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Molecular Characterization, Genomic Arrangement, and Expression of *bmpD*, a New Member of the *bmp* Class of Genes Encoding Membrane Proteins of *Borrelia burgdorferi*

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An expression library made with *Borrelia burgdorferi* DNA in the vector λZapII was screened with serum from a monkey infected with the Lyme disease agent. This serum killed *B. burgdorferi* in vitro by an antibody-dependent, complement-mediated mechanism and contained antibodies to at least seven spirochetal antigens, none of which were the major outer surface proteins OspA or OspB. Among several positive clones, a clone containing the *B. burgdorferi bmpA* gene encoding the immunodominant antigen P39 was obtained. Chromosome walking and DNA sequence analysis permitted the identification of two additional upstream genes homologous to the *bmpA* gene and its related companion, *bmpB*. The first of these was the recently characterized *bmpC* gene, and adjacent to it was the fourth and new member of this class, which has been designated *bmpD*. The gene product encoded by *bmpD* is 341 residues long, contains a signal sequence with a potential signal peptidase II cleavage site, and has 26% identity with TmpC of *Treponema pallidum*. Southern blotting confirmed the tandem arrangement of all four *bmp* genes in the chromosome of *B. burgdorferi* JD1. However, Northern (RNA) blotting revealed that *bmpD* is expressed as a monocistronic transcript, which indicates that it is not part of an operon at the *bmp* locus. The *bmpD* gene was found to be conserved in representative members of the three species of the *B. burgdorferi sensu lato* complex, suggesting that it serves an important biological function in the spirochete.

We have demonstrated previously that rhesus monkeys infected with *Borrelia burgdorferi*, the etiologic agent of Lyme disease, show signs that occur during both the acute and chronic phases of the disease in humans. These signs include erythema migrans, arthritis, and neuroborreliosis (14, 18). At the serological level, too, the time course and specificity spectrum of the antibody response to *B. burgdorferi* in rhesus monkeys were shown to be very similar to their counterparts in humans (14).

In monkeys, as well as in other experimental animals (15), an infection with *B. burgdorferi* via the natural route, i.e., through the bite of *Ixodes scapularis* nymphs, elicits no antibody response to the outer surface proteins OspA and OspB, as detected by Western blotting (immunoblotting) (14). Using serum obtained from tick-inoculated monkeys 3 weeks post-infection (p.i.), we demonstrated the existence of non-OspA or -B surface proteins that are targeted by the antibody-dependent complement-mediated killing mechanism in vitro (3). In some animals, these early serum samples recognized no more than seven bands on a Western blot of whole *B. burgdorferi* antigens. To identify and clone these potentially important antigens, the week-3 serum was used to screen expression libraries that were made from *B. burgdorferi* DNA.

Among several positive clones, we identified a clone expressing the immunodominant antigen P39, which has been previously described (21, 22). By chromosome walking upstream, we were able to identify two more genes that were homologous both to *bmpA* (*Borrelia* membrane protein A, P39) and to its related companion *bmpB*, which also encodes a potential membrane lipoprotein. The first of these two homologous genes was the recently characterized *bmpC* (2). Located further upstream was the fourth and new member of this class, which we have designated *bmpD*. In this study, we characterize *bmpD* and analyze its relationship to the other members of this class of chromosomally located genes encoding potential membrane lipoproteins.

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MATERIALS AND METHODS

Bacterial strains. *B. burgdorferi* JD1 (16) was cultured in BSK-H medium as previously described (17). The *Escherichia coli* strain used was XL1 Blue.

Serum samples. All serum samples were obtained from normal, uninfected rhesus monkeys or from rhesus monkeys infected with the JD1 strain of *B. burgdorferi* by tick (*Ixodes scapularis*) inoculation.

Construction and screening of *B. burgdorferi* expression libraries. Total DNA (genomic plus plasmid DNA) was prepared from passage-5 *B. burgdorferi* JD1 with sodium dodecyl sulfate (SDS) and proteinase K for lysis, after which two extractions with phenol and one with phenol-chloroform were performed. Following the digestion of RNA with 10 μg of RNase A per ml for 15 min at 37°C, DNA was precipitated with sodium acetate and ethanol (11).

An *Eco*RI-*Hin*fI library was prepared as follows. About 15 μg of total DNA was cut overnight with 50 U of *Hin*fI, and the ends were filled with Klenow and deoxynucleoside triphosphates (dNTPs) and then ligated with three different *Eco*RI linkers, one for each open reading frame (ORF). DNA fragments were digested with *Eco*RI, separated from the linkers with a Sepharose CL-6B column (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) and inserted into the *Eco*RI site of the vector λZapII DNA (Stratagene, La Jolla, Calif.). Packaging, plating, and amplification of the library was done by following the manufacturer's instructions. A second library with randomly sheared total DNA was constructed as follows. About 50 μg of total DNA (0.1 mg/ml in water) was randomly sheared to form 600- to 1,200-bp fragments by sonication with a Branson Sonifier 450. The ends were blunted with Klenow and dNTPs, and the blunt DNA fragments were methylated with *Eco*RI methylase. The ligation of the *Eco*RI linkers and the rest of the protocol were as described above.

About 9×10^9 plaques from the *Eco*RI-*Hin*fI library were screened with a 1:150 dilution of serum obtained from a tick-inoculated rhesus monkey, 3 weeks p.i. The nitrocellulose filters were incubated with biotinylated anti-human immunoglobulin G and immunoglobulin M and then with a complex of avidin-biotinylated horseradish peroxidase (Vector Laboratories, Burlingame, Calif.) and developed with 4-chloro-1-naphthol (Sigma Chemical Company, St. Louis, Mo.) with chromogen and hydrogen peroxide as the substrate (14). Several

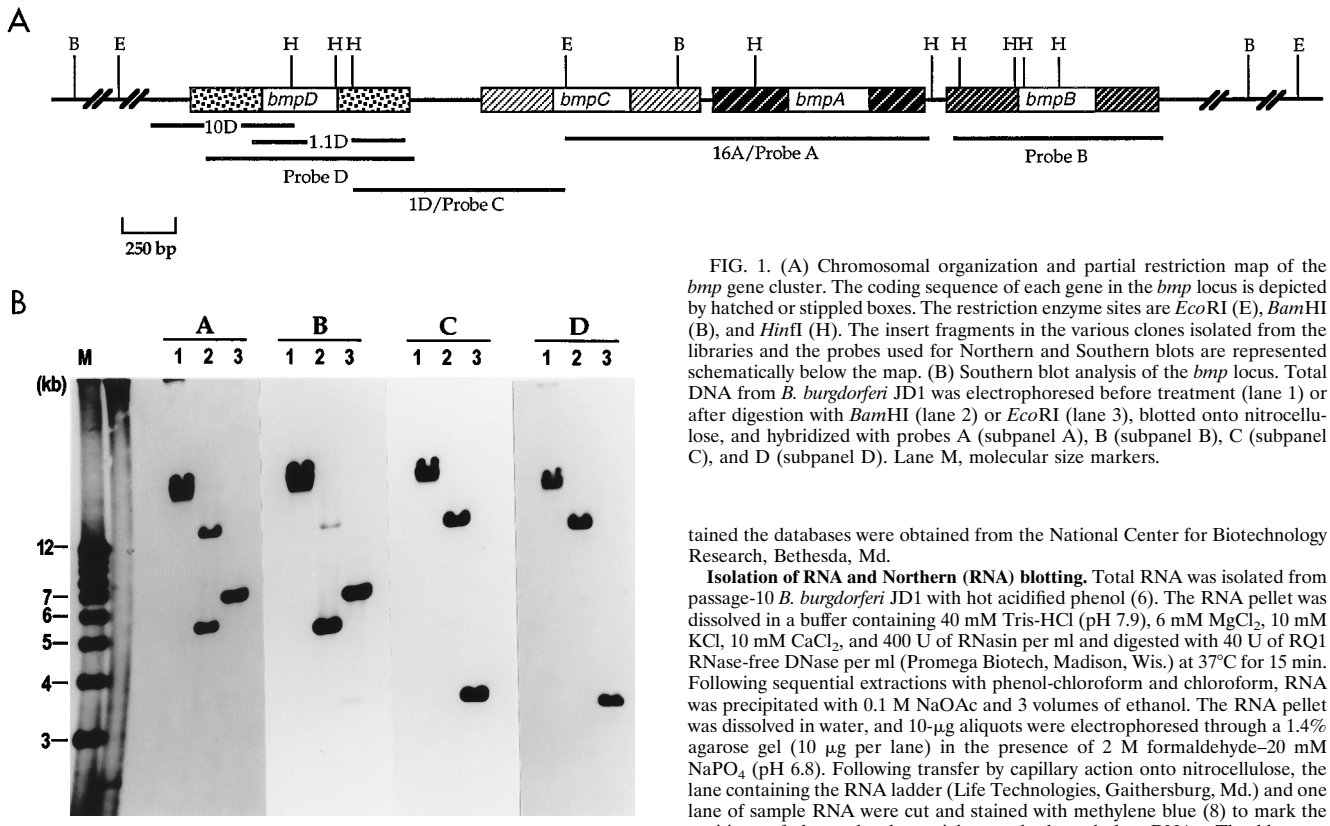

FIG. 1. (A) Chromosomal organization and partial restriction map of the *bmp* gene cluster. The coding sequence of each gene in the *bmp* locus is depicted by hatched or stippled boxes. The restriction enzyme sites are *Eco*RI (E), *Bam*HI (B), and *Hin*fI (H). The insert fragments in the various clones isolated from the libraries and the probes used for Northern and Southern blots are represented schematically below the map. (B) Southern blot analysis of the *bmp* locus. Total DNA from *B. burgdorferi* JD1 was electrophoresed before treatment (lane 1) or after digestion with *Bam*HI (lane 2) or *Eco*RI (lane 3), blotted onto nitrocellulose, and hybridized with probes A (subpanel A), B (subpanel B), C (subpanel C), and D (subpanel D). Lane M, molecular size markers.

positive clones were singled out and plaque purified. The corresponding inserts were rescued in the pBluescript plasmid with helper phage as per the manufacturer's instruction (Stratagene).

The libraries were also screened with peroxidase-labelled DNA probes with the ECL chemiluminescence kit (Amersham, Inc., Arlington Heights, Ill.). The nylon filters were hybridized, washed, and developed, all according to the manufacturer's instructions.

Identification of the recombinant clones. *E. coli* XL1 Blue containing the rescued plasmids were grown overnight in Luria broth supplemented with 50 μg of ampicillin per ml. Two colonies for each recombinant were tested. The overnight cultures were diluted 1:20 in fresh medium and allowed to grow up to mid-log phase, at which point the expression of fusion proteins was induced by adding 0.3 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and growth was continued for 2 h. Cells were harvested, lysed with SDS-polyacrylamide gel electrophoresis lysis buffer, and aliquots were electrophoresed through a 12.5% polyacrylamide gel (10). Proteins were transferred to nitrocellulose, and the blots were developed with week-3-p.i. serum as described above. Antibodies reacting with fusion proteins were affinity purified with nitrocellulose-bound fusion protein excised from adjacent undeveloped lanes (23). The *B. burgdorferi* JD1 antigens recognized by these affinity-purified antibodies were identified by Western blotting of extracts of whole *B. burgdorferi* organisms (14).

Circularization of *Hin*fI fragments and inverse PCR. To generate circular *Hin*fI templates for inverse PCR, total DNA from *B. burgdorferi* JD1 was digested with *Hin*fI restriction enzyme and precipitated with ethanol and amonium acetate following extraction with phenol and chloroform. The *Hin*fI fragments were circularized by ligation at a concentration of 10 μg of DNA per ml with T4 DNA ligase for 3 days at 16°C. Inverse PCRs were carried out with 100 ng of the circularized template DNA under the following conditions: 94°C for 1 min, 60°C for 30 s, and 72°C for 3 min for the first 5 cycles, after which annealing occurred at 55°C for the next 5 cycles, and for the last 25 cycles the annealing temperature was dropped to 50°C.

DNA sequence analysis. Plasmid DNAs were sequenced by the dideoxynucleotide chain termination method (20) with modified T7 DNA polymerase (Sequenase kit; United States Biochemical Corp., Cleveland, Ohio). The oligonucleotide primers for sequencing were synthesized at the Louisiana State University Medical Center Core Laboratories, New Orleans, La. DNA and protein sequences were analyzed with the MacVector software developed by International Biotechnology, Inc., New Haven, Conn. The BmpD amino acid sequence was compared with protein sequences in the GenBank databases by the BLAST algorithm with the PAM-250 scoring matrix (1). The compact discs that contained the databases were obtained from the National Center for Biotechnology Research, Bethesda, Md.

Isolation of RNA and Northern (RNA) blotting. Total RNA was isolated from passage-10 *B. burgdorferi* JD1 with hot acidified phenol (6). The RNA pellet was dissolved in a buffer containing 40 mM Tris-HCl (pH 7.9), 6 mM $MgCl_2$, 10 mM KCl, 10 mM $CaCl_2$, and 400 U of RNasin per ml and digested with 40 U of RQ1 RNase-free DNase per ml (Promega Biotech, Madison, Wis.) at 37°C for 15 min. Following sequential extractions with phenol-chloroform and chloroform, RNA was precipitated with 0.1 M NaOAc and 3 volumes of ethanol. The RNA pellet was dissolved in water, and 10-μg aliquots were electrophoresed through a 1.4% agarose gel (10 μg per lane) in the presence of 2 M formaldehyde–20 mM $NaPO_4$ (pH 6.8). Following transfer by capillary action onto nitrocellulose, the lane containing the RNA ladder (Life Technologies, Gaithersburg, Md.) and one lane of sample RNA were cut and stained with methylene blue (8) to mark the positions of the molecular weight standards and the rRNAs. The blot was hybridized with a strand-specific probe (probe D) (Fig. 1) generated by labelling the T11-B9 PCR fragment (see Fig. 2 for the locations of the T11 and B9 primers) with the bottom primer (B9), Klenow and [α-^{32}P]dATP. The blot was washed first with 0.3× SSPE (1× SSPE is 0.18 M NaCl, 10 M NaH_2PO_4, and 1 mM EDTA [pH 7.7])–0.1% SDS for 15 min at room temperature. Two additional 30-min stringent washes at 65°C were performed.

Southern blotting of total DNA. Total DNA (3 μg) was digested overnight with the appropriate restriction enzymes and electrophoresed through a 1% Tris-acetate-EDTA-agarose gel. DNA was transferred overnight onto nitrocellulose and hybridized with peroxidase-labelled DNA probes for signal detection by chemiluminescence with the ECL kit (Amersham). The probes used (depicted in Fig. 1) were as follows: probe A, the 1.7-kb insert from clone 16A; probe C, the 0.98-kb insert from clone 1D; probe D (see above); and probe B. Probe B contains the *bmpB* coding sequence between nucleotides 36 and 1112. It was generated by digesting a T2-B4 PCR product with *Hin*cII, which cuts at the 5′ end of the *bmpB* sequence. Oligonucleotides T2 (5′-TAAACAATAGGTTG GTTG-3′) and B4 (5′-TGTACTTCTATTTATTATAAC-3′) are located at the 3′ ends of the *bmpC* coding sequence and the *bmpB* flanking sequence, respectively (21).

Nucleotide sequence accession numbers. The *bmpD* DNA sequence data reported here has been deposited in the GenBank database under accession number U35450.

RESULTS

Cloning of *bmpD*. Approximately 9×10^9 plaques from the *Eco*RI-*Hin*fI expression library were screened with a 1:150 dilution of a serum obtained 3 weeks p.i. from a tick-inoculated monkey (3). Eighteen positive clones were obtained after three rounds of screening with the serum. The borrelia DNA inserts in these recombinant clones were rescued in pBluescript plasmid with *E. coli* XL1 Blue and ExAssist helper phage.

The proteins encoded by these positive clones were identified with the serum described above on Western blots of lysates that were prepared from *E. coli* XL1 Blue carrying the recombinant plasmids. For some clones, no recombinant protein was discernible in the Western blots, while for others the duplicate colonies expressed fusion proteins of different sizes, suggesting that certain *B. burgdorferi* sequences are unstable in the high-

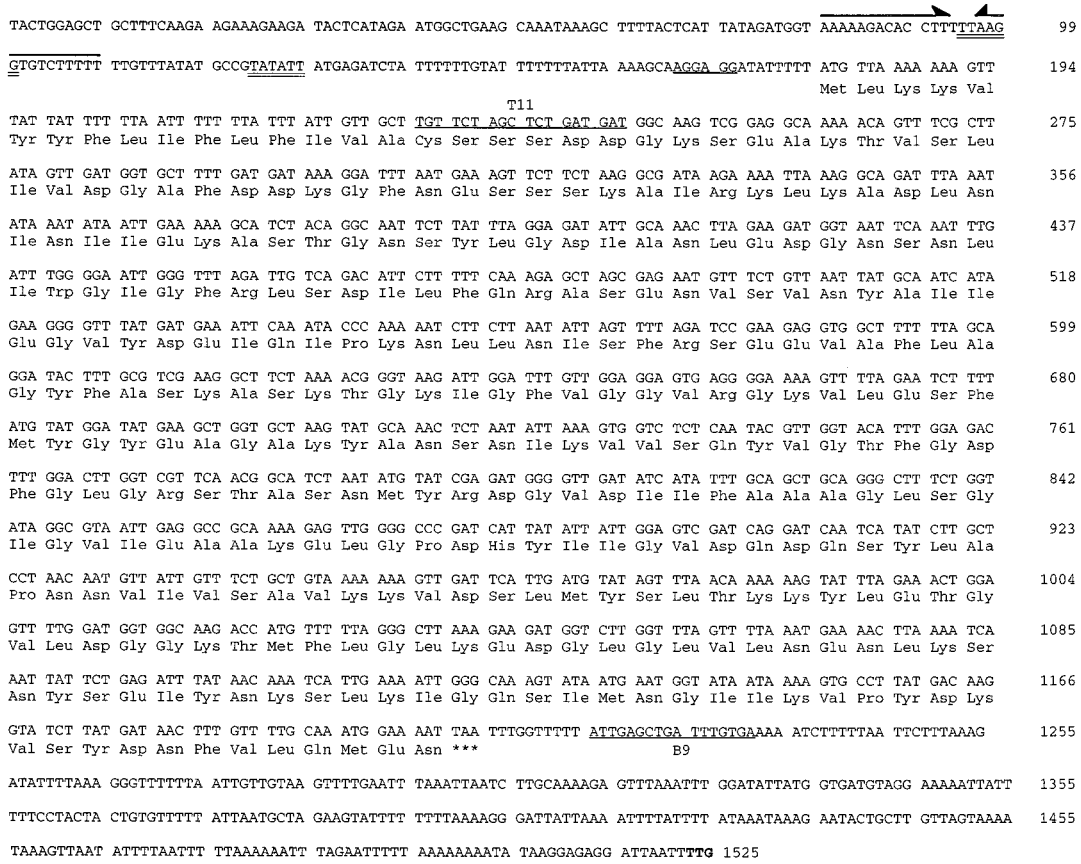

FIG. 2. Nucleotide and deduced amino acid sequences of *bmpD* and flanking regions. The putative promoter sequence (double underlined), the Shine-Dalgarno sequence (underlined), and the stop codon (asterisks) are marked. An inverted repeat is indicated by arrows, and the locations of primers T11 and B9 are marked. The start codon of the downstream *bmpC* gene is shown in boldface type.

copy-number pBluescript plasmid. To identify the *B. burgdorferi* antigens encoded by the recombinants, fusion-protein-reactive antibodies were affinity purified from the fusion proteins and bound to Western blots of whole *B. burgdorferi* antigens. Antibody eluted from the fusion protein of one clone, designated 16A, recognized a 39-kDa antigen. This antigen was studied further. Sequencing the insert fragment in 16A plasmid DNA confirmed the presence of a complete *bmpA* gene encoding the immunodominant antigen P39 at the 3′ end of the insert in this plasmid. The unique *Hin*fI site within the *bmpA* gene in this clone was missed by the *Hin*fI restriction enzyme during the construction of the library.

The sequence also included a partial ORF at the 5′ end of the insert. The remainder of this ORF was retrieved from the *Eco*RI-*Hin*fI library. We had prior knowledge, obtained from Southern blotting of total *B. burgdorferi* DNA digested with *Hin*fI and *Eco*RI-*Hin*fI, that there was a *Hin*fI site 1 kb upstream of the *Eco*RI site at the 5′ end of the insert fragment in clone 16A. Hence, the next upstream fragment in the library was expected to be a 1-kb *Hin*fI-*Eco*RI fragment. With the same information, the probe for screening the library was generated by an inverse PCR using circularized genomic *Hin*fI fragments as templates. The resulting 1.4-kb amplicon was digested with *Eco*RI and *Hin*fI to release the 1-kb *Hin*fI-*Eco*RI probe fragment. The library screening yielded several positive clones, and one of these (clone 1D) was rescued for further characterization. Shortly thereafter, Aron et al. reported the cloning and sequence of the same gene from *B. burgdorferi* 297, which they named *bmpC* (2). The sequence of the insert in clone 1D revealed, in addition to the expected 5′ half of *bmpC*, one other partial ORF of 82 residues with homology to the carboxyl terminus sequences of BmpA, BmpB, and BmpC. Therefore, it was of interest to complete the cloning of this ORF to determine if it showed extended homology to the previously characterized Bmps.

The rest of the ORF sequence was rescued from the randomly sheared DNA library in two overlapping clones. The first clone (1.1D) was obtained by screening with a 270-bp *Eco*RI-*Alu*I fragment derived from the 5′ end of the insert fragment in clone 1D. With the insert sequence from this clone as a probe to rescreen the library, a second clone (10D) that contained the complete 5′ end of the gene, including 179 bp of the flanking sequence, was isolated. On the basis of its extensive homology to the other three Bmps, the gene encoding this ORF has been designated *bmpD.*

Organization of the *bmp* gene cluster. The cloning and sequence analysis indicated that the gene order for three of the four *bmp* genes in *B. burgdorferi* JD1 was *bmpD-bmpC-bmpA*. To verify this result and to further map the location of *bmpB*, total *B. burgdorferi* DNA was digested with *Bam*HI or *Eco*RI and hybridized with four different probes spanning the entire *bmp* locus. There is a single site for each enzyme within the locus, and both sites occur in the *bmpC* sequence. All four probes hybridized to a total of just two *Bam*HI (5.5- and >12-kb) fragments and two *Eco*RI (7- and 4-kb) fragments, indicating that the locus is entirely contained within these

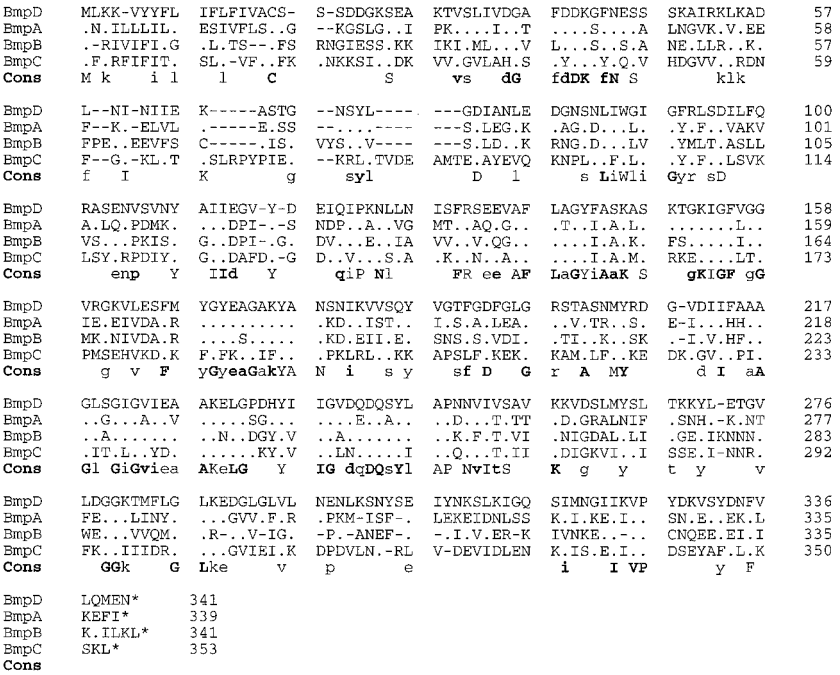

FIG. 3. Comparison of the deduced amino acid sequences of the Bmps. The alignment of the deduced amino acid sequences of BmpA, BmpB (21), BmpC, and BmpD of *B. burgdorferi* is shown. Gaps, indicated by dashes, are introduced into the sequences for optimal alignment. The consensus sequence (Cons) shows residues that are either identical in all four sequences (capital letters) or in three of the four sequences (lowercase letters), and those residues additionally conserved in the TmpC sequence of *T. pallidum* (25) are highlighted in boldface type.

fragments. More specifically, probes A and B recognized the same 7-kb *Eco*RI and 5.5-kb *Bam*HI fragments and probes C and D both hybridized to the same 4-kb *Eco*RI and >12-kb *Bam*HI fragments (Fig. 1B). Probe A additionally bound to the >12-kb *Bam*HI band because it spans the unique *Bam*HI site (Fig. 1A). The faint band corresponding to the >12-kb *Bam*HI fragment in Fig. 1B is the residual signal from probe A, as the same blot was used for probe B. These Southern blotting results are consistent with the cumulative cloning data for *bmpA*, *bmpC*, and *bmpD* and also demonstrate that *bmpB* is located downstream of *bmpA* (2, 21). A PCR strategy was used to confirm that *bmpB* in strain JD1 was located immediately downstream from *bmpA*, as occurs in strain Sh-2-82. With primers T2 (located at the 3′ end of *bmpC*) and B4 (located at the 3′ end of *bmpB*), *bmpA* and *bmpB* sequences were amplified in a single 2.4-kb fragment. The presence of the *bmpB* sequence in this amplicon was established by matching the profiles obtained by digestion with several restriction enzymes with those predicted from the *bmpB* sequence of *B. burgdorferi* Sh-2-82 (21) (data not shown).

DNA sequence analysis. The complete DNA sequence of *B. burgdorferi bmpD* and its flanking sequences, the 179-nucleotide upstream sequence and the 320-nucleotide downstream sequence (which includes the first codon of the downstream *bmpC* gene), are shown in Fig. 2. No plausible ORF was predicted to occur within the 320-bp intergenic region between *bmpD* and *bmpC*. The ORF corresponding to BmpD is 1,023 nucleotides long, starts with the initiator codon ATG at nucleotide position 180, and terminates at residue 1203 with TAA. A ribosomal binding site AAGGAG complementary to the 16S RNA of *B. burgdorferi* (7) is present 9 bases 5′ to the start codon of BmpD. A putative *E. coli* σ^{70}-type promoter exists upstream, with the −10 and the −35 consensus sequences located at nucleotides 124 to 129 and 95 to 100, respectively. The putative *bmpD* gene product is 341 amino acids long with a calculated molecular weight of 37,250 and an estimated pI of 5.14. The N terminus begins with a methionine and basic charged residues and is followed by a hydrophobic region, features that are typical of signal sequences of membrane proteins (28). Furthermore, the presence of a likely signal peptidase II site, LFIVAC (5), located at residues 12 to 17, suggests that BmpD may be a lipoprotein.

Comparison of the BmpD sequence with those of *B. burgdorferi* BmpA, BmpB, and BmpC and with the *Treponema pallidum* TmpC sequence. A homology search of sequences in the protein databases revealed that BmpD is closely related to the putative membrane proteins BmpA, BmpB, and BmpC of *B. burgdorferi* and to TmpC, a membrane lipoprotein of *T. pallidum* (24, 25). Pairwise comparisons of the coding nucleotide sequence of *bmpD* with those of *bmpA*, *bmpB*, and *bmpC* revealed an identity of 64, 56, and 57%, respectively. This translated to an identity of 46% (with BmpA), 43% (with BmpB), and 36% (with BmpC) at the amino acid level. This degree of relatedness is similar to those reported for BmpC and BmpA (39%), BmpC and BmpB (40%) (2), and BmpA and BmpB (52%) (21). BmpD is also less closely related to another membrane protein, TmpC (26% identity) from the spirochete *T. pallidum* (25). Alignment of all four Bmp sequences (Fig. 3) and that of TmpC showed that in the four Bmp sequences, 74 (22%) amino acid residues were identical, of which 25 residues were also conserved in TmpC. Furthermore, 82 residues were identical in three of the four Bmp sequences. Although these conserved residues are scattered throughout the length of the polypeptide, their distribution is more frequent in the middle half of the sequence.

Northern analysis. When total RNA from passage-10 *B. burgdorferi* JD1 was probed with the *bmpD* coding sequence, a 1.3-kb transcript was detected (Fig. 4). This 1.3-kb transcript is

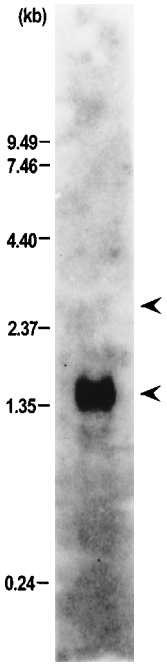

FIG. 4. Northern blot analysis of *bmpD* RNA. Total RNA isolated from passage-10 *B. burgdorferi* JD1 spirochetes was blotted onto nitrocellulose and hybridized with a [^{32}P]-labelled *bmpD* probe. The relative positions of the two rRNA bands are indicated by arrowheads.

bmpD specific, because the same probe did not cross-react with the other *bmp* genes in the Southern blotting experiments. In addition, the use of *bmpA*- and *bmpB*-specific probes in Northern blots resulted in distinctly different profiles (data not shown).

Considering that the coding sequence is only 1,023 nucleotides long, the mRNA seems unusually long. Since the 5′ untranslated sequence is expected to be short (about 40 bases) because the putative promoter lies 50 bp upstream of the initiation codon, it is reasonable to assume that the transcript has an extended 3′ untranslated sequence of about 240 nucleotides.

Presence of *bmpD* sequence in isolates from the three species of the *B. burgdorferi sensu lato* complex. Previous studies have demonstrated the presence of *bmpA* and *bmpC* in representative isolates of the three species of the *B. burgdorferi sensu lato* complex (2, 22). The conservation of *bmpD* in the selected members of these genospecies was evaluated by Southern blotting. Total DNAs from *B. burgdorferi* JD1, *Borrelia afzelii* 107 (19), and *Borrelia garinii* G2 (12) were individually digested with *Bam*HI and *Hin*dIII (both enzymes are known to cut outside the *bmpD* sequence, as derived from strain JD1), blotted onto nitrocellulose, and hybridized with the T11-B9 PCR probe (probe D) (Fig. 1), which comprises most of the coding sequence of *bmpD*. The probe hybridized to a single ~1.7-kb *Hin*dIII fragment in all the three isolates, to a >12-kb *Bam*HI fragment in *B. burgdorferi* JD1 and *B. garinii* G2, but to a >23-kb fragment in *B. afzelii* 107 (Fig. 5). This is a strong indication that all three genospecific groups contain *bmpD*.

DISCUSSION

We constructed a *B. burgdorferi* expression library and screened it with a serum sample obtained from a tick-inoculated monkey 3 weeks p.i. Our aim was to identify and clone genes encoding antigens that are targeted by the antibody-dependent complement-mediated killing mechanism (3). Several positive clones were obtained, and one of these, clone 16A, which expressed a 39-kDa antigen in *E. coli* and which antigenically cross-reacted also with a 39-kDa band in *B. burgdorferi*, was selected for further study. DNA sequencing revealed the presence of the complete *bmpA* gene, which encodes the immunodominant antigen P39 at the 3′ end of the 1.7-kb insert in this clone. In the same clone we recovered a second gene, upstream of *bmpA*, encoding a protein that was homologous to BmpA, BmpB, and TmpC. This gene was concomitantly identified in a different strain, 297, by Aron et al. (2) and designated *bmpC*. Similarly, during our effort to complete the cloning of *bmpC*, we discovered a third contiguous gene with homology to the three previously characterized *bmp* sequences and to that of *tmpC*. Accordingly, we have named this most recent member of the *bmp* class of homologous genes *bmpD*.

The three previously characterized genes, *bmpA*, *bmpB*, and *bmpC*, are all chromosomally encoded. Furthermore, *bmpA* and *bmpB* were shown to be located adjacent to each other in *B. burgdorferi* Sh-2-82 (21) and *bmpC* was localized upstream of *bmpA* in a different strain, *B. burgdorferi* 297 (2). In our study, we have comprehensively mapped the locations of all four *bmp* genes to the same locus within a single strain of *B. burgdorferi*, namely, JD1. Within this locus, the four genes are tandemly arrayed in the following order: *bmpD-bmpC-bmpA-bmpB*. While *bmpD* is expressed independently as a monocistronic transcript, the transcriptions of the others remain unknown. Putative promoters have been recognized upstream of *bmpC* (2) and *bmpA* but not *bmpB* (21). This would suggest that *bmpC*, like *bmpD*, forms a single transcriptional unit, whereas, on the other hand, *bmpA* and *bmpB* may be cotranscribed from a shared promoter located upstream of *bmpA*. In other words, the *bmp* locus as a whole does not appear to constitute an operon, although *bmpA* and *bmpB* likely are organized as one.

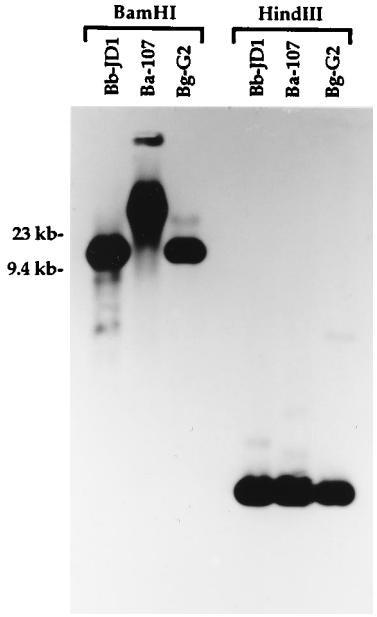

FIG. 5. Southern blot showing the presence of the *bmpD* sequence in representative isolates of the three genospecific *Borrelia* groups: *B. burgdorferi* (Bb), *B. afzelii* (Ba), and *B. garinii* (Bg).

The Bmps are nearly identical in size (with the exception of BmpC), they are closely related to each other in sequence, and they are tandemly arranged in the chromosome. Taken together, these findings suggest that the *bmp* genes evolved from the same parent gene by duplication but have since diverged considerably. However, unlike the plasmid-encoded outer surface lipoproteins OspA, OspB, and OspC, all of which show considerable sequence variations between strains (4, 9, 13, 26, 27, 29), the Bmp sequences appear to be more conserved. We found no changes between the BmpA sequences of *B. burgdorferi* JD1 and Sh-2-82 and a single conservative change (Val to Leu at position 179) (Fig. 3) in the BmpC sequence of *B. burgdorferi* JD1 compared with that of strain 297. The *bmpB* gene of strain JD1 was not sequenced to permit a similar comparison.

The genes encoding the Bmp molecules are conserved in the three distinct genospecies of *B. burgdorferi sensu lato*. The putative membrane localization of the Bmps and the conservation of their genes strongly suggest that these proteins serve an important biological function in the spirochetes.

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