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Ribosomal RNA genes in Mycoplasma

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ABSTRACT

Using Southern blotting analysis with labelled mycoplasmal ribosomal RNA as probe, two fragments (1 Kb and 5 Kb) were detected in an EcoR I digest of Mycoplasma capricolum DNA. This analysis revealed that the 5 Kb fragment carries both 16S rRNA sequences and the entire 23S rRNA gene of this mycoplasma. The 1 Kb fragment contains 16S rRNA sequences only. The 5 Kb EcoR I fragment has been cloned and used to characterize the structure of rRNA cistrons in various Mycoplasma strains.

These experiments clearly demonstrate a substantial homology of Mycoplasma capricolum rRNA sequences with the E. coli rRNA cistron on one hand, and with Mycoplasma mycoides subsp. capri and Acholeplasma laidlawii on the other hand. This analysis also reveals two rRNA cistrons in Mycoplasma mycoides subsp. capri and Acholeplasma laidlawii whereas one rRNA cistron is present in Mycoplasma capricolum.

INTRODUCTION

The control of rRNA synthesis in prokaryotes was studied extensively during the last two decades. However, the molecular mechanisms involved in the regulation of rRNA synthesis are still only partially understood. It is well established that the expression of ribosomal RNA genes is affected by changes in growth conditions (1). One of the control mechanisms activated by these changes is known as the stringent response (2). We have recently shown that ribosomal RNA synthesis in M. capricolum responds to amino acid starvation in a similar fashion to the E. coli system, that is cessation of stable RNA synthesis and accumulation of 5'-triphosphoguanosine-3'-diphosphate and 5'-diphosphoguanosine-3'-diphosphate (3). Obviously, information on the ribosomal RNA genes in mycoplasmas is required for their effective utilization as tools in studying ribosomal RNA synthesis and its control. The extreme structural and biochemical simplicity of mycoplasmas, the smallest self-replicating prokaryotes, has made them most useful models for studying basic problems in cell biology, particularly those concerning membrane structure

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and function (4). The fact that the genome size of most known mycoplasmas is about 5 x 10^8 daltons, the smallest recorded in self-replicating organisms (5), suggests that mycoplasmas may also serve as very useful experimental systems in molecular genetic studies, as they presumably possess only the minimal set of genes required for independent growth and replication (6). In support of this assumption Ryan and Morowitz (7) suggested long ago on the basis of hybridization saturation experiments between 16S and 23S rRNA and DNA of M. capricolum that this organism contains only one set of ribosomal RNA genes. Our results presented in this communication confirm this old suggestion, by utilizing cloning procedures, but show that other mycoplasmas may contain two ribosomal RNA cistrons. In addition, our hybridization data demonstrate substantial homology in the ribosomal RNA genes of several mycoplasmas, and of E. coli, indicating the marked conservation in structure of this part of the prokaryotic chromosome during evolution.

MATERIALS AND METHODS

Organisms and growth conditions: Two strains of Mycoplasma capricolum (California kid strain, ATCC 27343, and California goat strain) were obtained from J.G. Tully (NIH, Bethesda, MD). The identity of these strains was confirmed by specific fluorscein-labelled antibodies to M. capricolum. Acholeplasma laidlawii (oral strain) and Mycoplasma mycoides subsp. capri were from our laboratory collection. The organisms were grown in a modified Edward medium (8), supplemented with 3% horse serum. The cultures were incubated aerobically at 37°C and were harvested after 20-24 hr 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 by the Marmur procedure (9). Plasmids were isolated by the procedure of Clewell (10). Restriction endonucleases were purchased from New England Biolabs (Beverly, MA). Digestions by restriction enzymes were performed at 37°C in buffer mixtures recommended by the manufacturer. Transfection was carried out as described in Efstratiadis et al. (11). Digested DNA was electrophoresed on 0.8% agarose slab gels in Tris EDTA buffer (Tris Acetate 0.04M, pH 8.0, EDTA 0.001M) at 20mA for 18 hours. DNA fragments from gels were transferred to nitrocellulose sheets according to the method of Southern (12).

Nick translation and hybridization: Different probes were nick transla-

ted with [α^{32}P]dCTP (New England Nuclear) to a level of 2-4 x 10^8 cpm/µg (13). Hybridization with the labelled probes was according to Pollack et al. (14). ^{32}P labelled ribosomal RNA was isolated as previously described (15).

RESULTS

Hybridization of ^{32}P-labelled 23S ribosomal RNA of M. capricolum to an EcoR I digest of the DNA of the same mycoplasma revealed only a single strong band of 5 Kb (Fig. 1, lanes 3,4). However, two EcoR I bands were observed when 16S ribosomal RNA was used as probe, a strong 5 Kb fragment and a very weak hybridizing 1 Kb fragment (Fig. 1, lanes 1,2). The EcoR I digest of M. capricolum was cloned into pBR325 (16). Using the Hogness screening procedure (17) colonies which hybridized to a mixture of ^{32}P-labelled 16S and 23S M. capricolum rRNA were picked up.

Three colonies were further subcultured, characterized by their sensitivity to chloramphenicol (16) and used for plasmid preparation. The three isolated plasmids, designated pMC1, pMC5 and pMC9, were found to be of about the same size (∿ 11 kb). Upon digestion with EcoR I all three plasmids were shown to carry a 5 kb insert. The 5 kb fragments hybridized efficiently to 16S and 23S M. capricolum RNA (data not shown). Digestion of the plasmids with Hind III or double digestion with EcoR I and Hind III demonstrated that the 5 kb fragment is inserted in one orientation, in pMC1 and pMC9 and in the opposite orientation in pMC5 (data not shown). The plasmid pMC5 was used as probe for further analysis of the mycoplasmal rRNA genes. As can be seen in

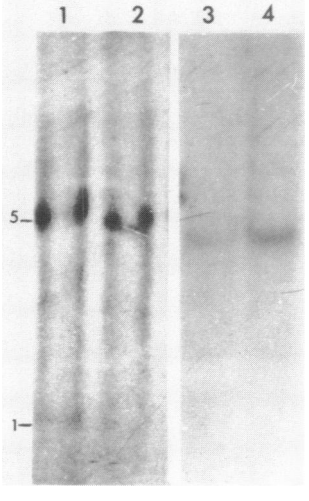

Fig. 1. Hybridization of ^{32}P-labelled M. capricolum ribosomal RNA to EcoR I digest of the DNA of the same organism. DNA isolation, digestion, fractionation and hybridization, were as described in the Methods. Lanes 1 and 2 were hybridized with 16S ribosomal RNA whereas lanes 3 and 4 were hybridized with 23S RNA.

Fig. 2, the nick-translated plasmid pMC5 hybridized to a single 5 kb band of the EcoR I digest of M. capricolum DNA, whereas two bands (8.8 kb and 5.2 kb) of EcoR I digest of Acholeplasma laidlawii DNA were revealed by the pMC5 probe (Fig. 2). In M. mycoides subsp. capri we could observe also two EcoR I bands of different size (9.5 kb and 6.8 kb). A control experiment with the nick translated cloning vehicle pBR325 used as probe did not show any nonspecific hybridization (data not shown).

As was previously shown by Boros et al. (18), none of the ribosomal RNA cistrons in E. coli carries a BamH I restriction site. We made use of this fact to visualize the seven rRNA cistrons of E. coli by molecular hybridization of a BamH I digest of E. coli DNA both to E. coli ^{32}P-labelled ribosomal RNA and to nick-translated pMC5 containing the 5 kb insert of M. capricolum DNA. As can be seen in Fig. 3, the seven bands are observed both with ^{32}P-labelled E. coli ribosomal RNA and with the nick translated pMC5.

These results, together with our finding that the 5 kb fragment of M. capricolum DNA cloned in pMC1, pMC5 and pMC9 hybridized both to 16S and 23S rRNA of E. coli (data not shown), raised the possibility of using pGG1 - a plasmid carrying the 5' end of the rrnB ribosomal cistron of E. coli (15) - as a probe, for analyzing the number of ribosomal RNA cistrons in the different mycoplasma species. As can be seen in Fig. 4, nick-translated pGG1 revealed two EcoR I bands in a M. capricolum EcoR I digest, and four EcoR I bands in M. mycoides subsp. capri and in A. laidlawii. Since pGG1 represents only the 5' end sequences of the E. coli rrnB cistron, it provides an

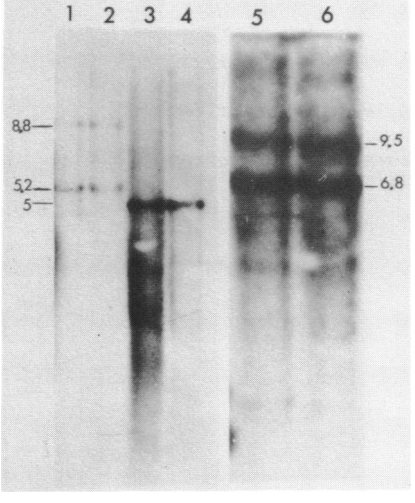

Fig. 2. Hybridization of EcoR I digest of DNAs of A. laidlawii (lanes 1 and 2), M. capricolum (lanes 3 and 4) and M. mycoides subsp. capri (lanes 5 and 6) to pMC5. The probe, pMC5, was prepared and nick translated as described in the Methods.

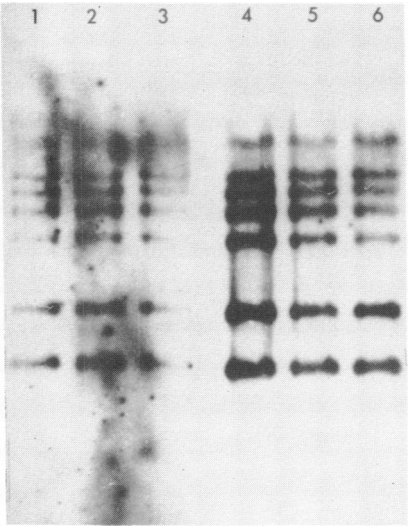

Fig. 3. BamH I DNA fragments coding for ribosomal RNA in E. coli:E. coli DNA was digested and blotted as described in the Methods. In lanes 1-3 hybridization was with ^{32}P-labelled 16S and 23S E. coli ribosomal RNA; In lanes 4-6 hybridization was with nick-translated pMC5, containing the 5 kb fragment of M. capricolum DNA.

ideal system for probing the 5' ends of the rRNA cistrons in Mycoplasma. The strong bands in Fig. 4 represent therefore the very extreme sequences at the 5' end whereas the weak bands represent rRNA sequences with which the pGG1 probe partially overlaps. Thus one strong band (1 kb) was observed with M. capricolum DNA (Fig. 4, lanes 5,6) and two strong bands (1 kb and 3.7 kb) are apparent with M. mycoides subsp. capri DNA (Fig. 4, lanes 1,2). It should be

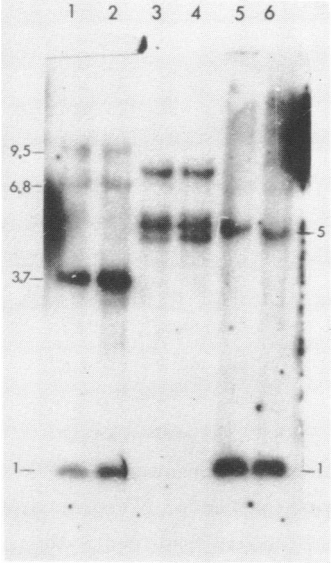

Fig. 4. Hybridization of pGG1 DNA to EcoR I digest of mycoplasmal DNA. Lanes 1,2 M. mycoides subsp. capri; lanes 3,4 A. laidlawii; lanes 5 and 6 M. capricolum.

pointed out that the two bands observed by pGG1 hybridization to EcoR I digest of M. capricolum DNA were also seen when ^{32}P-labelled M. capricolum 16S ribosomal RNA or E. coli 16S ribosomal RNA were used as probes (Fig. 1).

DISCUSSION

The data presented here suggest that while M. capricolum strains carry only one ribosomal RNA cistron, there are at least two ribosomal RNA cistrons in the genome of M. mycoides subsp. capri and in A. laidlawii. This conclusion is drawn from hybridization experiments with pMC5 (carries most rRNA sequences except for the 5' end of the cistron) , as described in Fig. 2. These results gain further support by the hybridization experiments with pGG1 as a probe to the different mycoplasma strains. pGG1, which carries only the 5' third of E. coli 16S ribosomal RNA cistron (15), was found to be an adequate probe for mapping of the mycoplasmal ribosomal RNA cistrons. This probe hybridized strongly to the 5' end of an EcoR I fragment of the ribosomal cistron and much less to a distal fragment, the one which carries most of the ribosomal RNA sequences (Fig. 4). Our results suggest that at least in all the mycoplasmas studied by us there is an EcoR I cut at the 5' end of the 16S ribosomal RNA gene. It should be noted, however, that both pMC5 and pGG1 hybridize to the rRNA genes of A. laidlawii at a lower efficiency than to the corresponding genes of M. capricolum or M. mycoides subsp. capri strains, suggesting a sifference in rRNA sequences between the Acholeplasma and the Mycoplasma species tested.

While we confirm the conclusion of Ryan and Morowitz (7) our results do not agree with those reported recently by Sawada et al. (19), who claimed for the presence of two cistrons in M. capricolum. In our experiments the ribosomal RNA cistron in M. capricolum carries a Bgl II site, so that by using pMC5 as a probe one could observe two bands (9.2 kb and 6.5 kb), similar to the bands described by Sawada et al. (19). Although M. mycoides subsp. capri is similar in its biological properties to M. capricolum, we could show that in M. mycoides subsp. capri there are two ribosomal RNA cistrons. This is also true for A. laidlawii in which the genome size is about double the size of the M. capricolum genome (20).

Our results with pMC5 as a probe indicate the great similarity in the ribosomal RNA genes of the different mycoplasmas studied. Moreover, they show that ribosomal RNA of E. coli is closely related to that of mycoplasma, a finding of great phylogenetic significance. These results combined with our previous findings that the control of ribosomal RNA synthesis in M. cap-

ricolum resembles that of E. coli (3), lead us to believe that M. capricolum with its single rRNA cistron provides a convenient system to study the control of rRNA synthesis in prokaryotes. The fact that E. coli, which has a genome size five times larger than that of M. capricolum, carries about seven ribosomal RNA cistrons (21), raises the possibility that the number of ribosomal RNA cistrons is grossly proportional to the size of the genome.

The reasons for the multiplicity of ribosomal RNA cistrons in E. coli and B. subtilis (19) are not clear. It can be speculated that in these bacteria, capable of growing in versatile habitats, the presence of a significant number of copies of ribosomal RNA cistrons amplifies RNA synthesis and enables thus fast growth even under adverse conditions. The mycoplasmas, on the other hand, are parasites well adapted to peculiar and constant ecological niches, and in this way are protected from drastic changes in growth conditions. Accordingly one or two copies of ribosomal RNA cistron may suffice for their relatively slow growth. The presence of multiple ribosomal RNA cistrons, and two tandem promoters in each ribosomal cistron studied (22, 23, 24, 25) suggest a complex fashion of control of these genes. We hope that the study of a smaller and apparently a simpler prokaryote will facilitate the elucidation of the control mechanisms involved in the expression of rRNA genes.

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