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Scattering of the rRNA Genes on the Physical Map of the Circular Chromosome of *Leptospira interrogans* Serovar Icterohaemorrhagiae

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***Leptospira interrogans* is a pathogenic bacterium with a low G+C content (34 to 39%). The restriction enzymes *Not*I, *Asc*I, and *Srf*I cut the chromosome of *L. interrogans* serovar icterohaemorrhagiae into 13, 3, and 5 fragments separable by one- and two-dimensional pulsed-field gel electrophoresis (PFGE). The genome is composed of a circular 4.6-Mbp chromosome and a 0.35-Mbp extrachromosomal element. A physical map of the chromosome was constructed for *Not*I, *Asc*I, and *Srf*I by using single and double digests, or partial *Not*I digests obtained at random or by cross-protection of *Not*I sites by *Fnu*DII methylase, and linking clones. rRNA genes were found to be widely scattered on the chromosome.**

The genus *Leptospira* is a member of the order *Spirochaetales*, an ancient group of eubacteria (24, 39). These organisms are aerobic bacteria of very characteristic structure: thin and spiral shaped (17). The pathogenic leptospires consist of seven species: *Leptospira interrogans* (sensu stricto), *L. santarosai*, *L. borgpetersenii*, *L. noguchi*, *L. weilii*, *L. inadai*, and *L. kirschneri* (27, 40). Very little is known about the pathogenesis of leptospirosis and the genetics and molecular biology of *Leptospira* species, in part because of the lack of any genetic transfer system for these organisms.

In a preliminary investigation by pulsed-field gel electrophoresis (PFGE), we found that the *Leptospira* genome contained a 5-Mbp circular chromosome (2). PFGE is now a well-established procedure for the analysis of large DNA fragments (31). It has been used for the physical mapping of the chromosomes of numerous microorganisms, first achieved for *Escherichia coli* (33).

Here we present a physical map of the genome of *L. interrogans* serovar icterohaemorrhagiae constructed by Southern blot analysis of restriction fragments separated by PFGE. This serovar of *L. interrogans* was chosen since it is one of the most commonly encountered pathogenic leptospires and strain Verdun is used as a vaccine for humans in France. Taylor et al. suggested that the *L. interrogans* chromosome is linear, fragmented, and approximately 3.1 Mbp (36). However, we found it to be circular and 4.6 Mbp, in agreement with our earlier results (2) and the report of Zuerner (41). Such a map will provide a point of reference from which the linkage of specific genes can be established. The localization of rRNA genes on the physical map was determined.

(Preliminary data were presented elsewhere [29]).

(This report is part of a thesis presented by C.B. in partial fulfilment of the requirements for the Dr.Sc. degree from the University of Paris.)

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MATERIALS AND METHODS

Bacterial strains, plasmids, and media. *L. interrogans* serovar icterohaemorrhagiae strain Verdun (National Reference Center, Paris, France) was grown in EMJH medium (7, 19). Cell numbers were determined by using a Petroff-Hauser chamber. *E. coli* GT869 (*thrB1004 pro thi hsdS lacZΔM15* F' *lacZΔM15 lacI*^{q} *traD36 proA*$^+$ *proB*$^+$) (23) was grown in Luria broth or Luria broth supplemented with 1.5% agar for solid media (22). Plasmid pBluescript+ (Stratagene) is a 2.96-kb pUC19 derivative. Plasmid pKNot was obtained by cloning the *Sac*I-*Pst*I fragment of the pBluescript polylinker containing the *Not*I site into pK19, which carries a kanamycin resistance gene (25). Plasmid pNEB193 (New England Biolabs, Beverly, Mass.) is a pUC19 derivative which carries a single *Asc*I site in the polylinker. All of these plasmids allow a blue/white color selection.

PFGE. Exponential-phase cells (6×10^9) were mixed with an equal volume of molten low-melting-temperature agarose (SeaPlaque; FMC), and the mixture was allowed to solidify in 100-μl rectangular molds. Genomic DNA was prepared in agarose plugs as described previously (1, 33). DNA corresponding to a third of a plug was digested by incubation with 5 to 20 U of restriction endonucleases such as *Apa*I, *Asc*I, *Not*I, *Srf*I, and *Sgr*AI for 16 h, using the conditions specified by the supplier (Boehringer, Mannheim, Germany; Pharmacia, Uppsala, Sweden; Stratagene, San Diego, Calif.; or New England Biolabs). Sequential digestions were performed by the method for two-dimensional PFGE as described by Bautsch (3) and modified by Tulloch et al. (37). PFGE was performed either in a CHEF-DRII apparatus (Bio-Rad Laboratories, Richmond, Calif.) or in a Pharmacia-LKB apparatus, using a hexagonal electrode array (Pulsaphor 2015). Agarose gels (1.0 to 1.5%, wt/vol) in 0.5× TBE buffer (1× TBE is 89 mM Tris-borate, 89 mM boric acid, and 2 mM EDTA, pH 8) were used. Various programs using different pulse times or pulse time ramps were used for the separations. Fragment sizes were determined by comparison of band mobilities with those of bacteriophage λ DNA multimers (monomer = 44.3 kb [5a]) and *Saccharomyces cerevisiae* chromosomes (Bio-Rad): 2,200, 1,600, 1,125, 1,020, 945, 850, 800, 770, 700, 630, 580, 460, 370, 290, and 245 kbp.

Fragments produced by digestion with a single restriction endonuclease are designated Nt, Sr, or As to identify the enzyme *Not*I, *Srf*I, or *Asc*I, respectively, with a capital letter suffix (A, B, C, etc.) in order of decreasing size. For fragments indistinguishable in size, the suffix is numbered (e.g., NtH1, NtH2). DNA bands corresponding to identified partial digests are designated, for example, NtH+J, indicating the linkage between NtH and NtJ. With partial digestions obtained by the use of the *Fnu*DII methylase prior to *Not*I digestion (26), the new bands obtained were designated NtB' and NtC', corresponding to the disappearance of the NtB and NtC bands, respectively.

Construction of *Not*I and *Asc*I linking clones. *Not*I-*Bam*HI and *Asc*I-*Bam*HI fragments were cloned to construct the map. *Leptospira* chromosomal DNA was isolated as described previously (32). Restriction enzyme digests, ligations, transformations, agarose gel electrophoresis, and small-scale plasmid isolations were carried out by standard procedures (21). *Leptospira Bam*HI-*Not*I fragments were ligated with the *Bam*HI- and *Not*I-digested plasmid vector pBluescript (Stratagene) or pKNot. Similarly, *Leptospira Bam*HI-*Asc*I fragments were ligated with the *Bam*HI- and *Asc*I-digested plasmid vector pNEB193. *E. coli* GT869 was then transformed with recombinant plasmids and plated on LB agar containing ampicillin (50 μg/ml) (for pBluescript derivatives and pNEB193) or kanamycin (25 μg/ml) (for pKNot derivatives), 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (40 μg/ml), and isopropyl-β-D-thiogalactopyranoside (0.2 mM). Plasmids were isolated from the Lac$^-$ transformants, digested with *Not*I and *Bam*HI, subjected to agarose gel electrophoresis to establish fragment size, and used as the *Not*I linking clones. *Bam*HI-*Asc*I linking clones were obtained in an analogous manner.

Probes. Several different probes carrying rRNA genes were used. Plasmid pKK3535 containing the whole *E. coli rrnB* ribosomal operon (5) was used. Probes for 16S+23S and 23S were obtained by subcloning *Bcl*I-*Pvu*II and *Sac*I-*Sac*II fragments, respectively, from pKK3535 into pKNot. A probe for 16S was obtained from *Clostridium perfringens* (14). A cold probe specific for 5S was synthesized in a polymerase chain reaction (28) which replaced dTTP by digoxygenin-11-dUTP (Boehringer) during amplification. Two oligonucleotides, 5'-GCTTTCAAGATTCCCGGTGAC TATAGAGA-3' and 5'-GAATTCCAACGTCCTACTCTCC C-3', were derived from the *rrf* (5S) sequence (12) and used as primers to synthesize the 5S probe.

DNA-DNA hybridizations. For blot analysis according to Southern (34), DNA fragments separated by PFGE were submitted to depurination and transferred to a Hybond-N nylon membrane (Amersham International, Amersham, England). Probes were radiolabeled with [^{35}S]dCTP (37 TBq/mmol; Amersham), using the multiprime method (8) (Amersham). Labeled DNA (50 ng) was added to the filter and allowed to hybridize at 65°C for 18 h. The filter was washed at 65°C in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M trisodium citrate, pH 7) and then 2× SSC, air dried, and exposed to X-ray film at room temperature. The same method was used for heterologous probes except that hybridization and washing were at 50°C.

A nonradioactive detection kit from Boehringer was also used. DNA was labeled by random priming with digoxygenin-dUTP and detected by enzyme immunoassay. Hybridization was performed overnight at 65°C in 5× SSC–0.1% *N*-sodium lauryl sarcosinate–0.02% sodium dodecyl sulfate (SDS)–1% blocking agent (Boehringer). The membrane was washed with 2× SSC–0.1% SDS and then with 0.1× SSC–0.1% SDS. Detection of the digoxygenin-labeled probe was with anti-digoxygenin-alkaline phosphatase, visualized by an enzyme-linked color reaction.

TABLE 1. Sizes of the *Not*I, *Asc*I, and *Srf*I restriction fragments of the *L. interrogans* serovar icterohaemorrhagiae genome

Band	Size of fragment (kbp)		
	*Not*I	*Asc*I	*Srf*I
A	1,200	1,800[a]	2,100
B	940	1,060	1,230
C	350[b]	350	550
D	320		420
E	300		350[a]
F	260[a]		
G	220		
H	130[a]		
I	90		
J	60		
Total	4,960	5,010	5,000

[a] Two fragments within the band.
[b] Three fragments within the band.

RESULTS

The strategy for mapping the *L. interrogans* chromosome involved the following steps: (i) digestion of the chromosome by *Not*I, resulting in 13 fragments that could be separated by one- or two-dimensional PFGE; (ii) isolation and analysis of *Not*I linking clones; (iii) further analysis using *Apa*I and *Sgr*AI digests, *Not*I partial digests, and cross-protection of *Not*I sites, used to resolve the map in regions that were not clearly defined by the approach described above; and (iv) localization of the *Asc*I and *Srf*I sites confirmed the *Not*I physical map.

***Not*I physical map of the *L. interrogans* chromosome.** The principal aim was to establish a physical map to facilitate the localization of cloned genes. Screening with a battery of endonucleases with dG+dC-rich recognition sites of six nucleotides or more showed that the *Not*I enzyme generated 10 large DNA bands (A to J) in a range of 1,200 to 60 kb (2) (Table 1). The multiplicity of the *Not*I bands was addressed by use of two-dimensional gels of sequential *Not*I-*Sgr*AI digests (Fig. 1). For the NtH band (130 kb), three fragments (130+90+40 kb) were obtained after *Sgr*AI digestion, indicating that one NtH fragment was digested by *Sgr*AI (Fig. 1A). The sum of the sizes of the six fragments (260+250+80+60+30+20 kb) derived from the NtC band after *Sgr*AI digestion was 700 kb, indicating the presence of two 350-kb fragments, NtC1 and NtC2 (Fig. 1C). Similarly, NtF was composed of a 260-kb fragment undigested by *Sgr*AI and another 260 kb cut into two fragments (140+120 kb) by *Sgr*AI. These results show that NtC, NtF, and NtH are at least doublets (Fig. 1B). There was no evidence that any other *Not*I band was multiple. The doublets were resolved by hybridization of linking clones to partial digests obtained by chance or by cross-protection (see below).

Identification of linking clones containing *Not*I sites. The strategy for mapping the *L. interrogans* chromosome involved the identification of linking clones carrying the rare *Not*I cutting site at one extremity and a *Bam*HI site at the other. First, 24 different *Bam*HI-*Not*I fragments varying in size (0.4 to 15 kb) and in restriction pattern were cloned into plasmid pKNot or pBluescript. Second, these clones were

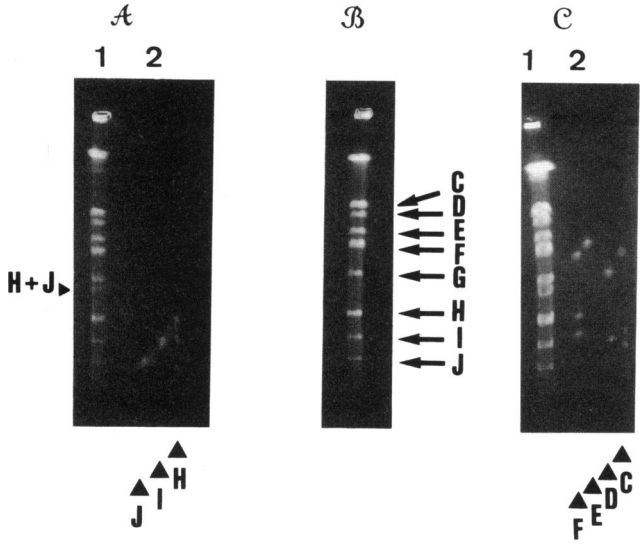

FIG. 1. Two-dimensional-PFGE gel of *L. interrogans* DNA digested first with *Not*I and then with *Sgr*AI. Agarose blocks corresponding to H-I-J (A, lane 2) and C-D-E-F (C, lane 2). *Not*I bands obtained in the first dimension were run in the second dimension after *Sgr*AI digestion. *Not*I digests were used as controls in lanes 1 of panels A and C and in panel B. The 8 *Not*I bands (out of 10) corresponding to a total digest in these pulse conditions are indicated (B), as are bands corresponding to partial digestions, such as NtH+NtJ (A and B). Ramping was for 5 to 35 s for 22 h.

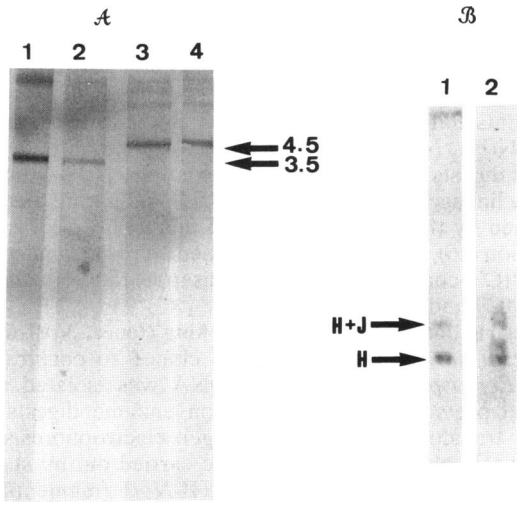

FIG. 2. Hybridization of *Bam*HI-*Not*I linking clones to *L. interrogans* *Bam*HI fragments and partial *Not*I digests. (A) Autoradiographs of *Bam*HI digests indicating complementary *Bam*HI-*Not*I fragments within a *Bam*HI fragment. Probes are BN98, BN93, BN92, and BN91 for lanes 1 to 4, respectively. (B) Autoradiographs of *Not*I partial digests with probes BN92 (lane 1) and BN98 (lane 2), attributed to the NtH doublet. Numbers correspond to the sizes (kilobases) of the DNA bands obtained.

hybridized by Southern blot analysis to *Not*I-generated blots. In addition, the *Bam*HI-*Not*I clones were also hybridized to *Bam*HI-generated blots. Hybridization of two different individually labeled recombinant plasmids carrying a *Bam*HI-*Not*I fragment with the same *Not*I DNA band indicated that they resided on the same *Not*I fragment except in the case of multiple fragments within a band.

Hybridization of individually labeled recombinant plasmids to the array of fragments produced by *Bam*HI allowed the determination of the complementary *Bam*HI-*Not*I fragments within each *Bam*HI fragment. An example of Southern blot analysis of a *Bam*HI digest probed with linking clones is shown in Fig. 2A. Fragments BN98 and BN93 are so-called complementary fragments (Fig. 2A, lanes 1 and 2), as are BN92 and BN91 (Fig. 2A, lanes 3 and 4; Table 2). This procedure was used with success for 18 clones (see the first 18 BN clones in Table 2). Four so-called linking clones (BN117, BN32, BN116, and BN125) hybridized with more than one *Not*I fragment and probably contain repetitive elements. One of them, BN117, indeed showed homology with insertion element IS*3* (unpublished results). These clones, which could not be attributed unambiguously to a given *Not*I band, were not used any further for construction of the map.

These results proved the contiguity of the following *Not*I fragments: I-B-H-J-H-E-F-A and G-D-F.

Individualization of DNA fragments within a DNA band by partial digestion and cross-protection. Ambiguities arose in that *Not*I bands such as NtC, NtF, or NtH consisted of multiple fragments. Means were thus devised to identify the individual fragments within a band.

Among the numerous *Not*I restriction patterns obtained, we sometimes visualized supplementary bands that corresponded to partial *Not*I digestion (Fig. 1A and C, lanes 1). A 190-kb supplementary band hybridized with probes BN92 and BN98, both specific for the NtH band (130 kb) (Fig. 2B), which indicates that NtH1 (bordered by BN92 and BN98) is adjacent to NtJ (60 kb). Analogously, a supplementary 560 kb hybridized with probes BN106 and BN95, specific for the NtF doublet (260 kb), and BN93, specific for the NtE band (300 kb). A higher additional band (580 kbp) hybridized with BN31, specific for the NtF doublet (260 kb), and BN122, specific for NtD (320 kb). This result confirmed the linkage of NtF2 (bordered by BN95 and BN106) and NtE on one hand and NtF1 (characterized by BN31) and NtD on the other.

TABLE 2. *Bam*HI-*Not*I linking clones in the *L. interrogans* serovar icterohaemorrhagiae chromosome

Linking clone	Linking clone size (kb)	*Bam*HI fragment size (kb)	Corresponding *Not*I band
BN110	3.2	5.8	B
BN96	0.3	1	B
BN126	15	18	H
BN92	2.7	4.5	H
BN98	1	3.5	H
BN3	2.6	5.8	H
BN120	2.9	18	J
BN91	1.8	4.5	J
BN114	5.1	6	D
BN122	0.75	3.5	D
BN106	1.3	10	F
BN31	0.8	6	F
BN95	5.5	9	F
BN118	3.5	9	E
BN100	2.7	3.5	G
BN90	0.7	1	I
BN119	7.5	10	A
BN93	2.5	3.5	E
BN97	4	24	G
BN101	3.8	22	C

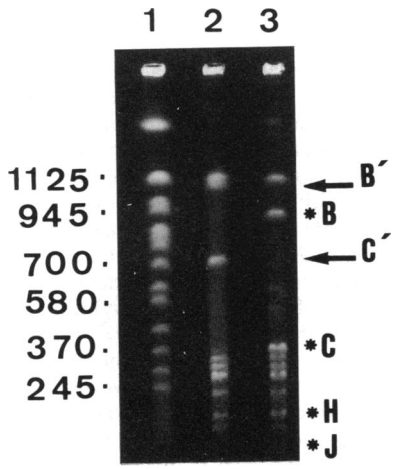

FIG. 3. In vitro cross-protection against *Not*I sites in *L. interrogans*. Lanes: 1, *S. cerevisiae* chromosomes as markers; 2, treatment with *Fnu*DII methyltransferase (twice with 20 U for 2 h each) prior to *Not*I digestion (ramping was for 30 to 120 s for 24 h); 3, digestion with *Not*I. *, changes within a given *Not*I band. Sizes are indicated in kilobases.

As an alternative to partial digestions which were obtained by chance, we used a method referred to as cross-protection, which involves blocking a defined subset of restriction target sites from cleavage at partly overlapping methyltransferase/restriction endonuclease sites. Pretreatment with *Fnu*DII methylase prior to *Not*I digestion identified those *Not*I sites (5′-GCGGCCGC-3′) overlapping with *Fnu*DII methylase sites (5′-mCGCG-3′). Bands NtB, NtC, and NtJ disappeared and were replaced by NtB′ and NtC′ at 1,100 and 700 kb (Fig. 3, lane 2 versus lane 3). This result indicated the linkage of NtC1 and NtC2. The disappearance of the NtJ band (60 kb) and the lower intensity of the NtH band (130 kb), corresponding to the disappearance of one NtH fragment of the doublet, were compatible with the linkage NtB-NtH2-NtJ.

Linkage of some fragments was confirmed by hybridization of *Bam*HI-*Not*I clones to *Apa*I digests (data not shown); hybridization to a single 400-kb *Apa*I band was obtained with BN93 (NtE), BN98 (NtH1), BN92 (NtH1), BN126 (NtH2), BN3 (NtH2), BN91 (NtJ), and BN120 (NtJ). These results indicated the close proximity of fragments NtJ, NtE, NtH1, and NtH2. Analogously, hybridization to a single 450-kb *Sgr*AI band was obtained with BN96 (NtB), BN90 (NtI), and BN97 (NtG), indicating the close proximity of NtI, NtG, and NtB.

The results for partial and total *Not*I digests and hybridization with *Not*I linking clones positioned the 13 *Not*I fragments of the *L. interrogans* genome as follows: F1-D-G-I-B-H2-J-H1-E-F2-A and C1-C2.

Localization of the *Asc*I and *Srf*I sites on the physical map and identification of an extrachromosomal element. Digestion of the *L. interrogans* genome with restriction endonuclease *Asc*I gave three bands (AsA, AsB, and AsC) of 2, 1, and 0.35 Mbp, respectively. Precise localization of the *Asc*I sites was performed by two-dimensional PFGE. Each *Not*I band was excised from a PFGE gel and submitted to another PFGE after digestion with *Asc*I. An *Asc*I site was found in each of the three following bands: NtB, NtC, and NtF (data not shown). The precise location of an *Asc*I site in a given fragment of the NtF or NtC doublet was addressed. The linking clone BN95, characteristic of NtF2, hybridized with a 100-kb fragment generated by the double digest *Asc*I-*Not*I. A similar result was obtained by hybridization with AB1, an *Asc*I-*Bam*HI linking clone (attributed to AsB). Analogously, the linking clone BN101, characteristic of NtC2, hybridized with the 100-kb fragment generated by the double digest *Asc*I-*Not*I. This finding allowed us to locate one *Asc*I site within NtF2 and another within NtC2. These results were compatible with the chromosome being composed of three *Asc*I fragments, AsA1 and AsA2 (1.8 Mbp) and AsB (1 Mbp). AsB was shown to contain NtJ (60 kb), NtH1 and NtH2 (130 kb), NtE (300 kb), a 100-kb fragment originating from NtF2 cut by *Asc*I, and a 300-kb fragment corresponding to NtB cut by *Asc*I. These results were confirmatory for part of the map.

Further analysis of the pattern obtained with the *Asc*I digest indicated that a unique extrachromosomal element, as suggested by others (36, 41), corresponded to the 0.35-Mbp band seen both with undigested DNA and after *Asc*I digestion (AsC). This extrachromosomal element should also be visible in the *Not*I digest pattern. In the cross-protection experiments described above, the NtC band (350 kb) disappeared and was replaced by the NtC′ band (700 kb). Close examination of the pattern obtained indicated that an additional DNA species (band P) resolved at the same molecular weight as did band NtC. Two-dimensional PFGE performed after *Sgr*AI digestion of the bands obtained after methylation and *Not*I digestion showed four fragments derived from band P, the sum of which was 350 kb (faint bands not shown). The fragments derived from band P are not visible in Fig. 1C, probably because of the low copy number of the plasmid. All of these results taken together are in agreement with the presence of an extrachromosomal element in the genome of *L. interrogans*.

Digestion of the *L. interrogans* chromosome with restriction endonuclease *Srf*I gave five bands of 2.1, 1.23, 0.55, 0.42, and 0.35 Mbp, respectively. Precise location of the *Srf*I sites was performed by two-dimensional PFGE. Each *Not*I band was excised from a PFGE gel and submitted to another PFGE after digestion with *Srf*I. One *Srf*I site was found in NtJ, three were found in NtA, and one was found in NtB (Fig. 4). These results are compatible with the chromosome being composed of five *Srf*I fragments, SrA, SrB, SrC, SrD, and SrE. Digestion of the *Srf*I fragments by *Sgr*AI or *Not*I gave further information. SrE was a doublet containing two fragments, one of which was cut by *Sgr*AI and corresponded to the plasmid (band P) mentioned above.

In addition, SrA was shown to contain, after *Not*I digestion, the following *Not*I fragments: NtI, NtG, NtD, NtF, NtC, and a 440-kb fragment from NtB. These data taken together allowed us to demonstrate linkage of NtC2 to NtF1 and to deduce the linkage of NtC1 to NtA. The circular map of the *L. interrogans* serovar icterohaemorrhagiae chromosome as thus determined is shown in Fig. 4.

Dispersion of the rRNA genes on the physical map. By using heterologous or homologous probes carrying *rrs* (16S) and *rrf* (5S), hybridization was obtained with NtG and NtF, respectively. When the whole *E. coli rrnB* operon or a probe derived from *rrnB* specific for 16S+23S rRNA was used, hybridization was with NtA, NtB, and NtG. NtF did not hybridize with the whole *rrnB* operon as would have been expected from its positive signal with *rrf* (5S). Possible explanations are the low homology of the *L. interrogans rrf* (5S) gene with its *E. coli* counterpart and its small size compared with the whole ribosomal operon. We can therefore conclude that there are at least two rrl (23S) genes, one

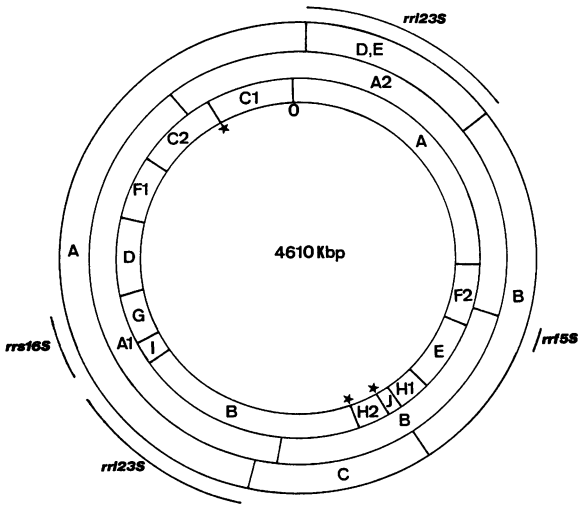

FIG. 4. *Not*I physical map of the chromosome of *L. interrogans* serovar icterohaemorrhagiae. The 13 *Not*I, 3 *Asc*I, and 5 *Srf*I fragments are indicated by a letter followed by a number, if necessary, from inner circle to outer circle. The methylated *Not*I sites are indicated by stars. Locations of rRNA genes are indicated.

located on NtA and the other on NtB, plus at least one rrf (5S) gene on NtF and one *rrs* (16S) gene on NtG (see below). More precise localization was obtained with *Asc*I and *Srf*I (Fig. 4).

DISCUSSION

In this study, restriction endonucleases *Not*I, *Srf*I, and *Asc*I were found to produce DNA of fragment sizes useful for mapping the *L. interrogans* chromosome. The *L. interrogans* chromosome size determined by PFGE of *Not*I, *Srf*I, and *Asc*I digests was estimated to be approximately 4.6 Mbp, which is close to the size of 5 Mbp obtained previously (2). These results suggest that the chromosome size of *L. interrogans* is of the same order of magnitude as that of *E. coli*, which correlates with their ability to grow in a defined medium; other fastidious spirochetes such as *Treponema pallidum* and *Borrelia burgdorferi* have chromosomes about one-fifth of this size (1, 6, 9, 38).

In contrast to other bacteria, the chromosome of *B. burgdorferi* is linear (1, 6, 9). Recently, Taylor et al. suggested that the chromosome of *L. interrogans* could be linear (36). One possible explanation for these results could be the quality and quantity of DNA loaded on the pulsed-field gel. We find that highly concentrated or slightly degraded undigested DNA results in the presence of linear molecules of around 2,000 kb. However, the physical map constructed in this study demonstrates the circular nature of the *L. interrogans* chromosome. In addition, during the course of these studies, the physical map of the chromosome of *L. interrogans* serovar pomona strain Kennewicki was reported and shown to be circular (41). Serovars pomona and icterohaemorrhagiae belong to the same *L. interrogans* sensu stricto species as defined by Yasuda et al. (40). However, the physical maps obtained are completely different. This difference can be correlated to PFGE studies showing a specific *Not*I fingerprint for each of the numerous serovars of the pathogenic leptospires (among them serovars pomona and icterohaemorrhagiae) (15, 16). Variation in size or organization between serovars may result from rearrangements due to recombination between repetitive sequences (42). It should be emphasized that the *Not*I PFGE pattern shown and the 350-kb plasmid are found in all clinical isolates (total of 45) of *L. interrogans* serovar icterohaemorrhagiae studied (our unpublished results). These results indicate that the laboratory strain Verdun analyzed in this study is very similar to recently isolated strains from nature.

In agreement with the results of others (36, 41), our analysis of the whole genome of *L. interrogans* showed the presence of an extrachromosomal element. A 350-kb band is seen in undigested preparations and in *Asc*I digests of *L. interrogans*. It is hidden in *Not*I and *Srf*I digests because of its size, identical to that of NtC and SrE, respectively. This giant plasmid may be homologous to the extrachromosomal element reported in other *Leptospira* serovars (36, 41). However, further study is needed to ascertain its circular or linear structure, as conflicting results have been reported. The 350-kb plasmid found in *L. interrogans* serovars canicola, pomona, icterohaemorrhagiae, and grippotyphosa was reported to be linear (36). The physical map of plasmid pLIN1 (350 kb) found in *L. interrogans* serovar pomona is circular (41). pLIN1 has a copy number of 1 or 2 per chromosome (41).

Fukunaga et al., in their studies of *L. interrogans* rRNA genes, showed that there are two *rrs* (16S) genes, two *rrl* (23S) genes, and only one *rrf* (5S) gene and that all of the rRNA genes in *L. interrogans* are unlinked (10–12). Our data are in agreement with this finding (although the two *rrs* [16S] genes, located on NtG, could not be separated) and indicate that the *Leptospira* rRNA genes are widely scattered on the chromosome. This is in contrast with the organization in numerous operons of the rRNA genes for *E. coli*, *Bacillus subtilis*, *C. perfringens*, and *Lactococcus lactis* and their clustering on their respective chromosomes (4, 14, 18, 37). However, in another spirochete, *B. burgdorferi*, and in *Pirullela marina*, the *rrs* (16S) gene is separated from the *rrl* (23S) and *rrf* (5S) genes (6, 13, 20, 30). In *Mycoplasma pneumoniae*, the *rrf* (5S) gene is separated from the *rrl* (23S) and *rrs* (16S) genes (35). It seems that different organizations of the rRNA genes are possible among the eubacteria.

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