JOURNAL OF BACTERIOLOGY, Apr. 1984, p. 376–378
0021-9193/84/040376-03$02.00/0
Copyright © 1984, American Society for Microbiology
Vol. 158, No. 1

Mycoplasmas (*Mollicutes*) Have a Low Number of rRNA Genes

DORIT AMIKAM,[1] GAD GLASER,[2] AND SHMUEL RAZIN[1]*

Department of Membrane and Ultrastructure Research[1] and Department of Cellular Biochemistry,[2] The Hebrew University-Hadassah Medical School, Jerusalem 91010, Israel

Received 25 August 1983/Accepted 29 December 1983

DNA from *Mycoplasma*, *Ureaplasma*, *Acholeplasma*, and *Spiroplasma* species digested by restriction endonucleases was hybridized with probes consisting of portions of the *rrnB* rRNA operon of *Escherichia coli* and the rRNA operon of *Mycoplasma capricolum*. The results indicate the presence of only one or two sets of rRNA genes in the genome of *Mollicutes* linked in the procaryotic fashion, 16S-23S-5S.

The small genome size (5×10^8 to 1×10^9 daltons) of the mycoplasmas constituting the class *Mollicutes* and the low guanine-plus-cytosine content of their DNA (13) led Ryan and Morowitz (9) to predict long ago a low number of copies of rRNA genes in these organisms. Recent studies by Sawada et al. (11), employing the Southern procedure (12) to detect hybridization of labeled *Mycoplasma capricolum* rRNA species with DNA fragments produced by restriction endonuclease digestion supported the presence of two sets of rRNA genes in this mycoplasma. Our studies (1) with a similar blotting analysis of mycoplasmal DNA digests with a cloned portion of one of the rRNA gene sets of *M. capricolum* and of the *rrnB* rRNA operon of *Escherichia coli* also indicated a low number (one or two) of rRNA genes in several mycoplasmas. It seemed of interest to extend the investigation on the number of rRNA genes to other species of the class *Mollicutes*, a broad class of wall-less procaryotes containing organisms of diverse genetic makeup (13), probably arising along different lines of evolutionary descent (17).

The *Mycoplasma* and *Acholeplasma* species were grown in a modified Edward medium (8), except for *Mycoplasma pneumoniae*, which was grown in Hayflick's medium (5). *Ureaplasma urealyticum* (serotype not determined) was cultivated in a similar medium supplemented with 7 mM urea, and the spiroplasmas were grown in the medium of Saglio et al. (10). The organisms were harvested and washed as described before. DNA was prepared by the method of Marmur (6). Plasmids were isolated by the method of Clewell (3). Digestion by restriction endonucleases (New England Biolabs, Beverly, Mass.) was performed at 37°C for 2 h in buffer mixtures recommended by the manufacturer. Digested DNA was electrophoresed on 0.8% agarose slab gels as described before. DNA fragments from gels were transferred to nitrocellulose sheets by the method of Southern (12).

Plasmid pKK3535 containing the intact rRNA operon *rrnB* of *E. coli* (2) was digested by *Eco*RI to give three fragments, designated probes 2, 3, and 4 in Fig. 1. The fragments were eluted from the gel by the method of Vogelstein and Gillespie (15) and were used as probes after being nick translated as described below. An additional probe was obtained by digestion of pKK3535 with *Ava*I, resulting in a 4,500-base-pair (bp) fragment containing 127 bp of the 23S rRNA region, all the 5S rRNA, and the region of the terminators (probe 5 in Fig. 1). Another plasmid (pGG1; probe 1 in Fig. 1) carrying the 5′ end of the *rrnB* rRNA operon of *E. coli* (4) and plasmid pMC5 (Fig. 1) carrying a 4.8-kilobase (kb) insert of *M. capricolum* DNA coding for the 5S, 23S, and part of the 16S rRNAs (1) were also included in the battery of probes employed. The probes were nick translated with α-^{32}P to a level of 2×10^8 to 4×10^8 cpm/μg of DNA (16). Hybridization with the labeled probes was done by the method of Pollack et al. (7).

Table 1 summarizes the hybridization data obtained with *Eco*RI-digested DNA. In some cases, additional restriction enzymes were used to clarify and support data obtained with *Eco*RI. Thus, digestion of the *Mycoplasma orale* DNA with *Bgl*II yielded on hybridization with pMC5 and probe 4 one 6.8-kb band only. When the DNA was digested by both *Eco*RI and *Bgl*II, a single 5.0-kb hybridization band was observed with probe 4, supporting the conclusion that *M. orale* has a single set of rRNA genes. The *Eco*RI-digested *Mycoplasma pneumoniae* and *Spiroplasma citri* DNA gave only one hybridization band with the probes used (Table 1). Since the size of the chromosomal segment hybridized may suffice to accommodate two rRNA gene sets arranged in tandem, the DNA of these mollicutes was digested with *Bgl*II and *Pst*I. The hybridization data obtained failed, however, to provide a definite answer, so that the estimated number of rRNA operons in these organisms was left at one or two. The results obtained with *Mycoplasma gallisepticum* A5969 (Table 1) are more difficult to interpret, as the hybridization tests produced the bands presented schematically in Fig. 2. These results can be explained by assuming that *M. gallisepticum* possesses two rRNA operons, which differ somewhat in their nucleotide sequence, presumably at the 16S region, a difference responsible for the presence of an *Eco*RI restriction site in only one of the operons.

The organisms examined by us belong to different families and genera within the class *Mollicutes* and are genetically unrelated to each other, as indicated by DNA base composition and hybridization studies (13). Nevertheless, our data show that they resemble each other in carrying a low number of rRNA genes. Although the relatively small number of species tested so far cautions against sweeping generalizations, a tentative conclusion stating that the genomes of the mollicutes contain only one or two rRNA gene sets or operons appears justified. Our present hybridization data with *Eco*RI-digested *M. capricolum* DNA support the conclusion of Sawada et al. (11) that this organism carries two sets of rRNA genes. In our earlier experiments, we failed, apparently for technical reasons, to observe the 20-kb hybridization band with probes pMC5 and pGG1, leading to the erroneous conclusion that *M. capricolum* carries only one rRNA gene set (1).

* Corresponding author.

TABLE 1. Number and size of chromosomal segments of various mollicutes hybridizing with different probes of RNA genes[a]

Organism	Probe designation	No. of hybridization bands	Size of chromosomal segment hybridized (kb)	Estimated no. of rRNA operons
Mycoplasma orale (ATCC 23714)	pMC5	1	5	
	1	2	5;6.6	1
	3	1	5	
	4	1	5	
Mycoplasma capricolum (ATCC 27343)	pMC5	2	4.8;20	
	1	3	1;4.8;20	2
	2	3	1;4.8;20	
	3	2	4.8;20	
	4	2	4.8;20	
	5	2	4.8;20	
Mycoplasma pneumoniae (strain FH and M129-B16)	pMC5	1	9	
	1	1	9	
	2	1	9	1 or 2
	3	1	9	
	4	1	9	
Mycoplasma arginini (G-230)	pMC5	2	4;7	
	1	4	4;4.2;7;7.2	2
	3	2	4;7	
	4	2	4;7	
Mycoplasma gallisepticum (strain A5969)	pMC5	3	8;8.2(weak);9	
	1	3	8;8.2;9(weak)	
	2	3	8;8.2;9(weak)	2
	3	3	8;8.2(weak);9	
	4	2	8;9	
Mycoplasma gallisepticum (strain S6)	pMC5	1	8.0	
	1	1	8.0	1
	4	1	8.0	
	5	1	8.0	
Acholeplasma laidlawii (oral strain)	pMC5	2	5.2; 8.8	
	1	4	4.8;5.2;8.0;8.8	2
	3	2	5.2;8.8	
	4	2	5.2;8.8	
Acholeplasma axanthum	pMC5	2	6.4;7.0	
	1	4	2.0;6.4;7.0;8.0	2
	5	2	6.4;7.0	
Acholeplasma granularum	pMC5	2	6.9;9.6	
	1	4	6.9;8.0;8.4;9.6	2
	5	2	6.9;9.6	
Ureaplasma urealyticum	pMC5	4	2.5;6.2;8.2;12.0	
	1	2	2.5;6.2	2
	4	2	8.2;12.0	
	5	2	8.2;12.0	
Spiroplasma citri (Maroc R8A2)	pMC5	1	13	
	1	1	13	
	2	1	13	1 or 2
	3	1	13	
	4	1	13	
Spiroplasma sp. B31 (serogroup IV)	pMC5	2	5.6;6	
	1	3	5.6;6;10.4	2
	3	2	5.6;6	
	4	2	5.6;6	
Spiroplasma sp. BC-3 (serogroup I-2)	pMC	1	4.1	
	1	2	1.1;4.1	1
	4	1	4.1	
	5	1	4.1	
Spiroplasma sp. MQ-1 (serogroup VII)	pMC5	1	7	1
	1	2	7;1.6	
	4	1	7	
	5	1	7	

[a] The DNA of the mollicutes was digested with *Eco*RI before hybridization with the probes. For probe designation see Fig. 1.

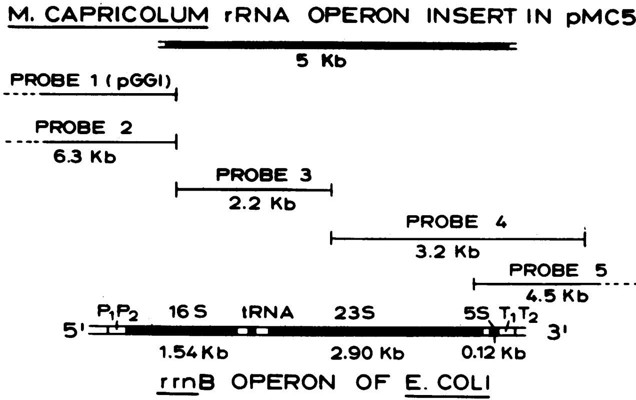

FIG. 1. Probes used to study rRNA genes in mollicutes.

Our findings, that the different probes consisting of consecutive and defined portions of the *rrnB* rRNA operon of *E. coli* (Fig. 1) hybridized well with the DNA fragments carrying the corresponding rRNA genes of all mollicutes tested by us, support the existence of a marked sequence homology along the entire rRNA operon of *E. coli* and the presumed rRNA operons of the mollicutes. Furthermore, our data indicate that the rRNA genes in the operon are linked together in all the mollicutes tested by us. Moreover, restriction mapping of the two rRNA gene sets of *M. capricolum* done by us (unpublished data) showed that the rRNA genes in this organism are linked in the typical procaryotic fashion, that is, 5′-16S-23S-5S-3′. In contrast, in the wall-less archaebacterium *Thermoplasma acidophilum*, previously included in the class *Mollicutes*, the rRNA genes appear unlinked, with large spacers separating them (14).

Our data show clearly that every mollicute tested by us has different flanking sequences adjacent to its rRNA operon(s), as the *Eco*RI-digested DNA of each of the tested species yielded a species-specific and highly reproducible hybridization pattern. The ability to identify a mycoplasma according to its hybridization pattern with highly labeled rDNA probes, such as pMC5, can be used to detect and identify mycoplasmas in contaminated cell cultures (S. Razin, M. Gross, M. Wormser, Y. Pollack, and G. Glaser, In Vitro, in press).

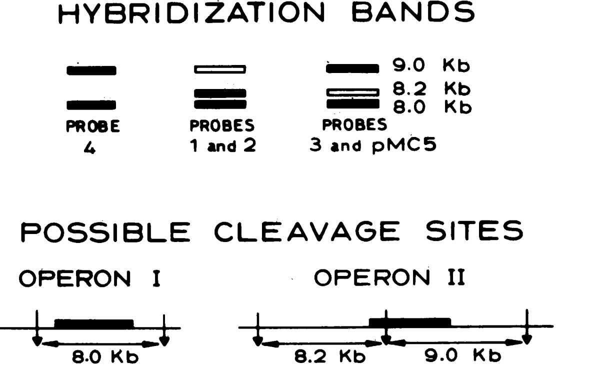

FIG. 2. Schematic presentation of the hybridization bands of *Eco*RI-digested *M. gallisepticum* A5969 DNA with various probes of rRNA genes and the possible cleavage sites in or adjacent to the proposed two rRNA operons of this organism.

We gratefully acknowledge the excellent technical assistance of Miriam Gross and Mordechai Wormser. We thank R. F. Whitcomb for the supply of *Spiroplasma* strains and Aharon Razin and Howard Cedar for their advice.

This study was supported by Public Health Service grant GM 25286, awarded to G.G., and by a grant from the United States-Israel Binational Agricultural Research Development Fund awarded to S.R.

LITERATURE CITED

1. **Amikam, D., S. Razin, and G. Glaser.** 1982. Ribosomal RNA genes in mycoplasma. Nucleic Acids Res. **10**:4215–4222.
2. **Brosius, J., A. Ullrich, M. A. Raker, A. Gray, T. J. Dull, R. R. Gutell, and H. F. Noller.** 1981. Construction and fine mapping of recombinant plasmids containing the *rrnB* ribosomal RNA operon of *E. coli*. Plasmid **6**:112–118.
3. **Clewell, D. B.** 1972. Nature of the colE1 plasmid replication in *Escherichia coli* in the presence of chloramphenicol. J. Bacteriol. **110**:667–676.
4. **Glaser, G., L. Enquist, and M. Cashel.** 1977. ColE1 cloning of a ribosomal RNA promoter region from λrifd18 by selection for lambda integration and excision functions. Gene **2**:159–172.
5. **Hayflick, L.** 1965. Tissue cultures and mycoplasmas. Texas Rep. Biol. Med. **23**(suppl. 1):285–303.
6. **Marmur, J.** 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. **3**:208–218.
7. **Pollack, Y., R. Stein, A. Razin, and H. Cedar.** 1980. Methylation of foreign DNA sequences in eukaryotic cells. Proc. Natl. Acad. Sci. U.S.A. **77**:6463–6467.
8. **Razin, S., and S. Rottem.** 1976. Techniques for the manipulation of mycoplasma membranes, p. 3–26. *In* A. H. Maddy (ed.), Biochemical analysis of membranes. Chapman and Hall, London.
9. **Ryan, J. L., and H. J. Morowitz.** 1969. Partial purification of native rRNA and tRNA cistrons from *Mycoplasma* sp. (Kid). Proc. Natl. Acad. Sci. U.S.A. **63**:1282–1289.
10. **Saglio, P., D. LaFlèche, C. Bouissol, and J. M. Bové.** 1971. Isolement, culture et observation au microscope électronique des structures de type mycoplasme asociées à la maladie du stubborn des agrumes et leur comparaison avec les structures observées dans le cas de la maladie du Greening des agrumes. Physiol. Veg. **9**:569–582.
11. **Sawada, M., S. Osawa, H. Kobayashi, H. Hori, and A. Muto.** 1981. The number of ribosomal RNA genes in *Mycoplasma capricolum*. Mol. Gen. Genet. **182**:502–504.
12. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
13. **Stanbridge, E. J., and M. E. Reff.** 1979. The molecular biology of mycoplasmas, p. 157–185. *In* M. F. Barile and S. Razin (ed.), The mycoplasmas, vol. 1. Academic Press, Inc., New York.
14. **Tu, J., and W. Zillig.** 1982. Organization of rRNA structural genes in the archaebacterium *Thermoplasma acidophilum*. Nucleic Acids Res. **10**:7231–7245.
15. **Vogelstein, B., and D. Gillespie.** 1979. Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. U.S.A. **76**:615–619.
16. **Weinstock, R., R. Sweet, M. Weiss, H. Cedar, and R. Axel.** 1978. Intragenic DNA spacers interrupt the ovalbumin gene. Proc. Natl. Acad. Sci. U.S.A. **75**:1299–1303.
17. **Woese, C. R., J. Maniloff, and L. B. Zablen.** 1980. Phylogenic analysis of the mycoplasmas. Proc. Natl. Acad. Sci. U.S.A. **77**:494–498.