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THE NUCLEOTIDE SEQUENCE OF NUCLEAR 4.8S RNA OF MOUSE CELLS

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SUMMARY: The nucleotide sequence of nuclear 4.8S RNA has been determined. The 4.8S RNA consists of 108 nucleotide residues with one mole each of m^2G, m^6A and Gm, 3 moles each of Ψ and Am and 4 moles of Cm as modified nucleosides. This RNA has pppG as the 5'-terminal nucleotide and contains a sequence complementary to some of the splice junctions of mRNA precursors.

INTRODUCTION

The nuclei of eukaryotic cells contain small molecular weight (4 to 8S) RNAs (snRNAs)(1,2). The nucleotide sequences of some of them have been determined (3-6), but little is known about their function. Recently, Lerner et al. reported that autoantibodies produced by patients with systemic lupus erythematosus (SLE) react with nuclear ribonucleoproteins (RNPs) which contain snRNAs. Since one snRNA (U1a) has a sequence complementary to the terminal sequences of the intervening regions, it has been suggested that these snRNPs may be involved in the splicing of heterogeneous nuclear RNAs (hnRNAs)(7,8). We recently determined the nucleotide sequences of 4.5S RNAs associated with poly(A)-containing RNAs of rodent cells. These RNAs also contain a sequence which is complementary to the terminal sequences of the intervening regions (9).

To clarify whether other snRNAs have similar sequences complementary to the splice junctions, we determined the nucleotide sequence of nuclear 4.8S RNA.

MATERIALS AND METHODS

Materials. Enzymes were obtained from the following sources : RNases T1, T2 and U2 from Sankyo Co. Ltd,; RNase A from Sigma Chemical Co.; snake venom phosphodiesterase and bacterial alkaline phosphatase from Worthington Biochemical Corp. Silkworm nuclease was a gift from Dr. J. Mukai of Kyushu University, Fukuoka. Nuclease P1 was a gift from Dr. A. Kuninaka of the Research Laboratory, Yamasa Shoyu Co., Choshi. Nuclease S1 was a gift from Dr. T. Ando of the Institute of Physical and Chemical Research, Wako-shi. [5'-^{32}P]pCp was obtained from RCC, Amersham.

Purification of the 4.8S RNA. Mouse lymphoma cells (L1210/C)(10) were labeled for 24 hours with [^{32}P]phosphate and nuclear RNA was extracted as described previously (11). snRNAs were further purified by chromatography

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on DEAE-Sephadex A-50 (12) and then were fractionated by two dimensional polyacrylamide gel (2-D gel) electrophoresis by a modification (11) of the method of Ikemura et al. (13,14).

Unlabeled 4.8S RNA was isolated from the livers of ICR mice as follows. Nuclear RNA was extracted from the purified nuclei (15) by the hot phenol-SDS method (16) and was fractionated by gel filtration on a Sephadex G-100 column. The snRNA fraction was subjected to electrophoresis on 10% polyacrylamide gel and snRNAs were located by staining with methylene blue. The 4.8S RNA fraction was eluted, labeled with [5'-^{32}P]pCp at the 3'-end (17) and purified by 2-D gel electrophoresis.

Sequence analysis. Standard procedures (9,18,19) were used for enzymatic digestion of purified 4.8S RNA, fingerprinting and identification of oligonucleotides from fingerprints. Modified nucleotides were identified by their mobilities on two dimensional thin layer chromatography relative to unmodified marker nucleotides, with reference to published mobilities (20, 21).

Large oligonucleotide fragments of the 4.8S RNA were obtained by partial digestion with RNase T1 (9) and their sequences were identified (21). Nuclease S1 was also used for obtaining large fragments (22). The total sequence of the 4.8S RNA was confirmed by the chemical sequence method (23) using 3'-end-labeled 4.8S RNA.

RESULTS

2-D gel electrophoresis of snRNAs. Uniformly ^{32}P-labeled snRNAs of L1210 cells were analyzed by 2-D gel electrophoresis. As shown in Figure 1, 17 distinct spots were obtained. These snRNAs were assigned as follows by fingerprint analysis. (i) Spot 1 is 4.5S RNA$_I$ (3). (ii) Spots 3 and 4

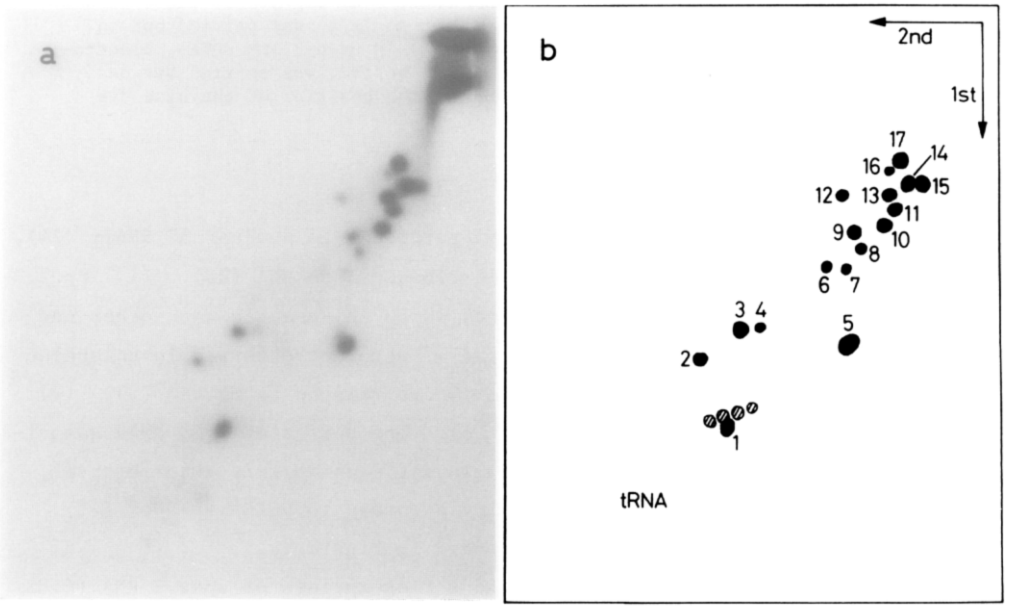

Figure 1. (a) 2-D gel electrophoretic pattern of ^{32}P-labeled snRNAs from L1210 cells. (b) Schematic presentation of (a). The positions of the series of 4.5S RNAs associated with poly(A)-containing RNAs are indicated by cross-hatched circles.

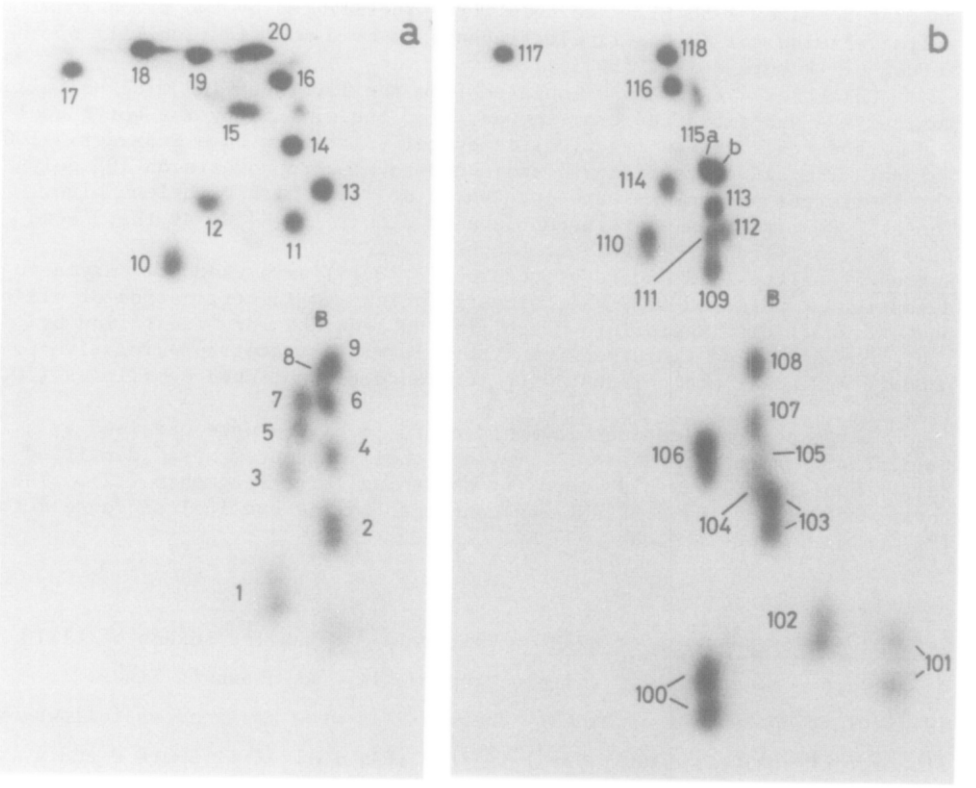

Figure 2. Fingerprints of RNase T1(a) and RNase A(b) digests of the 4.8S RNA. Electrophoresis in the first dimension, right to left, was carried out on cellulose acetate in pyridine acetate (pH 3.5)-7M urea-2.5mM EDTA. Electrophoresis in the second dimension, from top to bottom, was carried out on DEAE-cellulose in 7% formic acid. B denotes the position of the blue dye marker (xylene cyanol FF).

resemble each other and give similar fingerprints to nuclear 5S RNA$_{III}$ (24), although less complex. (iii) Spot 5 is ribosomal 5S RNA (25). (iv) Spot 9 is ribosomal 5.8S RNA (26). (v) Spots 10 to 13 resemble each other and are related to U1 RNA (4). Spots 11 and 12 were not observed in unlabeled mouse liver snRNAs. (vi) Spots 14 and 15 are related to U2 RNA (5). (vii) Spots 16 and 17 are related to U3 RNA (6). The series of 4.5S RNAs associated with poly(A)-containing RNAs (27) is not seen in this autoradiogram, because these 4.5S RNAs are specifically adsorbed to DEAE-Sephadex (11). The positions of these 4.5S RNAs are added in Figure 1b.

Figure 2 shows RNase T1 and RNase A fingerprints of spot 2 RNA (4.8S RNA). Since the mobility of each RNase T1 oligonucleotide of the 4.8S RNA corresponds to that of U6 RNA (7), we concluded that the 4.8S RNA is identica to U6 RNA.

Complete RNase T1 and RNase A digestion products of the 4.8S RNA. The oligonucleotides eluted from the RNase T1 and RNase A fingerprints (Fig. 2a and b) were each digested with various enzymes and their structures were determined (Tables I and II). Modified nucleotides were identified by two dimensional chromatography after digestion of oligonucleotides with RNase T2 or nuclease P1.

In oligonucleotide 20, about 40% of the U at position 7 from the 5'-end was modified to Ψ (Table I).

Alignment of the oligonucleotides. The partial RNase T1 and nuclease S1 digestion products of the 4.8S RNA are summarized in Figure 3. No large oligonucleotide fragments for linking positions 33 and 34 or 68 and 69 were obtained. These discontinuous points were confirmed by the chemical

Table I. Analysis of RNase T1 End Products

Spot No.	Sequence	Molar Yield Measured	Theoretical
1	Gp, m^2Gp	3.8	4
2	C-Gp	2.4	2
3	A-Gp	1.0	1
4	C-A-Gp	0.9	1
5	Am-A-Gp	0.8	1
6	C-A-Am-Gp	0.9	1
7	A-A-Gp	1.0	1
8	A-A-C-Gp	0.8	1
9	A-Cm-A-C-Gp	0.7	1
10	U-Gp	2.1	2
11	C-U-C-Gp	1.0	1
12	A-U-Gp	1.1	1
13	Cm-C-Cm-Cm-U-Gp	0.9	1
14	A-Ψ-A-C-m^6A-Gp	1.0	1
15	C-U-U-C-Gp	1.1	1
16	C-A-A-A-U-Ψ-C-Gp	1.0	1
17	pppGp	1.2	1
18	U-U-C-C-A-U-A-U-U-U-U-U	1.2	1
19	A-U-U-U-Am-Gm-C-A-U-Gp	1.0	1
20[a)]	C-A-C-A-U-A-U*-A-C-U-A-A-A-A-Ψ-U-Gp	0.9	1

a) U* in oligonucleotide 20 means partial substitution of U to Ψ.

Table II. Analysis of RNase A End Products

Spot No.	Sequence	Molar Yield Measured	Theoretical
100	Up,Ψp	14.6	12
101	Cp	5.4	5
102	A-Cp	3.0	3
103	G-Cp	4.3	5
104	Am-Gm-Cp	1.0	1
105	Cm-Cm-Up	0.7	1
106	A-Up	5.0	5
107	A-G-Cp	1.1	1
108	G-A-Cm-A-Cp	1.0	1
109	A-A-A-Up	1.1	1
110	G-Up	2.3	2
111	G-G-Cp	1.0	1
112	G-G-Cm-Cp	0.7	1
113	A-A-A-A-Ψp	1.0	1
114	G-A-Ψp	1.3	1
115a	G-G-A-A-Cp	1.0	1
115b	G-A-A-G-Cp	1.0	1
116	A-Am-G-m^2G-A-Up	1.0	1
117	pppG-Up	1.2	1
118	m^6A-G-A-G-Am-A-G-A-Up	1.1	1

sequence method (23) using 3'-end-labeled 4.8S RNA (Figure 4). In this method, m^2G, m^6A and other 2'-O-methylated nucleosides reacted with chemicals and were cleaved off equally as unmodified nucleosides. A possible secondary structure of the 4.8S RNA is shown in Fig. 5.

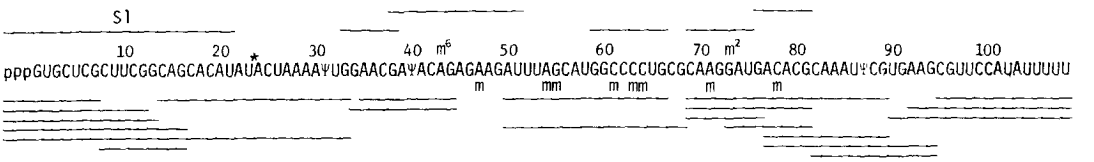

Figure 3. Nucleotide sequence of the 4.8S RNA. The lines above the sequence are overlapping sequences deduced by comparison of the oligonucleotide catalogues shown in Tables I and II and the nuclease S1 digestion product. The lines below the sequence represent partial RNase T1 digestion products.

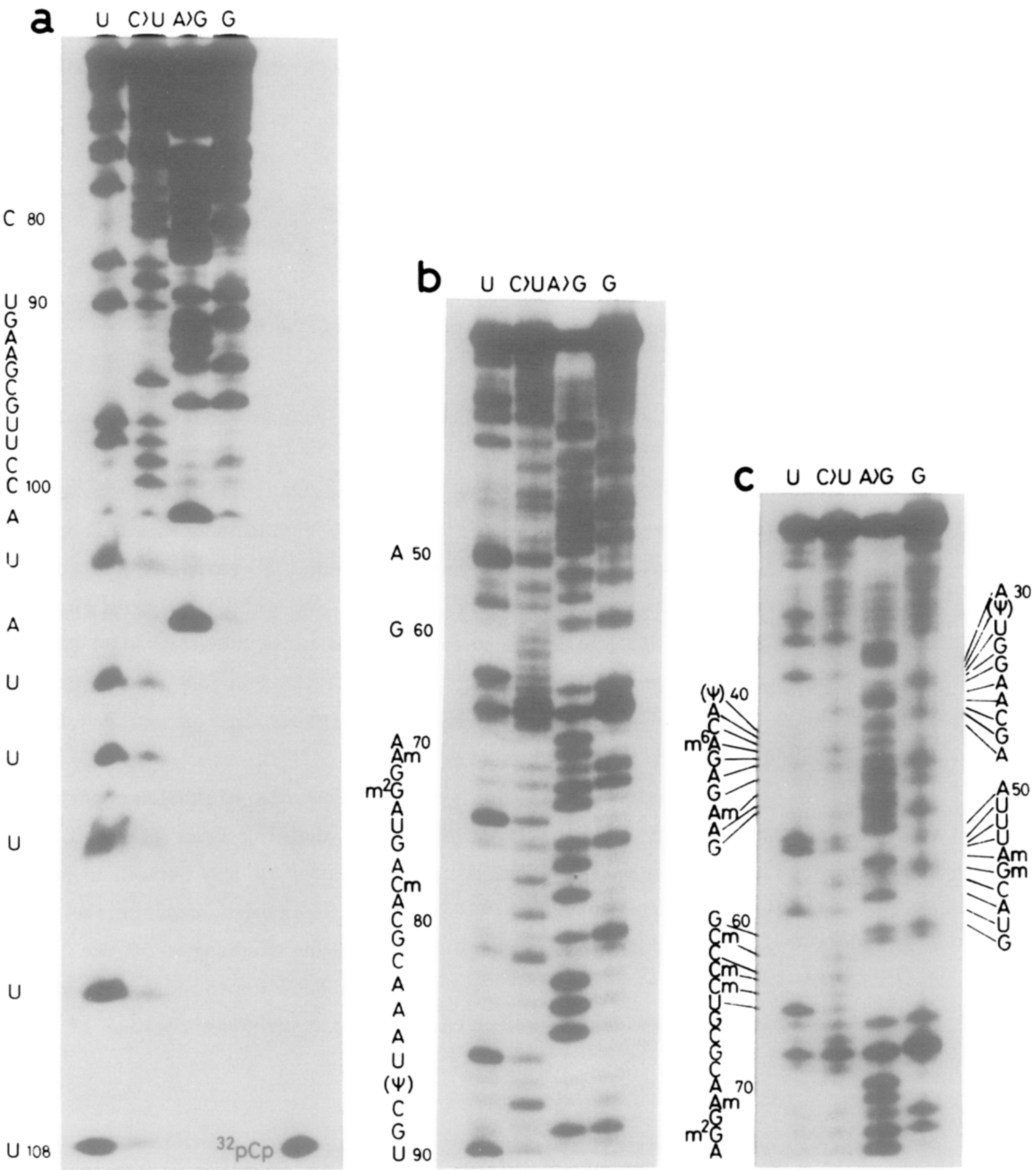

Figure 4. Sequencing gels of 3'-end-labeled 4.8S RNA. RNA was digested chemically (23) and subjected to electrophoresis on polyacrylamide gels (40 X 20 X 0.06cm). (a) 20% gel at 700V for 7.5 hr. (b) 15% gel at 600V for 24 hr. (c) 15% gel at 500V for 44 hr.

DISCUSSION

The mouse 4.8S RNA contains 25 G (including Gm and m^2G), 24 C (including 4 Cm), 32 A (including m^6A and 3 Am) and 27 U (including 3Ψ). Modified nucleosides are concentrated in the center of the molecule. U2

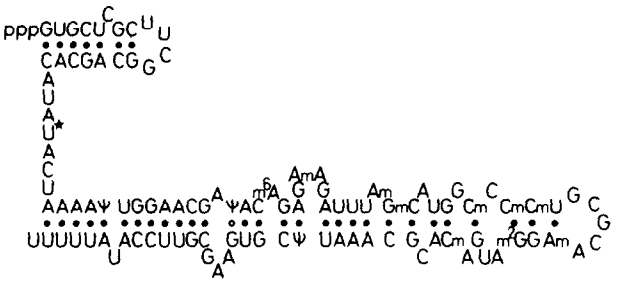

Figure 5. A possible secondary structure of the 4.8S RNA.

RNA also contains many modified nucleosides, but they are distributed in the 5'-terminal third (5).

Busch and his colleagues isolated similar snRNA (4.5S RNA_{III}) from Novikoff hepatoma ascites cells. 4.5S RNA_{III} also contains one mole each of m^2G and m^6A and 3 moles of Ψ (28), but its 5'-and 3'-terminal nucleotides and most of its alkaline resistant dinucleotides are different from those of our 4.8S RNA (29). It should be noted that we also isolated 4.8S RNA from cultured normal rat kidney cells. This rat 4.8S RNA had the same mobility on 2-D gel and gave the same RNase T1 fingerprint as mouse 4.8S RNA (F. Harada, unpublished observations).

Lerner et al. reported that the serum of patients with SLE reacts with nuclear RNPs which contain six snRNAs (7). Judging from RNase T1 fingerprints, U6 RNA, one of the snRNAs precipitated by the serum, might be the 4.8S RNA. Therefore, it was interesting to examine whether the 4.8S RNA has sequences complementary to the terminal sequences of the intervening regions of hnRNAs (8). The 4.8S RNA does not have a sequence complementary to the consensus sequences of the intervening regions of

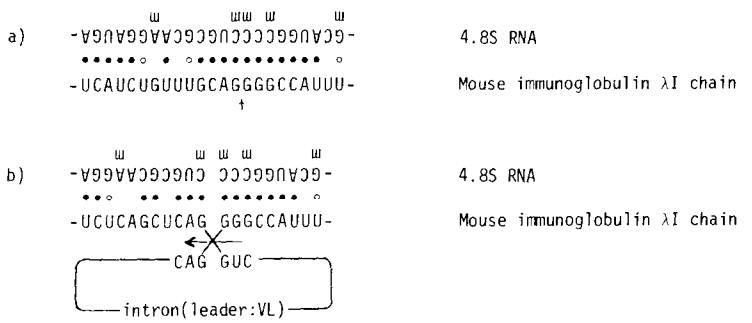

Figure 6. Possible hybrid structures between the 4.8S RNA and the 3'-junction(a) or both junctions(b) of the intron(leader:VL) of mouse immunoglobulin λI chain (30). Arrows indicate splice positions.

hnRNAs (8). However, this RNA possesses a sequence complementary to some 3'-junctions of introns such as mouse immunoglobulin λI(leader:VL) and λII (small) chains (30), the γII b chain (CH1:hinge and CH2:CH3)(31,32) and rat insulin (small and large)(33). Figure 6a shows a possible base pairing interaction between 4.8S RNA (residues 57 to 77) and the 3'-junction of mouse immunoglobulin λI chain (leader:VL)(30). In this case, the 5'-junction also contains a complementary sequence to 4.8S RNA and it is possible to construct a hybrid structure between 4.8S RNA and both ends of the exons (Figure 6b). A similar hybrid structure can be constructed between 4.8S RNA and splice junctions of mouse immunoglobulin λII chain (small)(30). Elucidation of the function of the 4.8S RNA must await further biological and biochemical studies.

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