JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1993, p. 2577–2583
0095-1137/93/102577-07$02.00/0

Vol. 31, No. 10

Identification of a Protein in Several *Borrelia* Species Which Is Related to OspC of the Lyme Disease Spirochetes

RICHARD T. MARCONI,* D. SCOTT SAMUELS, TOM G. SCHWAN, AND CLAUDE F. GARON

Laboratory of Vectors and Pathogens, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, Montana 59840

Received 21 April 1993/Returned for modification 8 June 1993/Accepted 29 June 1993

Using oligonucleotide probes which have previously been shown to be specific for the *ospC* gene found in the Lyme disease spirochete species *Borrelia burgdorferi*, *B. garinii*, and group VS461, we detected an *ospC* homolog in other *Borrelia* species including *B. coriaceae*, *B. hermsii*, *B. anserina*, *B. turicatae*, and *B. parkeri*. In contrast to the Lyme disease spirochetes, which carry the *ospC* gene on a 26-kb circular plasmid, we mapped the gene in other *Borrelia* species to linear plasmids which varied in size among the isolates tested. Some isolates carry multiple copies of the gene residing on linear plasmids of different sizes. The analyses conducted here also demonstrate that these *Borrelia* species contain a linear chromosome. Northern (RNA) blot analyses demonstrated that the gene is transcriptionally expressed in all species examined. High levels of transcriptional expression were observed in some *B. hermsii* isolates. Transcriptional start site analyses revealed that the length of the untranslated leader sequence was identical to that observed in the Lyme disease spirochete species. By Western blotting (immunoblotting) with antiserum (polyclonal) raised against the OspC protein of *B. burgdorferi*, we detected an immunoreactive protein of the same molecular weight as the OspC found in Lyme disease spirochete species. The results presented here demonstrate the presence of a protein that is genetically and antigenically related to OspC which is expressed in all species of the genus *Borrelia* tested.

Four outer surface proteins, OspA, OspB (6), OspC (12), and OspD (23), have been described for Lyme disease bacteria. OspA and OspB have been found to vary in molecular weight (5, 8, 13), isoelectric point (20), immunoreactivity (1, 5, 11, 13, 20, 35, 36), and sequence (9, 11, 13, 20, 27) among the three spirochete species, *Borrelia burgdorferi*, *B. garinii*, and group VS461 (1, 7, 16–18, 21, 34), associated with Lyme disease. OspA and -B are cotranscribed (6) and carried on a linear plasmid (3, 4) of 50 kb in *B. burgdorferi*, 55 kb in *B. garinii*, and 56 kb in group VS461 isolates (29). The *ospD* gene has been mapped in some isolates to a 38-kb linear plasmid (23). Recently, we have mapped the *ospC* gene to a 26-kb circular plasmid in the Lyme disease spirochete species (22). *ospC* represents the first *Borrelia* gene to be mapped to a circular plasmid.

The cellular function of OspC and its potential contributions to the pathogenicity of the Lyme disease spirochetes are unknown. The immunological response of patients who had Lyme disease to this protein has been suggested to be more common in European than in North American patients (12). The immune response to OspC is primarily an immunoglobulin M response (38). Since the host immune response to OspC occurs early in the infection, it has been suggested that this protein may prove highly useful as an antigen for the serodiagnosis of Lyme disease (12).

In this report, we demonstrate that a protein with properties similar to those of OspC from Lyme disease spirochete species is carried by other species of the genus *Borrelia* including the relapsing fever spirochetes, *B. hermsii*, *B. parkeri*, and *B. turicatae*; the putative agent of epizootic bovine abortion, *B. coriaceae* (15); and *B. anserina*. We refer to this protein as an OspC homolog. In contrast to Lyme disease spirochetes, the *ospC* gene in other *Borrelia* species resides on linear plasmids. The transcriptional expression of this gene is most pronounced in some *B. hermsii* isolates. By primer extension analysis, we have found that the *ospC* 5′ untranslated leader sequence observed in the species investigated here has a length equivalent to that of the untranslated leader sequence of transcripts derived from the P1 *ospC* promoter in the Lyme disease spirochetes (22). Western blot (immunoblot) analysis with a polyclonal anti-OspC antibody provided further evidence for the expression of this gene and for the conservative nature of this protein among *Borrelia* species. The detection of this gene and its gene product in *Borrelia* species not associated with Lyme disease suggests that careful consideration must be given to the suggested use of OspC as an antigen for the serodiagnosis of Lyme disease (12, 25a).

MATERIALS AND METHODS

Bacterial isolates and cultivation. All isolates were cultivated in BSKII medium (2) at 34°C and are described in Table 1. All isolates investigated in this study have been passaged in the laboratory over an extended period of time. Hence, while the exact passage number is not known, they are considered to be of high passage.

DNA and RNA isolation. After pelleting of the cells by centrifugation, the cells were washed three times with phosphate-buffered saline (PBS). DNA was isolated as previously described (5) except that the lysozyme treatment was omitted from the protocol. RNA was isolated as described by Selden (32) except that the lysozyme treatment step was omitted. Briefly, the cells were resuspended in lysis buffer (10 mM Tris-HCl [pH 8.0], 10 mM NaCl, 1 mM Na citrate, 1.5% sodium dodecyl sulfate [SDS]), incubated for 5 min at 37°C, and then chilled on ice. Saturated NaCl (0.5 volume) was added, and incubation on ice was continued for 10 min. Each sample was then microcentrifuged for 10 min at high speed; ethanol precipitation of the supernatant followed. The resulting pellets were resuspended in H_2O, treated with

* Corresponding author.

TABLE 1. *Borrelia* species and isolates used in this study

Species and isolate	Origin	Geographic origin
Borrelia hermsii HS1	*Ornithodoros hermsii*	Washington State
Borrelia hermsii MAN-1	Human blood	California
Borrelia hermsii YOR-1	Human blood	California
Borrelia hermsii FRO	Human blood	Washington State
Borrelia turicatae	*Ornithodoros turicata*	Unknown
Borrelia turicatae 91E135	*Ornithodoros turicata*	Texas
Borrelia parkeri	*Ornithodoros parkeri*	Unknown
Borrelia coriaceae CO53	*Ornithodoros coriaceus*	California
Borrelia anserina BA-2	Unknown	Unknown
Borrelia garinii VS102	*Ixodes ricinus*	Switzerland
Borrelia burgdorferi DN127 cl9/2[a]	*Ixodes pacificus*	California

[a] This is a cloned isolate from DN127. All isolates are high passage.

RQ1-DNase (Promega Biotec) at 37°C for 0.5 h, extracted with phenol-chloroform, ethanol precipitated, and resuspended in H_2O. All reagents used in RNA isolation were either treated with diethylpyrocarbonate or prepared with diethylpyrocarbonate-treated H_2O.

Transcriptional start site mapping. The length of the 5′ untranslated leader sequence of the *ospC* transcript was determined by reverse transcriptase primer extension analysis (22). The identity of the transcriptional start site was confirmed with two different extension primers, pC13 (5′-GTC-ATT-AAT-ATC-GCA-CTT-AAT-G) and pC52 (5′-CCC-TGA-ATT-ATT-ACA-AGA). The primers and methods have been previously described (22).

Northern (RNA) blot analysis. Total cellular RNA was fractionated in 1.2% agarose–formaldehyde gels as previously described (33). Following electrophoresis, the gels were soaked for 10 min in diethylpyrocarbonate-treated H_2O and then vacuum blotted onto GeneScreen membranes (DuPont) with the VacuGene system (Pharmacia) as instructed by the manufacturer, with some modifications. The H_2O washing step was increased to 10 min. Either 2 or 20 μg of total cellular RNA was loaded per lane, as indicated in the legend to Fig. 3. Blots were probed at 37°C with the pC13 5′-end-labeled oligonucleotide probe as described above with hybridization solutions previously described (17). Oligonucleotides were radiolabeled by standard methods. Blots were washed twice at 37°C with 2× SSC–0.1% SDS for 10 min each (1× SSC is 150 mM NaCl and 15 mM Na citrate, pH 7.0) and once for 1 h at 37°C with 0.1× SSC–0.2% SDS. The RNA was fixed on the membranes via UV cross-linking with a Stratalinker (Stratagene) as instructed by the manufacturer.

TAFE and Southern blotting. DNA in agarose plugs was prepared as described by Ferdows and Barbour (10) with modifications described elsewhere (29). DNA was fractionated in 1% agarose gels by transverse alternating field gel electrophoresis (TAFE) in 0.25× TBE (22 mM Tris, 22 mM borate, 0.5 mM EDTA) with a GeneLine II system (Beckman) at 13°C. Electrophoresis was at 350 mA with pulse times of 1 s for 1 h, 2 s for 2 h, 3 s for 2 h, 4 s for 2 h, 5 s for 2 h, 6 s for 2 h, 7 s for 2 h, 8 s for 2 h, 15 s for 1 h, 60 s for 2 h, 75 s for 3 h, 90 s for 3 h, 105 s for 3 h, 120 s for 2 h, and 150 s for 1 h. Molecular weight standards were Low Range PFE Markers (New England Biolabs) and *Saccharomyces cerevisiae* YNN 295 chromosomal DNA (Beckman). Plasmid sizes were determined with an NA2 nucleic acid analyzer (Bethesda Research Laboratories). We have previously postulated that size determination of DNA fractionated in TAFE gels is slightly greater than the actual size (29); hence, the sizes presented are approximate.

For Southern blot analysis, DNA fractionated by TAFE was transferred onto Hybond-N membranes (Amersham) by vacuum blotting as instructed by the manufacturer, with some modifications. The depurination and denaturation steps were extended to 10 min each. After transfer, the DNA was fixed on the membrane and hybridized with the 5′-end-labeled pC13 probe as described above.

Two-dimensional gel electrophoresis. The conformation of the DNA molecules carrying the *ospC* gene was determined by two-dimensional gel electrophoresis as previously described (22, 28). Briefly, isolated DNA was electrophoresed in a 0.35% gel in the first dimension for 18 h at 0.4 V/cm in standard 1× TAE buffer. The gel was then soaked in 15 μM chloroquine in 1× TAE, rotated 90° (relative to the direction of electrophoresis in the first dimension), and electrophoresed for 18 h at 0.4 V/cm. Prior to ethidium bromide staining, the chloroquine was removed from the gel by soaking in H_2O. The fractionated DNA was transferred onto GeneScreen membranes for hybridization analysis with the pC13 oligonucleotide probe as described above.

Protein profile and Western blot analysis. Cells were pelleted by centrifugation, washed twice with PBS, and resuspended in PBS, and the optical density was determined at A_{540}. Cells were resuspended at 1 optical density unit per 100 μl in solubilizing solution (14). The solubilized proteins (20 μl) were then fractionated by electrophoresis through SDS–12.5% polyacrylamide gels at 50 mA until the dye front reached the bottom of the gel. The gels used for protein profile analysis were then stained with Coomassie brilliant blue (R-250) and destained by standard procedures. For Western blotting, one gel was transferred onto a nitrocellulose membrane by electroblotting as previously described (31). The blot was screened with a monospecific rabbit polyclonal antiserum raised against gel-purified OspC from the *B. burgdorferi* isolate Sh-2-82 (30) at a 1:500 dilution as previously described (31).

RESULTS

Detection and mapping of *ospC* gene homolog. Undigested DNA was fractionated by TAFE and subjected to Southern blot analysis with the pC13 oligonucleotide probe. Previously, we have shown that the pC13 target sequence is conserved among the three species of Lyme disease spirochetes as inferred from the ability of the probe to hybridize with the *ospC* gene in all isolates tested (22). Southern blotting demonstrated that a sequence complementary to the pC13 probe occurred in the spirochete species *B. hermsii*, *B. parkeri*, *B. turicatae*, and *B. coriaceae* (Fig. 1). We were able to detect only weak hybridization with *B. anserina* with the pC13 probe. However, as discussed below, we were able to detect *ospC* transcript in *B. anserina* with the same probe. With the exception of the *B. garinii* positive control, the *ospC*-carrying DNA fragments migrated ahead of the chromosomal DNA, suggesting that the gene(s) is carried on plasmids (Fig. 1). These plasmids appear to be linear in conformation on the basis of their migration in the TAFE gels. Circular plasmids have been demonstrated to migrate anomalously, near the chromosome, in TAFE gels (10, 22). The 26-kb circular plasmid which carries the *ospC* gene in Lyme disease spirochetes (22) migrates with an apparent size of 1.5 megabases, as shown in Fig. 1 for the *B. garinii* isolate G1. Southern blotting of the TAFE-fractionated DNA demonstrated the presence of multiple copies of the gene in

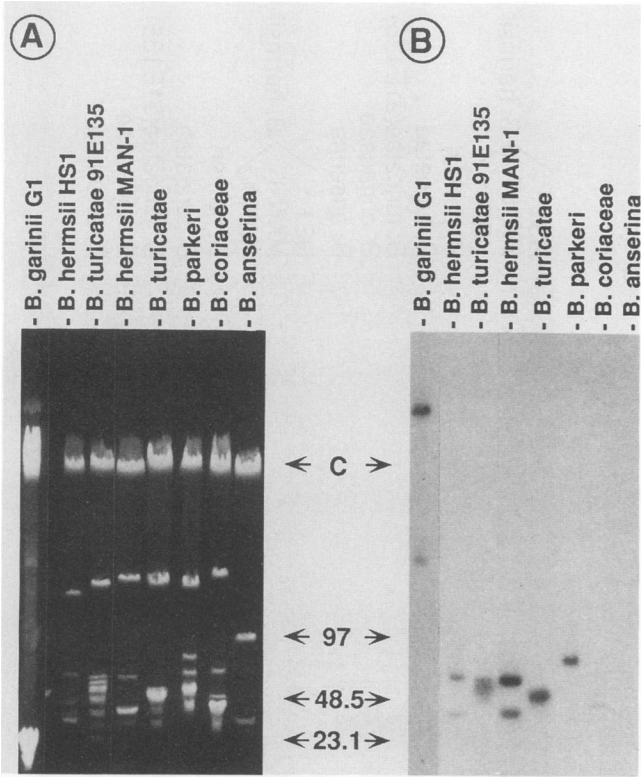

FIG. 1. DNA fractionation by TAFE and subsequent Southern blot analysis. The DNA from several *Borrelia* species and isolates was fractionated in TAFE gels (A), blotted to hybridization membranes, and probed with radiolabeled pC13 oligonucleotide (B) as described in the text. The location of the linear chromosome is indicated by the letter C. Molecular size markers (in kilobases) are indicated between panels.

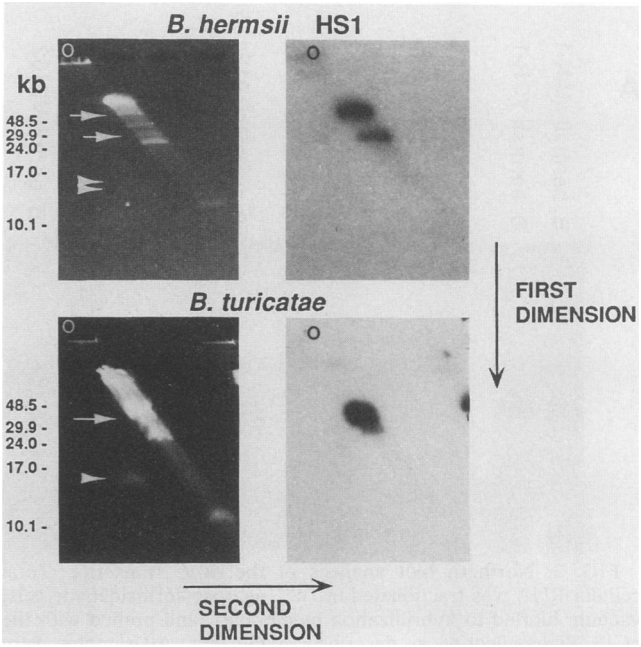

FIG. 2. DNA fractionation by two-dimensional gel electrophoresis and subsequent Southern blot analysis. Isolated DNA from *B. hermsii* HS1 and *B. turicatae* was fractionated by two-dimensional gel electrophoresis, blotted to hybridization membranes, and probed with radiolabeled pC13 oligonucleotide as described in the text. Prior to electrophoresis in the second dimension, the gel was soaked in 15 μM chloroquine in 1× TAE buffer. The direction of electrophoresis in each dimension is indicated in the figure. The panels on the left depict the ethidium bromide-stained gels, and the subsequent Southern blots are depicted on the right. The locations of the loading wells are indicated by an "o." The ethidium bromide-stained bands which correspond with the hybridization-positive bands are indicated by arrows, and the locations of circular plasmids are indicated by arrowheads.

some *Borrelia* species. In *B. hermsii* isolates, the sizes of the hybridizing molecules were 62 and 37 kb in isolate HS1 and 62 and 39 kb in isolate MAN-1. In the *B. hermsii* YOR-1 isolate, two bands of 85 and 58 kb were seen (data not shown). Variability in the size of the plasmids carrying *ospC* was also found among *B. turicatae* isolates. While four hybridizing bands (62, 58, 54, and 52 kb) were observed in the 91E135 isolate, only one was observed in a second *B. turicatae* isolate (in the text, we refer to this isolate as the RML isolate to differentiate it from isolate 91E135) of approximately 52 kb. *B. parkeri* and *B. coriaceae* exhibited one hybridizing band each of 80 and 45 kb, respectively. Consistent with that reported for the Lyme disease spirochetes (10, 29), the *Borrelia* species investigated here were also found to possess a linear chromosome (Fig. 1).

Two-dimensional gel electrophoresis. To confirm further that the *ospC* gene was carried on linear plasmids, we performed two-dimensional gel electrophoresis. By this approach, linear plasmids can be differentiated from circular plasmids (22, 28). Upon electrophoresis in the second dimension in the presence of chloroquine, some bands were found to migrate with retarded mobility, demonstrating the presence of circular plasmids. The two-dimensional ethidium bromide-stained gels and the subsequent Southern blots of two representative species (*B. hermsii* and *B. turicatae*) are presented in Fig. 2. The locations of the circular plasmids are indicated by arrowheads, and the locations of the hybridizing linear plasmids are indicated by arrows. As judged by ethidium bromide staining, the copy number and presence of the circular plasmids varied among species. Southern blotting with the pC13 probe revealed that the migration of the hybridizing bands was unaffected by chloroquine treatment, therefore confirming their linear conformation (Fig. 2). To further confirm that the plasmids carrying the *ospC* homolog were linear in conformation, we excised the two hybridizing bands of the *B. hermsii* MAN-1 isolate from a TAFE gel and examined them by transmission electron microscopy (data not shown). By this approach, the linear conformation of these plasmids was clearly elucidated.

Northern blot analyses. When the pC13 probe was used to screen Northern blots, transcript was detected in all *Borrelia* species tested (*B. hermsii*, *B. parkeri*, *B. turicatae*, *B. anserina*, *B. coriaceae*, *B. burgdorferi*, *B. garinii*, and group VS461). The *ospC* transcript in *B. hermsii*, *B. turicatae*, and *B. anserina* was approximately 730 bases in length (Fig. 3), the same size as that observed in *B. burgdorferi*, *B. garinii*, and group VS461 isolates (22). However, the transcript detected in *B. coriaceae* and *B. parkeri* was approximately 1,150 nucleotides in length. *ospC* transcript was not detected in *Leptospira interrogans* serovar hardjo or in a recently characterized spirochete isolate, CT11616 (24) (ATCC 43811) (data not shown). The relative autoradiographic signal intensity varied widely among the species and isolates of a given species (Fig. 3), implying different levels of transcription of this gene. Increasing the stringency of hybridization

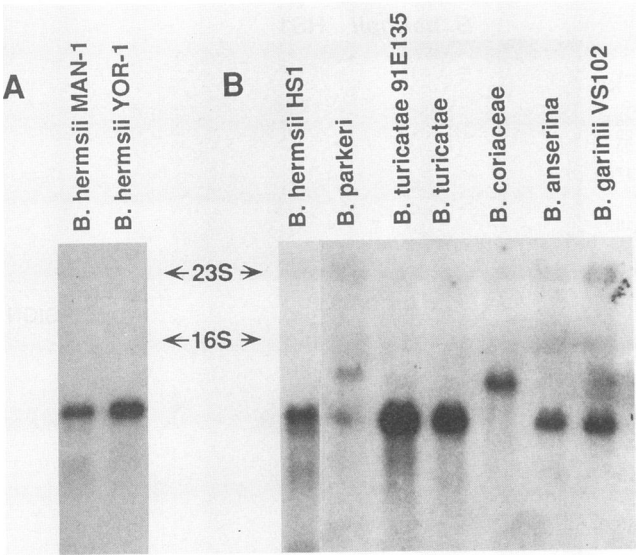

FIG. 3. Northern blot analysis of the *ospC* transcript. Total cellular RNA was fractionated in 1.2% agarose–formaldehyde gels, vacuum blotted to hybridization membranes, and probed with the pC13 oligonucleotide as described in the text. All samples were electrophoresed in the same gel. A total of 2 μg of RNA was loaded for each isolate in panel A while 10 μg of RNA was loaded for *B. garinii* VS102 and 20 μg was loaded for all remaining *Borrelia* isolates in panel B. Prior to exposure of the blot to film, the portion of the blot carrying the *B. hermsii* isolates MAN-1 and YOR-1 was cut off and exposed to film separately. Panel A was exposed for 2 h while panel B was exposed for 28 h. The positions of the 16S and 23S rRNAs are indicated between the panels.

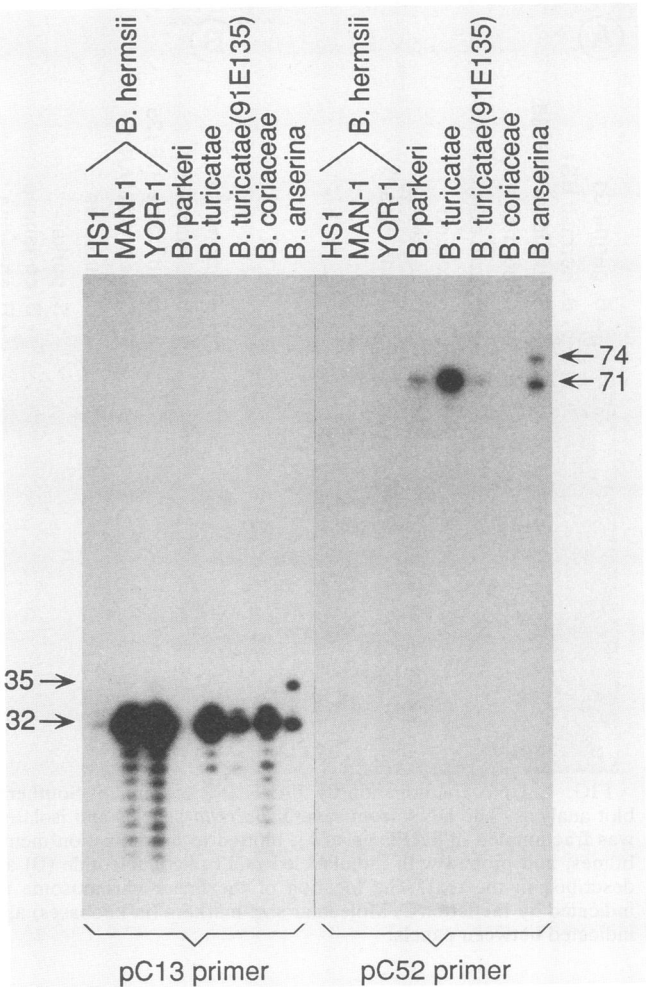

FIG. 4. *ospC* transcriptional start site analyses. The length of the 5′ untranslated leader sequence and, hence, the transcriptional start site were determined by the primer extension method as described in the text. The pC13 and pC52 probes were used as primers in the left and right panels, respectively. With prolonged exposure, extension products are also visible when the pC52 primer is used for all *B. hermsii* isolates but not for *B. coriaceae*. The numbers indicated on both the left and the right of the figure indicate the length of the extension products (excluding the length of the nucleotide) for each primer.

had no effect on the relative autoradiographic signal observed among species, and since signal intensity did not appear to vary significantly among isolates in Southern blots (with the exception of *B. anserina*), the difference in signal in the Northern blots most likely reflects differences in transcript level rather than variability in the probe target sequence. The *B. hermsii* isolates MAN-1 and YOR-1 produced significantly more *ospC* transcript than was observed in *B. hermsii* HS1 or in other *Borrelia* species. To obtain a balanced autoradiograph, only 2 μg of total RNA was loaded per lane for these isolates (1/10 the amount loaded for other isolates). In addition, to achieve similar signal intensity the blot in panel A of Fig. 3 was exposed to film for only 2 h while the blot in panel B required a 28-h exposure.

Transcriptional start site mapping. All *Borrelia* isolates tested were found to exhibit primer extension products of the same length. With the pC13 primer, a major extension product of 32 nucleotides was observed in most isolates (Fig. 4). In *B. anserina*, a second minor extension product of 35 nucleotides was also observed (the extension product lengths referred to in this, and all subsequent discussions, do not include the length of the primer). To verify further that the transcripts detected were derived from the *ospC* gene, a second primer, pC52, was used in primer extension reactions. This primer yielded a major extension product of 71 nucleotides and a second minor product of 74 nucleotides (Fig. 4). Hence, the results obtained with this primer are consistent with that observed with the pC13 primer in that both primers indicate a 5′ untranslated leader sequence of 20 nucleotides in length. We note, however, that primer extension products were not observed for all isolates with both primers (Fig. 4), indicating some degree of sequence variability in the 5′ region of the gene. Nonetheless, the size of the extension products observed in the *Borrelia* species not associated with Lyme disease is consistent with that observed for the *ospC* 5′ untranslated leader sequence in *B. garinii*, *B. burgdorferi*, and group VS461 isolates, which is also approximately 20 nucleotides in length (22).

Western blot analyses. Analysis of protein profiles revealed that in several species a protein with a molecular weight similar to that of the OspC protein of Lyme disease spirochetes was expressed (Fig. 5A). By Western blotting, with a polyclonal antibody preparation generated against gel-purified *B. burgdorferi* OspC, an immunoreactive protein analogous to OspC was observed in some isolates (Fig. 5B). That this polyclonal antibody preparation specifically recognizes OspC has also been demonstrated by Norris et al. (23). *B. hermsii* isolates FRO and MAN-1, *B. parkeri*, and *B. anserina* each possessed proteins of approximately 24 kDa that

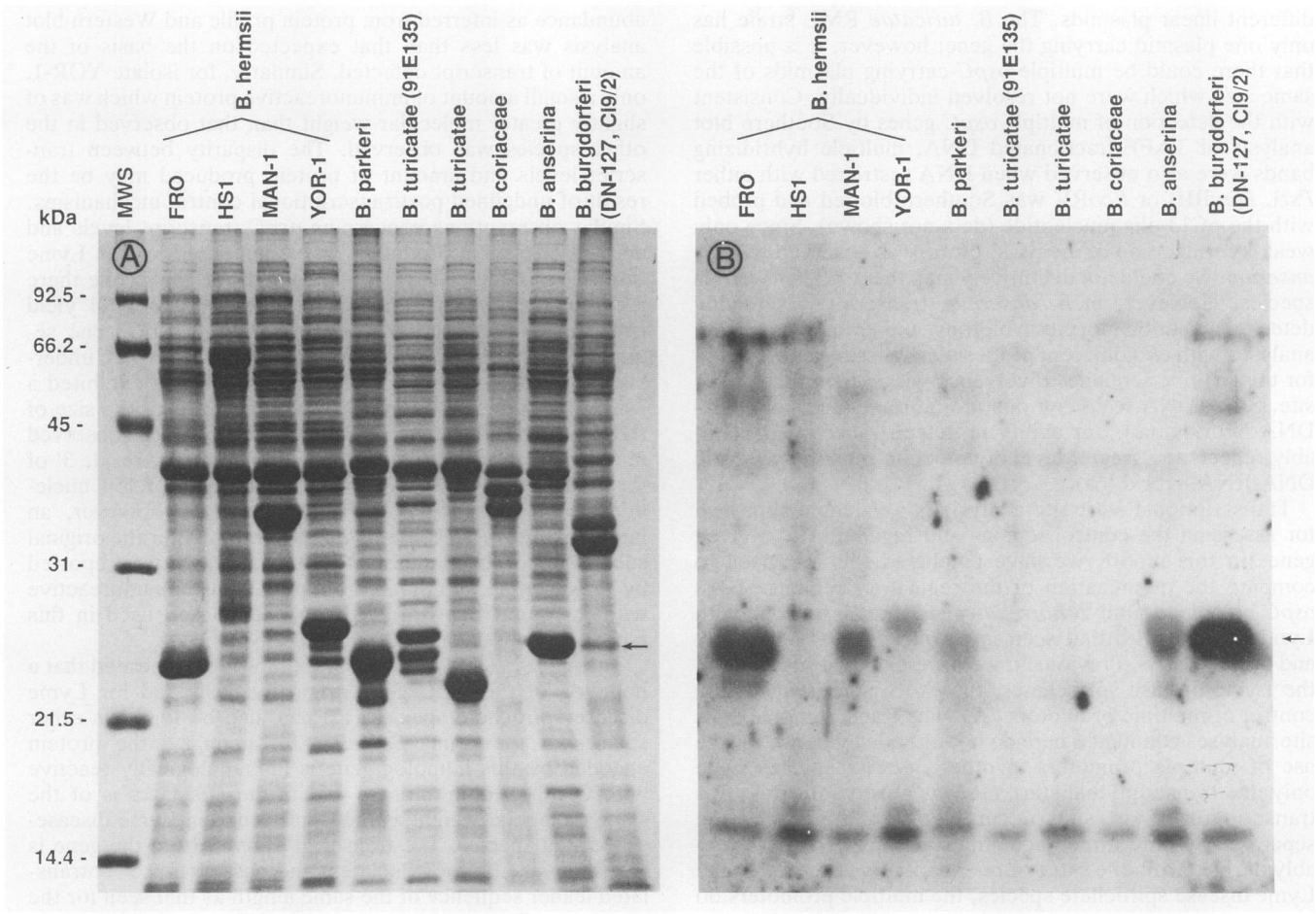

FIG. 5. Protein profile (A) and Western blot analysis (B) using an OspC polyclonal antibody. All methods were as described in the text. The *B. hermsii* FRO isolate, which was not subjected to the other analyses described in this report, is included here for additional information. The *B. burgdorferi* isolate was included as a positive control. The location of OspC is indicated by arrows.

reacted with the sera. In the *B. hermsii* isolate YOR-1, an immunoreactive protein of approximately 26 kDa was observed. Reactivity with a protein of approximately 14 kDa was observed in all species including the *B. burgdorferi* positive control. The identity of this protein is currently unknown.

DISCUSSION

In this study, we have identified and mapped a gene in *B. hermsii*, *B. turicatae*, *B. parkeri*, *B. coriaceae*, and *B. anserina* which codes for a protein genetically related and antigenically similar to OspC found in Lyme disease spirochetes. Although the overall degree of sequence relatedness between the *ospC* homolog detected here and the *ospC* gene of Lyme disease spirochetes is not known, we will refer to the homolog simply as *ospC* throughout this discussion. In contrast to the Lyme disease spirochetes, which carry a single copy of the *ospC* gene on a circular plasmid (22), the gene was found to be carried on one or more linear plasmids which vary in size among the *Borrelia* species not associated with Lyme disease. The oligonucleotide probes used in this study were designed on the basis of the sequence reported for the *ospC* gene of the group VS461 isolate PKo (12). When data bases were searched for sequences complementary to these probes, no genes other than *ospC* were found. However, the probes did share some similarity with the *vmp* genes of *B. hermsii*, which are carried on linear plasmids (26). The linear conformation of the plasmids carrying the *ospC* homolog genes was confirmed by three independent approaches including TAFE analysis, two-dimensional chloroquine gel electrophoresis, and transmission electron microscopy. It is interesting to note that the presence of this gene on either a linear or a circular plasmid is coincident with the evolutionary lines of divergence among *Borrelia* species (17). 16S rRNA phylogenetic analyses have demonstrated a deep branching between those *Borrelia* species carried by hard-bodied ticks (*B. burgdorferi*, *B. garinii*, and group VS461) and those carried by soft-bodied ticks (*B. hermsii*, *B. anserina*, *B. coriaceae* [17], *B. parkeri*, and *B. turicatae* [19]). All *Borrelia* species that we have analyzed which are carried by soft-bodied ticks carry the *ospC* gene on one or more linear plasmids, while species carried by hard-bodied ticks carry the gene on a single circular plasmid (22).

Multiple copies of the *ospC* gene were found to occur in some species. In a previous study of 30 Lyme disease spirochete isolates, all were found to possess one copy of the gene by restriction fragment length analysis (22). *B. hermsii* isolates carry the *ospC* gene on two different plasmids, while the *B. turicatae* isolate 91E135 carries the gene on four

different linear plasmids. The *B. turicatae* RML strain has only one plasmid carrying the gene; however, it is possible that there could be multiple *ospC*-carrying plasmids of the same size which were not resolved individually. Consistent with the detection of multiple *ospC* genes by Southern blot analysis of TAFE-fractionated DNA, multiple hybridizing bands were also observed when DNA restricted with either *Pst*I, *Hin*dIII, or *Eco*RV was Southern blotted and probed with the pC13 oligonucleotide (data not shown). Since only weak hybridization of the pC13 probe was observed with *B. anserina*, we could not definitively map the *ospC* gene in this species. However, in *B. anserina*, transcript was readily detected by both Northern blotting and primer extension analyses with two different probes. One possible explanation for this may be sequence divergence within the probe target site. Since DNA-RNA hybrids are more stable than DNA-DNA hybrids (6a), our ability to detect transcript presumably reflects the greater level of detection sensitivity for the DNA-RNA hybrid (6a).

Transcriptional start site analysis is a valuable approach for assessing the control regions and regulation of a given gene. In this report, we have employed this approach to compare the organization of the regulatory regions of the *ospC* homolog found in *Borrelia* species not associated with Lyme disease with that seen in *B. burgdorferi*, *B. garinii*, and group VS461. Previously, we have demonstrated that in the Lyme disease spirochetes, the *ospC* gene is under the control of multiple promoters (22). The transcriptional start site analyses conducted here do not provide evidence for the use of multiple promoters in other *Borrelia* species since only one transcriptional start site was observed. While two transcriptional start sites occur in *B. anserina*, they are separated by only three nucleotides and hence are presumably derived from the same or overlapping promoters. In the Lyme disease spirochete species, the multiple promoters do not overlap and are separated by approximately 25 nucleotides. The *ospC* gene in *Borrelia* species not associated with Lyme disease is driven by a promoter with spacing from the translational start codon equivalent to that of the *ospC* promoter P1 in Lyme disease spirochetes (22). All *Borrelia* species and isolates that we have tested exhibit an *ospC* 5′ untranslated leader sequence of approximately 20 nucleotides. The size of the *ospC* transcript in *B. hermsii*, *B. turicatae*, and *B. anserina* (730 nucleotides) is also consistent with that reported for the Lyme disease spirochetes (22). In contrast, *B. parkeri* and *B. coriaceae* exhibited *ospC* transcripts that are approximately 400 nucleotides longer than that seen in the other *Borrelia* species. It is not known whether the *ospC* transcripts in these species possess additional coding capacity. Since the 5′ untranslated leader sequences are the same length in all isolates tested, the additional 400 nucleotides must be contained either in the coding sequence or 3′ of the gene. While several isolates were found by Southern blot analysis to possess more than one copy of the homolog gene, it is not known at this time whether all copies of the gene are complete and transcriptionally active or whether some may exist in silent loci, as has been demonstrated for the *vmp* genes of *B. hermsii* (25).

While several isolates were found to produce *ospC* transcript, not all of those produced an apparent OspC protein. As inferred from Northern blot analyses, the *B. hermsii* isolates MAN-1 and YOR-1 both transcribed the gene at levels more than 100-fold higher than that seen in other isolates (excluding the Lyme disease spirochete isolates VS102 and DN127, which served as positive controls). While the MAN-1 isolate did exhibit an immunoreactive protein, its abundance as inferred from protein profile and Western blot analysis was less than that expected on the basis of the amount of transcript detected. Similarly, for isolate YOR-1, only a small amount of immunoreactive protein which was of slightly greater molecular weight than that observed in the other species was observed. The disparity between transcript levels and amount of protein produced may be the result of undefined posttranscriptional control mechanisms. Similar observations concerning *ospC* transcript levels and amount of protein produced have been noted for the Lyme disease spirochetes (22). It is also possible that while there are multiple copies of the gene, they may not all yield translatable transcript. An analysis of the *ospC* gene sequences in these species should provide a further understanding of these observations. *B. parkeri* also exhibited a 24-kDa immunoreactive protein. On the basis of the size of the *B. parkeri* OspC, the additional 400 nucleotides observed in the *ospC* transcript in this isolate presumably reside 3′ of the coding sequence. An *ospC* transcript of 1,150 nucleotides was also observed in *B. coriaceae*; however, an immunoreactive protein was not observed. After the original submission of the manuscript for this article, it was reported by Wilske et al. (37) that a protein which is immunoreactive with the same OspC-directed polyclonal sera used in this report was observed in *B. hermsii* HS1.

Through several approaches, we have demonstrated that a homolog of the *ospC* gene originally described for Lyme disease spirochete species (12) can also be found in other species of the genus *Borrelia*. In summary, the protein encoded by this homolog gene is immunologically reactive with OspC-specific antisera and in most isolates is of the same molecular weight as the OspC found in Lyme disease-associated species. The transcript derived from the gene is the same size (in most isolates) and possesses a 5′ untranslated leader sequence of the same length as that seen for the *ospC* gene. The *ospC* homolog gene differs from that seen in the Lyme disease spirochetes in that it is carried on linear plasmids rather than on circular plasmids. The mapping of the gene to linear plasmids provides further insight into the coding capacity of these extrachromosomal genetic elements in species of the genus *Borrelia*. The results presented here suggest that detection of anti-OspC antibodies in suspected Lyme disease patients may not be a reliable indicator of infection with one of the three Lyme disease-associated spirochete species.

ACKNOWLEDGMENTS

We thank J. Rawlings for providing isolates, M. Konkel and J. Hinnebusch for critical review of the manuscript, and G. Hettrick and R. Evans for graphic assistance. T.G.S. thanks M. Schrumpf for technical assistance.

REFERENCES

1. **Baranton, G., D. Postic, I. Saint-Girons, P. Boerlin, J.-C. Piffaretti, M. Assous, and P. A. D. Grimont.** 1992. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. Int. J. Syst. Bacteriol. **42**:378–383.
2. **Barbour, A. G.** 1984. Isolation and cultivation of Lyme disease spirochetes. Yale J. Biol. Med. **57**:521–525.
3. **Barbour, A. G., and C. F. Garon.** 1987. Linear plasmids of the bacterium *Borrelia burgdorferi* have covalently closed ends. Science **237**:409–411.
4. **Barbour, A. G., and C. F. Garon.** 1988. The genes encoding major surface proteins of *Borrelia burgdorferi* are located on a plasmid. Ann. N.Y. Acad. Sci. **539**:144–153.
5. **Barbour, A. G., R. A. Heiland, and T. R. Howe.** 1985. Hetero-

geneity of major proteins in Lyme disease borreliae: a molecular analysis of North American and European isolates. J. Infect. Dis. **152:**478–484.

6. **Bergström, S., V. G. Bundoc, and A. G. Barbour.** 1989. Molecular analysis of linear plasmid-encoded major surface proteins, OspA and OspB, of the Lyme disease spirochaete *Borrelia burgdorferi*. Mol. Microbiol. **3:**479–486.

6a. **Bishop, J. O.** 1972. Molecular hybridization of ribonucleic acid with a large excess of deoxyribonucleic acid. J. Biochem. **126:**171–185.

7. **Boerlin, P., O. Peter, A.-G. Bretz, D. Postic, G. Baranton, and J.-C. Piffaretti.** 1992. Population genetic analysis of *Borrelia burgdorferi* isolates by multilocus enzyme electrophoresis. Infect. Immun. **60:**1677–1683.

8. **Bundoc, V. G., and A. G. Barbour.** 1989. Clonal polymorphisms of the outer membrane protein OspB of *Borrelia burgdorferi*. Infect. Immun. **57:**2733–2741.

9. **Eiffert, H., A. Ohlenbusch, W. Fehling, H. Lotter, and R. Thomssen.** 1992. Nucleotide sequence of the *ospAB* operon of a *Borrelia burgdorferi* strain expressing OspA but not OspB. Infect. Immun. **60:**1864–1868.

10. **Ferdows, M. S., and A. G. Barbour.** 1989. Megabase-sized linear DNA in the bacterium *Borrelia burgdorferi*, the Lyme disease agent. Proc. Natl. Acad. Sci. USA **86:**5969–5973.

11. **Fikrig, E., S. W. Barthold, D. H. Persing, X. Sun, F. S. Kantor, and R. Flavell.** 1992. *Borrelia burgdorferi* strain 25015: characterization of outer surface protein A and vaccination against infection. J. Immunol. **148:**2256–2260.

12. **Fuchs, R., S. Jauris, F. Lottspeich, V. Preac-Mursic, B. Wilske, and E. Soutschek.** 1992. Molecular analysis and expression of a *Borrelia burgdorferi* gene encoding a 22 kDa protein (pC) in *Escherichia coli*. Mol. Microbiol. **6:**503–509.

13. **Jonsson, M., L. Noppa, A. G. Barbour, and S. Bergström.** 1992. Heterogeneity of outer membrane proteins in *Borrelia burgdorferi*: comparison of *osp* operons of three isolates of different geographic regions. Infect. Immun. **60:**1845–1853.

14. **Laemmli, U. K., and M. Favre.** 1973. Maturation of the head of bacteriophage T4. I. DNA packaging events. J. Mol. Biol. **80:**575–599.

15. **Lane, R. S., W. Burgdorfer, S. F. Hayes, and A. G. Barbour.** 1985. Isolation of a spirochete from the soft tick, *Ornithodoros coriaceus*, possible agent of epizootic bovine abortion. Science **230:**85–87.

16. **Marconi, R. T., and C. F. Garon.** 1992. Development of polymerase chain reaction primer sets for diagnosis of Lyme disease and for the species-specific identification of Lyme disease isolates by 16S rRNA signature nucleotide analysis. J. Clin. Microbiol. **30:**2830–2834.

17. **Marconi, R. T., and C. F. Garon.** 1992. Phylogenetic analysis of the genus *Borrelia*: a comparison of North American and European isolates of *B. burgdorferi*. J. Bacteriol. **174:**241–244.

18. **Marconi, R. T., and C. F. Garon.** 1992. Identification of a third genomic group of *Borrelia burgdorferi* through signature nucleotide analysis and 16S rRNA sequence determination. J. Gen. Microbiol. **138:**533–536.

19. **Marconi, R. T., and C. F. Garon.** Unpublished data.

20. **Marconi, R. T., M. E. Konkel, and C. F. Garon.** 1993. Variability of *osp* genes and gene products among species of Lyme disease spirochetes. Infect. Immun. **61:**2611–2617.

21. **Marconi, R. T., L. Lubke, W. Hauglum, and C. F. Garon.** 1992. Species-specific identification of and distinction between *Borrelia burgdorferi* genomic groups by using 16S rRNA-directed oligonucleotide probes. J. Clin. Microbiol. **30:**628–632.

22. **Marconi, R. T., D. S. Samuels, and C. F. Garon.** 1993. Transcriptional analyses and mapping of the *ospC* gene in Lyme disease spirochetes. J. Bacteriol. **175:**926–932.

23. **Norris, S. J., C. J. Carter, J. K. Howell, and A. G. Barbour.** 1992. Low-passage-associated proteins of *Borrelia burgdorferi* B31: characterization and molecular cloning of OspD, a surface-exposed, plasmid-encoded lipoprotein. Infect. Immun. **60:**4662–4672.

24. **Paster, B. J., F. E. Dewhirst, W. G. Weisburg, L. A. Tordoff, G. J. Fraser, R. B. Hespell, T. B. Stanton, L. Zablen, L. Mandelco, and C. R. Woese.** 1991. Phylogenetic analysis of the spirochetes. J. Bacteriol. **173:**6101–6109.

25. **Plasterk, R. A., M. I. Simon, and A. G. Barbour.** 1985. Transposition of structural genes to an expression sequence on a linear plasmid causes antigenic variation in the bacterium *Borrelia hermsii*. Nature (London) **318:**257–263.

25a. **Preac-Mursic, V., B. Wilske, E. Patsouris, S. Jauris, G. Will, E. Soutschek, S. Reinhardt, G. Lehnert, U. Klockmann, and P. Mehraein.** 1992. Active immunization with pC protein of *Borrelia burgdorferi* protects gerbils against *Borrelia burgdorferi* infection. Infection **20:**342–349.

26. **Restrepo, B. I., T. Kitten, J. Carter, D. Infante, and A. G. Barbour.** 1992. Subtelomeric expression regions of *Borrelia hermsii* linear plasmids are highly polymorphic. Mol. Microbiol. **6:**3299–3311.

27. **Rosa, P. A., T. G. Schwan, and D. Hogan.** 1992. Recombination between genes encoding major outer surface proteins A and B of *Borrelia burgdorferi*. Mol. Microbiol. **6:**3031–3040.

28. **Samuels, D. S., and C. F. Garon.** 1993. Coumermycin A_1 inhibits growth and induces relaxation of supercoiled plasmids in *Borrelia burgdorferi*, the Lyme disease agent. Antimicrob. Agents Chemother. **37:**46–50.

29. **Samuels, D. S., R. T. Marconi, and C. F. Garon.** Variation in the size of the *ospA*-containing linear plasmid, but not the linear chromosome, among the three *Borrelia* species associated with Lyme disease. J. Gen. Microbiol., in press.

30. **Schwan, T. G.** Unpublished data.

31. **Schwan, T. G., W. Burgdorfer, and C. F. Garon.** 1989. Antibody response in white-footed mice (*Peromyscus leucopus*) experimentally infected with the Lyme disease spirochete (*Borrelia burgdorferi*). Infect. Immun. **57:**3445–3451.

32. **Selden, R. F.** 1991. Preparation and analysis of RNA, p. 4.4.4-4.4.5. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology. Greene Publishing Associates and Wiley-Interscience, New York.

33. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77:**5201–5205.

34. **Welsh, J., C. Pretzman, D. Postic, I. Saint Girons, G. Baranton, and M. McClelland.** 1992. Genomic fingerprinting by arbitrarily primed polymerase chain reaction resolves *Borrelia burgdorferi* into three distinct phyletic groups. Int. J. Syst. Bacteriol. **42:**370–377.

35. **Wilske, B., V. Preac-Mursic, G. Schierz, and K. V. Busch.** 1986. Immunochemical and immunological analysis of European *Borrelia burgdorferi* strains. Zentralbl. Bakteriol. Mikrobiol. Hyg. Ser. A **263:**92–102.

36. **Wilske, B., V. Preac-Mursic, G. Schierz, R. Kuhbeck, A. G. Barbour, and M. Kramer.** 1988. Antigenic variability of *Borrelia burgdorferi*. Ann. N.Y. Acad. Sci. **539:**126–143.

37. **Wilske, B. V., V. Preac-Mursic, S. Jauris, A. Hofmann, I. Pradel, E. Soutschek, E. Schwab, G. Will, and G. Wanner.** 1993. Immunological and molecular polymorphisms of OspC, an immunodominant major outer surface protein of *Borrelia burgdorferi*. Infect. Immun. **61:**2182–2191.

38. **Wilske, B. V., V. Preac-Mursic, G. Schierz, G. Liegl, and W. Gueye.** 1989. Detection of IgM and IgG antibodies to *Borrelia burgdorferi* using different strains as antigen. Zentralbl. Bakteriol. Suppl. **18:**299–309.