Volume 9 Number 20 1981 Nucleic Acids Research

The nucleotide sequence of 5S rRNA from Mycoplasma capricolum

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Received 12 August 1981

ABSTRACT

The nucleotide sequence of 5S rRNA from *Mycoplasma capricolum* is UUGGUGGUAUAGCAUAGAGGUCACACCUGUUCCCAUGCCGAACACAGAAGUUAAGCUCUAUUACGGUGAAGAUAUUA CUGAUGUGAGAAAAUAGCAAGCUGCCAGUU$_{OH}$. The length is 107 nucleotides long, and the shortest in all the 5S rRNAs so far known. The sequence is more similar to those of the gram-positive bacteria than those of the gram-negative bacteria.

INTRODUCTION

The mycoplasmas, that have been well known as one of the smallest self-replicating organisms (1), have a genome of 0.67 x 10^9 daltons in size (one-quarter as large as *E. coli*) (2), which includes only two rRNA cistrons (3). These characteristics have often been taken as evidence suggesting that *Mycoplasma* is a primitive prokaryote emerged in an early stage of bacterial evolution (1). As we already pointed out (4,5), the 5S rRNA sequences can be used for deducing the phylogenic relationships among widely separated organisms. We have therefore determined the sequence of 5S rRNA from *M. capricolum* (= KID, (6)) and compared it to other eubacterial 5S rRNAs.

MATERIALS AND METHODS

The 5S rRNA was purified by the phenol method from the 70S ribosomes of *M. capricolum* ATCC 27343 as previously described (3,7). The sequencing of 5S rRNA was performed mainly by the rapid sequencing procedure of Peattie (8). The method involves the chemical degradation of RNA, of which the 3' or 5'-terminus was post-labelled with ^{32}P (8,9), followed by the electrophoresis and autoradiography of the degradation products. Details of this procedure have been already described (7). To check the sequences obtained by this method and the presence of minor bases, the formamide-fragment analysis (10) was also used. The oligonucleotide fragments (5'-end labelled) were digested with nuclease P_1, and the resulted radioactive mononucleotides were chromatographed on Avicel TLC plates for autoradiographic identification (11).

RESULTS

(1) Size of 5S rRNA

The 5S rRNA of *M. capricolum* migrated definitely faster than the 5S rRNA of *B. subtilis* (116 nucleotides long) in a 12% polyacrylamide-7M urea gel, suggesting a shorter chain length of this 5S rRNA.

(2) 5'- and 3'-terminal analyses

When the RNase T_2-digest of the [3'-^{32}P] 5S rRNA was developed on an Avicel TLC plate, the main radioactive nucleotide was U* (* radioactive), while [5'-^{32}P] 5S rRNA was digested with nuclease P_1, p*U was the main, indicating that the 3'- and 5'-terminal bases were both U.

(3) Sequence analyses by post labelling procedures

The 104 nucleotide sequences from the 3'-terminus of the 5S rRNA were determined by the method of chemical degradation of [3'-^{32}P] RNA (8). The 3 residures from the 5'-terminal could not be sequenced under these conditions. To find these missing residues, the partial digest of the [5'-^{32}P] RNA by RNase A, T_1, PhyI or U_2 was analysed by the electrophoresis. The 106 nucleotide sequences from the 3'-terminus were further confirmed by the formamide fragment analyses (10,11). No minor bases were found with these analyses. The primary sequence of *M. capricolum* 5S rRNA so obtained is shown in Fig. 1, together with the sequences of *E. coli* (12) and *B. subtilis* (13) 5S rRNAs for comparison.

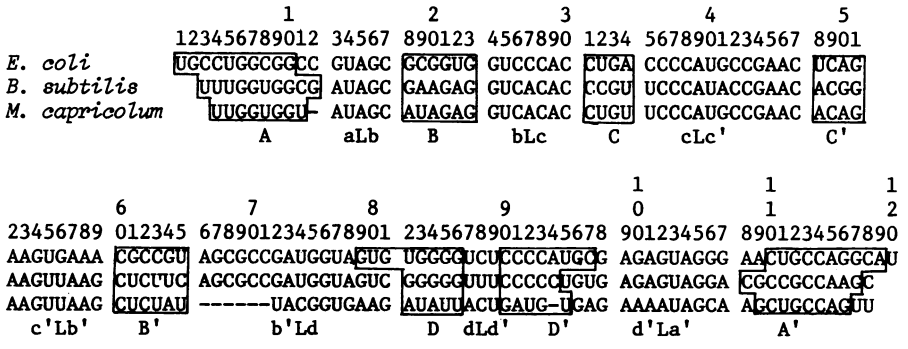

Fig. 1. Comparison of the sequences of *Mycoplasma capricolum*, *E. coli* and *B. subtilis* 5S rRNAs. The squared-off sequences correspond to the helix region in the secondary structure (Fig. 2), and A,A',B,B',C,C' and D,D' in the lowest line are symbols for them. aLb, bLc, cLc', c'Lb', b'Ld, dLd' and d'La' are symbols for loop regions (see ref. 4).

DISCUSSION

The *M. capricolum* 5S rRNA has the lowest G+C content (42%; G:C:A:U=23:19:30:28) among the 5S rRNAs yet sequenced. The base composition of 5S rRNA of this species is close to that of *M. hominis* (G:C:A:U=22:21:29:28) (16). The G+C contents of most of the genes in *Mycoplasma* must be low, because the average G+C content of the genome DNA is only 25% (14). There exists some exceptions for this. For example, *M. capricolum* tRNAs have 53% G+C (16), and the initiator tRNA of *M. mycoides* sp. *capri* has 68% G+C (17). The G+C content of the 5S rRNA is indeed low as compared with the 5S rRNAs from other organisms, but is still higher than the average G+C content of the entire genome of this species.

Total length of *M. capricolum* 5S rRNA is 107 nucleotides long, the shortest so far known. Fig. 1 shows the sequence of the *M. capricolum* 5S rRNA in comparison with *E. coli* and *B. subtilis* 5S rRNAs. Gaps must be inserted into *M. capricolum* sequence as shown. This indicates that the *M. capricolum* 5S rRNA has a long deletion in the 5' half of the b'Ld region. The 3' half of the b'Ld of the eubacteria is well conserved and probably interacts with 23S rRNA (18) or with the cLc' loop (19). The *M. capricolum* 5S rRNA has UACGGUGAAG sequence in this region, which is similar to the corresponding sequence of other eubacterial 5S rRNAs.

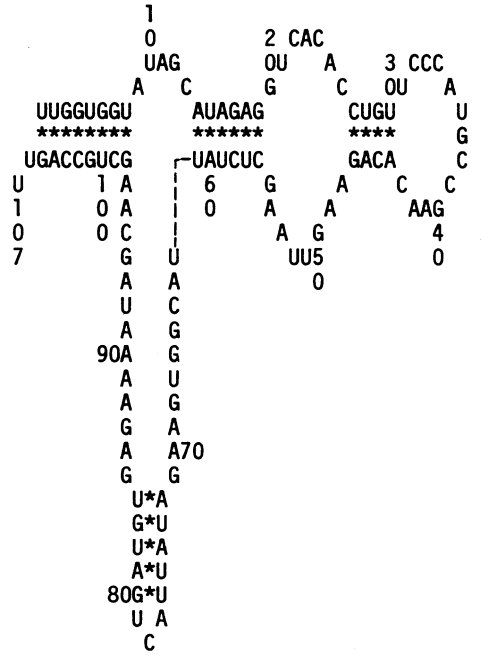

Fig. 2. Secondary structure model of *Mycoplasma capricolum* 5S rRNA.

The secondary structure of the *Mycoplasma* 5S rRNA (Fig. 2) is more related to those of the gram-positive bacteria (the 116-N type (4)) than the gram-negative bacteria (the 120-N type). For example, the regions aLb, B, bLc, C, C' and c'Lb' have the sequences specific to the 116-N type. The *M. capricolum* 5S rRNA reveals 63 ~ 70% and 50 ~ 65% identities to the gram-positive and the gram-negative bacterial 5S rRNAs, respectively, when the comparisons were made with the non-gapped regions. Thus, *M. capricolum*

is closer to the gram-positive bacteria than to the gram-negative both in the secondary structure and in overall nucleotide sequence of the 5S rRNA. Comparisons of *Mycoplasma* tRNA sequences (20, 21) with other gram-positive and negative tRNA sequences also reveal the same relationships. Thus, no evidence has been obtained for the ancient origin of *Mycoplasma*.

ACKNOWLEDGEMENTS

This work was supported by the Grant-in-Aid for Scientific Research of Japan (No. 5657018).

1. Manoloff, J. and Morowitz, H. (1972) Bacteriol. Rev. 36, 263-290.
2. Wallace, D.C. and Morowitz, H. (1973) Chromosoma (Berl.) 40, 121-126.
3. Sawada, M., Osawa, S., Kobayashi, H., Hori, H. and Muto, A. (1981) Mol. Gen. Genet. in press.
4. Hori, H. and Osawa, S. (1979) Proc. Natl. Acad. Sci. USA 76, 381-385; Hori, H. and Osawa, S. (1979) Proc. Natl. Acad. Sci. USA 76, 4175.
5. Osawa, S. and Hori, H. (1979) in Ribosomes, Chambliss, G. et al. Eds., pp. 333-355, Univ. Park Press, Baltimore.
6. Tully, J.G., Barilie, M.F., Edward, D.G., Theodore, T.S. and Ernø, H. (1974) J. Gen. Microbiol. 85, 102-120.
7. Hori, H, Osawa, S., Murao, K. and Ishikura, H. (1980) Nucl. Acids Res. 8, 5423-5426.
8. Peattie, D.A. (1979) Proc. Natl. Acad. Sci. USA 76, 1760-1764.
9. Silberklang, M., Prochiantz, A., Haenni, A.L. and RajBhandary, U.L. (1977) Eur. J. Biochem. 72, 465-478.
10. Stanley, J. and Vassilenko, S. (1978) Nature 274, 87-89.
11. Kuchino, Y., Kato, M., Sugisaki, H. and Nishimura, S. (1979) Nucl. Acids Res. 6, 3459-3469.
12. Brownlee, G.G., Sanger, F. and Barell, B.G. (1967) Nature 215, 735-746.
13. Marotta, C.A., Varrichio, F., Smith, I., Weissman, S.M., Sogin, M.L. and Pace, N.R. (1976) J. Biol. Chem. 251, 3122-3127.
14. Jones, A.S. and Walker, R.T. (1963) Nature 198, 588-589.
15. Johnson, J.D. and Horowitz, J. (1971) Biochim. Biophis. Acta 247, 262-279.
16. Razin, S. (1973) Adv. Microb. Physiol. 10, 1-80.
17. Walker, R.T. and RajBhandary, U.L. (1978) Nucl. Acids Res. 5, 57-70.
18. Herr, W. and Noller, H.F. (1975) FEBS Letters 53, 248-252.
19. Erdmann, V.A. (1981) Presented at the "Workshop on Archaebacteria" (München).
20. Chang, S.H., Brum, C.K., Silberklang, M., RajBhandary, U.L., Hecher, L.I. and Barnett, W.E. (1976) Cell 9, 717-723.
21. Kilpatrick, M.W. and Walker, R.T. (1980) Nucl. Acids Res. 8, 2783-2786.