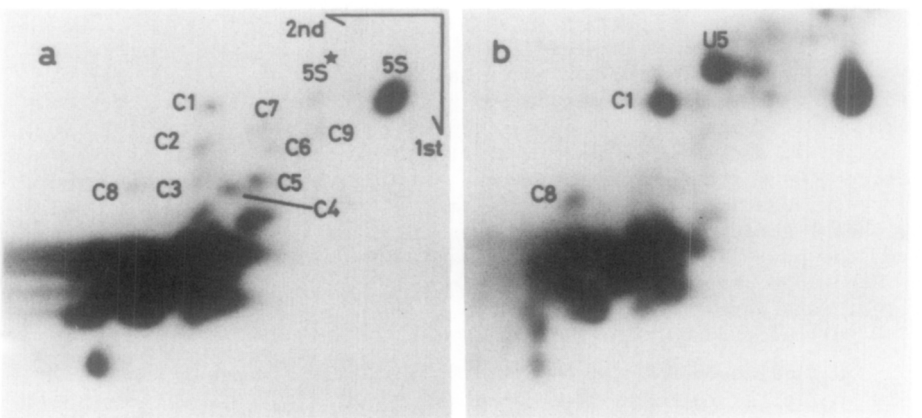


Figure 1. 2-D gel electrophoretic patterns of cytoplasmic (a) and nuclear (b) 4S-5S RNAs from HeLa cells. The nuclear sample was exposed for 20 times longer than the cytoplasmic sample. 5S* is an RNA with a very similar RNase T1 fingerprint to that of 5S rRNA (designated as "5S" in the figure).

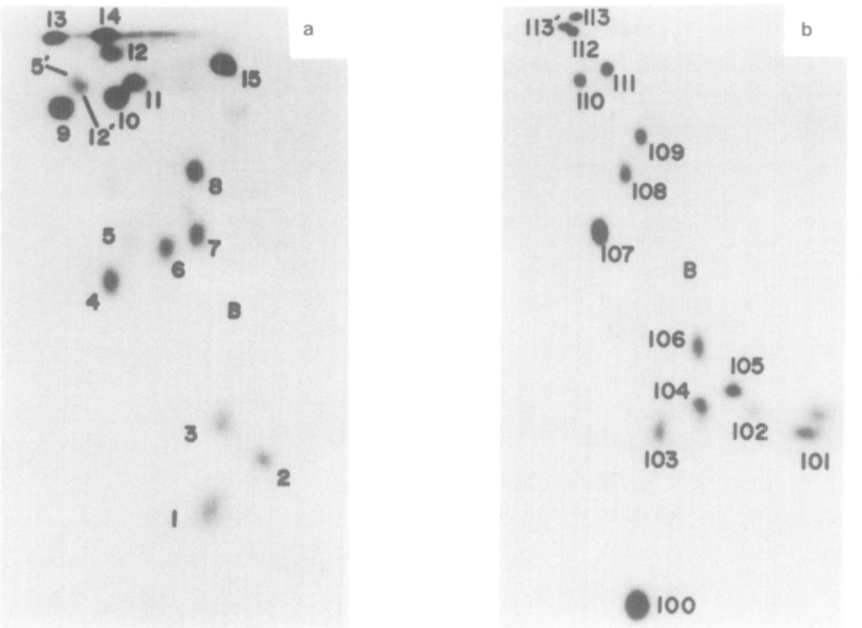


Figure 2. Fingerprints of RNase T1 (a) and RNase A (b) digests of cytoplasmic C8 RNA. The nuclear C8 RNA gave the same fingerprints as the cytoplasmic C8 RNA. Electrophoresis in the first dimension, from right to left, was carried out on cellulose acetate in pyridine acetate (pH 3.5)-7M urea-2.5 mM EDTA. Electrophoresis in the second dimension, from top to bottom, was carried out on DEAE-cellulose in 7 % formic acid. B denotes the position of the blue dye marker (xylene cyanol FF).

Complete RNase T1 and RNase A digestion products of C8 RNA. Figure 2a and b show RNase T1 and RNase A fingerprints of cytoplasmic C8 RNA. Since the nuclear C8 RNA gave the same fingerprints as cytoplasmic C8 RNA (data not shown), the two C8 RNAs must be the same molecule.

The oligonucleotides eluted from the RNase T1 and RNase A fingerprints were each digested with various enzymes and their structures were determined (Tables I and II). C8 RNA did not contain any modified nucleotides. The 5'-terminus of this RNA was pppA-G-Up and a small amount of partially dephosphorylated oligonucleotide (ppA-G-Up) was also obtained. The 3'-terminus of this RNA was heterogeneous in the number of uridylate residues. The 3'-terminus was mainly U-U-U with a small amount of U-U-U-U.

Total nucleotide sequence of the C8 RNA. The total nucleotide sequence of the C8 RNA was determined by analysis of products of nuclease S1 digestion of uniformly ^{32}P-labeled C8 RNA (Figure 4) and by the chemical sequence method (14) using 3'-end-labeled C8 RNA (Figure 3), by comparison with the results of sequencing of complete RNase T1 and RNase A digestion products (Tables I and II). The 3'-end-labeled C8 RNA was separated into species I (U-U-U as the 3'-terminus) and II (U-U-U-U as the 3'-terminus) by re-electrophoresis and the two species were separately sequenced (Figure 3).

Table I. RNase T1 Digestion Products of C8 RNA

Spot No.	Sequence	Molar Yield Measured	Molar Yield Theoretical
1	GP	4.1	3
2	C-Gp	1.0	1
3	A-Gp	1.3	1
4	U-Gp	2.0	2
5	U-U-U	0.7	1
5'	U-U-U-U	0.3	
6	C-U-Gp	1.0	1
7	U-C-C-Gp	0.9	1
8	A-C-U-A-Gp	0.8	1
9	U-U-Gp	2.8	3
10	C-U-U-Gp	2.1	2
11	U-U-A-A-Gp	0.9	1
12	pppA-Gp	0.7	1
12'	ppA-Gp	0.2	
13	U-U-A-U-U-Gp	1.0	1
14	A-U-U-U-A-A-C-A-U-U-Gp	0.7	1
15	U-C-U-C-C-C-C-C-C-A-C-A-A-C-C-Gp	0.8	1

Table II. RNase A Digestion Products of C8 RNA

Spot No.	Sequence	Molar Yield Measured	Molar Yield Theoretical
100	Up	16.5	18-19
101	Cp	9.4	10
102	A-Cp	1.1	1
103	A-Up	2.4	2
104	G-Cp	2.9	3
105	A-A-Cp	1.5	2
106	A-G-Cp, G-A-Cp	1.9	2
107	G-Up	5.3	5
108	G-A-Up	1.3	1
109	A-A-G-Up	1.1	1
110	G-G-Up	1.3	1
111	G-A-G-Up	1.0	1
112	G-G-G-Up	0.8	1
113	pppA-G-Up	0.8	1
113'	ppA-G-Up	0.4	

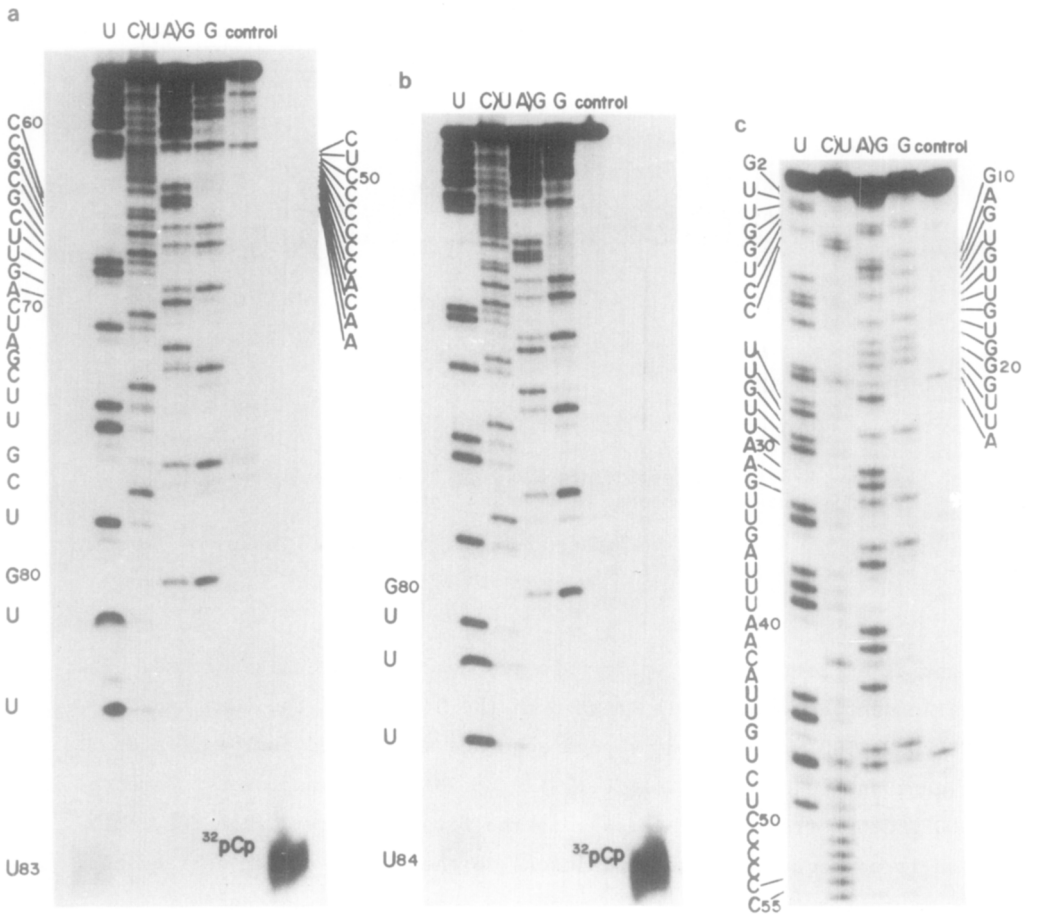


Figure 3. Sequencing gels of 3'-end-labeled C8 RNA_I and C8 RNA_{II}. RNAs were digested chemically (14) and subjected to electrophoresis on polyacrylamide gels (40 x 20 x 0.06 cm for a and b and 60 x 20 x 0.06 cm for c). (a) C8 RNA_I, 20 % gel at 2.5 kV for 2 hrs. (b) C8 RNA_{II}, 20 % gel at 2.5 kV for 2 hrs. (c) C8 RNA_I, 12 % gel at 2.5 kV for 8 hrs.

Heterogeneity between species I and II was only observed in the number of uridylate residues at the 3'-terminus.

The total sequence and nuclease S1 fragments are shown in Figure 4 and a possible secondary structure is shown in Figure 5.

DISCUSSION

C8 RNA consists of 83 or 84 nucleotide residues containing 31 or 32U, 20G, 18C and 14A. This RNA is rich in uridylate residues (about 38 %) and does not contain modified nucleotide. Since C8 RNA contains triphosphates at its 5'-terminus and oligo U at its 3'-terminus, it must be the primary transcript of RNA polymerase III and not a degradation product of higher molecular weight RNA. Recently, there have been many reports on transcription initiation by RNA polymerase III (for review, see 18). According to these reports, RNA polymerase promoter is intragenic and the

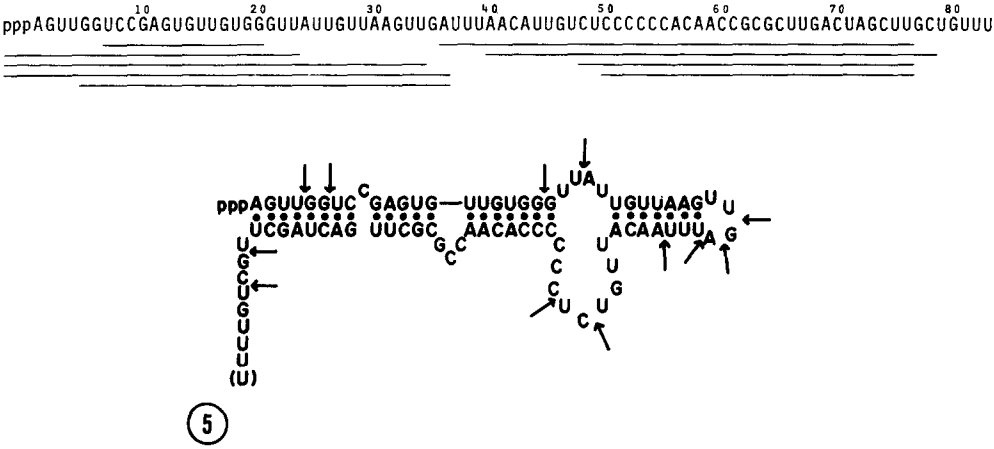


Figure 4. Total nucleotide sequence of C8 RNA. The lines below the sequence represent nuclease S1 digestion products.

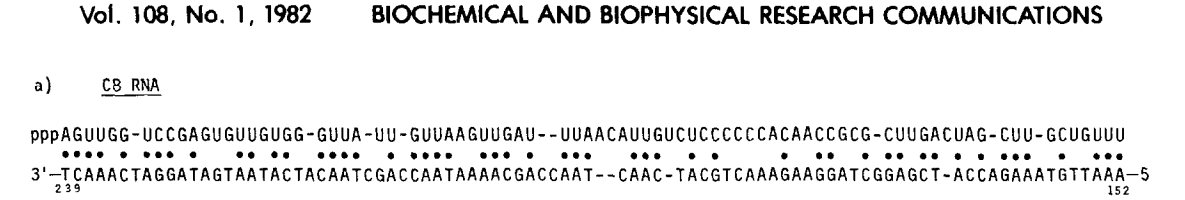
Figure 5. A possible secondary structure of C8 RNA. The arrows denote nuclease S1 digestion sites.

essential nucleotides are split into two conserved sequence blocks. The first conserved sequence is located on the 5'-side of the gene and is GTGGPyNNPuGTGG, whereas the second sequence is located on the 3'-side of the gene and reads GGGTTCGAANCC (19). C8 RNA has an analogous sequence, GUUGGUCCGAGUG (residues 2 to 14), to the first conserved sequence, but there is no sequence closely analogous to the second conserved sequence.

Recently, Hendrick et al. reported that anti-Ro and anti-La antibodies from patients with systemic lupus erythematosus precipitate two classes of small RNA-protein complexes (RNP)(20). hY5, a human RNA precipitated as a small RNP by anti-Ro or anti-La antibodies, shows the same RNase T1 fingerprint, the 5'-terminus and the cellular localization as C8 RNA (20). Therefore, these two RNAs must be the same molecule. Anti La antibody also precipitates 4.5S RNA_H as a small RNP from mouse cells (20). In a preliminary experiment, we found that a part of C8 RNA is associated with mRNA of HeLa cells. Thus, 4.5S RNA_H and C8 RNA show very similar modes of the existence, and it is probable that these 4.5S RNAs have a common cellular function in human and rodent cells. However, the primary structures of these RNAs are not related to each other. Elucidation of the function of these 4.5S RNAs requires further biological and biochemical studies.

Since C8 RNA is associated with mRNA, it is probable that the sequence of C8 RNA, like that of 4.5S RNA_H (6-8), shows homology with repetitive DNA sequences. Two human interspersed repeated sequences, Alu family (21) and EC1 (22), do not show homology with C8 RNA. On the other hand, human Hind III 1.9 Kb family (23) has an AT rich sequence and shows

a) C8 RNA



Hind III 1.9 kb family (23)

b)

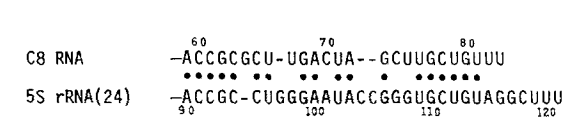


Figure 6. (a) A possible hybrid structure between C8 RNA and human Hind III 1.9 Kb repeated DNA (23). Dots between the sequences denote complementary bases. (b) Sequence homology in the 3'-terminal portions of C8 RNA and 5S rRNA (24). Dots between the sequences denote homologous bases.

appreciable homology with C8 RNA. Figure 6a shows a hybrid structure between C8 RNA and 1.9 Kb family (residues 152 to 239). The overall homology of this hybrid is 55 % (51 of 92 nucleotides) and the 5'-side of C8 RNA (residues 1 to 46) is more complementary (62 %, 32 of 52 nucleotides) than the 3'-side. Although there is no information about the transcript of this gene family yet, it is possible that a member of this family is used as the template for C8 RNA. Another possibility is that mRNA containing the sequence of this family forms a hybrid with C8 RNA. It is also noteworthy that the 3'-terminus (residues 59 to 81) of C8 RNA shows homology (69 %, 18 of 26 nucleotides) with that (residues 90 to 114) of human 5S rRNA (24)(Figure 6b), but its significance is not yet known.

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