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The Ribosomal Genes of *Mycoplasma capricolum*

AKIRA MUTO, D.Sc., HIROSHI HORI, M.D., MAKOTO SAWADA, M.D.,*
YASUSHI KAWAUCHI, M.S.,* MASAFUMI IWAMI, B.S.,
FUMIAKI YAMAO, D.Sc., AND SYOZO OSAWA, D.Sc.

Laboratory of Molecular Genetics, Department of Biology, Faculty of Science, Nagoya University, Nagoya, Japan

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The nucleotide sequence of 5S rRNA from *Mycoplasma capricolum* is more similar to that of the gram-positive bacteria than that of the gram-negative bacteria.

The presence of two copies of rRNA genes in *M. capricolum* genome has been demonstrated. The two different rRNA gene clusters have been cloned in *E. coli* plasmid vectors and analyzed for the rRNA gene organizations, demonstrating that the gene arrangement is in the order of 16S, 23S, and 5S rDNA.

The ribosomes of *M. capricolum* contain about 30 species of proteins in 50S and 20 in 30S subunits. The number and size of the ribosomal proteins are not significantly different from those of other eubacterial ribosomes.

INTRODUCTION

The ribosome is an essential component in the cell, serving as a site of protein biosynthesis; thus all self-replicating cells have their own ribosomes. Many biochemical and genetic studies of ribosomal components have been done using various organisms, showing that the ribosomes exhibit divergent evolution. It is, therefore, advantageous to use ribosomes or ribosomal genes for studying evolution, since the results of one organism can be directly compared with those of others. The present paper summarizes our recent work on the ribosome of *Mycoplasma capricolum*. Several lines of evidence have suggested that the mycoplasma is phylogenetically related to gram-positive bacteria.

MATERIALS AND METHODS

M. capricolum ATCC27343 (KID) was used throughout the experiments. The detailed experimental procedures were described in the separate papers [1-3].

RESULTS AND DISCUSSIONS

The Nucleotide Sequence of 5S rRNA

The sequences of 5S rRNAs from over two hundred organisms have been reported and used for deducing phylogenic relationships among them [4,5]. We have determined the total nucleotide sequence of *M. capricolum* 5S rRNA mainly by the rapid chemical degradation procedure of Peattie [6] and reported in a separate paper [1].

*On leave from Department of Biochemistry and Biophysics, Research Institute for Nuclear Medicine and Biology, Hiroshima University, Hiroshima, Japan.

Address reprint requests to: Dr. Akira Muto, Laboratory of Molecular Genetics, Dept. of Biology, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Japan 464

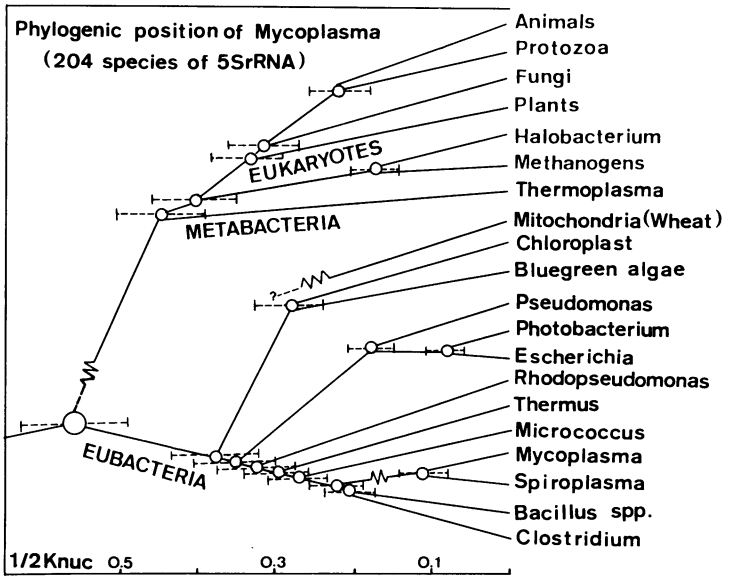

FIG. 1. Phylogenic tree of 5S rRNAs. **Knuc** represents the rate of nucleotide substitution (see [5]).

The length is 107 nucleotides, the shortest of all the 5S rRNAs so far known. The 5S rRNA has the lowest G + C content (42 percent among the 5S rRNAs yet sequenced, but still higher than the average G + C content of the total genome DNA (25 percent) of this species. The nucleotide sequence of *M. capricolum* 5S rRNA is more similar to that of the gram-positive bacteria than the gram-negative. Figure 1 shows the phylogenic relationship of *M. capricolum* to other organisms deduced by the sequence homologies of the 5S rRNAs (see [5]). Comparisons of the mycoplasma tRNA sequences with other bacterial tRNAs also revealed the same relationship [7]. Walker et al. [8] have sequenced 5S rRNA of *Spiroplasma* sp. BC3, indicating that the spiroplasma is closely related to the mycoplasma. On the other hand, *Thermoplasma acidophilum,* whose 5S rRNA sequence was determined by Luhrsen et al. [9], has been shown to be a member of Metabacteria [5].

The Organization of rRNA Genes

It has been previously shown that *M. capricolum* has only one set of rRNA genes by hybridization-saturation experiments between 16S and 23S rRNAs, and DNA [10]. We reexamined the copy number of rRNA genes by the Southern blotting analysis [11]. The total DNA was completely digested either with *Eco*RI, *Bg*lII, or *Xba*I endonucleases, separated by agarose gel electrophoresis, and submitted to hybridization with ^{32}P-3′-end-labeled 16S, 23S, and 5S rRNAs, respectively. The results have clearly shown that the *M. capricolum* genome carries at least two sets of genes for 16S, 23S, and 5S rRNAs [2]. For example, the *Bg*lII digested DNA gel gave two distinct bands of 6.8 and 9.8 kilobases (Kb) long, equally with 16S, 23S, and 5S rRNA. The results also suggest that each one of the copies for 16S, 23S, and 5S rRNA genes are clustered on the chromosome. To see the rRNA gene organization, we have cloned several DNA fragments containing *M. capricolum* rRNA genes to the *Escherichia coli* plasmids pBR322 or pBR325. The gene organization in the cloned DNA fragments was analyzed by the Southern blotting method. Figure 2 shows the physical maps of two of these hybrid plasmid DNAs. The plasmids pMCB221 and pMCB339, respectively, contain 6.8 and 9.8 Kb DNA fragments

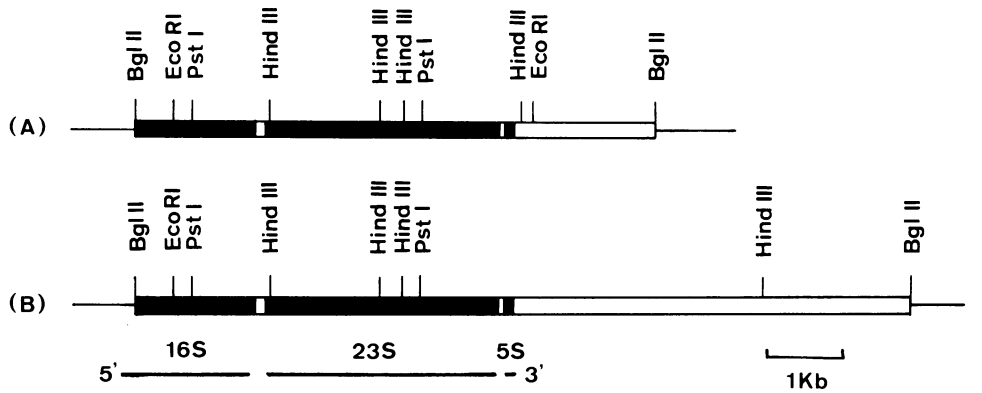

FIG. 2. Physical maps of *M. capricolum* rRNA genes. The DNA fragments generated by *Bg*lII digestion of *M. capricolum* genome were cloned in the plasmid vector pBR322. The hybrid plasmids (**A**) pMCB221 and (**B**) pMCB339, respectively, contain 6.8 and 9.8 Kb DNA fragments including rRNA genes. Thick lines represent the *M. capricolum* DNA. The vector DNA is shown as thin lines. The dark zones represent rRNA-coding regions.

generated by *Bg*lII digestion, each including a different set of rRNA genes. In both DNAs, the rRNA genes are arranged in the order of 16S, 23S, and 5S rDNA. Thus the arrangement of *M. capricolum* rRNA genes is common to other prokaryotic rRNA gene clusters so far known. We have determined the nucleotide sequences of several parts of the cloned rRNA genes, including the spacer region between 16S and 23S rRNA genes. The results have revealed that the coding sequences of 16S and 23S rRNA genes of *M. capricolum* are highly similar to those of *Bacillus subtilis* and *E. coli,* suggesting that the rRNA genes are well conserved in these prokaryote genomes. On the other hand, the spacer sequences between 16S and 23S rRNA genes have little resemblence between *M. capricolum* and *B. subtilis* or *E. coli.* We have so far not detected any tRNA like-sequence in the spacer region.

The isolation of the two different clones, each containing a set of rRNA genes, strongly supports the idea that the *M. capricolum* genome carries two sets of rRNA genes [2]. Contrary to this, Amikam et al. [12] recently reported that *M. capricolum* contains only one set of rRNA genes, while there are two sets in *M. mycoides* subsp. *capri* and *Acholeplasma laidlawii.* The reason for this discrepancy is not clear. The genome size of mycoplasmas is one-quarter to one-fifth of that of *E. coli* or *B. subtilis.* It is known that the rRNA gene copies are at least seven in *E. coli* [13] and ten in *B. subtilis* [14]. These facts suggest that the copy number of rRNA genes varies from one bacterial species to another roughly in proportion to their genome size.

Ribosomal Proteins

The total and ribosomal proteins of *M. capricolum* were analyzed by two-dimensional (2D) gel electrophoresis, and compared with those of *E. coli* and *B. subtilis* [3]. First, the total proteins were separated by O'Farrell's 2D gel system [15,16] to estimate the approximate number of proteins in the cell. The number of protein spots detected was about 350, indicating that the *M. capricolum* genome contains at least about 350 genes for proteins. The number of whole cell protein spots of *E. coli* or *B. subtilis* detected under the same conditions is about 1,100.

Second, the ribosomal proteins were analyzed by the 2D gel system of Kaltschmidt and Wittmann [17]. The number of the *M. capricolum* ribosomal protein species is

at least 30 for 50S and 20 for 30S subunits with average molecular weight of about 15,000 daltons. This shows that the number and size of *M. capricolum* ribosomal proteins are not significantly different from other eubacterial ribosomal proteins in contrast to a great reduction of the number of total proteins. This suggests that the number and size of the genes for ribosomal proteins are conserved in the mycoplasma genome in spite of the limited genetic capacity. The protein profiles of both 30S and 50S subunits in 2D gel electrophoresis, as a whole, resemble those of *B. subtilis* or *B. stearothermophilus* rather than *E. coli.* Several characteristic features of ribosomal proteins from gram-positive bacteria, for example, the lack of protein S1 (see [18,19]), are also seen in *M. capricolum.* These observations are consistent with the idea that the mycoplasma is phylogenetically close to *Bacilli.*

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