

Complete Nucleotide Sequence of a Circular Plasmid from the Lyme Disease Spirochete, *Borrelia burgdorferi*

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We have determined the complete nucleotide sequence of a small circular plasmid from the spirochete *Borrelia burgdorferi* Ip21, the agent of Lyme disease. The plasmid (cp8.3/Ip21) is 8,303 bp long, has a 76.6% A+T content, and is unstable upon passage of cells in vitro. An analysis of the sequence revealed the presence of two nearly perfect copies of a 184-bp inverted repeat sequence separated by 2,675 bp containing three closely spaced, but nonoverlapping, open reading frames (ORFs). Each inverted repeat ends in sequences that may function as signals for the initiation of transcription and translation of flanking plasmid sequences. A unique oligonucleotide probe based on the repeated sequence showed that the DNA between the repeats is present predominantly in a single orientation. Additional copies of the repeat were not detected elsewhere in the Ip21 genome. An analysis for potential ORFs indicates that the plasmid has nine highly probable protein-coding ORFs and one that is less probable; together, they occupy almost 71% of the nucleotide sequence. Analysis of the deduced amino acid sequences of the ORFs revealed one (ORF-9) with features in common with *Borrelia* lipoproteins and another (ORF-2) having limited homology with a replication protein, RepC, from a gram-positive plasmid that replicates by a rolling circle (RC) mechanism. Known collectively as RC plasmids, such plasmids require a double-stranded origin at which the Rep protein nicks the DNA to generate a single-stranded replication intermediate. cp8.3/Ip21 has three copies of the heptameric motif characteristically found at a nick site of most RC plasmids. These observations suggest that cp8.3/Ip21 may replicate by an RC mechanism.

Lyme disease, a tick-borne infection caused by the spirochete *Borrelia burgdorferi*, has become a serious health threat in North America, Europe, Eurasia, and Japan (31). The causative agent was first isolated and cultured in 1982 from the midgut of an *Ixodes scapularis* (*dammini*) deer tick harvested on Shelter Island, N.Y. (4, 12). Subsequent research identified what appeared to be identical spirochetes in *Ixodes ricinus* ticks in Europe and *Ixodes persulcatus* ticks in Eurasia (11).

All *Borrelia* spp. are distinguished by their characteristic morphology and motility, as well as a very high ($\geq 70\%$) A+T content in their DNA (7, 32). One of the most notable features of this genus is the finding that borrelias possess a linear chromosome of about 1,000 kb rather than circular chromosomes (13, 15, 21, 45). In addition to this "maxichromosome" (10), all *Borrelia* isolates have a complex mixture of extrachromosomal elements: covalently closed linear and circular plasmids, some of which are thought to carry genes coding for factors that contribute to virulence (5, 6, 20, 24, 29, 48, 51). The linear plasmids, or minichromosomes, range in size from 16 to about 50 kb (6). Genes for the abundant outer membrane lipoproteins OspA and OspB are encoded by tandemly arrayed genes that are part of a single transcription unit on the largest of the linear plasmids, lp49 (9). OspD, another outer surface lipoprotein, is encoded on a 38-kb linear plasmid, lp38, present in low-passage B31 cells (41). Recently, two groups (37, 44) determined that the gene for OspC, an additional major outer surface lipoprotein (22, 55), is on a 27-kb covalently closed

circular plasmid, cp27. Interestingly, under laboratory conditions all the linear plasmids seem to be completely dispensable, whereas cp27 and another circular plasmid, cp30, appear to be stable with respect to their replication and partitioning to daughter cells (28, 44).

The stability of cp27 and cp30 in vitro is in contrast to the instability of circular *Borrelia* plasmids of less than 10 kb. Several small 8- to 9-kb supercoiled circular plasmids have been detected in fresh, low-passage isolates of North American strains of *B. burgdorferi*; however, they are unstable and are lost completely after a maximum of 30 passages in vitro (24, 29, 47, 51). Their loss correlates with reduced infectivity in the white-footed mouse, *Peromyscus leucopus* (47, 51). Even though the presence of these smaller plasmids has been correlated with virulence and, therefore, they are of potential medical significance, not much is known about the genes of these plasmids or about their mechanisms of replication. Attempts to clone the smallest of the supercoiled circular plasmids, pBBC1, from the infectious strain Sh-2-82 were unsuccessful; only 1.6 kb of the starting 8.4-kb *EcoRI*-linearized DNA was recovered in *Escherichia coli* (47, 51).

During our studies on the phylogenetic relationship between geographically diverse *Borrelia* isolates (19), we compared the plasmid profiles from several low-passage North American and Eurasian isolates. One strain we examined, Ip21, which originated near Saint Petersburg, Russia, had a DNA band that migrated during agarose gel electrophoresis with the expected mobility of a circular plasmid of less than 10 kb that was recovered in diminishing amounts upon passage in vitro. To further characterize this plasmid, we decided to take a direct approach and determine its entire nucleotide sequence. Determining the nucleotide sequence of this small, unstable

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TABLE 1. *B. burgdorferi* strains and plasmids

Strain or plasmid	Source or description ^a	Reference
Strains		
B31	<i>I. scapularis</i> , Shelter Island, N.Y.	4, 10
Ip3	<i>I. persulcatus</i> , Leningrad province, Russia	34
Ip21	<i>I. persulcatus</i> , Leningrad province, Russia	34
Ip90	<i>I. persulcatus</i> , Khabarovsk territory, Russia	34
DK29	Skin of a patient with erythema migrans, Denmark	54
Plasmids		
cp8.3/Ip21	Native, cryptic plasmid	This work
pJD5851	<i>HincII</i> B fragment of cp8.3/Ip21 in the <i>Bam</i> HI site of pBR322, Ap ^r	This work
pJD5852	<i>HincII</i> C fragment of cp8.3/Ip21 in the <i>Bam</i> HI site of pBR322, Ap ^r	This work
pJD5853	<i>HincII</i> D fragment of cp8.3/Ip21 in the <i>Bam</i> HI site of pBR322, Ap ^r	This work
pJD5854	Subfragment of <i>HincII</i> A of cp8.3/Ip21 from nt 259 to the <i>Hae</i> III site at nt 2147 in pCR II, Ap ^r Km ^r	This work
pJD5855	Subfragment of <i>HincII</i> A of cp8.3/Ip21 from the <i>Hae</i> III site at nt 2148 to nt 3776 in pCR II, Ap ^r Km ^r	This work
pJD5856	Subfragment of <i>HincII</i> A of cp8.3/Ip21 from the <i>Hae</i> III site at nt 2148 to nt 3776 in the <i>Bam</i> HI site of pBR322, Ap ^r	This work

^a Ap, ampicillin; Km, kanamycin.

plasmid and the larger, more stably maintained *Borrelia* plasmids would be first steps toward a thorough understanding of their genetic organization and should help us understand how they replicate. Prior to this work, no *Borrelia* plasmid has been sequenced in its entirety. In this article, we present the complete nucleotide sequence of this small plasmid and analysis of its open reading frames (ORFs) and other features in the sequence. A striking feature of the sequence is the presence of two long inverted repetitive elements having some features in common with transposable elements. These data suggest that this plasmid may eventually have use as a vector for gene transfer in borrelia.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. Table 1 lists the *B. burgdorferi* strains used. Spirochetes were grown in BSK II medium at 32°C (4, 46). Commercial preparations of frozen, competent *E. coli* DH5 α [F⁻ ϕ 80d *lacZ* Δ M15 *endA1* *recA1* *hsdR17* (r_K⁻ m_K⁻) *supE44* *thi-1* *gyrA96* Δ (*lacZYA-argF*)U169] (26) (GIBCO BRL, Gaithersburg, Md.) and One-Shot INV α F' cells [F' ϕ 80d *lacZ* Δ M15 *endA1* *recA1* *hsdR17* (r_K⁻ m_K⁻) *supE44* *thi-1* *gyrA96* *relA1* Δ (*lacZYA-argF*)U169 *deoR*] (Invitrogen, San Diego, Calif.) cells were used as the recipients for recombinant plasmids according to the supplied protocols. *E. coli* Tap90 [*pro* *leuB6* *rpsL* *lacY1* *tonA1* *supE44* *supF58* *hsd*(r_K⁻ m_K⁺) *thi-1* *recD1903::mini-tet*] (43) was used as the host for infection with the λ ZapII vector (Stratagene). It was grown in glucose-free medium supplemented with maltose (52), a condition known to stimulate synthesis of the λ receptor. Transformed *E. coli* strains were cultured at 37°C as previously described (52). For antibiotic selection, ampicillin and kanamycin were added at final concentrations of 40 and 50 μ g/ml, respectively.

Recombinant DNA methods. Plasmid DNA was isolated from 25- to 250-ml samples of *Borrelia* cells taken at both early and late stages of logarithmic growth by using Qiagen-tip 100 cartridges (Qiagen Inc., Chatsworth, Calif.) according to the manufacturer's plasmid Midi protocol. This procedure, which involves alkaline-sodium dodecyl sulfate (SDS) lysis of cells in the presence of RNase followed by chromatography of a high-salt-concentration supernatant on a proprietary resin, gave reproducible preparations of linear and circular *Borrelia* plasmids, as judged by electrophoresis on 0.3% agarose gels in the presence 0.5 μ g of ethidium bromide per ml. The same procedure was used to purify plasmid subclones from overnight

E. coli cultures. Most other DNA manipulations were performed as described by either Maniatis et al. (36) or Ausubel et al. (2). DNA modification enzymes were obtained from several sources (GIBCO BRL; New England Biolabs, Beverly, Mass.; and Stratagene, La Jolla, Calif.) and were used according to the instructions of the manufacturers. *E. coli* single-stranded DNA-binding protein was purified by standard techniques after expression from the cloned gene in a bacteriophage T7-based expression system (52). Plasmid DNA was incubated with purified bacteriophage T7 gene 3 endonuclease, the gift of F. W. Studier, Brookhaven National Laboratory, at 37°C in an assay mixture containing 50 mM Tris-HCl (pH 8.0), 10 mM MgSO₄, 1 mM dithiothreitol, 50 μ g of bovine serum albumin, and 5 μ g of tRNA per ml. Under these conditions gene 3 endonuclease has a strong preference for supercoiled DNA over double-stranded linear DNA, and the enzyme converts supercoiled plasmids to a mixture of nicked circles and then to full-length linear double-stranded DNAs (17). Purified λ Zap arms and λ packaging extract were gifts of B. Burr (Brookhaven National Laboratory). *Borrelia* cells were embedded in low-melting-temperature agarose plugs as described elsewhere (21) by using cells harvested from early-stationary-phase cultures. Oligonucleotide primers were synthesized in a Milligen 7550 DNA synthesizer (Millipore) and purified by using Poly-Pak purification cartridges (Glen Research Corp., Herndon, Va.) according to the manufacturers' specifications. Hexamer primers were purchased from Research Genetics (Huntsville, Ala.).

PCR amplifications were carried out in 100- μ l reaction volumes containing 2.5 U of AmpliTaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, Conn.), each primer at 0.5 μ M and \approx 0.1 μ g of template DNA. The reaction mix also contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3 mM MgCl₂, 0.28 mM dATP, 0.28 mM dTTP, 0.12 mM dCTP, and 0.12 mM dGTP (the deoxynucleoside triphosphate concentrations used reflect the known DNA base content of \approx 29% G+C for *B. burgdorferi* [32]). Reactions were carried out for 32 cycles in a 9600 DNA thermal cycler (Perkin-Elmer Cetus), with each cycle consisting of 1 min at 94°C, 1 min at 47°C, and 4.5 min at 72°C. Amplification was completed by a final incubation at 72°C for 10 min. The amplified products were purified by electrophoresis on 0.7% low-melting-temperature agarose gels (Bethesda Research Laboratories).

DNA sequencing. The complete nucleotide sequence of cp8.3/Ip21 was determined on both strands by the chain

termination method using Sequenase 2.0 essentially according to the manufacturer's instructions (United States Biochemicals); however, the ratios of dATP and dTTP to their corresponding dideoxy analogs were doubled to balance the signal in all four lanes and prevent loss of signal in the A and T lanes on the AT-rich *Borrelia* templates. The chemical method of Maxam and Gilbert (38) was used to sequence short stretches of nucleotide sequence near the *Eco*RI and *Sau*3A sites of the plasmid. Subclones in pBR322 or TA vectors (pCR II; Invitrogen) were sequenced directly after heat denaturation of the double-stranded DNA and annealing with suitably positioned forward and reverse primers for each vector. Direct sequencing of PCR products in the presence of 25 μ g of *E. coli* SSB per ml was routinely performed with primers that anneal to the ends of the amplicons or to internal sites (primer walking). Before sequencing, the PCR-amplified templates were purified from low-melting-temperature agarose gels by using β -agarase I to digest the melted agarose and release the DNA according to the suppliers' instructions. New primers, typically 15 to 24 nucleotides (nt) long, based on cp8.3/Ip21 sequence information, were synthesized to extend the sequence into unknown regions of the DNA. Primer walking with strings of contiguous hexamers in the presence of saturating amounts of *E. coli* SSB protein also was employed under conditions essentially similar to those described previously (33). The determination of the relative orientation of each subclone and the junction sequence between each pair was determined by PCR analysis and sequencing of the amplified DNAs. No discrepancies between sequences determined on cloned DNA and those determined on PCR-generated templates were found.

Hybridization. Southern hybridization was performed in situ (2) in 0.7% agarose gels that had been run in Tris-acetate-EDTA buffer at 4°C for 14 to 16 h at 1 V/cm. DNA was denatured by gently rocking the gel in 2.5 volumes of 0.5 M NaOH–1 M NaCl for 20 min at room temperature. After the denaturation step was repeated, the gel was washed briefly with two changes of water, neutralized twice with 2.5 volumes of 0.5 M Tris-HCl (pH 7.2)–3 M NaCl for 30 min, and then washed in distilled water for 20 min. The gel was dried on a vacuum apparatus at 60°C for 1 h and then rehydrated overnight at 42°C in a hybridization solution (5 \times SSC [1 \times SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 20 mM Na₂HPO₄ [pH 7.4], 5 \times Denhart's solution, 250 μ g of sonicated calf thymus DNA per ml, and 0.1% SDS). Hybridization with 5'-³²P-labeled oligonucleotide probe ($\approx 10^6$ cpm/ml) was carried out in the fresh hybridization buffer for 18 h at 42°C. The unhybridized probe was removed by washing the gel three times in 6 \times SSC–0.1% SDS and once with 2 \times SSC–0.1% SDS and then exposed to X-ray film. Oligonucleotide probes radiolabeled with [γ -³²P]ATP (specific activity of 6,000 Ci/mmol) and T4 polynucleotide kinase were passed through a 1-ml Sephadex G-25 spin column equilibrated with Tris-EDTA buffer to remove free ATP beforehand. To differentiate between double- and single-stranded DNAs, electrophoretically resolved DNAs were transferred to nitrocellulose with or without denaturation with NaOH prior to transfer by diffusion with 20 \times SSC (53). For some experiments, 0.5% low-melting-temperature agar plugs (250 μ l) containing $\approx 10^7$ *Borrelia* cells were used as the source of total genomic DNA. Cells were lysed (21), and the plugs were thoroughly washed with 10 mM BisTris-HCl (pH 6.5)–100 mM NaCl, melted at 68°C, cooled to 40°C, and then incubated with β -agarase I (2 U/100 μ l) overnight. *Hind*III (20 U/100 μ l) and 1/10 volume of 10 \times *Hind*III restriction buffer (New England BioLabs) were added, and after a 2-h incubation at 37°C, the digests were fractionated by electrophoresis on 0.7% agarose gels. Blots were

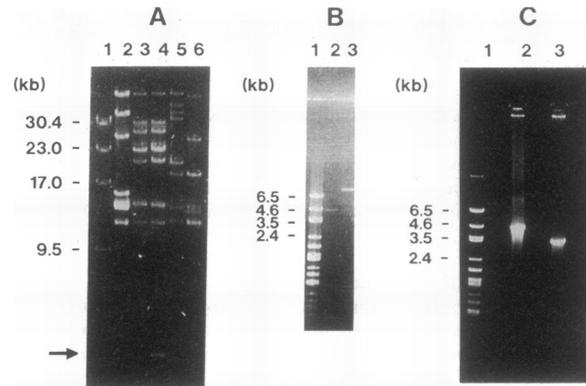


FIG. 1. (A) Agarose gel electrophoretic profiles of *Borrelia* plasmid preparations of different isolates. Lane 1, T7 *Mlu*I and *Nhe*I restriction fragment size markers (18); lanes 2 to 6, plasmids from isolates B31, Ip3, Ip21, Ip90, and DK29, respectively. Samples were analyzed on a 0.3% agarose gel. Arrow, cp8.3/Ip21 DNA band. (B) Restriction enzyme digestion of cp8.3/Ip21 analyzed on a 0.7% agarose gel. Lane 1, T7 *Hae*II digest (18); lanes 2 and 3, purified cp8.3/Ip21 DNA before and after digestion with *Eco*RI, respectively. (C) Agarose gel analysis of PCR-amplified cp8.3/Ip21. Lane 1, T7 *Hae*II digest; lanes 2 and 3, amplification products using primers pairs 1801 plus 1649 and 1802 plus 1648, respectively. Gels were stained with ethidium bromide and photographed with UV illumination. The positions and sizes of marker bands are indicated on the left of each panel.

exposed to X-ray film, or images were obtained by using a Molecular Dynamics PhosphorImager system and ImageQuant software.

Computer analysis of the DNA sequence. DNA sequence data were assembled by using software packages developed by S. Zhang at Brookhaven National Laboratory. The homologies of DNA and protein sequences were analyzed by using Fasta and the BLAST service of the National Center for Biotechnology Information (1).

Nucleotide sequence accession number. The cp8.3/Ip21 nucleotide sequence data reported here have been deposited in the GenBank data base under accession number U03641.

RESULTS AND DISCUSSION

Plasmid analysis of *Borrelia* spp. We compared several methods for preparing endogenous linear and circular plasmids from *Borrelia* sp. cultures and found that the Qiagen Midi kit procedure invariably gave excellent recovery and highly reproducible results. As expected, each *Borrelia* strain listed in Table 1 harbors a minimum of six plasmids ranging from about 16 kb to more than 40 kb, on the basis of their electrophoretic mobility relative to linear bacteriophage T7 marker DNAs (Fig. 1A). Although each strain has a unique plasmid profile, some strains have patterns that are very similar. In particular, the plasmid patterns of strains Ip3 and Ip21 are nearly identical except for the presence in Ip21 of a much smaller plasmid that migrates on agarose gels near the position of a linear 4.6-kb marker. This small plasmid was not seen in the plasmid pattern of a B31 strain that has been passaged repeatedly in our laboratory. After three passages in BSK II medium the small Ip21 plasmid was no longer visible in plasmid preparations, and the Ip21 and Ip3 plasmid patterns became essentially indistinguishable (data not shown).

Cloning and sequencing strategy. Initially, we surveyed several restriction endonucleases for their ability to cleave preparations of the plasmid after it had been purified from

agarose gels. No sites were found for *AccI*, *ApaI*, *AvaI*, *BamHI*, *BclI*, *BglII*, *BstYI*, *KpnI*, *PstI*, *SmaI*, or several other restriction enzymes which should cut infrequently a high-A+T-content DNA. However, the plasmid appeared to have one recognition site for *EcoRI*, producing a band with a mobility approximately equal to that of an 8.3-kb linear DNA (Fig. 1B). When the purified plasmid was incubated with T7 gene 3 endonuclease, an enzyme known to convert covalently closed supercoiled circles to double-stranded linear molecules (17), a band with the same mobility as that of the *EcoRI* digestion product was produced. From these data and the observation that before cleavage the plasmid did not enter a 5% polyacrylamide gel, we concluded that this DNA is a double-stranded circular plasmid; we have named it cp8.3/Ip21.

Our first approach was to attempt to clone the *EcoRI*-linearized DNA into λ ZapII with *E. coli* Tap90, a strain believed to minimize loss of sequences from genomic libraries (43), as the host strain. However, two attempts to identify phage containing an insert in the expected 8-kb size range were unsuccessful. Very few plaques were positive when screened with a randomly primed, ³²P-labeled cp8.3/Ip21 whole-DNA probe, and the resulting lysates they produced either had inserts that were considerably smaller than the starting DNA or appeared to have lost the insert entirely (data not shown). These results are very similar to those reported by Simpson et al. (51), who also were unsuccessful in cloning a similar-sized plasmid from the North American *Borrelia* strain Sh-2-82 in a λ Zap vector. In all likelihood, this failure was due to the presence of sequences in each plasmid that prevent stable cloning in *E. coli*. Therefore, we decided to try two approaches to circumvent this problem: direct sequencing of PCR-amplified cp8.3/Ip21 DNA and subcloning of individual restriction fragments of the plasmid.

Before primer pairs could be designed to amplify the cp8.3/Ip21 sequence, limited sequence information had to be obtained. This was accomplished by chemical sequencing in both directions from the single *EcoRI* site in cp8.3/Ip21 and from a *Sau3A* site situated about 40% around the plasmid. Two sets of primer pairs—primers 1801 and 1649 (GATAC AATAGTCGCTGTAATTCAC and GGTTTATATAATAT ACTACGTC) and primers 1802 and 1648 (GTGAATTAC AGCAGCTATTGTATC and GAGCGATATCCTTAAAA TTGCC)—which, subsequent analysis showed, were able to amplify fragments of 4,772 and 3,555 bp, respectively (Fig. 1C), were synthesized on the basis of the known sequences. Together, the two amplified fragments represent the entire cp8.3/Ip21 DNA molecule with a 24-bp region of overlapping sequence.

Since cloning PCR fragments in this size range could conceivably result in selecting DNA molecules with amplification errors, we used several approaches to subclone cp8.3/Ip21 DNA that had been purified from low-melting-temperature agarose gels. Cutting cp8.3/Ip21 with *HincII* generated four fragments (A to D in order of decreasing size from approximately 3.3 kb to just under 1 kb) which could be resolved on agarose gels. Three of the *HincII* sites are also sites for *HpaI*. After *BamHI* linkers were added and cut with *BamHI* to generate sticky ends, *HincII* fragments B to D could be subcloned directly into the *BamHI* site of pBR322 and propagated in the *E. coli* host strain, DH5 α . Repeated attempts to subclone *HincII* fragment A using this strategy were unsuccessful. Further analysis indicated that this region of the DNA contained a single recognition site for *HaeIII* situated nearly in the middle of the fragment. Two related approaches were used to try to subclone these *HaeIII* subfragments. The first involved digesting agarose gel-purified *HincII* A with *HaeIII*, adding

BamHI linkers, and ligating with *BamHI*-digested pBR322. The second approach took advantage of the template-independent terminal transferase activity of *Taq* DNA polymerase to add an adenosine to the 3' end of DNA fragments (14). Such molecules are conveniently cloned into so-called T vectors by virtue of a 1-nucleotide complementary overhang. *HaeIII*-digested *HincII* A was incubated with *Taq* DNA polymerase in the presence of dATP and then ligated to pCR II. Each ligation mixture was used to transform competent INV α F' cells, and the resulting colonies were screened for the presence of inserts by digesting the plasmid preparations with *BamHI* or *EcoRI*, respectively. Clones of each *HincII*-*HaeIII* subfragment were obtained when the T vector was used, but for unknown reasons, clones of the slightly larger subfragment were not recovered in the pBR322 vector (Table 1).

Complete sequence of cp8.3/Ip21. Figure 2 shows the complete nucleotide sequence of cp8.3/Ip21, and a physical map of the plasmid is presented in Fig. 3. The total length is 8,303 bp, in close agreement with the size predicted from the mobility of the linearized DNA on agarose gel electrophoresis. The overall G+C content is 23.4%, a range typical for *Borrelia* DNA. The longest uninterrupted run of A's is 10, beginning at nt 4960; the longest run of uninterrupted C's is 7, beginning at nt 1353. Direct sequencing of PCR-amplified DNA revealed two closely spaced *HpaI* sites, 10 bp apart, between fragments D and A, that had been overlooked during initial construction of the physical map. All of the restriction endonuclease cleavage sites shown in Fig. 3 have been confirmed, and there are no recognition sites in the nucleotide sequence for enzymes that are known not to cut the DNA. *Sau3A* cleaves the two sites in cp8.3/Ip21 nonrandomly. The *Sau3A* site at nt 7163 is cleaved more slowly than the site at nt 3537; this difference is not due to methylation of the C residue in the GATC recognition sequence, since both sites are followed by the same 3'-bordering sequence, TAA. Interestingly, the 5' border of the GATC recognition sequence at nt 7163 is GAT. Perhaps the *Sau3A* binds to the 5' trinucleotide GAT sequence and, in the process, occludes recognition of the adjacent 4-base site.

Comparison of the long inverted repeats of cp8.3/Ip21. One of the most striking features of the sequence is the presence of two long, nearly perfect, inverted elements, IR-A and IR-B (Fig. 2 to 4). These elements also contain a putative promoter similar to the *E. coli* promoter consensus sequence (27). The -35 sequence in the putative promoter (TTGTAA) differs by 2 bases from the *E. coli* consensus sequence (TTGACA), and the -10 sequence (TATATT) differs by 1 base from the consensus sequence (TATAAT). If active, each promoter would direct transcription outward into flanking unique sequences of the plasmid. The resulting transcripts each would contain a Shine-Dalgarno sequence (AGGAG) capable of uninterrupted pairing with nucleotides near the 3' end of *Borrelia* 16S rRNA (49) upstream of a suitably positioned AUG initiation codon that could initiate translation for polypeptides 266 (ORF-4) or 88 (ORF-8) amino acids long. In each case, the inverted repeat includes sequences 12 codons past the start codon of the ORF. Therefore, the deduced amino acid sequences at the N termini of polypeptides 4 and 8 are nearly identical for the first 13 residues; codon 10 of ORF-4 is AAA (Lys), and in ORF-8 it is GAA (Glu). The portions of IR-A and IR-B that may function in translation seem to have little potential to form stem-and-loop structures, and therefore any RNA derived from the putative promoters would have a high probability of serving as a functional mRNA. In contrast, the other end of each inverted repeat has the potential to form several long stem-and-loop structures (Fig. 5). Inspection of the sequence immediately adjacent to the inverted repeats

TTCTTCATCATAAAGATTTCCTCTTTAAACCGGATTATAAAGGGCATAAGAACAAATTCGGAGTATTTTATATATAITAGACGTAGTATATTATATAAACCGAGCGATATCCTTAAA 120
 ATTGCCATTAAAGTTTATTATATCCATCCACAACCCGAATTTTGGCAAATATGCGGGGCCATTGGTTAATAAAAATGTTATTAGTTTTGGTACAATTTGAATTGTAATTTTATTAAC 240
 TAAATGTTAACGTATGTTAACGTATAGAAAAAGTCAATTTTGCATACATCAATATTTATTGATTAAATAAAAAATCTGCAAATTTACCCCGTAAAGATAGTAAAAATTAACAAA 360
 AACCATTAATGGAACAAAAATTTTTAAATTCCTAAATTAGGATAGTAAATAAATAAATCTTTTTATAAGTGTACAACCTTACAAGTTATTTTGGTTGGAGAATTTATTTT 480
 *****-10 *****-35
 Asn Lys Tyr Phe Asn Asp Asp Ile Gln Cys Phe Ser Cys Lys Arg Leu Ile Lys Asn Tyr Asp Glu Ile Cys Val Ala Cys Gly Ala Lys Asn Arg Gln Asn Lys Gln Ser Tyr Leu Val
 GAACAAATAATTTTAAATGATGATATACAGTGC TTTTCTGGTAAAAGATTAATAAAAAATTATGACGAAATTTGTGTCGCTTGTGGGGCTAAAAATAGACAAAATAACAAATCTTATTTGGT 600
 Leu Ile Thr Phe Leu Leu Cys Leu Ser Phe Gly Tyr Val Gly Leu His Asn Leu Tyr Leu Gly Asn Lys Ile Ala Phe Thr Phe Leu Leu Ser Leu Phe Ser Phe Leu Leu
 ATTAATAACATTTTTATTGTTTATCTTTTGGCTACGTAGGACTTCATAATTTGACCTAGGCAATAAAATCAAATCGCTTTTACATTTTTATTATTATCTCTTTTTTCTTTTTTACT 720
 Val Val Leu Leu Gln Lys Thr Asn Lys Thr Asn *** -35***** -10*****
 TGTGCTATTACTGCAAAAACTAACAAAACCAATTAATAACAGCTTATTAGTTTTTATGAGATTGTTTTTATGGATTAAATCTATAAAAAATAAATATATAAACACACAAA 840
 AGTTAGTGGATTTTAAACAAAAATTAGTAGCAAAAATCCACA AAAATGGGGATACCCCTACAGTATCTATAAAGGATAGAGTCAAAAATATATAAATCAATATATCCCCCTTCATTT 960
 TAAAGTTTTTGTATATTTAGAATAACGTTTATGTAGCTACATTTATGTTCTCTTAAAAATGTTTTTCTACTTACTACAGCTATTCTAGCTCGAATTTTGCACACAAATTAACATTTTT 1080
 TAGTTTGACTAATATTGATAATCTTGTGATAAATTTGAGGTGAAATTAATAAATGATATTATTTTATTGATATTTAATTTTGGTTGATTTAAAAGTGGAGTTAATTAATGGAAAT 1200
 ORF-6 Met Glu Ile
 Asn Asp Tyr Leu Asp Phe Lys Lys Asn Thr Ala Lys Val Leu Leu Lys Ile His Asp Asp Tyr Gln Lys Ile Leu Gln Ile Ile Asp Lys Asn Lys Thr Leu Lys Asn Lys Ile Lys Lys
 TAATGATTATCTAGATTTTAAAAAACACGTCGAAAAGTGTACTTAAGATTCACGATGATTATCAAAAATATTACAAATAATAGATAAAAAATAAACCTTAAAAAACAGATAAAAAA 1320
 Ser Thr Glu Asn Lys Gln Glu Asn Ser Lys Thr Pro Pro Lys Leu Tyr Leu Asn Pro Lys Thr Asn Gln Leu Ile Ile Lys Cys Val Lys Ile Leu Lys Gln Ile Asp Pro Ile Ser Gly
 ATCAACTGAAAATAACAAGAAAATCTAAAACCCCCCAAACTGTACTTAAATCCAAAACAAATCAATTAATAATAAATGTTCAAAAATCTTAAACAAAATGACCCCAATATCTGG 1440
 *****-10 *****-35
 Trp Phe Val His Leu Leu Thr Ile Ser Gly Cys Arg Gly Ala Glu Leu Gln Lys Val Lys Met Gln Asp Ile Ser Ser Phe Leu Ser Thr Thr Gly Lys Thr Leu Tyr Asn Ile Lys Val
 CTGTTTGTACTATTACTGACAAATAAGTGGTGTAGGGGCGCGAAGTCAAAAAGTAAAATGCAAGATTTTTCATCCCTTTTAAAGCAACCGGAAAACCTTTATACACATAAAAGT 1560
 Asn Val Val Lys Lys Arg Ile Asn Thr Cys Val Arg Glu Phe Val Ile Asn Ser Lys Glu Phe Asn Ser Ile Gln Lys Val His Glu Asp Tyr Phe Lys Glu Lys Asn Phe Asn Thr Asn
 AAATGGTAAAAAAAAGAAATTAATACTTGTGTAGAGAATTTGTTATAAATCAAAGAATTTAATCTATTCAAAGTACATGAAGATTTTAAAGAAAAAATTTTAATACAAA 1680
 Arg Thr Tyr Phe Phe Gln Lys Thr Lys His Arg Phe Lys Asp Asn Arg Ile Ser Ile Asp His Ile Ala Lys Lys Phe Lys Lys Leu Leu Lys Lys Ser Gly Phe Lys Ala Asn Lys Ser
 CCGCACTTATTTTTTCAAAAACCAAGCATAGATTTAAGATAATCGAATTAGCATTGACCATTTGCTAAAAAATTTAAAAGTTGCTTAAAAATCAGGATTTAAAGCAATAAATC 1800
 Leu His Leu Cys Arg Asn Leu Phe Ile Phe Asn Leu Lys Ala Asn Gly Tyr Asn Ser Phe Gln Ile Lys Glu Leu Met Lys Tyr Ser Ser Thr Tyr Glu Ile Asp Asn Ile Tyr Gly Leu
 TCTTCATTTGTGTAGAAAATTTGTTTATTTTAAATTTAAAGCCAACGGTTACAATCTTTCCAAAATTAAGAACCTTATGAATATTCTCAACATATGAATGATAATATCTATGGAC 1920
 Ser Ser Ala Ser Lys Ile Gln Ala Tyr Glu Cys Val Lys Asn Ser Ile Gly Leu ***
 ATCTTCGCAAGTAAAATTCGAAGGTATGAGTGCCTAAAAAATAGCATTGGTTATAAATCTCCTATTTAGGCTTTAAATGATGAATACCTTTATTTTGACATATAATCTATATTAAC 2040
 TATTTTTTAAACAATTTTTCCGTGTCCTTATCAACGGGACACGGGCATTATTTGAAAACGCTATGTTATTAGAAAAGAATACTTTTCCACTTGTGGTAAATAGGCCCTTTAATCTAT 2160
 *** Glu Val Pro Val Arg Ala Asn Asn Ser Phe Ala Ile Asn Asn Ser Phe Phe Val Lys Gly Ser Thr Pro Leu Leu Gly Lys Leu Arg Asp
 CTGATATTTCCCTTTTATTAGGGTCATAATGAAACATAAATCTCTAAATTTATAATTTACATTTAATTAAGTGGATATTTACGTATGATTTCAATTTTAAATTTAAGTAATCTT 2280
 Ser Ile Glu Arg Lys Asn Pro Asp Tyr Asn Phe Met Phe Glu Arg Phe Lys Tyr Asn Val Asn Asn Ile Leu Pro Tyr Lys Arg Ile Ile Glu Asp Ile Lys Ile Lys Leu Leu Glu Lys
 TAAITGGTTTTCTTTTTTCTGTTTTCAITTTTGAATTTTATTAGTTCTACTTTTAGATTTTTAATTTCAAAAAGAGAACACTCAAGATTTACAGGGGACTGTTCAAGTTTAACTTAA 2400
 Ile Pro Lys Glu Lys Lys Arg Asn Glu Asn Gln Ile Lys Asn Leu Glu Val Lys Leu Asn Lys Ile Glu Phe Leu Ser Cys Glu Leu Asn Val Ala Ser Gln Glu Thr Lys Ile Lys Leu
 GGCTATATAATCAACATATTCTACAAATCTTTTCCAGTCAAACCTCAACCCGGAAACAATAAAAATCACTCTTTTCTACTAACTCATCAACAAAATTTATAAAGTATAATTAATCA 2520
 Asp Ile Tyr Asp Val Tyr Glu Val Phe Glu Asn Ile Trp Asp Phe Glu Val Gly Ser Cys Tyr Phe Asp Ser Lys Glu Val Leu Glu Asp Val Phe Asn Ile Phe Thr Tyr Asn Asp Asp
 CATTATGATATTAATAAATGACCCGATTTGTTTTTCATAATTTTATTTCTCAAATGATAATTTCAATTCACATTTTATAACAAATCTAACACTTTGATATTAAGCATCATTTT 2640
 Asn His Ser Ile Leu Leu Asn Val Ala Asn Asn Lys Met Ile Lys Asn Arg Leu Asn Ile Ile Glu Leu Glu Cys Lys Asn Val Phe Glu Leu Val Lys Ser Ile Leu Ala Asp Asn Lys
 TCTTTATCTTACAATTAATAGGTGCTGCACCTATTAAGAAGAATAAATACAATTTCAAGTCCAGTACATGCTAGCTGCTATTGTGCTGCACGTAGTACTTGA AAAAATACTTACTAC 2760
 Lys Ile Lys Cys Asn Ile Pro Ala Ala Gly Ile Leu Phe Phe Leu Asn Cys Asn Glu Leu Gly Thr Cys Ala Leu Glu Met Gln Ala Gln Val Tyr Tyr Lys Phe Phe Tyr Lys Ser Ser
 TTAAGAAATGGCATTTTTATTGACTTATCAATAGCGCTACTCATATAATAGAATCACTACTCTTAATCTCTAAAAGCTCTAAATCACCATTATTATTAATAACCAACCGTCAATTG 2880
 Leu Phe Asn Gly Asn Lys Asn Tyr Lys Asp Ile Ala Ser Ser Met Tyr Asn Ser Asp Ser Ser Lys Ile Glu Leu Leu Glu Leu Asp Gly Asn Asn Asn Ile Phe Thr Gly Asp Ile Thr
 TTGAGCCCACTAAAGTTTTGAATTTCCCACTTTTTTAAAAATAGTTATACTTATCAACACCATTAGCATATTTATTTTTGTACAAAACCGCAATATTATACCGGTGCTTTTATAAAT 3000
 Ser Gly Val Leu Thr Lys Ser Asn Gly Val Lys Lys Phe Tyr Asn Tyr Lys Asp Val Gly Asn Ala Tyr Lys Asn Lys Tyr Leu Val Ala Ile Asn Asp Gly His Ala Lys Ile Phe Glu
 CTCTGAATCCCAAAATTTCTAACTTTGCCCCTTAAGCATATATAAATCTCTCTAAAAGGCATACTTATACCAAAATATTTAAGCACTCTATTTACCATTAAATCTTTTACGCCCTACAC 3120
 Arg Phe Gly Leu Asn Glu Leu Lys Gly Lys Leu Met Tyr Leu Asn Glu Glu Phe Pro Met Ser Ile Gly Phe Tyr Lys Leu Val Arg Asn Val Met ORF-7 *** Val
 CACCAATAAGAATATCTCTACTGCTAGCGCGTATCTATCAAGTTTGTCTTTGTAATCAAAATCTTGATTTCCGGTGAATTTAAACACTCTTGACTACTTATCTCGGTAATTT 3240
 Val Leu Leu Phe Ile Gly Val Glu Ser Ala Gly Tyr Arg Asp Leu Lys Asn Arg Gln Ile Ser Phe Asp Gln Asn Arg Asp Phe Lys Phe Cys Glu Gln Ser Ser Ile Arg Pro Leu Lys

FIG. 2. Complete nucleotide sequence of cp8.3/Ip21 beginning with the first T residue of the single *EcoRI* site. Every 10th residue is marked (.). The deduced amino acid sequences of the predicted ORFs are shown above or below the DNA sequence, depending on their orientation. The Met residue for the potential start of ORF-7b is underlined. The major inverted repeats IR-A and IR-B are indicated (open arrows). Possible locations for plus origins of replication are boxed. Potential -35 and -10 promoter regions (asterisks) and regions with potential to form stem-loop structures (arrows over the sequence) are indicated.

TTGCCTATCTTGCTTAATTTGCTTTTTCTTTAGTTTGACTAACTGATTTTCAAGTCTATAAAAATTTCAAACCAATAAAAATTTGGTGTGGTGTATGTTGATTAAITCTTGTTG 3360
 Lys Gly Ile Lys Ser Leu Lys Ser Lys Glu Lys Thr Lys Val Gln Asn Glu Leu Glu Ile Phe Asn Glu Phe Gly Ile Phe Asn Pro Gln Lys Tyr Thr Gln Asn Ile Glu Gln Pro

ATTATTGTTAAAGTTTTGCATTTTTATCTCCTAGAACATTAGATTTTTTCTTATATAAGAATATAGCAAAAACATTTTTTACAAGTTTTTACAAAAATTTTGAAAAACAT 3480
 Asn Asn Asn Phe Asn Gln Met ORF-8 *****-10 *****-35

AGGGCTTTACCAAATTCATCTTTAAAGAACTTAGTAAAGCCCTAATTTAATAGCTGATCTAACGTTCACTAGAAAATAATGAAAATATTATCATTTCACAAAACCATTTTTAGTATAG 3600
 TTCAAATTTTAAATAGAACTAATCTTATTTATAAGATACAATAGTCGCTGTAATTCACAAAAATTTTGTATTTTTTAAAACATTTACTTTTTAAATTAATTTGTGATATATATATTTCT 3720

ORF-1 Met Lys Asn Leu Ser Asn Val Asn Lys Thr Arg Lys Asn Thr Leu Cys Cys Asn Lys Leu Gln Gln Lys Leu Ile Val Leu Val
 TAATAGAATGTAATCTAACGTTAAGGAGCTATAAATGAAAAATTTATCTAATGTTAACAAAACCTGTA AAAACACACTTTGTTGCAATAAAATGCAACAAAAATTAATAGTTCTTGTT 3840
 Ser Thr Leu Ala Tyr Ile Asn Thr Lys Tyr Lys Lys Tyr Thr Gln Lys Thr Ile Leu Tyr Tyr Phe Asn Glu Asn Leu Lys Arg Asn Gly Gln Ala Thr Thr Lys Leu Arg Thr Leu Gln
 CAACACTGGCTATATAAACACCAATATAAAAAATATAACAAAAACCTACTCTATCTACTTTAATGAAAATCTAAAAAGAAATGGTCAAGCTACTACTAAATTAAGAACATTTGCAAA 3960
 Lys Tyr Leu Tyr Arg Leu Glu Lys Asp Phe Lys Val Thr Thr Asn Tyr Tyr Lys His Leu Gly Val Asn Leu Gly Thr Glu Ile Tyr Tyr His Leu Asn Phe Glu Lys Asn Lys Cys His
 AATATCTTTACAGATTAGAAAAGATTTTAAAGTAACAACCACTACTAACAACATTTGGGTGTAATTTGGGCACGAAATTTACTATCACCTTAATTTGAAAAAAAACAAATGCCACT 4080
 Phe Lys Ile Asn Gln Tyr Phe Gln Glu Lys Lys His Leu Arg Phe Thr Thr Arg Val Lys Asn Tyr Leu Lys Asp Lys Ser Leu Lys Lys Gly Ser Val Glu Leu Gly Glu Cys Phe Asn
 TTAATAACCAACATATTTTCAAGAAAAAACCACTTACGATTTACAACCTAGAGTTAAGAACTACCTTAAAGACAAATCTTAAAAAGGGTAGTGTAGAGTTGGGGGAGGTTTTAATA 4200
 Asn Lys Asn Asn Asn Ile Lys Glu Glu Lys Asn Lys Lys Ile Glu Asn Phe Gln Ile Ile Lys Tyr Gly Asn Lys Cys Asn Phe Lys Cys Lys Glu Ile Leu Ser Phe Ile Leu Asn Leu
 ATAAAAATAAATAATAAAGAAAGAAAAAGATAAAAAGATAGAAAATTTTCAATAATAAATATGGCAATAAATGCAACTTAAATGTAAAGAAATCTTTCTTTTATTTTAAATTTAG 4320
 Glu Leu Asn Lys Asp Leu Lys Ile Lys Met Leu Lys Phe Ala Lys Ile Ile Glu Ile Lys Leu Leu Lys Tyr Lys Asn Ile His Phe Asn Lys Ser Cys Phe Lys Asp Lys Gln Asn Lys
 AACTTAATAAGACTTGAATTAATAATGCTTAAATTCGCAAAAATAATGAGATTAACCTACTAAAGTATAAAAATATACATTTCAATAAATCTTGCTTTAAAGATAAGCAAAAACAAAT 4440
 Leu Lys Glu Ile Leu Glu Asn Ile Gln Lys Gln Leu Glu Lys Lys Gly Tyr Asn Ala Glu Gln Leu Glu Thr Glu Phe Lys Lys Ile Tyr Glu Asn Tyr Lys Asn Lys Pro His Phe Ile
 TAAAAGAAATCTAGAAAATTTCAAAAACAAATAGAAAAAAGGATACAATGCTGAACAATTAGAAAACAGAATTTAAAAAATATATGAAAACACAAAATAAGCCGCATTTTATTA 4560
 Ile Glu Asn Gln Lys Tyr Asn Asp Leu Gly Lys Ile Met Phe Lys Leu Glu Lys Ser Ile Glu Leu Lys Lys Glu Asn Ser Gln Lys Asn Tyr Glu His Ile Lys Thr Asn Ile Phe Asn
 TTGAAAATCAAAAATATAACGATTTAGGCAAAAATAATGTTTAAATTAGAAAATCAATCGAATTTGAAAAAGAAAATTCACAAAAAATTTAGGACATATAAGACAAAACATTTTCAATA 4680
 Ile Leu Ile Glu Gln Leu Gly Lys Thr Ala Asn Ile Glu Ile Leu Lys Pro Ile Ile Lys Lys Tyr Leu Asn Ser Lys Lys Lys Leu Glu Tyr Asn Asn Val Phe Val Thr Tyr Tyr Tyr
 TACTGATTGACCAATTAGGAAAAACAGCAATATTGAAATCTAAAGCCAATATAAAAAATATTTGAATAGCAAAAAGAAAATAGAATACAATAATGTTATTGTTACATATTATTATG 4800
 Glu Leu Leu Glu Leu Ile Lys Asn Gln Lys Ser Leu Leu Asn Leu Lys Glu Leu Ala Lys Lys Val Ile *** ORF-2 Met Glu Asn Leu Leu Lys Thr Thr Lys Thr Glu Glu Lys
 AATTATTAGAACTAATAAAAATCAAAAAGTTTACTTAAATTTAAAGAAATAGCTAAAAAGTCATATAAGGATTAACATGAGAAATTTATTGAAAACACAAAACCTGAAGAAAAA 4920
 Lys Phe Asp Phe His Lys Ala Ser Ile Phe Leu Met Lys Glu Lys Lys Asn Asp Arg Asn Ile Tyr His Thr Lys Ile Leu Lys Glu Ile His Lys Val Val Ser Asp Lys Lys His Gly
 AGTTTGACTTTCATAAGCAAGATTTTTCTTAATGAAAGAAAAAAAATGATAGAAATTTATCATACAAAAATACATAAGGAAATTCATAAAGTAGTAAGGATAAAAACACGGGT 5040
 Cys Ile Val Phe Phe Lys Lys Leu Phe Asn Glu Ser Glu Ile Gln Asn Leu Cys Leu Tyr Pro Met Gln Glu Gly Asn Lys Phe Leu Gly Ile Phe Tyr Gly Tyr Lys Lys Pro Ile Lys
 GTATAGTTTTTTTTAAAAATTTTCAATGAGTCAGAAAATTCAAAATCTTTGCTATATCTCTATGCAAGAAGGGAATAAATTTTTAGGGATTTTTTATGGTTATAAAAACCAATAAAAA 5160
 Asn Val Leu Val His Tyr Glu Leu Asn Gln Val Lys Lys Ala Tyr Thr Phe Ser Lys Thr Tyr Tyr Ile Glu Phe Arg Phe Lys Thr Gly Ser Val Phe Cys Tyr Leu Arg Gly Leu Phe
 ATGTTCTTGACATTATGAATTAATGGTGTAAAAAAGCATATACATTTTCAAAAACATATTACATAGAATTTAGATTTAAAACCTGGTAGTGTTTTTGCTATTTAAAGGGATTTGTTT 5280
 Arg Leu Leu Lys Lys Glu Lys Ile Asp Thr Pro Tyr Asn Lys Ala Leu Val Asn Lys Phe Leu Asn Leu Glu Lys His Val Tyr Glu Phe Tyr Asn Lys Lys Tyr Ser Asp Lys Gly Leu
 GATTACTAAAAAAGAAAGATAGATACACCTTATAAATAAGCACTTGTGAATAAATTTTAAATTTGAAAAACATGTAATGAATTTTACAATAAAAATATTCTGATAAGGGACTAA 5400
 Ile Ile Lys Trp Ile Lys Lys Asn Leu Lys Gln ***
 TTATTAATGGATTTTAAAAATCTAAAGCAATAAACAATTTGTGCTAATTAAGGACATTTGTGATAAAATTTTTTATATGATGCAAAAATGATTTTCAAGAAAGTTTTCTAGAAAATAAG 5520

ORF-3 Met Glu Ile Lys Phe Lys Arg Val Leu Thr Lys Lys Ile Asn Thr Asn Val Glu Gln Asn Ser Ile Ile Asp Glu Ser Lys Ile Ser Tyr Tyr Lys Thr Leu Lys Glu
 GAGATTTTTTATGGAATAAAATTCAAAAAAGAGTTTTAACAAAAAAAATAAATACTAATGTTGAACAAAATTCAAATTAAGATGAAAGTAAAAATTTCTATTACAAAACACTAAAGGA 5640
 Lys Leu Lys Thr Asn Phe Lys Lys Glu Ile Phe His Lys Val Glu Asn Ile Arg Ile Leu Lys Glu Ile Lys Asp Asn Glu Tyr Tyr Lys Phe Asp Gly Tyr Lys Asn Phe Leu Asp Phe
 AAAATTA AAAACCAATTTCAAAAAGAAAATTTCCACAAAGTGAAAAATTCAGAATTTTAAAAGAAATAAAAGATAATGAATTTAATAATTTGATGTTATAAAAAATTTTTGGATT 5760
 Val Lys Asn Phe Asn Val Ala Lys Ser Gln Ala Tyr Lys Tyr Leu Lys Leu Ala Thr Ala Leu Gln Asn Gly Val Leu Asn Glu Asn Tyr Val Ile Glu Asn Gly Ile His Asn Ser Phe
 TGTAAAAACCTTAATGTTGCAAAAAGTCAAGCATACAAGTATTTAAAATTTAGCAACCCACTGCAAGATGGTCTTTACGAGAAATTAAGTAATAGAAAATGGTATTCTATAATCTTT 5880
 Asn Tyr Ile Lys Asp Lys Glu Ser Pro Ser Leu Lys Lys Ser Lys Glu Asn Pro Ile Lys Pro Leu Arg Leu Lys Leu Lys Thr Gln Glu Ser Tyr Asp Phe Tyr Lys Ser Lys Ala Lys
 TAATTATATAAAGATAAAGAAAGTCCATCATTAAAAAATCTAAGAAAACCAATAAAACCTTTAAGATTA AAAACTTAAAACCTCAAGAAAGTACGATTTTACAAAAGCAAGGCTAA 6000
 Phe Thr Ser Phe Met Met Asn Glu Ile Phe Glu Asn Gln Lys Asp Leu Ile Asn Lys Leu Met Lys Lys Tyr Asp Glu Leu Lys Ile ***
 ATTTACTAGTTTTTATGATGAATGAAATTTTGAATAACAAAAGATTTAATTAATAGCTTATGAAGAAATATGACGAACTAAAATAATAATTTAGTTCAATACAATTTGATTATGATT 6120
 TTGATATAGATTATCCAACACTACTAGTTATATAATTTTCAATTTATTATTACAATCGTTAAATATTGGTTTTAAATCCATAGGGCTTTATTAAGTTCTTTTAAAATAGAATTTGGTAAAG 6240

CCCTATAGTTTTTACAAAAATTTTTTGAAAAAACCTTGTA AAAAATAGTTTTGCTATATTATATAAAGAAAAAATCTAATGTTCTAGGAGATAAAAATGCAAAAACCTTTAAC 6360
 Asn Asn Pro Gln Lys Ile Asn Gln Ala Glu Ile His Phe Ile Ser Asp Met Lys Thr Leu Arg Met Asn Leu Ala Gly Ile Asp Lys Asn Leu Lys Gly Tyr Gly Tyr Lys Tyr Gln Asn
 AATAATCCAAAAAATTAATCAAGCAGAAATTCACCTTTATAAGTGATATGAAAACCTAAGAATGAACCTTAGCGGGGATGATAAAAATCTTAAAGGATATGGATACAAGATATCAGAA 6480

FIG. 2—Continued.

Phe Asn Glu Ile Val Arg Glu Ile Lys Asn Val Ile Asn Lys His Asn Leu Tyr Leu Asp Phe Lys Gln Phe Pro Thr Phe Thr Val Val Glu Gly Gln Gln Val Leu His Val Val Arg
 TTTAATGAAAAGTAGTAGAGAAATAAAAAAGCTTATTAATAGCACAATTATACCTTGATTTTAAAGCAATTCGGACTTTTACAGTTGGAGGGTCAACAAGTTCTACATGTTGTAGA 6600

Thr Thr Phe Tyr Ser Thr Asn Thr Gly Tyr Lys Asp Ser Phe Asp Thr Pro Ile Leu Thr Glu Asn Leu Lys Trp Tyr Asn Glu Asn Gly Ala Lys Asn Val Asn Thr Val Pro Gln Leu
 ACTACATTTTATAGTACAAACTACTGGTTATAAGAGCTCAATTGATAGCGCAACTACTAGCGAAAATTTAAATAGGTATAATGAAAATGGCGCTAAAAATGTAATACAGTGCCTCAATA 6720

Val Gly Ser Ser Ile Thr Tyr Phe Lys Arg Tyr Ala Leu Val Ala Tyr Leu Asn Ile Glu Ser Glu Val Asp Thr Asp Ala Ala Pro Ile Asn Asn Asn Tyr Lys Asn Glu Asn Ser Asn
 GTAGTTCAATCAATTACTTTTCCAAAAGATACGCTTTAGTAGCTTATCTTAACTAGAAAAGTGAAGTGGATCTGATGCGAGCGCTTAAACAATAATATAAAAAATGAAAATTTCTAAT 6840

Met Pro Ser Lys Gln Val Ile Val Asn Gln Glu Gln Lys Lys Asp Ile Asn Gln Asn Gln Asn Gln Ile Lys Asn Phe Asp Lys Lys Leu Lys Ile Gly Lys Ser Tyr Cys Tyr Glu Ile
 ATGCCTAGCAACAAGTTATGTTAATCAAGAACAACAAAAAGACATAAATCAAAACCAAAATCAATAAAAACTTTGATAAAAAATGAAAATGGAAAATCTTATTGCTATGAAAT 6960

Phe Arg Asn Ala Leu Phe Asn Met Lys Asn Trp Val Asn Asp Gly Thr Glu Lys Lys Asn Ile Asn Ala Leu Ile Arg Ala Leu Cys Val Asp Asn Asp Val Leu Asp Asn Ile Phe
 TTTAGAAATGCATGTTTAAATGAAAAATGGGTAATGATGGTACAGAAAAAAGAATAAATGCCCTAATAAGGGCGTTATGTTGATAATGATGATGTTTAGATAATATTTT 7080

Glu Lys Asp Phe Lys Leu Lys Ser Ile Glu Tyr Trp Gly Lys Asn Phe Lys Arg Leu Phe Gln ***
 GAAAAAGATTTAAGCTTAAAGCATAGAATATTGGGGTAAAAATTTAAAGATTTTCAATAAAACTAATAAATGGATGATCAATAAAGTTGAAAATTTATGCTTGATAAAGA 7200

GTCCATCTATGAAACAAGTGTATTAATAATTTTTTGTGTTTAAAGAAGAAGACAAATTTGATTATATTTTTTATTGTAATTATTAGATTAAATACCAATATTTCCCTTATTAGGG 7320

AAATATTAACGTATTAGTGAACATTTATATCTAAATCAAAGATTATAGAATAATAGTTCACACTATCTATTTTTTAAATTCCTTTCTAAAGTTCTGTTTTGTAATTTACAGT 7440

GTCTAGCGGAGCTTTAAATATTCTCTACACTATCTTTTCATCATTATAATTTCAAATAAATGAGATAGTGTCTTATGAAAACCTTATAAATTAAGAGGCAATTAATAATGAA 7560

Lys Phe Phe Thr Leu Ile Leu Ile Phe Gly Leu Thr Ile Gln Met Phe Ala Thr Asn Asn Lys Lys Asp Ile Ile Asp Lys Glu Leu Gln Val Trp Phe Ile His Glu Tyr Arg Tyr Lys
 AAAATTTTTTACATTAATACTAATTTTTGGGTTAAACAATTCAAATGTTGCTACAAAAATAAAGAAGATATAATTGATAAAGAGCTTCAGGTGGTATTATTCATGAAATATCGATACAA 7680

Tyr Arg Lys Glu Ile Asn Arg Asp Asp Gly Lys Leu Lys Met Phe Tyr Gln Lys Val Ile Lys Tyr Asn Leu Leu His Glu Leu Lys Asn Phe Phe Asn Ser Tyr Ile ***
 GTATAGAAAAGAGATAAATAGAGATGATGAAAACCTAAATGTTTTATCAAAAAGTATTAATATAAACCCTACTACAGGTTAAAGAATTTCTTTAACTCGTACATATAAATTAATA 7800

CATTTTCAATCTTCATAAATTTTTGATATAACTACTATAACTTAAGATAACTTAAGTATTATAGAAAAATCCTAAAAATTTGATGAACAACACTAGCATTATTTCTAAATTTTCG 7920

*** Asn Glu Leu Asn Arg

AATGTGAGTTCTTGACGACTATATCTAGAATTACAAGATTCTATTAGCTATTTCTGCTCTTTATGAATTTACAAGAAGTATTTTCTCATCTATTATTCCTGGATATATTCTACA 8040

Ile Thr Leu Glu Gln Arg Ser Tyr Arg Ser Asn Cys Ser Glu Ile Leu Glu Asn Glu Thr Glu Lys His Ile Lys Cys Ser Thr Ile Lys Glu Asp Ile Ile Gly Pro Tyr Ile Arg Cys

AGAATTTATAACACAACAAAAACATAAAAAATAAACATTTTGGTTTTGCATGAATTTCTCCAAACCTTAAAAAGTAAAGTAAAGATAGATAATTGCAATGTCTATGTTTTAAAAATAAA 8160

Ser Asn Ile Phe Val Val Phe Val Tyr Phe Ile Phe Met Lys Ser Lys Met ORF-9

AAGAAAAAATCAAGAAACAGTTTATTTTATTTAGGGCAATTAATTAACAATTTTCAAGGATTTAAAAACCAATTTTATTACATTGTGGTTATGATTTTAAACAACCTGCAAGATG 8280

AATGCTAATGAAATTTTTAGAA 8303

FIG. 2 —Continued.

failed to reveal the presence of direct repeats which could have served as target sequences for insertion of the DNA segment bounded by IR-A and IR-B.

Hybridization analysis. Recombination between inverted repeats is a mechanism for inversion isomerization. The extent of isomerization can be quantified by cutting the DNA with a restriction enzyme(s) that cuts at least once on both sides of a repeat. In the orientation shown in Fig. 3, cutting cp8.3/Ip21 with *Hind*III would generate fragments 7,266 and 1,037 bp long; however, inversion via recombination between IR-A and IR-B would generate *Hind*III fragments 4,876 and 3,427 bp long, each having a single copy of the IR element. To test for inversion, we cut Ip21 total plasmid preparations, gel purified cp8.3/Ip21 with *Hind*III, and analyzed them by Southern hybridization using a radioactive oligonucleotide probe designed to detect both *Hind*III DNA fragments because it is complementary to positions 3477 to 3497 within IR-A as well as positions 6228 to 6248 within IR-B (Fig. 4). Figure 6 shows autoradiograms of dried gels containing Ip21 DNA. In lanes containing uncut DNA, most of the hybridization signal was at the position of covalently closed supercoiled cp8.3/Ip21 and relaxed cp8.3/Ip21. A small signal was associated with a band migrating slightly faster than covalently closed cp8.3/Ip21. Subsequent analysis demonstrated that this faster band contains irreversibly denatured forms of the cp8.3/Ip21 plasmid. In *Hind*III-digested samples, only two fragments hybridized with the probe, and they had mobilities consistent with their being

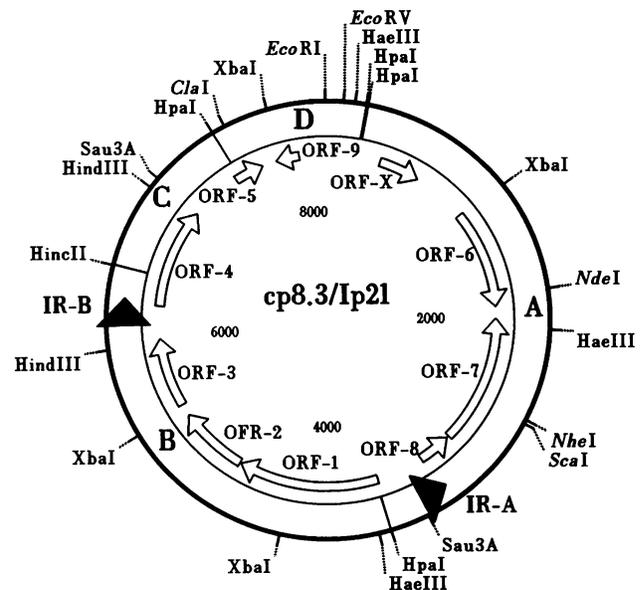


FIG. 3. Circular map of cp8.3/Ip21. The locations and orientations of the inverted repeats and ORFs are indicated (solid arrowheads and open arrows, respectively). Diagnostic restriction sites are marked along with the locations of *Hinc*II fragments A to D; the small 10-bp fragment between C and D has been omitted for clarity. Unique restriction sites are italicized.

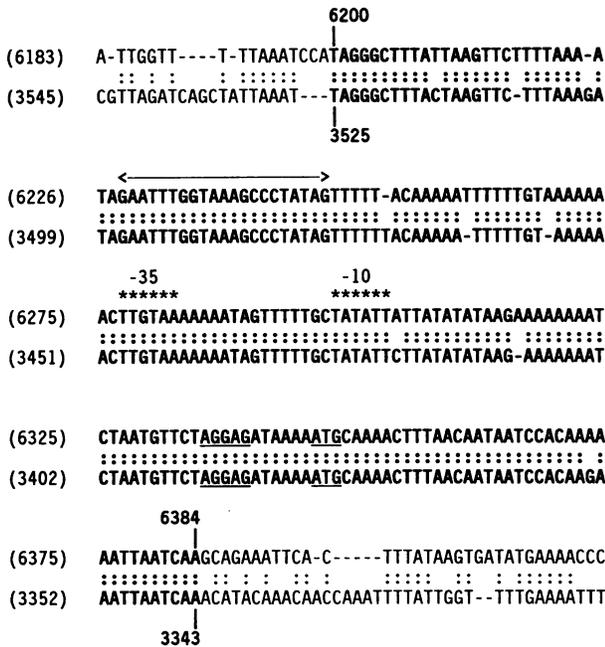


FIG. 4. Nucleotide alignment of IR-A (lower sequence) and IR-B (upper sequence). The regions of cp8.3/Ip21 DNA containing IR-A (nt 3525 to 3343) and IR-B (nt 6200 to 6384) are shown. Both sequences are written 5' to 3'. The repeated sequences are boldfaced. ., identity; dashes, gaps. Disregarding the six single-nucleotide gaps (two in IR-B and four in IR-A), 178 of the remaining 181 nucleotide residues are identical. Ribosome binding sites and appropriately positioned ATG initiation triplets are underlined. Potential -35 and -10 promoter regions (asterisks) and the position of oligonucleotide 2443 (GAATTTGGTAAAGCCCTATAG), used for probing within the inverted repeats (double-headed arrow), are indicated.

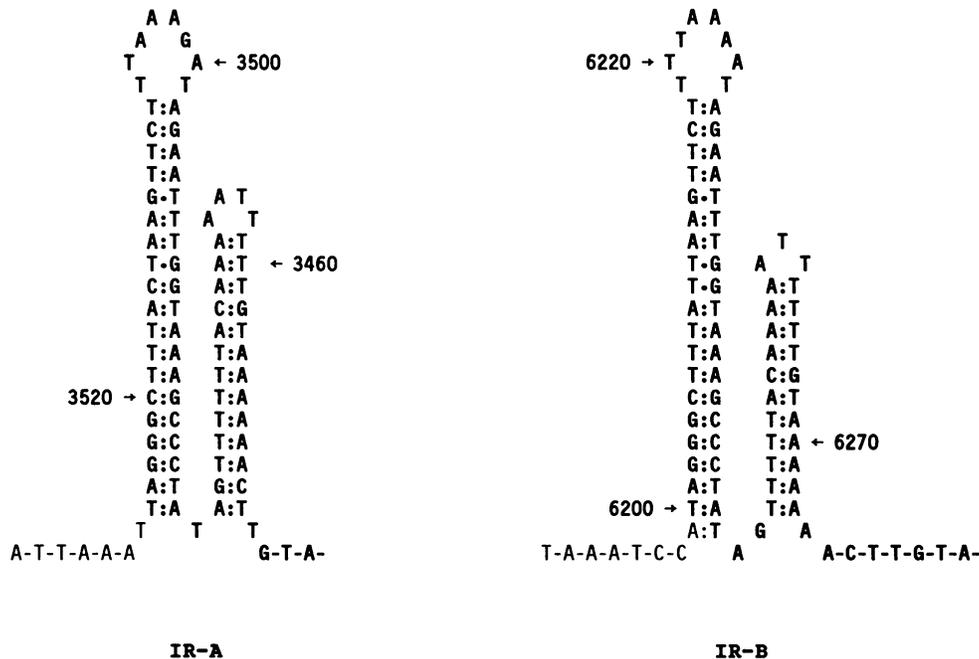


FIG. 5. Potential stem-and-loop structures at the ends of IR-A and IR-B. Both sequences are written 5' to 3', and the repeated sequences are boldfaced.

7.3 and 1 kb, respectively. Thus, there was no indication of isomerization of cp8.3/Ip21. Because no additional bands were observed when total genomic DNA was analyzed by hybridization with this probe, we concluded that there are no additional copies of this particular IR element in any of Ip21's other plasmids or in its linear chromosome. We also surveyed the other strains in Table 1 for the presence of this particular repetitive DNA element. Southern blots of total genomic DNA that had been cut with *Hind*III in liquified agarose plugs gave no indication of the cp8.3/Ip21 repetitive element in any of the strains tested.

Polypeptides predicted by the most probable ORFs. Analysis of the sequence in all possible phases revealed 10 ORFs of more than 50 codons that seem likely to be expressed. All use ATG as the potential initiation triplet; none begin with GTG. Of these ORFs, nine appear to have a characteristic ribosome binding sequence capable of uninterrupted pairing with the 3' end of 16S rRNA positioned a favorable distance in front of the putative translational start site (Table 2). ORF-7 may encode two overlapping polypeptides, 7a and 7b, which would begin at initiation sites 60 nucleotides apart and end at the same termination site. The 10th ORF (ORF-X) does not have an ideal ribosome binding sequence upstream of the initiation codon, and if the polypeptide is expressed, the mRNA may interact atypically with the ribosomes. Table 2 summarizes the major characteristics of these ORFs, and the deduced polypeptide sequences are shown in Fig. 2 with the DNA sequence. The putative polypeptides all have basic pI's, and together their coding sequences occupy 70.7% of the nucleotide sequence. The total percent G+C content is slightly higher in the potential coding regions than in the noncoding regions, 24.19 versus 21.41%.

Computer searches were performed to look for similarity between these ORFs and any known proteins in the GenPept, EMBL, and Swiss-Prot data bases. In addition, these sequences were examined by using a computer program devel-

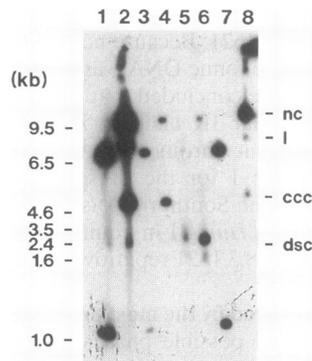


FIG. 6. In situ Southern blot of agarose gel-separated total and purified plasmid DNA from *B. burgdorferi* Ip21 DNA hybridized with oligonucleotide 2443 as the probe. Lanes: 1 and 2, purified total linear and circular plasmid preparations digested with *Hind*III or undigested, respectively; 3 and 4, gel-purified cp8.3/Ip21 digested with *Hind*III or undigested, respectively; 5 and 6, gel-purified denatured cp8.3/Ip21 digested with *Hind*III or undigested, respectively; 7 and 8, total genomic Ip21 DNA prepared in agarose plugs digested with *Hind*III or undigested, respectively. The sizes are indicated on the left; the forms of the DNA are indicated on the right. ccc, covalently closed supercoiled circle; nc, nicked circle; dsc, denatured supercoiled circle; l, linear plasmid.

oped by S. Zhang for known structural motifs in the Prosite data base (3). These comparisons revealed that the 62-amino-acid polypeptide predicted by ORF-9 begins with an N-terminal sequence that has several features in common with the signal sequences of bacterial lipoproteins. The initiating methionine is followed closely by two positively charged residues, a hydrophobic core, and a sequence (I-N-S-C) that resembles the canonical lipidation and proteolytic processing site for signal peptidase II (L-X-Y-C), where X and Y generally are amino acids with small, nonpolar side chains (56). Further work will show whether asparagine in the sequence (I-N-S-C) hinders lipidation or proteolytic processing of the nearby cysteine during translation of ORF-9. Cleavage at this cysteine residue by signal peptidase II would produce a polypeptide 45 amino acids long that has no sequence homology with the known *Borrelia* lipoproteins, OspA, OspB, OspC, and OspD, or the variable major proteins of *B. hermsii*.

Recently, Giladi et al. (24) screened a recombinant *Borrelia* library for sequences that could facilitate export of an alkaline

phosphatase fusion protein to the periplasmic space of *E. coli*. They identified a new gene, pBb244, that is present on a small circular plasmid, cp9, found in a low-passage, virulent *B. burgdorferi* B31 strain. DNA sequence analysis of pBb244 indicates that the gene product should be present in or between the *Borrelia* cell's inner and outer membranes, since the encoded protein begins with an amino acid sequence that has features characteristic of bacterial leader peptidase I signal sequences. None of cp8.3/Ip21's ORFs shows homology with pBb244, and except for ORF-2, none of the predicted polypeptides showed significant homology with known proteins. ORF-2 encodes a 184-amino-acid polypeptide with limited homology to the replication protein, RepC, of pCB101, a plasmid found in the gram-positive bacterium *Clostridium butyricum* (accession number X62684 in GenBank). This homology (Fig. 7) is most pronounced at the putative active site of RepC, which itself shows significant conservation of amino acid sequence with the active site present in Rep proteins of the pC194 group of plasmids (50), plasmids known to replicate by a rolling circle (RC) mechanism. Therefore, we searched the cp8.3/Ip21 sequence for the nick site sequence CTTGATA, which is found at the double-stranded origin of most members of the pC194 group of plasmids. Exact matches to this heptameric motif were found at three positions (Fig. 2): twice on one strand of the DNA (nt 1104 to 1110 and 7190 to 7196) and once on the other strand (nt 3159 to 3165). This result suggests that double-strand synthesis may be initiated on both strands of cp8.3/Ip21.

We attempted to detect single-stranded cp8.3/Ip21 plasmid DNA in our plasmid preparations by the method of te Riele et al. (53), in which DNA is electrophoresed on agarose gels, transferred to nitrocellulose without prior denaturation, and then probed with a radiolabeled oligonucleotide. In the absence of denaturation, only single-stranded nucleic acids bind to the filter. Two different complementary 24-nt oligonucleotides (1801 and 1802 [Fig. 1C]) were used in an attempt to determine the polarity of any positive signal. Equal amounts of hybridization signal were associated with a single band migrating faster than covalently closed cp8.3/Ip21 with each oligonucleotide probe (data not shown). Because the signal was in the same region of the gel to which irreversibly denatured forms of the cp8.3/Ip21 plasmid would migrate, we decided to look for a similar signal in total DNA preparations obtained by lysing cells embedded in agarose using conditions which should not denature supercoiled plasmids. The results (Fig. 6, lanes 7 and 8) indicate that such whole-cell DNA preparations do not

TABLE 2. Most-probable ORFs in cp8.3/Ip21

ORF	Potential ribosome binding site ^a	Product (amino acids)	Stop codon	Predicted mol of product	Predicted pI of product	Location (nt)	% G+C content
1	<u>TTT</u> AAGGAGCTATAAA <u>ATG</u>	371	TAA	44,769	10.67	3756→4871	21.56
2	ATATA <u>AAGG</u> ATTAAC <u>ATG</u>	184	TAA	22,213	10.65	4881→5435	21.92
3	<u>TAA</u> AAGGAGATTTT <u>ATG</u>	186	TAA	22,350	10.32	5531→6091	20.97
4	<u>TTCT</u> AAGGAGATAAAA <u>ATG</u>	266	TAA	31,245	9.96	6346→7146	26.07
5	<u>AGAGG</u> CAATATTAAT <u>ATG</u>	78	TAA	9,780	10.53	7556→7792	23.93
6	AAGT <u>GGAG</u> TTAATTA <u>ATG</u>	261	TAA	30,558	10.77	1193→1978	24.90
7a	GGCTAAAAGATT <u>ATG</u>	343	TAG	39,977	9.50	2069←3100	26.82
7b	TGAAGAGAATTTATA <u>ATG</u>	323	TAG	37,543	9.51	2069←3040	26.93
8	<u>TTCT</u> AAGGAGATAAAA <u>ATG</u>	88	TAG	10,349	10.02	3115←3381	28.41
9	<u>GT</u> TTGGAGAAAATTC <u>ATG</u>	62	TAA	7,425	9.43	7903←8091	24.19
X	TAAATTC <u>TTTT</u> TAA <u>ATG</u>	108	TAA	12,466	10.63	431→757	24.69

^a Nucleotides that would be expected to pair with the end of *B. burgdorferi*'s 16S rRNA sequence (3' OH-UUCCUCCACUA · · · 5') (49) and the initiation codon are underlined.

φX174	Ala Lys Tyr Val Asn Lys Lys Ser Asp
pTD1	Ala Lys Tyr Ser Val Lys Ala Thr Asp
pCB101	Phe Lys Tyr Met Thr Lys Val Thr Gly
cp8.3/Ip21	Lys Lys Tyr Ser Asp Lys Gly Leu Ile

FIG. 7. Alignment of ORF-2 with the amino acid sequences at the active site of Rep proteins of the pC194 group of plasmids. The consensus sequence at the active site is GluXXLysTyrXValLysXXAsp (50).

contain irreversibly denatured forms of cp8.3/Ip21 DNA; the majority of the cp8.3/Ip21 appears to have been converted to a band with the mobility of a relaxed circle. Furthermore, no signal was apparent from the region of the gel below the position of the supercoiled monomeric plasmid, a result which indicates that, if single-stranded plasmid DNA is present normally, it is below the level needed to give an easily detectable hybridization signal.

Other features of the nucleotide sequence. In addition to the putative promoters found in IR-A and IR-B, close matches to appropriately separated -35 and -10 consensus sequences were found at three other locations (Fig. 2). Positioned between ORF-6 and ORF-7 is a potential transcription terminator-like sequence with a GC-rich region of dyad symmetry capable of forming a 9-bp stem-loop structure which is preceded by and then followed by a string of T-rich residues. Interestingly, ORF-6 is encoded by the DNA strand opposite to that which encodes ORF-7. Termination at this site may be necessary to balance the expression of ORF-6 and ORF-7. Other regions of the sequence having the potential to form stem-loop structures are found mainly between bp 7710 and 250. Such structures are characteristic of minus origins of replication in plasmids which replicate by an RC-type mechanism.

Conclusion and discussion. We isolated and characterized at the nucleotide level a small cryptic plasmid, cp8.3/Ip21, found in *B. burgdorferi* Ip21, which shares several features with plasmid pBB1, studied by Schwan and coworkers (47, 51). In addition to having similar sizes, both plasmids contain a single *EcoRI* site and appear to be unclonable in λ vectors. We do not know if there is extensive homology between these plasmids; however, because there is no *AccI* site in cp8.3/Ip21 while pBB1 contains at least two, these plasmids should be able to be readily differentiated in isolates. Both cp8.3/Ip21 and pBB1 are also similar in size to a circular plasmid, pCT1, isolated from *I. scapularis* (*dammini*) collected in northern Wisconsin (29). Restriction enzyme analysis indicates that pCT1 is a dimer composed of two tandem copies of a 4.6-kb DNA. Although no sequence information is available, the pCT1 restriction map is markedly different from those of cp8.3/Ip21 and pBB1. Further work will be needed to determine if these plasmids and the 9.0-kb plasmid cp9, recently reported for virulent *B. burgdorferi* B31 (24), are all part of a related family of plasmids sharing regions of sequence homology and function. Our analysis did not reveal significant homology between the pBb244 gene on cp9 and any ORF of cp8.3/Ip21. This result may portend significant differences in coding sequences carried by these plasmids in terms of geographic locations of the isolates and the tick species in which they were found. In this regard it should be noted that strains Ip21 and B31 belong to separate genospecies (8) and that strain Ip21 should be considered to be a member of the *B. burgdorferi* sensu lato complex of spirochetes.

Currently, there is no known mechanism for genetic transfer in borrelia, and plasmid shuttle vectors that could be used for gene transfer have not been developed. Presumably, integrative or replicating plasmids able to transform borrelia could be constructed by using as the DNA backbone all or part of one of borrelia's linear plasmids or cryptic circular plasmids. Such vectors would be extremely useful and allow the use of powerful molecular approaches to evaluate the contributions of individual genes to the physiology and pathogenicity of this organism. Although many plasmids from gram-negative and gram-positive bacteria have been sequenced and their mechanisms of replication have been elucidated, there has been only one reported complete sequence analysis of a plasmid from a spirochete. MacDougall et al. (35) determined the complete nucleotide sequence of a 2,647-bp cryptic plasmid, pTD1, from the oral spirochete *Treponema denticola* (30). They showed that pTD1 has several features in common with plasmids typically found in gram-positive cells which usually replicate by an RC mechanism via single-stranded intermediates rather than the theta-form mechanism most gram-negative plasmids use to replicate (16, 25, 50). However, whether pTD1 can be used to develop a vector for gene transfer to borrelia is problematic. Even if pTD1 encodes all the proteins needed to initiate its own replication, these proteins might not be expressed in borrelia, because the ribosome binding sites upstream of pTD1's two ORFs probably would not function in borrelia. The sequence at the 3' end of the 16S rRNA of *T. denticola* (3' OH-UUUGCAUGGA ··· 5' [42]) is quite different from the sequence at the end of *B. burgdorferi* 16S rRNA (3' OH-UUUCUCCAC ··· 5' [49]).

Ip21's cp8.3/Ip21 plasmid appears to be lost rather readily on routine passage in vitro, and before this plasmid can be used as a genetic tool, several obstacles will have to be overcome. If cp8.3/Ip21 does belong to the large family of double-stranded circular DNAs that replicate via a single-stranded DNA intermediate, it probably uses an RC pathway that first generates a circular plus-strand DNA which is then converted to double-stranded DNA by complementary-strand synthesis initiated at an origin located elsewhere on the single-stranded DNA circle. This RC mode of replication is known to lead to high frequencies of both homologous recombination and illegitimate recombination (39, 40). Furthermore, if two plus-strand origins are present on the same replicon, initiation can occur at one origin and termination can take place at the other, generating two smaller molecules that may or may not contain active signals for minus-strand synthesis or further rounds of plus-strand synthesis. This feature of RC plasmid replication may be responsible for loss of cp8.3/Ip21 in vitro in the absence of selective pressure. If regions leading to instability can be identified, perhaps they can be modified, thereby stabilizing the inheritance of the plasmid. Alternatively, an antibiotic resistance gene or other selectable marker can be introduced into the DNA to allow positive selection for cells that maintain the plasmid in culture. This may be feasible since the plasmid contains several unique restriction sites. A stabilization cassette, such as the *parB* locus of plasmid R1, also may have to be added to stabilize the plasmid (23). Eventually, it should be possible to use this plasmid as a vector for gene disruption or, alternatively, if it can be shown that the IR elements of cp8.3/Ip21 promote insertion or recombination of plasmid DNA with other molecules, as a vehicle to begin genetic mapping in borrelia. In ongoing experiments we are examining the mechanism by which cp8.3/Ip21 is lost on in vitro passage and attempting to devise techniques to select integrants in the resulting population of spirochetes.

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