JOURNAL OF BACTERIOLOGY, June 1990, p. 3478–3480
0021-9193/90/063478-03$02.00/0

Vol. 172, No. 6

Unique Ribosome Structure of *Leptospira interrogans* Is Composed of Four rRNA Components

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Received 5 December 1989/Accepted 26 February 1990

All known ribosomes of procaryotic organisms are made up of three rRNA components that are 23, 16, and 5S in size. We now report that in some *Leptospira interrogans* strains, the classical 23S rRNA is further processed to generate 14 and 17S rRNAs. This processing step was previously known to occur only in some eucaryotes and in a small group of procaryotes. The implications of this finding are discussed.

Translation of mRNA into proteins is mediated by the most complex ribonucleoprotein, ribosome. A classical procaryotic ribosome is made up of three species of rRNA and 52 different ribosomal proteins (22). A ribosome is the center of polypeptide synthesis. In addition to having peptidyl transferase activity, which mediates the polypeptide chain elongation step, ribosomes also interact with mRNA, charged tRNAs, and a large number of other factors to ensure accurate translation (7, 17, 21). Because of the critical role of the ribosome in every organism and because of its complex nature, the genes for rRNA in all organisms are highly conserved not only in the nucleic acid sequence but also in the basic and secondary structures. This feature of rRNA genes makes them a good molecular clock for the study of phylogenetic relationships, even for organisms which are only remotely related (15).

The rRNA in most procaryotic cells is typically transcribed from a ribosomal gene operon(s) as a precursor molecule, which is then processed by a number of RNases into 16, 23, and 5S matured rRNA components (14). The rRNA of the eucaryotic cells is also processed in a similar way during maturation, but it has an extra cleavage site near the 5′ end of the large-subunit rRNA to generate 18, 28, 5, and 5.8S rRNAs. The 18S rRNA is homologous to the procaryotic 16S rRNA (small-subunit rRNA), and the 28 and 5.8S rRNAs together are homologous to the 23S rRNA (large-subunit rRNA) of the procaryotic cells (3, 4, 9).

Though most eucaryotic large-subunit rRNAs contain only one extra cleavage site compared with procaryotic large-subunit rRNAs, some eucaryotic organisms have been reported to contain more cleavage sites. These organisms include protostomes, protozoa, and some coelenterates (2, 8, 20). In these organisms, there are one or more short stretches of spacer sequence located in the 28 or 5.8S rRNA molecules which are removed during maturation, resulting in the fragmented rRNA molecules (1, 10, 13, 16, 19, 20). Similarly, *Salmonella typhimurium* and two strains of *Salmonella paratyphi* B have been reported to contain fragmented 23S rRNA (18). In this report we describe an additional rRNA cleavage site in two closely related procaryotic organisms. The additional cleavage site results in the generation of discontinuous 23S rRNA molecules.

rRNAs of various *Leptospira interrogans* serovars were examined directly by lysing the cells with one-half volume of 5% sodium dodecyl sulfate at 65°C and then fractionating the nucleic acid molecules by agarose gel electrophoresis. Electrophoresis was carried out on a 0.9% agarose gel at 6 V/cm for 3 h, and the RNA molecules were stained with ethidium bromide before being visualized on a UV transilluminator. The organism used for each serovar was the reference strain, with the exception of serovar hardjo, for which the reference strain hardjoprajitno and genotype hardjobovis were both studied. We discovered that serovar hardjo genotype hardjobovis and serovar balcanica do not contain the typical 23S rRNA. Instead, they contain two smaller rRNAs, 14 and 17S, respectively, in addition to the 16S rRNA (Fig. 1A). When the heating step was eliminated and the lysis was performed at room temperature by adding 5% sodium dodecyl sulfate to the cells, only small amounts of the 14 and 17S fragments were generated, and the classical-sized 23S rRNA appeared (Fig. 1B). This result suggested that the 14 and 17S fragments were probably held together by a number of hydrogen bonds, as predicted from the secondary structure of the rRNA. To confirm the identity of the 14 and 17S RNA fragments, the nucleic acid fragments were transferred to nitrocellulose filter paper and hybridized to a radioactively labeled oligonucleotide probe (3′-ccaagtcttgcagcactctgt caagccagggatag-5′) that was designed according to the recently reported 23S rRNA gene sequence of *L. interrogans* serovar canicola, which contains the classical 23S rRNA (5). The probe represents a highly conserved region (positions 2639 to 2673) at the 3′ portion of the 23S rRNA gene. The results of the hybridization showed that the 17S fragment hybridized to the oligonucleotide probe (Fig. 1C), indicating that the 17S fragment is homologous to the 3′ portion of the classical 23S rRNA. Thus, the 17 and 14S fragments are probably generated by a specific cleavage site located near the middle of the 23S rRNA molecule. The 23S rRNA of *L. interrogans* serovar canicola and *Escherichia coli* also hybridized to the oligonucleotide probe as expected.

We are confident that the 17 and 14S rRNAs found in *L. interrogans* serovar hardjo genotype hardjobovis and serovar balcanica are not artificially degraded products of the 23S rRNA for several reasons. First, the high sodium dodecyl sulfate concentration (1.7%) would be expected to inhibit any enzymatic reaction that might degrade the RNA molecules; second, over 20 strains of bacteria representing many of the major phylogenetic groups have been examined in this laboratory by the same procedures. Only these two closely related *L. interrogans* strains were found to contain discontinuous 23S rRNA. To further demonstrate that the fragmentation of the 23S rRNA is not an artifact caused by

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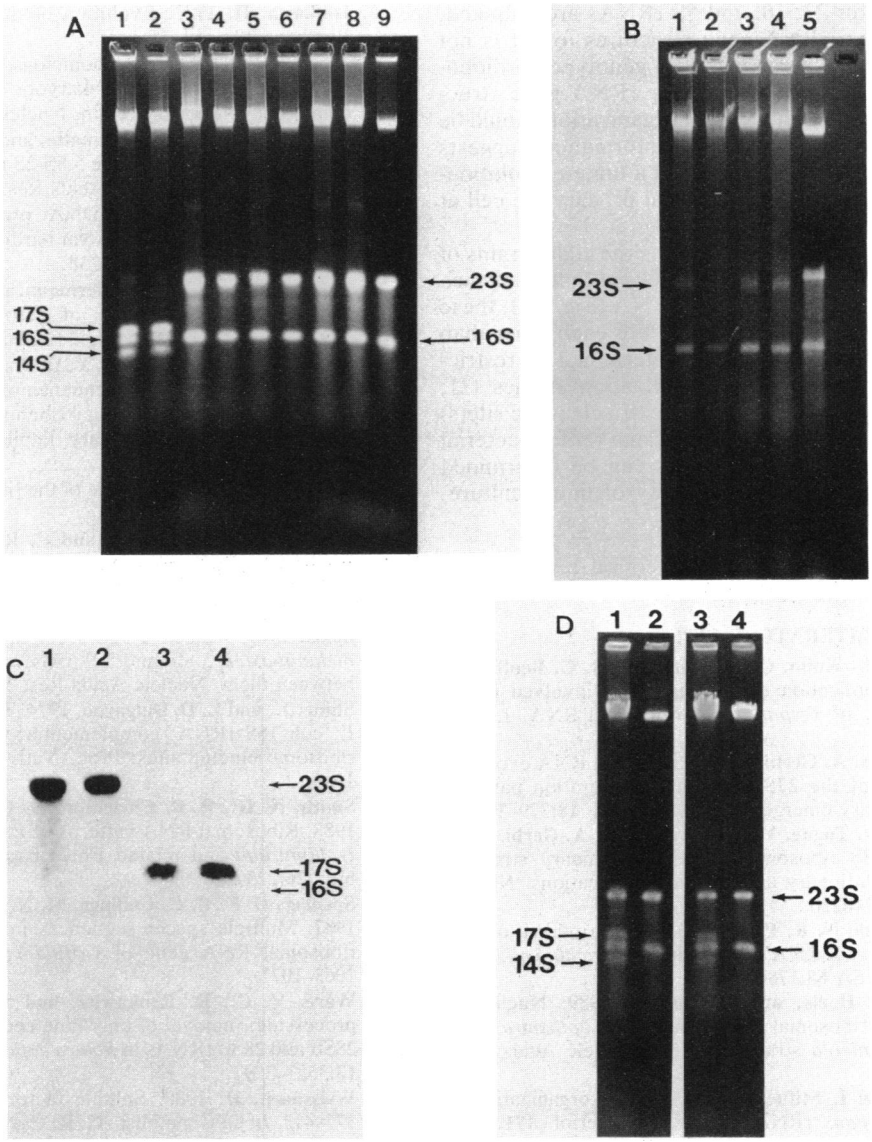

FIG. 1. rRNA molecules of different *L. interrogans* serovars and *E. coli*. (A) Electrophoresis on a nondenaturing agarose gel. The cells were lysed with one-half volume of 5% sodium dodecyl sulfate solution at 65°C for 30 min. Lane 1, serovar hardjo genotype hardjobovis; lane 2, serovar balcanica; lane 3, serovar hardjo reference strain hardjoprajitno; lane 4, serovar canicola; lane 5, serovar pomona; lane 6, serovar icterohaemorrhagiae; lane 7, serovar grippotyphosa; lane 8, serovar kennewickii; lane 9, *E. coli* JM83. (B) Electrophoresis on nondenaturing agarose gel, with the heating step in cell lysis eliminated. Lane 1, *L. interrogans* serovar hardjo genotype hardjobovis; lane 2, serovar balcanica; lane 3, serovar canicola; lane 4, serovar hardjo reference strain hardjoprajitno; lane 5, *E. coli* JM83. (C) Northern (RNA) blot hybridization. Radioactively labeled oligonucleotide specific for the 3′ portion of the 23S rRNA was used as a probe for the hybridization. Lane 1, *E. coli* JM83; lane 2, serovar canicola; lane 3, serovar balcanica; lane 4, serovar hardjo genotype hardjobovis. (D) Electrophoresis of *E. coli* JM83 and *L. interrogans* serovar canicola mixed with *L. interrogans* serovar balcanica before lysis. Lane 1, *E. coli* JM83 mixed with *L. interrogans* serovar balcanica at a 1:5 ratio before lysis; lane 2, *E. coli* JM83 lysed alone; lane 3, *L. interrogans* serovar canicola mixed with *L. interrogans* serovar balcanica at a 1:1 ratio before lysis; lane 4, *L. interrogans* serovar canicola lysed alone.

nuclease cleavage after cell lysis, two bacterial strains containing classical 23S rRNA (*L. interrogans* serovar canicola and *E. coli*) were mixed with a bacterial strain containing the fragmented 23S rRNA (*L. interrogans* serovar balcanica) before being lysed. The lysates of the mixed bacteria were analyzed on agarose gel. The results show that both *L. interrogans* serovar canicola and *E. coli* yielded classical 23S rRNA when they were lysed with *L. interrogans* serovar balcanica (Fig. 1D). These results exclude the possibility that extraordinarily aggressive nucleases were responsible for the fragmentation of 23S rRNA after the cells were lysed. Future work will involve cloning the 23S rRNA gene and determining its nucleic acid sequence. Of particular interest will be the comparison of the DNA sequence with other known rRNA gene sequences, because the added cleavage site has been shown to provide valuable information in studying evolutionary events (2, 8).

It is important to note that the closely related *L. interrogans* serovar canicola, which contains classical ribosomes, has been reported to have a unique rRNA gene organization that is different from rRNA gene organization in all procaryotic and eucaryotic cells. In *L. interrogans* serovar

canicola, the genes for 23, 16, and 5S rRNAs are unlinked, contrary to all known rRNA gene structures (6). It is not known if *L. interrogans* serovar hardjo genotype hardjobovis and serovar balcanica have similar rRNA gene structures, but the information on the gene organization should be forthcoming. However, all available information suggests that *L. interrogans* probably represents a unique evolutionary branch that evolved from a classical procaryotic cell at an early stage.

It is also worthwhile to note that among the eight strains of *L. interrogans* examined, only the hardjobovis and balcanica strains contained discontinuous large rRNA (Fig. 1A); these two strains are also more closely related to each other than to the rest of strains in the group, as determined by restriction endonuclease analysis and hybridization studies (11, 12). There is a good possibility that the extra cleavage site(s) in the rRNA can be used as an important marker for bacterial identification. The sizes of the rRNAs can be determined from a single colony or a small quantity of liquid culture, usually within less than 3 h.

We are grateful to John Ingraham for critical review of the manuscript and valuable advice.

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