The 23S/5S ribosomal RNA genes (rrl/rrf) are separate from the 16S ribosomal RNA gene (rrs) in Borrelia burgdorferi, the aetiological agent of Lyme disease

MASAHITO FUKUNAGA,1* YASUTAKE YANAGIHARA2 and MASAKO SOHNAKA1

1Faculty of Pharmacy and Pharmaceutical Sciences, University of Fukuyama, Fukuyama, Hiroshima 729-02, Japan
2Department of Microbiology, School of Pharmaceutical Sciences, University of Shizuoka, Yada, Shizuoka 422, Japan

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DNA fragments containing the rRNA genes for Borrelia burgdorferi strain B31 were cloned in bacteriophage λ EMBL3. A restriction map of the fragments was constructed and the organization of the rRNA genes was determined by Southern hybridization. One genomic DNA fragment contained a single copy of the rrs sequence and another cloned fragment contained both rrl and rrf sequences. The results revealed that the rrs gene is located separately from the set of rrl/rrf genes, suggesting that these rRNA genes are expressed independently in B. burgdorferi.

Introduction

Borrelia burgdorferi, the causative agent of Lyme disease, was discovered about 10 years ago (Burgdorfer et al., 1982), and the organisms have been isolated from humans, animals and ticks in different areas of the world (Hughes & Johnson, 1990; Postic et al., 1990). Lyme borreliosis is a widespread zoonanthroposiosis which is transmitted by ticks (Steere et al., 1983; Johnson et al., 1984). Since the first isolation of the organism, immunological, biochemical and molecular biological studies have been reported (Barbour & Hayes, 1986; Barbour & Garon, 1987; Bergstrom et al., 1989; Howe et al., 1985; Hyde & Johnson, 1984). B. burgdorferi has a small (about 1000 kb) linear chromosome and linear plasmids (Baril et al., 1989; Ferdows & Barbour, 1989).

We are interested in the organization and regulation of the genes coding for the rRNA of spirochaetes. The organization of the rRNA genes in leptospirae is unique (Fukunaga & Mifuchi, 1989a, b; Fukunaga et al., 1990a, b). Organization and transcription of genes for rRNA have been highly conserved among prokaryotes because the synthesis of rRNA is an essential process (Nomura & Morgan, 1977; Nomura et al., 1984).

This work is dedicated to Professor Ichiji Mifuchi.

* Author for correspondence. Tel. 849 36 2111 ext. 5216; fax 849 36 2213.

Abbreviations: rrl, rrs, rrf, genes for 23S, 16S and 5S rRNA, respectively.

The number of rRNA genes in B. burgdorferi has been deduced from the results of genomic hybridization using Escherichia coli rRNA as a probe, and DNA relatedness among Borrelia strains has also been examined by DNA–DNA hybridization (Postic et al., 1990). It was of interest to determine the number and organization of rRNA genes in B. burgdorferi and to compare the structures of these genes in various spirochaetes. Analysis of the genetic organization and nucleotide sequences of the rRNA genes in comparison with those of other organisms are very useful from the evolutionary and taxonomic viewpoints. In this paper we describe the cloning of rRNA genes of B. burgdorferi, physical maps, and structural relationship to other spirochaetal rRNA genes.

Methods

Bacterial strains and media. B. burgdorferi strain B31 was used. Cells were taken from a late exponential culture in 100 ml of BSKII medium (Barbour, 1984). E. coli strains HB101 and NM539 were grown in Luria medium, either in liquid form or solidified with 2% (w/v) agar (Miller, 1972). L-Agar plates supplemented with 100 μg ampicillln ml−1 were used to isolate E. coli transformants.

Preparation of DNA, and genomic- and plaque-hybridization. Total cellular DNA was extracted as described previously (Fukunaga & Mifuchi, 1989a). DNA was partially digested with Sau3AI and cloned into the BamHI sites of bacteriophage λ EMBL3 as described by Fukunaga & Mifuchi (1989b). DNAs were cloned by the standard method of Sambrook et al. (1989) by using plasmid vector pUC18 or
Fig. 1. Pattern of hybridization and physical map of the DNA fragment containing the \( rrs \) gene. Recombinant \( \lambda \) phage DNA containing the \( rrs \) gene was digested with the enzymes indicated, electrophoresed in 1\% agarose gels, transferred and hybridized to the treponemal rRNA probe (a). The 6.2 kb \( BamH I \) fragment was isolated and ligated into pUC18 DNA. The leptospiral \( rrs \) gene probe (\( BanII \) fragment) was used to determine the orientation of the gene in the fragment (b). The enzymes used were: (a) \( BamH I \) (lane 1), \( BamH I + EcoR I \) (lane 2), \( BamH I + Mlu I \) (lane 3), \( BamH I + SacI I \) (lane 4), \( HindII I \) (lane 5), \( HindII I + BanH I \) (lane 6), \( HindII I + Mlu I \) (lane 7), and \( HindII I + SacI I \) (lane 8); (b) \( Mlu I \) + \( Sal I \) (lane 1) and \( Sal I \) + \( SacI I \) (lane 2). Restriction fragment sizes were estimated by using size marker DNA. The location of the \( rrs \) gene was determined and is indicated together with restriction cleavage sites (c). The leptospiral \( rrs \) gene probe used in this experiment is also indicated. All of the other experimental conditions are described in the text.

Probes used in this study. The DNA fragment generated by \( BanII \) digestion of the \( rrs \) gene of \( Leptospira \) interrogans strain Moulton (nucleotides 220 to 590, \('rrs\) gene probe'; Fukunaga et al., 1990c) was used as an \( rrs \) gene probe. The fragment including the 3' part of the \( rrl \) gene of the same organism generated by \( BamH I + BglII \) digestion (nucleotides 1587 to 2839, \('rrl\) gene probe'; Fukunaga et al., 1989) was used as an \( rrl \) gene probe. The DNA fragment including the entire sequence of the \( rrf \) gene from strain Moulton (\( EcoR I - HincII \) fragment, \('rrf\) gene probe'; Fukunaga et al., 1990a) was used as an \( rrf \) gene probe. For the genomic hybridization, the DNA fragment generated by \( Mlu I + SacI I \) digestion of the \( rrs \) gene (Fig. 1) and the DNA fragment generated by \( Sty I \) digestion of the \( rrl \) gene of \( B. burgdorferi \) (Fig. 3) were used as probes to determine the number of \( rRNA \) genes in this organism. Each DNA fragment was labelled with deoxycytidine 5'-\( \alpha \)-\( 32P \)triphosphate (6000 Ci mmol\(^{-1}\); 222 TBq mmol\(^{-1}\); New England Nuclear) and a random primer labelling kit (Takara Shuzo Co.). Treponemal rRNA (a mixture of \( rrs \) and \( rrl \)) was partially hydrolysed as described by Suzuki et al. (1987) and labelled with T4-polynucleotide kinase and adenosine 5'-\( \gamma \)-\( 32P \)triphosphate (6000 Ci mmol\(^{-1}\); 222 TBq mmol\(^{-1}\); New England Nuclear) and used as an \( rRNA \) probe.
Results

Southern hybridization patterns and physical maps of λ recombinant DNA containing the rrs gene for B. burgdorferi

The recombinant λ EMBL3 phages were obtained by plaque hybridization with a treponemal rRNA probe. Clone b2, carrying the rrs gene of B. burgdorferi, was selected by using the leptospiral rrs gene probe and used for further study. Recombinant phage DNA was purified, digested with several restriction enzymes, and electrophoresed in an agarose gel. The pattern of Southern hybridization for clone b2 is shown in Fig. 1(a). The physical map is shown in Fig. 1(c). This physical map was constructed by digestion, partial digestion and double digestion of recombinant DNA with each enzyme. The location of the rrs gene was determined by Southern hybridization using the treponemal rRNA probe.

The BamHI fragment (6.2 kb) which carries the rrs gene was isolated and ligated into the pUC18 BamHI site. The orientation of the rrs gene was determined by using the leptospiral rrs gene (BanII fragment) as a probe. The rrs gene probe hybridized solely with 3.1 kb (right half of the BamHI fragment) fragment generated by digestion with MluI+Sall, but the same probe hybridized with both the 4.1 kb (left 2/3 of the BamHI fragment) and the 5.4 kb (right 1/3 of the BamHI fragment + pUC18 DNA) fragments by SacI+SacII double digestion (Fig. 1b). All of these results clearly indicate the location and orientation of the rrs gene.

Southern hybridization pattern and physical map of insert DNA containing rrl and rrf genes

Recombinant phages which hybridized with the rrl gene probe were isolated. Clone 16.1 was selected and used for further experiments. DNA was digested, electrophoresed, blotted and hybridized with the treponemal rRNA probe. The pattern of hybridization and the location of the rrl gene are shown in Fig. 2(a, b). Genomic Southern hybridization with the rrf gene probe after HindIII digestion gave a single radioactive band of 2.0 kb (data not shown). Digestion of recombinant phage DNA with HindIII generated a 2.0 kb fragment that hybridized with both rrl and rrf probes. To determine the location and linkage of the rrl and rrf genes in B. burgdorferi, the 2.0 kb HindIII fragment of clone 16.1 was electroeluted from an agarose gel slice, purified, and subcloned into plasmid pUC18 (Fig. 2b).

Linkage of the rrl and rrf genes

The DNA fragment which carries the rrl gene and the entire rrf gene(s) for B. burgdorferi was ligated into the pUC18 HindIII site. Bacterial clones harbouring recom-
Bacillus subtilis plasmid DNA were selected and used for further experiments. Recombinant plasmid DNA was extracted and digested with several restriction enzymes, electrophoresed, blotted and hybridized with the probe. The pattern of hybridization of clone 16.1H3b with the rrf gene probe is shown in Fig. 3(a). The hybridization pattern with the rrl gene probe for clone 16.1H5a, which contains the same 2.0 kb HindIII fragment but in the opposite direction, is shown in Fig. 3(b). The restriction cleavage map and the results of hybridization are shown in Fig. 3(c). The results clearly show that the rrl gene(s) is located between the EcoRV and EcoRI restriction sites of the fragment. For analysis of the 3' end of the rrl gene, a 1.2 kb BamHI–BglII fragment (nucleotides 1587 to 2839, leptospiral rrl gene) was labelled and used as a probe. As shown in Fig. 3(b), the largest fragment which solely hybridized with the probe was the 1.5 kb fragment generated by EcoRI+EcoRV digestion. The results indicate that the 3' end of the rrl gene exists around the EcoRV cleavage site. Thus, all of these results indicate that the sequences for the rrl and rrf genes are close to each other.

**Number of rRNA genes in B. burgdorferi**

We determined the number of rrs and rrl genes in B. burgdorferi by hybridization of several restriction endonuclease digests of genomic DNA to the rrs and/or rrl gene probes. (Fig. 4). There are two radioactive bands in the rrs probe hybridization with the HindIII digest (lane 3). The results indicate that there are HindIII sites inside the gene, and that the rrs probe (Mlu–SacII fragment, Fig. 1c) hybridizes with both HindIII fragments. Only one radioactive band appeared in all the other digests using the rrs or rrl probes. These results indicate that there is one gene for both rrs and rrl in this organism. The number of rrf genes is estimated as one because the rrf
genes.

there are two rrf genes in saprophytic leptospires but one that the leptospiral genome has two genes for leptospires were determined previously (Fukunaga Reiter, three rRNA genes are arranged in the order 1989a, 6; Fukunaga & et al., 1990a, 1990b). We found that the leptospiral genome has two genes for rrs and rrl; there are two rrf genes in saprophytic leptospires but one rrf gene in parasitic leptospires (Fukunaga et al., 1990a, b). Furthermore, there is no linkage among those rRNA genes.

In one of the rRNA gene sets for T. phagedenis strain Reiter, three rRNA genes are arranged in the order rrs–rrl–rrf (Fukunaga et al., 1992). The sequences corresponding to the three rRNAs are all contained in a 5.3 kb fragment, implying that the three rRNA genes are closely linked. The other gene sets, including the genes of the pathogenic treponema T. pallidum, also contain at least one copy of the rrs, rrl and rrf gene sequences arranged in the same order. This arrangement is identical to that found in other common eubacteria.

The organization of the rRNA genes in B. burgdorferi, described here, is different from that in any other spirochaete. There is no linkage between the rrs gene and the rrl/rrf genes in this organism. Recent reports have given evidence of an rRNA gene organization similar to that of B. burgdorferi in the thermophilic eubacterium Thermus thermophilus and the plantcymote Pirellula marina. In these organisms, the rrs gene has been shown to be locally and transcriptionally separate from the rrl/rrf genes (Hartmann & Erdmann, 1989; Hartmann et al., 1991; Liesack & Stackebrandt, 1989). The coincidence of the organization of rRNA genes in these organisms is surprising because they are members of three different eubacterial phyla (Woese, 1987).

Spirochaetes are identified by their characteristic spiral shape and axially coiled fibrils. Despite these morphological similarities, there are differences among them regarding habitat, physiological and biochemical characters, and DNA base composition (Canale-Perola, 1984; Johnson, 1981; Johnson & Faine, 1984). Molecular methods have given new insight into the taxonomy of spirochaetes. Oligonucleotide cataloguing using a partial rrs sequence has shown that the Leptospiraceae and other spirochaetes, such as the genera Treponema and Borrelia, constitute a discrete phylogenetic branch (Fox et al., 1980.; Paster et al., 1984; Woese et al., 1983; Woese, 1987). Paster et al. (1991) recently showed that the members of the genus Borrelia are more closely related to the spirochaetes than to the treponemes.

The results reported here and our earlier work, therefore, confirm the phylogenetic situation of Borrelia and Treponema in the evolution of the spirochaetes. In this study, we found that B. burgdorferi has single rrl and rrs genes and that the usually closely linked rrs and rrl genes are separate in this organism. For T. thermophilus the existence of two different transcription units for rrs and rrl/rrf has been proven (Hartmann & Erdmann, 1989; Hartmann et al., 1991); our results suggest that this is also likely for B. burgdorferi.

We have cloned the rrs and rrl genes of B. burgdorferi in different λ clones. The recombinant DNA fragment including the rrs gene hybridized with the rrs gene probe but not with the rrl gene probe, and the rrl-containing fragment hybridized only with the rrl gene probe. The results described in this paper, therefore, indicate that the rrs gene and the rrl gene are located separately on the


rRNA genes in B. burgdorferi


