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Physical mapping of the ribosomal RNA genes of *Mycoplasma capricolum*

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ABSTRACT

Physical mapping of the rRNA genes of Mycoplasma capricolum was done by digestion of the mycoplasmal DNA with EcoRI, PstI and BglII and hybridization with nick-translated probes consisting of defined portions of the rrnB ribosomal RNA operon of Escherichia coli. The results indicate that the rRNA genes in the chromosome of M. capricolum are arranged in two clusters, each organized in the order 5'-16S-23S-5S-3', resembling the order of the genes in the rrnB operon, with no large spacer regions separating the genes in each cluster.

INTRODUCTION

Mycoplasmas, the smallest wall-less prokaryotes, appear to carry only one or two rRNA sets of genes, as was recently shown by us on examination of 13 species belonging to different taxonomic groups within the class Mollicutes (Amikam, Glaser and Razin, J. Bacteriol., in press). This conclusion has been based on results of experiments in which mycoplasmal DNA cleaved by restriction endonucleases was hybridized by the Southern blotting procedure (1) with nick-translated probes consisting of E. coli rRNA genes or with pMC5, a clone containing part of the 16S, and the entire 23S and 5S rRNA genes of M. capricolum (2). The marked sequence homology revealed between the rRNA genes of E. coli and the corresponding rRNA genes of mycoplasmas enabled the use of defined portions of the rrnB operon of E. coli for physical mapping of the two rRNA gene sets of M. capricolum. The data summarized in the present communication indicate that the rRNA genes in M. capricolum form two clusters arranged in the same order as in other prokaryotes, that is 5'-16S-23S-5S-3'. Each of the two clusters functions presumably as an operon. The term operon will be used in this paper as a matter of convenience, though no evidence is available as yet to substantiate the use of this term in the strict sense.

MATERIALS AND METHODS

Organism and growth conditions: Mycoplasma capricolum (California kid strain, ATCC 27343) was used throughout this study. The mycoplasma was grown in

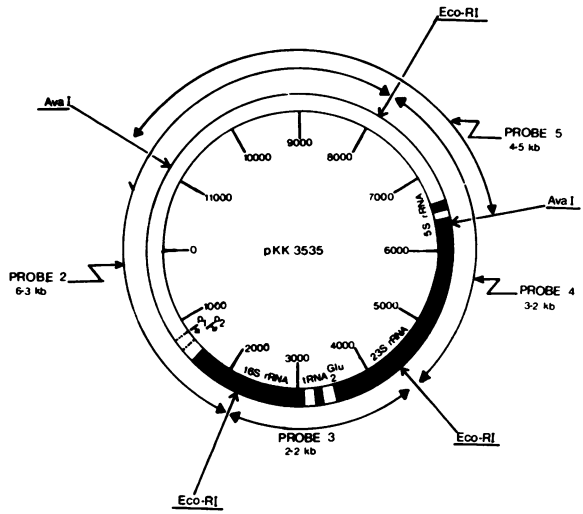

Fig. 1. Probes for rRNA genes derived by digestion of the plasmid pKK3535 that carries the rrnB operon of E. coli.

a modified Edward medium (3) supplemented with 3% horse serum for 20-24 hr at 37°C. The organisms were harvested by centrifugation at 12000 xg for 30 min in the cold and were washed once in cold 0.25M NaCl containing 0.1M EDTA. The pellets of washed cells were kept at -20°C until used for preparation of DNA.

DNA preparation and cleavage: DNA was prepared according to Marmur (4). Plasmids were isolated by the procedure of Clewell (5). Restriction endonucleases were purchased from New England Biolabs (Beverly, MA). Digestion by restriction enzymes was performed at 37°C for 2 hr in buffer mixtures recommended by the manufacturer. Digested DNA was electrophoresed on 0.8% agarose slab gels in buffer containing 0.04M Tris-acetate, pH 8.0 and 0.001M EDTA at 30 mM for 18 hr. DNA fragments from gels were transferred to nitrocellulose sheets according to the method of Southern (1).

Probes: The plasmid pKK3535 containing the intact ribosomal RNA operon rrnB of E. coli (6) was digestd by EcoRI to give three fragments of the following sizes and composition: 1) a fragment of 6300 base pairs (bp) containing the DNA coding for the promoters P1 and P2 and for part of 16S rRNA (probe 2 in Fig. 1); 2) a fragment of 2200 bp containing the DNA coding for the rest of the 16S rRNA, the tRNA and some of the 5'-region of the 23S rRNA (probe 3 in Fig. 1); 3) a fragment of 3200 bp containing the DNA coding for the rest of the 23S rRNA, the 5S rRNA and the terminators region (probe 4 in Fig. 1). The fragments were

Table 1. Size (in Kb) of M. capricolum chromosomal segments hybridizing with different probes of ribosomal RNA genes*

Probe \ Restriction enzyme	EcoRI	BglII	PstI	EcoRI + PstI	EcoRI + BglII	PstI + BglII
Probe 1 (5' end of rrnB operon)	1.0;4.8; 20	1.5;1.8; 6.6;8.8	2.8;3.0; 8.0	1.0;1.8; 2.8;7.8	0.5;1.0; 1.8;4.8 20	0.7;1.5; 1.8;2.8
Probe 2 (5' end of rrnB operon)	1.0;4.8 20	1.5;1.8 6.6;8.8	2.8;3.0 8.0	--	--	--
Probe 3 (Central portion of rrnB)	4.8;20	6.6;8.6	2.8;3.0 8.0	2.8	--	--
Probe 4 (Distal portion of rrnB)	4.8;20	--	2.8;3.0	1.8;3.0 2.8	--	--
Probe 5 (3' end of rrnB operon)	4.8;20	--	--	1.8;3.0	--	--
pMC5 (The 4.8Kb cloned fragment of operon A of M. capricolum)	4.8;20	6.6;8.6	2.8;3.0	--	4.8;8.1	--

*The underlined values represent DNA fragments producing heavier hybridization bands.

eluted from the gel by the method of Vogelstein and Gillespie (7) and were used as probes after being nick-translated as described below. An additional probe was obtained by digestion of pKK3535 with AvaI, resulting in a 4500 bp fragment containing 127 bp of the 23S rRNA region, all the 5S rRNA and the terminators region (probe 5 in Fig. 1). Another plasmid, pGG1, was used as a probe. This plasmid, designated probe 1 in the present communication, carries the same 5'-end of the rrnB operon of E. coli as probe 2 (8). The plasmid pMC5 (Fig. 3) carrying a 4.8 kb insert of M. capricolum DNA coding for the 5S, 23S and part of the 16S rRNAs (2) was also included in the battery of probes employed.

Nick translation and hybridization: The probes were nick-translated with [α^{32}P] deoxycytosinetriphosphate (New England Nuclear, Boston, MA) to a level of 2-4 x 10^8 cpm/µg DNA (9). Hybridization with the labeled probes was done according to Pollack et al. (10).

RESULTS AND DISCUSSION

M. capricolum DNA cleaved by EcoRI yielded two hybridization bands of 4.8 kb and 20 kb with probes 3, 4, 5 and with pMC5 (Table 1). Hybridization of the same blots to probes 1 or 2 yielded an additional heavy band of 1 kb. When the DNA was cleaved by Bgl II, probes 3 and pMC5 yielded two hybridization bands of 6.6 kb and 8.6 kb (Table 1). These results concur with those of Sawada et al. (11) obtained with labeled rRNAs as probes, and support the presence of two sets (or operons) of rRNA genes in this mycoplasma.

Probes 1 or 2, carrying the 5' end of the E. coli ribosomal RNA operon rrnB, yielded four bands with BglII digested M. capricolum DNA (1.5; 1.8; 6.6 and 8.6 kb, Table 1). Since the 1.5 and 1.8 kb bands were observed only with these probes, it can be deduced that the small DNA fragments (1.5 and 1.8 kb) belong to the proximal part of the rRNA operon. Digestion of the DNA with PstI and hybridization with the probes containing the 5' end of the E. coli operon (probes 1 or 2) or with the probe carrying the central part of the operon (probe 3), yielded three bands, which differed in intensity with the different probes. Probes 1 or 2 gave strong 3 kb and 8 kb bands and a weak 2.8 kb band, while probe 3 gave a strong 2.8 kb band and weak 3 and 8 kb bands. Probe 4 gave two hybridization bands only, of 2.8 kb and 3 kb.

Double digestion of the DNA with combinations of the enzymes helped to achieve a finer mapping of the two operons, as presented schematically in Fig. 2. The 6.6 kb BglII fragment obtained with probes 1, 2, 3 and pMC5, was placed in operon A, carrying the 4.8 kb EcoRI fragment. This was verified by digestion with BglII and EcoRI, where the 6.6 kb fragment disappeared and a 4.8 kb fragment was produced, which hybridized with pMC5. In the same experiment the 8.6 kb BglII fragment placed in operon B was cut only slightly, to give an 8.1 kb fragment hybridizing with pMC5 (Table 1). Fine mapping of the 5' ends of the operons was achieved by double digestions and hybridizations with probe 1 (Table 1 and Fig. 2). Thus, the presence of an EcoRI cut in the 1.5 kb BglII fragment, the digestion of the 3 kb PstI fragment with BglII and EcoRI, and the size of fragments obtained (Table 1) helped us in the final positioning of the different restriction sites of the two operons.

The plasmid pMC5 which carries the 4.8 kb insert of operon A of M. capricolum cloned into pBR 325 (2) was tested for restriction sites, using the plasmid pBR 325 as a control. Based on the data obtained with PstI and EcoRI, a restriction map of pMC5 was constructed (Fig. 3). The existence of a 0.2 kb fragment produced by digestion with EcoRI and PstI, though not detected in our gels, is supported by the detection of a 0.7 kb fragment on hybridization of

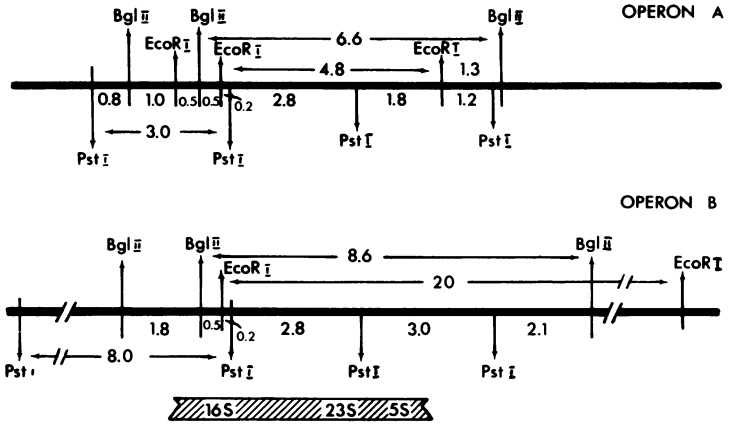

Fig. 2. Physical map of the two ribosomal RNA operons of M. capricolum.

PstI + BglII digested DNA with probe 1 (Table 1 and Fig. 2).

The main conclusion that may be drawn from our data is that the two rRNA gene sets of M. capricolum are organized as a cluster 5'-16S-23S-5S-3', that is in the same order as in the rRNA operons of E. coli. It can also be presumed that the order of transcription of the M. capricolum genes follows that of E. coli. There is apparently no large spacer regions between the genes in each cluster, unlike the findings with the rRNA genes in Thermoplasma acidophilum

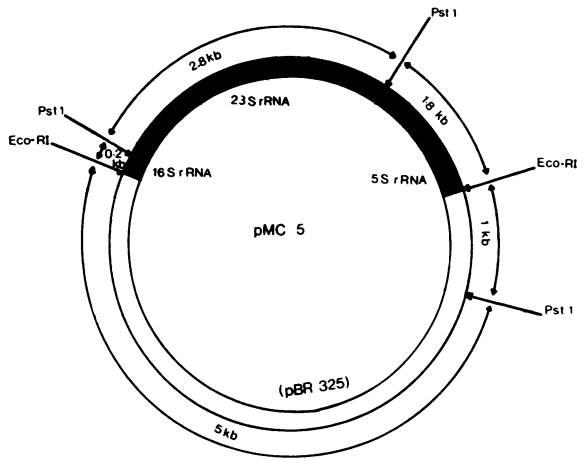

Fig. 3. Restriction map of pMC5 with PstI and EcoRI.

(12). It also appears that the two rRNA gene sets, which most probably function as operons, are not arranged in tandem, but are separated from each other.

In light of our knowledge of prokaryotic rRNA operons, the 3 kb and 8 kb PstI fragments at the 5' end of the two M. capricolum operons most probably carry the promoters region. Experiments are now under way to characterize these regions, and elucidate the elements participating in the control of rRNA transcription in M. capricolum. As shown by us previously (13) rRNA transcription in this mycoplasma is subject to stringent control.

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