J. Biochem. 122, 1202-1207 (1997)

Probing the Secondary Structure of MCS4 RNA of *Mycoplasma capricolum*[1]

Chisato Ushida,[2] Shinpei Sasamura, and Akira Muto

Department of Biology, Faculty of Science, Hirosaki University, Bunkyo-cho 3, Hirosaki, Aomori 036

Received for publication, July 28, 1997

MCS4 RNA is one of the small stable RNAs found in *Mycoplasma capricolum*. Its function is unknown. The conformation of MCS4 RNA (125 nucleotides in length) in solution was investigated using chemical and enzymatic probes. Single and double-stranded regions were estimated by means of diethyl pyrocarbonate (DEPC) and by dimethyl sulfate (DMS) modifications. Ribonuclease V_1 was also used to identify paired or stacked nucleotides. Based on these data, a secondary structure model for MCS4 RNA containing four stem-structures was constructed.

Key words: chemical probing, MCS4 RNA, *Mycoplasma capricolum*, RNA secondary structure, small stable RNA.

Mycoplasmas are small wall-less eubacteria, which are phylogenetically related to Gram-positive bacteria such as *Bacillus* spp. and *Clostridium* spp. (*1*). They are parasitic in eukaryotic tissues and organs, and their genomes are the smallest in all self-replicating organisms. Recently, the total genome sequences from two mycoplasma species, *Mycoplasma genitalium* and *M. pneumoniae*, were determined (*2, 3*).

M. capricolum is parasitic in goats, and its genome is about 1,100 kbp (*4-6*). The genome contains only two sets of rRNA genes (*7, 8*), 30 genes for 29 tRNA species (*9, 10*) and about 500 genes for proteins (*11*). Previously, we have reported that the *M. capricolum* cells contain six small stable RNA species, designated MCS1 through MCS6 RNA, besides tRNAs and rRNAs (*12*). Among them, MCS1, MCS5, and MCS6 RNAs are the homologs of the *Escherichia coli* 4.5S RNA, M1 RNA (RNase P RNA), and 10Sa RNA (tmRNA), respectively (*10, 13, 14*). However, homologous RNAs to the other three RNAs, MCS2, MCS3, and MCS4 RNAs, have not been found even in the genomes of *M. genitalium* and *M. pneumoniae*, suggesting that these are present only in *M. capricolum* (*15*).

MCS4 RNA is of special interest because its sequence reveals extensive similarity to that of eukaryotic U6 snRNAs (*16*). This RNA (125 nucleotides in length) is as abundant as 5S rRNA in the cell. For functional analysis of MCS4 RNA, it is important to know the structural features of this molecule. In the present study, we probed the base-paired sequences by chemical and enzymatic methods, and we constructed a secondary structure model for MCS4 RNA.

[1] This work was supported by a Grant-in-Aid from The Ministry of Education, Science, Sports and Culture of Japan.

[2] To whom correspondence should be addressed. Tel: +81-172-39-3592, Fax: +81-172-39-3593, E-mail: cushida@cc.hirosaki-u.ac.jp

Abbreviations: DEPC, diethyl pyrocarbonate; DMS, dimethyl sulfate; A, adenine; C, cytosine; RNase, ribonuclease.

MATERIALS AND METHODS

Chemicals and Enzymes—DEPC and aniline were purchased from Sigma (St. Louis, MO); DMS was from Aldrich (Milwaukee, WI); hydrazine was from Honen (Tokyo); RNase V_1, T_1, U_2, and RNA sequencing kit were purchased from Pharmacia (Piscataway, NJ). Radioactive [5′-^{32}P]pCp at 3,000 Ci/mol and [γ-^{32}P]ATP at 6,000 Ci/mol were from Amersham (Little Chalfont, Buckinghamshire, England). The other enzymes were obtained from Takara (Tokyo) and Wako Pure Chemical Industries (Osaka).

Culture of M. capricolum—*M. capricolum* strain Kid (ATCC27343) was grown at 37°C in modified Edward medium (MEM) (*17*). Cells were collected at the mid-log growth phase by centrifugation at 6,000 rpm for 20 min at 4°C, and were stored at −20°C.

Preparation of MCS4 RNA—Crude preparations of small RNAs were obtained by the direct phenol extraction method followed by precipitation with 1 M NaCl and isopropyl alcohol as described previously (*16*). About 250 μl of 5 mg/ml total small RNAs was mixed with an equal volume of gel loading buffer (10 M urea, 89 mM Tris-borate, pH 8.3, 10 mM EDTA, xylene cyanol, and bromophenol blue) and loaded on a 12% polyacrylamide-7 M urea gel (0.2×20×40 cm) made in TBE-buffer (89 mM Tris-borate, pH 8.3, 10 mM EDTA). Electrophoresis was performed in TBE buffer at 500 V for 12 h. The gel was stained with ethidium bromide, and the MCS4 RNA band was cut from the gel. The RNA was extracted from the gel in elution buffer (0.5 M ammonium acetate, 10 mM magnesium acetate, 1 mM EDTA, 0.1% SDS) and recovered by ethanol precipitation. Purified MCS4 RNA was labeled at the 3′ end with [5′-^{32}P]pCp by T4 RNA ligase or at the 5′ end with [γ-^{32}P]ATP by polynucleotide kinase after phosphatase treatment. Labeled RNAs were purified by gel electrophoresis (12% polyacrylamide-7 M urea gel) as described (*16*). The sequence of the RNA was determined using the RNA

sequencing kit.

Chemical Probing—Chemical modifications were performed essentially according to the method described by others (*e.g.*, Ehresmann *et al.* 1987; Peattie and Gilbert 1980; Krol and Carbon 1989) (*18-20*).

Unpaired A residues in the 3′ end-labeled MCS4 RNA were modified with 2 μl of DEPC in 200 μl of reaction mixture containing 10 μg of carrier yeast tRNA under the following conditions: (i) denaturing: 200 mM Hepes (pH 8.0), 1 mM EDTA at 90°C, (ii) semidenaturing: 200 mM Hepes (pH 8.0), 1 mM EDTA at 37°C, (iii) native: 200 mM Hepes (pH 8.0), 1 mM EDTA, 10 mM, or 100 mM $MgCl_2$ at 37°C. The RNAs were then precipitated by ethanol, washed once with cold 70% ethanol, and dried.

Unpaired C residues in the 3′ end labeled MCS4 RNA were modified with 0.5 μl of DMS in 300 μl of reaction mixture under the same conditions as DEPC modification. RNAs were precipitated with ethanol, washed once with cold 70% ethanol, and dried. The precipitate was suspended in 10 μl of 50% hydrazine-50% water and kept on ice for 5 min. The RNAs were again precipitated with ethanol, washed with cold 70% ethanol, and dried.

The modified MCS4 RNAs were dissolved in 20 μl of 1.0 M aniline/acetate buffer (pH 4.5), incubated in the dark at 60°C for 20 min, and then lyophilized. The sample was dissolved in 20 μl of water and lyophilized again. After repeating the last step 3 times, the sample was dissolved in 2 μl of water and mixed with 2 μl of gel loading buffer. The sample was heated at 90°C for 30 s and quickly chilled on ice. The electrophoresis were performed on a 12% polyacrylamide-8 M urea gel and on a 20% polyacrylamide-8 M urea gel.

Structure Probing by RNase V_1—The 5′ or 3′ end-labeled MCS4 RNA was incubated in 10 μl of the reaction mixture [10 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 100 mM KCl] containing 1 μl of RNase V_1 at 37°C for 10 min. The RNA was precipitated with ethanol, washed with cold 70% ethanol, and dried. The sample was dissolved in 2 μl of water and mixed with 2 μl of gel loading buffer. Electrophoresis was performed on a 12% polyacrylamide-8 M urea gel as described above.

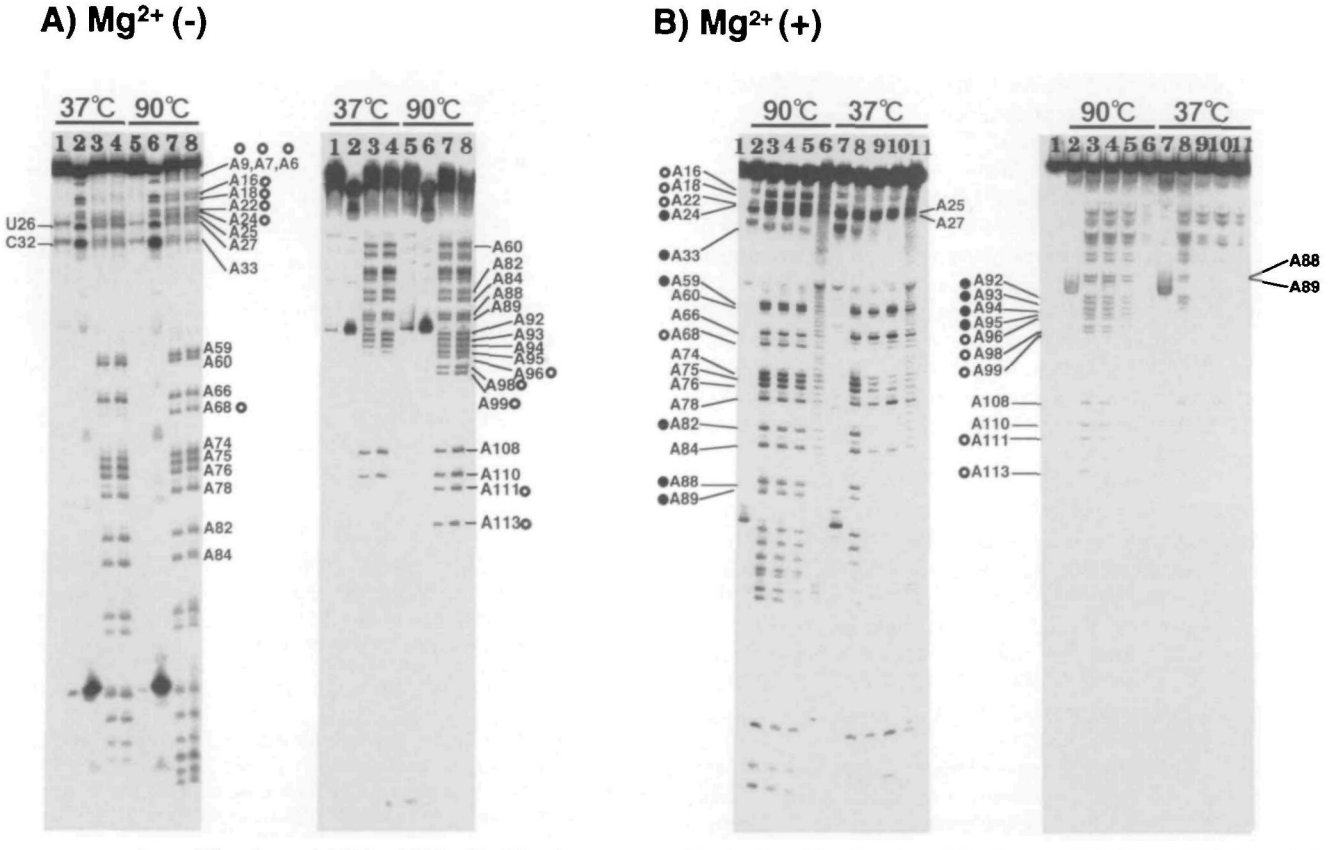

Fig. 1. **DEPC modification of MCS4 RNA.** Modifications were performed under denaturing, semidenaturing, or native conditions in the absence (A) or presence (B) of Mg^{2+} ions. (A) The 3′ end-labeled MCS4 RNA without incubation (lanes 1 and 5); incubation at 37°C for 60 min (lane 2) or at 90°C for 30 s (lane 6) without modification; DEPC modification at 37°C for 60 min (lane 3) or for 120 min (lane 4); modification at 90°C for 30 s (lane 7) or for 60 s (lane 8). Gel electrophoresis was carried out at 1,200 V for 2 h on 20% polyacrylamide-8 M urea gel (left panel) and for 3 h on 12% polyacrylamide-8 M urea gel (right panel). (B) The 3′ end-labeled MCS4 RNA without incubation (lane 1); incubation at 90°C for 30 s (lane 2) or at 37°C for 60 min (lane 7) without modification; modification at 90°C for 30 s in the presence of increasing amounts of $MgCl_2$, 0 mM (lane 3), 1 mM (lane 4), 10 mM (lane 5), and 100 mM (lane 6); modification at 37°C for 60 min with $MgCl_2$, 0 mM (lane 8), 1 mM (lane 9), 10 mM (lane 10), and 100 mM (lane 11). The positions of the A residues at which the cleavage occurred to produce the bands are indicated. The positions protected from modification under semidenaturing or native conditions are shown by open circles. The positions protected from modification only under native conditions are indicated by closed circles.

RESULTS AND DISCUSSION

Chemical Modification—DEPC carbethoxylates the N-7 position of A's that are not involved in the stacked regions in helices or in long-range interactions. DMS alkylates the N-3 position of C's that are not involved in Watson-Crick pairings. Modifications were performed under three different conditions: denaturing (at 90°C without Mg^{2+}), semidenaturing (at 37°C without Mg^{2+}), and native (at 37°C with Mg^{2+}). Aniline treatment of RNAs after DEPC or DMS modification induces β-elimination, generating cleavage of the chain at the position of the modification (*18-20*). The cleavage products were then run on a denaturing polyacrylamide gel alongside T_1 and U_2 ribonuclease RNA sequencing reaction products of MCS4 RNA.

Figure 1 shows the results of DEPC modification of the 3′ end-labeled MCS4 RNA under different conditions. Under the denaturing conditions, all the A's, except for A1, A3, A5, and A125 located in the extreme 5′ and 3′ ends, could be identified (Fig. 1A, lanes 7 and 8). Among them, 13 A's (A6, A7, A9, A16, A18, A22, A24, A68, A96, A98, A99, A111, A113) were protected from DEPC modification under semidenaturing conditions (Fig. 1A, lanes 3 and 4), indicating that these positions are involved in base-pairings or stacked regions. Under native conditions, 9 additional A's (A33, A59, A82, A88, A89, A92, A93, A94, A95) were protected from DEPC modification (Fig. 1B, lanes 9, 10, and 11). The modifications of A74, A75, and A76 were weakened under native conditions.

Figure 2 shows the results of DMS modification of C's of MCS4 RNA. Among 18 C's, 14 C's located in positions 50 to 102 were analyzed under semidenaturing conditions (Fig. 2A, lanes 7 and 8), and 6 C's in positions 79 to 102 were analyzed under the native conditions (Fig. 2B, lanes 9, 10, and 11). Under semidenaturing conditions, 5 C's (C71, C72, C97, C100, C102) were protected from DMS modification. When Mg^{2+} was present (native conditions), two additional C's (C90 and C91) were protected from modification by DMS.

V_1 Nuclease Cleavage of MCS4 RNA—RNase V_1 from cobra venom cuts double-stranded or structured regions with base stacking, such as pseudoknots. There is no strong sequence preference (*21, 22*). Figure 3 shows representative cleavage patterns of the 5′ and 3′ end-labeled MCS4 RNA generated by RNase V_1 digestion at 37°C in the presence of Mg^{2+} ions. Specific cleavages were observed at A7-U10, A18-A24, G54-G56, C72-C73, C100-U104, and A110-U122, indicating that these regions form base-pairings.

Secondary Structure Model—The results of chemical and V_1 nuclease probing experiments are summarized in Table I. The chemical probing data are essentially consistent with the enzymatic probing data, although some ambiguities are seen, probably due to mechanistic and size differences between the two probes. The data allowed discrimination between the single- and double-stranded regions within MCS4 RNA. In Table I, the bases indicated by the probing data to be in single-stranded or double-stranded regions are represented by S and D, respectively.

Figure 4 presents a secondary structure model for MCS4 RNA, based on the above probing data as well as base complementarities within the molecule. The model contains four helices (H1, H2, H3, and H4) and four loops. H1 and H4 could be predicted by the relatively long base-complementarities in the regions (9 base-pairs in H1 and 13 base-pairs in H4). The presence of most of the base-pairs within the two helices is supported by both chemical and enzymatic probing data. The existence of H3 is suggested by both DMS modification and nuclease V_1 cleavage patterns. There is a complementary sequence stretch that is

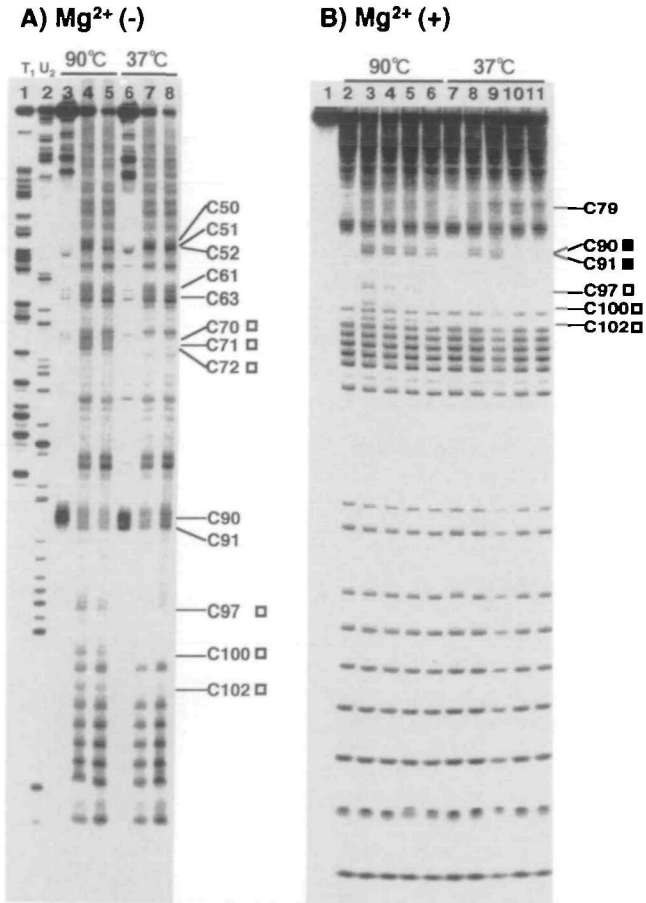

Fig. 2. **DMS modification of MCS4 RNA.** Modifications were performed under denaturing, semidenaturing, or native conditions in the absence (A) or presence (B) of Mg^{2+} ions. (A) The 3′ end-labeled MCS4 RNA was partially digested with ribonuclease T_1 (lane 1) or U_2 (lane 2); incubation at 90°C for 30 s (lane 3) or at 37°C for 60 min (lane 6) without modification; DMS modification at 90°C for 30 s (lane 4) or for 60 s (lane 5); modification at 37°C for 60 min (lane 7) or 120 min (lane 8). (B) The 3′ end-labeled MCS4 RNA without incubation (lanes 1); incubation at 90°C for 30 s (lane 2) or at 37°C for 60 min (lane 7) without modification; modification at 90°C for 30 s in the presence of increasing amounts of $MgCl_2$, 0 mM (lane 3), 1 mM (lane 4), 10 mM (lane 5), and 100 mM (lane 6); modification at 37°C for 60 min with $MgCl_2$, 0 mM (lane 8), 1 mM (lane 9), 10 mM (lane 10), and 100 mM (lane 11). Gel electrophoresis was carried out at 1,200 V for 2 h on 20% polyacrylamide-8 M urea gel (A) and for 3 h on 12% polyacrylamide-8 M urea gel (B). The positions of the C residues at which the cleavage occurred to produce the bands are indicated. The positions protected from modification under semidenaturing or native conditions are shown by open squares. The positions protected from modification only under the native conditions are indicated by closed squares. The bands other than C's, which can be seen under all conditions, are the results of uridine cleavage at the hydrazine step, and do not reflect RNA conformation.

TABLE I. **Summary of chemical modifications and enzymatic cleavage of MCS4 RNA.** "+" shows DEPC modifications, DMS modifications or nuclease V_1 digestion. "−" indicates no modification (protected from modifications) or digestion. The degrees of modification or digestion are classified as strong (+++), moderate (++), or weak (+). "S" or "D" represents the nucleotides predicted to be in a single-stranded region, or in a double-stranded region, respectively. "(D)" represents the nucleotides probably involved in long-range interactions. ND, not determined.

Base	DEPC			DMS			V1	S or D
	Den	Semi	Native	Den	Semi	Native		
A1	ND	ND	ND				ND	
U2							ND	
A3	ND	ND	ND				ND	
U4							ND	
A5	ND	ND	ND					
A6	+++	-	ND					
A7	+++	-	ND				++	D
U8							+++	D
A9	+++	-	ND				++	D
U10							++	D
C11				ND	ND	ND		
U12								
U13								
G14								
U15								
A16	+++	-	-					D
U17								
A18	+++	-	-				+++	D
U19							+++	D
U20							+++	D
U21							+++	D
A22	+++	-	-				+++	D
U23							+++	D
A24	+++	+++	-				+++	D
A25	+++	+++	+++					S
U26								
A27	+++	+++	+++					S
U28								
U29								
G30								
G31								
C32				ND	ND	ND		
A33	+++	+++	-				+	(D)
G34								
U35								
G36								
U37								
G38								
G39								
U40								
U41								
C42				ND	ND	ND		
U43								
U44								
G45								
C46				ND	ND	ND		
U47								
G48								
G49								
C50				+++	++	ND		S
C51				+++	+++	ND		S
C52				+++	+++	ND		S
U53								
G54								
G55								
G56							+	(D)
U57								
G58								
A59	+++	++	-					(D)
A60	+++	+++	+++					
C61				+++	+++	ND		S
U62								
C63				+++	+++	ND		S

Base	DEPC			DMS			V1	S or D
	Den	Semi	Native	Den	Semi	Native		
U64								
G65								
A66	+++	+++	+++					S
G67								
A68	+++	-	-					(D)
U69								
C70				+++	-	ND		
C71				+++	-	ND		
C72				+++	-	ND	+++	D
G73							+++	D
A74	+++	+++	++					S
A75	+++	+++	++					S
A76	+++	+++	++					S
G77								
A78	+++	+++	+++					S
C79				+++	ND	+++		S
G80								
G81								
A82	+++	+++	-					(D)
G83								
A84	+++	+++	+++					S
U85								
U86								
G87								
A88	+++	+++	-					D
A89	+++	+++	-					D
C90				+++	+++	-		D
C91				+++	+++	-		D
A92	+++	+++	-					D
A93	+++	+++	-					D
A94	+++	++	-					D
A95	+++	+	-					D
A96	+++	-	-					D
C97				++	-	-		D
A98	+++	-	-					D
A99	+++	-	-					D
C100				++	-	-	++	D
U101							+++	D
C102				+++	-	-	+++	D
U103							++	D
U104							++	D
U105								
U106								
U107								
A108	+++	+++	++					S
U109								
A110	+++	+++	++				++	S
A111	+++	-	-				++	D
G112							+++	D
A113	+++	-	-				+++	D
G114							+	D
U115							+++	D
U116							++	D
G117							++	D
U118							++	D
U119							+++	D
U120							+++	D
U121							++	D
U122							++	D
U123							ND	
U124							ND	
A125	ND	ND	ND				ND	

capable of forming 5 base-pairs (H2: between G38-C42 and G87-C91). A88, A89, C90, and C91 are protected from DEPC or DMS modifications in the presence of Mg^{2+} (Figs. 1 and 2), suggesting the presence of H2. However, the protections of these bases are not seen in the absence of Mg^{2+}, and nuclease V_1 did not cleave the complementary

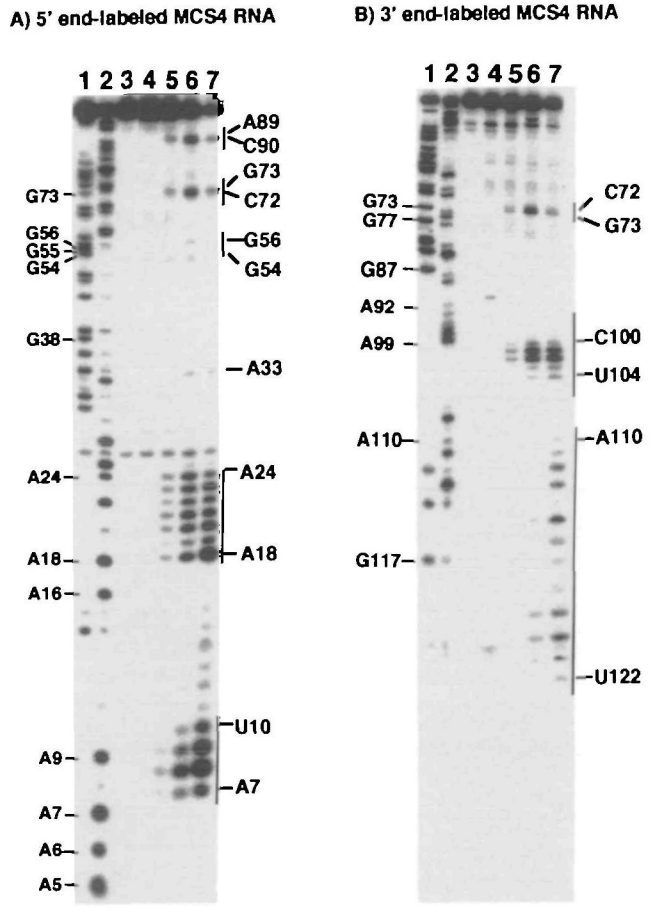

Fig. 3. **Nuclease V_1 cleavage of MCS4 RNA.** The 5′ end-(A) or the 3′ end-(B) labeled MCS4 RNA (lane 3) was incubated with ribonuclease T_1 (lane 1), U_2 (lane 2), or increasing amounts of nuclease V_1, 0, 10^{-7}, 10^{-6}, and 10^{-5} units/μl (lanes 4, 5, 6, and 7, respectively) at 37°C for 10 min. The positions of representative G and A residues are shown (left of the panel), and the regions cut by nuclease V_1 are indicated by bars (right of the panel).

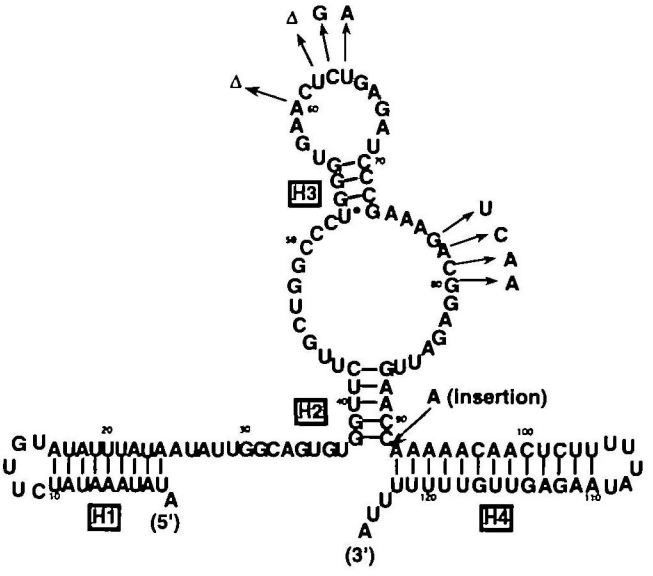

Fig. 4. **The secondary structure model for MCS4 RNA.** The bases in MCS4b RNA that are different from those of the major MCS4 RNA are indicated by arrows. The triangle shows a deleted base.

stretches of H2 (Fig. 3). These results suggest that the base-pairs in H2 are not as stable as they are in the other three helices. It is also known that nuclease V_1 access is limited due to steric hindrance by an additional structure in the RNA molecule (*21, 22*).

Chemical and nuclease probing showed that most of the remaining bases were located in single-stranded regions in the model. However, DEPC probing experiments showed that A33, A59, and A82 are protected from modification under both native and semidenaturing conditions and that A68 is protected under native conditions (Fig. 1), although these are not involved in the double-stranded regions in the model (Fig. 4). Similarly, nuclease V_1 weakly, but significantly, cut A33 in spite of its location in a single-stranded region in the model. These discrepancies between the model and the probing data may be partly due to tertiary folded structure, including base-pairings between the single-stranded regions.

Southern hybridization of MCS4 RNA with the *M. capricolum* genome showed that it is encoded by two genes (*16*). The sequence of the second gene, whose expression in the cell has not yet been identified, is slightly different from that of the major MCS4 RNA (Ushida *et al.*, unpublished). The second MCS4 RNA, designated MCS4b RNA, contains six base changes, two deletions and one insertion as compared with the major MCS4 RNA sequence, as indicated in Fig. 4. All these changes occur in the single-stranded regions in the secondary structure model, showing that MCS4b RNA can be folded in the same secondary structure as that of the major MCS4 RNA.

There are some similarities between the secondary structures of MCS4 RNA and U6 snRNAs: the 5′-terminal region of U6 snRNAs can be folded into a stem-loop structure like H1 in MCS4 RNA; internal stem-loops such as H2 and H3 in MCS4 RNA are also proposed for yeast U6 snRNA structures (*23*). However, it is unknown whether these similarities reflect functional similarities between MCS4 RNA and U6 snRNAs, since the function of MCS4 RNA is not established.

MCS4 RNA was found to interact with several proteins *in vivo* (*16*) and *in vitro* (our unpublished results). The secondary or tertiary structure of the RNA may be important for specific interaction with the proteins.

We wish to thank the staff of the Radioisotope Center, Faculty of Agriculture, Hirosaki University, for allowing us to use their radioisotope facility.

REFERENCES

1. Weisburg, W.G., Tully, J.G., Rose, D.L., Petzel, J.P., Oyaizu, H., Yang, D., Mandelco, L., Sechrest, J., Lawrence, T.G., Van Etten, J., Maniloff, J., and Woese, C.R. (1989) A phylogenetic analysis of the mycoplasmas: basis for their classification. *J. Bacteriol.* 171, 6455-6467
2. Fraser, C.M., Gocayne, J.D., White, O., Adams, M.D., Clayton, R.A., Fleischmann, R.D., Bult, C.J., Kerlavage, A.R., Sutton, G., Kelley, J.M., Fritchman, J.L., Weidman, J.F., Small, K.S., Sandusky, M., Fuhrmann, J., Nguyen, D., Utterback, T.R., Saudek, D.M., Phillips, C.A., Merrick, J.M., Tomb, J.-F., Dougherty, B.A., Bott, K.F., Hu, P.-C., Lucier, T.S., Peterson, S.N., Smith, H.O., Hutchison III, C.A., and Venter, J.C. (1995) The minimal gene complement of *Mycoplasma genitalium*.

Science **270**, 397-403
3. Himmelreich, R., Hilbert, H., Plagens, H., Pirkl, E., Li, B.-C., and Herrmann, R. (1996) Complete sequence analysis of the genome of the bacterium *Mycoplasma pneumoniae*. *Nucleic Acids Res.* **24**, 4420-4449
4. Whitley, J.C., Muto, A., and Finch, L.R. (1991) A physical map for *Mycoplasma capricolum* Cal. kid with loci for all known tRNA species. *Nucleic Acids Res.* **19**, 399-400
5. Bove, J.M. (1993) Molecular features of Mollicutes. *Clin. Infect. Dis.* **17** (Suppl. 1), S10-31
6. Bork, P., Ouzounis, C., Casari, G., Schneider, R., Sander, C., Dolan, M., Gilbert, W., and Gillevet, P.M. (1995) Exploring the *Mycoplasma capricolum* genome: a minimal cell reveals its physiology. *Mol. Microbiol.* **16**, 955-967
7. Sawada, M., Osawa, S., Kobayashi, H., Hori, H., and Muto, A. (1981) The number of ribosomal RNA genes in *Mycoplasma capricolum*. *Mol. Gen. Genet.* **182**, 502-504
8. Sawada, M., Muto, A., Iwami, M., Yamao, F., and Osawa, S. (1984) Organization of ribosomal RNA genes in *Mycoplasma capricolum*. *Mol. Gen. Gent.* **196**, 311-316
9. Andachi, Y., Yamao, F., Muto, A., and Osawa, S. (1989) Codon recognition patterns as deduced from sequences of the complete set of transfer RNA species in *Mycoplasma capricolum*. Resemblance to mitochondria. *J. Mol. Biol.* **209**, 37-54
10. Muto, A., Andachi, Y., Yuzawa, H., Yamao, F., and Osawa, S. (1990) The organization and evolution of transfer RNA genes in *Mycoplasma capricolum*. *Nucleic Acids Res.* **18**, 5037-5043
11. Kawauchi, Y., Muto, A., and Osawa, S. (1982) The protein composition of *Mycoplasma capricolum*. *Mol. Gen. Genet.* **188**, 7-11
12. Ushida, C. and Muto, A. (1993) Small stable RNAs in *Mycoplasma capricolum*. *Nucleic Acids Symp. Ser.* **29**, 157-158
13. Ushida, C., Izawa, D., and Muto, A. (1996) RNase P RNA of *Mycoplasma capricolum*. *Mol. Biol. Rep.* **22**, 125-129
14. Ushida, C., Himeno, H., Watanabe, T., and Muto, A. (1994) tRNA-like structures in 10Sa RNAs of *Mycoplasma capricolum* and *Bacillus subtilis*. *Nucleic Acids Res.* **22**, 3392-3396
15. Ushida, C. and Muto, A. (1995) Novel small stable RNAs of *Mycoplasma capricolum*. *DNA Res.* **2**, 229-230
16. Ushida, C. and Muto, A. (1993) A small RNA of *Mycoplasma capricolum* that resembles eukaryotic U6 small nuclear RNA. *Nucleic Acids Res.* **21**, 2649-2653
17. Razin, S. and Rottem, S. (1976) Techniques for the manipulation of mycoplasma membranes in *Biochemical Analysis of Membranes* (Maddy, A.H., ed.) pp. 3-26, Chapman and Hall, London
18. Ehresmann, C., Baudin, F., Mougel, M., Romby, P., Ebel, J.-P., and Ehresmann, B. (1987) Probing structure of RNAs in solution. *Nucleic Acids Res.* **15**, 9109-9128
19. Peattie, D.A. and Gilbert, W. (1980) Chemical probes for higher-order structure in RNA. *Proc. Natl. Acad. Sci. USA* **77**, 4679-4682
20. Krol, A. and Carbon, P. (1989) A guide for probing native small nuclear RNA and ribonucleoprotein structures in *Methods in Enzymology* (Dahlberg, J.E. and Abelson, J.N., eds.) Vol. 180, pp. 212-227, Academic Press, New York
21. Lowman, H.B. and Draper, D.E. (1986) On the recognition of helical RNA by cobra venom V_1 nuclease. *J. Biol. Chem.* **261**, 5396-5403
22. Knapp, G. (1989) Enzymatic approaches to probing of RNA secondary and tertiary structure in *Methods in Enzymology* (Dahlberg, J.E. and Abelson, J.N., eds.) Vol. 180, pp. 192-212, Academic Press, New York
23. Fortner, D.M., Troy, R.G., and Brow, D.A. (1994) A stem/loop in U6 RNA defines a conformational switch required for pre-mRNA splicing. *Genes Dev.* **8**, 221-233