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Organization of Genes Encoding Two Outer Membrane Proteins of the Lyme Disease Agent *Borrelia burgdorferi* within a Single Transcriptional Unit

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OspA and OspB are major outer membrane proteins and antigens of the Lyme disease spirochete, *Borrelia burgdorferi*. We examined the organization of *ospA* and *ospB*, the genes encoding these proteins. The location and direction of transcription of each *osp* gene was determined by subcloning, deletion analysis, and transposon Tn*5* mutagenesis. Transposon Tn*5* insertions within the *ospA* gene abrogated expression of *ospB*, suggesting that these genes are transcribed from a common promoter. Northern blot analysis of mRNA from *B. burgdorferi* with two DNA probes individually specific for *ospA* or *ospB* identified a 2.2-kilobase transcript that hybridized with each probe. These studies indicate that the two *osp* genes of *B. burgdorferi* constitute a single transcriptional unit.

Lyme disease is a complex of clinical disorders caused by the tick-borne spirochete *Borrelia burgdorferi* (11, 22). Distinctive forms of the infection include erythema chronicum migrans (9, 34), acrodermatitis chronica atrophicans (1), lymphocytic meningoradiculitis (30, 34), and Lyme arthritis (33–35). Chronic manifestations of Lyme disease appear to be the consequence of the persistence of viable borreliae in the host (32, 33).

Immunochemical analyses of the Lyme disease agent, *B. burgdorferi*, have identified several antigens (2, 4, 12, 38), two of which are abundant cellular proteins with apparent molecular weights of 31,000 (31K protein) and 34,000 (34K protein) in the type strain of *B. burgdorferi*, B31 (6, 8). The 31K and 34K proteins have been designated OspA and OspB, respectively (21). These proteins are exposed at the surface of the borrelia and are embedded in its fluid outer membrane (6, 8).

Examination of several isolates of *B. burgdorferi* from the United States and Europe revealed variation among strains in the apparent sizes and antigenicities of the Osp proteins (5). Whether variation in Osp proteins among isolates may be responsible for differences in clinical manifestations of infection remains to be determined (5), but antigenic differences in Osp proteins provide a basis, at least, for serotyping *B. burgdorferi* isolates, thus facilitating studies of the pathogenesis of Lyme disease.

We have reported the isolation and preliminary characterization of a recombinant plasmid, pTRH32, which produces both OspA and OspB in *Escherichia coli* (21). This recombinant provides a foundation for a variety of studies concerning potential roles for the Osp proteins in the pathogenesis, immunobiology, and serodiagnosis of Lyme disease. In the present study, we determined the organization of the two *osp* genes within pTRH32. The manipulation of the individual *osp* genes for high-level expression in *E. coli* would be aided by the determination of gene location and direction of transcription. In addition, information on gene organization in one strain provides a basis for using gene-specific nucleic acid probes to examine the gene organization in *B. burgdorferi* strains with antigenically different Osp proteins. This approach was recently used to examine the heterogeneity of OspA proteins among North American and European isolates (5) and should ultimately lead to an understanding of the mechanisms that regulate expression of these abundant, surface-exposed antigens.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *E. coli* ED8654 (*met gal supE supF hsdR*) (27) served as the recipient for pTRH32, its deletion derivatives, and Tn*5* insertion derivatives. *E. coli* XAcSu$^-$ (28) carrying pTRH32 was used to obtain Tn*5* insertions within pTRH32. Subclones of pTRH32 in plasmid vectors were selected and maintained in *E. coli* JM101 [Δ(*lac-pro*) *supE thi* F′ *traD36 proAB lacI^{q}Z*, ΔM15] (Bethesda Research Laboratories, Inc. [BRL], Gaithersburg, Md.). *E. coli* recipients were routinely cultivated in L medium [LB] broth or on LB agar plates (25). Cells used for plasmid transformation were grown in SOB medium (18). Media were supplemented with ampicillin at 50 μg/ml for the selection and maintenance of plasmids. Kanamycin sulfate (25 μg/ml) was used to select and maintain Tn*5* insertions within pTRH32. *B. burgdorferi* B31 (ATCC 35210) was grown in BSKII medium (3).

Plasmids. The plasmid pTRH32 contains a 6-kilobase (kb) DNA fragment of *B. burgdorferi* B31 inserted into the *Bam*HI site of the vector pBR322. The isolation and preliminary characterization of this recombinant have been described previously (21). Subclones were constructed in vectors pUC8 and pUC9 (37).

Recombinant techniques. Plasmid DNA was routinely obtained by alkaline lysis (10). Plasmid DNA was further purified by ethidium bromide-cesium chloride density gradient centrifugation (25) for certain applications. Restriction enzymes and T4 DNA ligase were obtained from BRL or New England BioLabs, Inc., Beverly, Mass., and were used according to the conditions recommended by the supplier. Competent cells were prepared as described by Hanahan

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(18). Subclones in pUC vectors were plated on media supplemented with isopropylthio-β-galactoside (IPTG; BRL) and 5-bromo 4-chloro-3-indolyl-β-D-galactoside (BRL). Restriction endonuclease-generated DNA fragments were purified from agarose gels by electroelution onto DE81 paper (Whatman, Inc., Clifton, N.J.) as described by Dretzen et al. (14). DNA fragments were further purified by RCP5-analog chromatography (Elutip; Schleicher & Schuell, Inc., Keene, N.H.) before ligation or radiolabeling.

Transposon mutagenesis. Tn*5* insertions in pTRH32 were obtained essentially as described by de Bruijn and Lupski (13). Briefly, *E. coli* carrying pTRH32 was infected with λ 467 (λ v221 *rex*::Tn*5* *c*I857 *Oam*29 *Pam*80) at a multiplicity of infection of 1 to 10 (28). After incubation at room temperature for 60 min, transfectants were selected on LB agar supplemented with ampicillin and kanamycin. Plasmid DNA was prepared from pools of greater than 10^5 colonies and used to transform *E. coli* ED8654. Colonies were scored for resistance to kanamycin and ampicillin.

Solid-phase radioimmunoassay. Transformants in some experiments were screened for expression of *osp* genes by a solid-phase radioimmunoassay. Two murine monoclonal antibodies were used to identify Osp proteins. Antibody H5332 binds to an epitope of the OspA protein of strain B31 (9). Antibody H6831 recognizes the OspB protein of strain B31 (7).

In the assay, 1 ml of an overnight culture was pelleted, suspended in 100 μl of a 2% solution of sodium dodecyl sulfate (SDS) containing 1 μg of phenylmethylsulfonyl fluoride per ml, and boiled for 10 min. Disks punched from Whatman no. 54 filter paper were placed in flat-bottom microtiter plate wells. Cell lysates (10 μl) were applied to these disks, which were dried for 30 min at 42°C. The disks were then blocked with a solution of 50 mM Tris hydrochloride (pH 7.4)–150 mM NaCl–5 mM EDTA containing 0.05% Tween 20 (TSE/Tween) and incubated at room temperature for 60 min with hybridoma supernatants diluted 1:10 in TSE/Tween. After antibody incubation, the disks were washed twice with TSE/Tween, and then 50 μl of ^{125}I-labeled protein A (New England Nuclear Corp., Boston, Mass.) (5×10^5 cpm/ml in TSE/Tween) was added to each well. After incubation for 30 min, disks were washed four times with TSE/Tween, twice with water, and once with methanol and then dried. The disks were counted in a Gamma 4000 counter (Beckman Instruments, Inc., Fullerton, Calif.). Samples were examined in triplicate, and the mean count was determined. Lysates of *E. coli* ED8654 with and without plasmid pTRH32 served as positive and negative controls, respectively. A positive assay was defined as a mean count at least fivefold greater than and 2,000 cpm higher than the mean count obtained with negative controls.

Polyacrylamide gel electrophoresis and Western blot analysis. Whole-cell lysates of *E. coli* containing recombinant plasmids were prepared from overnight cultures. Cells were pelleted, washed once with phosphate-buffered saline, suspended in 1/10 volume of 2% SDS sample buffer, and boiled for 10 min. Ten microliters of each whole-cell lysate was applied to a polyacrylamide gel and electrophoresed under conditions described previously (8); the acrylamide concentration in the separating gel was 10%. Proteins in the gel were stained with Coomassie blue or electrophoretically transferred to nitrocellulose membranes as described previously (8). Blots were incubated with 2% bovine serum albumin (fraction V; Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.) in phosphate-buffered saline for 1 h and then incubated overnight in a 1:10 dilution of hybridoma supernatant in phosphate-buffered saline–bovine serum albumin. Subsequent washings and ^{125}I-labeled protein A incubation were performed in phosphate-buffered saline with 0.05% Tween 20. Blots were exposed to XAR-5 film (Eastman Kodak Co., Rochester, N.Y.) for 16 h.

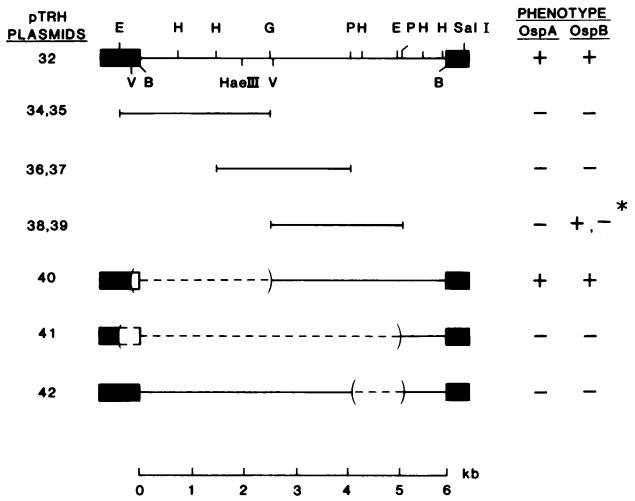

FIG. 1. Physical and genetic map of pTRH32 and its derivatives. The rectangular areas represent sequences in pBR322 flanking the 6-kb cloned insert. ——, Subcloned regions; (– – –), regions deleted from pTRH32. Restriction sites within pTRH32: B, *Bam*HI; E, *Eco*RI; G, *Bgl*II; H, *Hin*dIII; P, *Pst*I; V, *Eco*RV. The asterisk indicates that production of OspB was noted only when recombinant pTRH38 was grown in the presence of IPTG.

Northern blot analysis. Borreliae were grown to the late exponential phase and collected by centrifugation (9,000 × *g* for 20 min at 25°C). Freshly harvested cells were lysed in a solution of 4 M guanidine thiocyanate–25 mM sodium citrate (pH 7.0)–2% 2-mercaptoethanol–2% sodium lauryl sarcosinate, and total RNA was extracted with hot acidic phenol (15, 26). Transfers of mRNA species from formaldehyde-containing agarose gels to nylon membranes (Biodyne A, 1.2-μm pores; ICN Pharmaceuticals Inc., Irvine, Calif.) were performed by a modification (26) of the method of Seed (31). Nucleic acid probes were radiolabeled with [α-^{32}P]ATP with a commercial nick translation kit (BRL). The prehybridization and hybridization solutions were 50% formamide, 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt solution (26), 0.1% sodium PP_i, and 0.5% SDS. The blots were prehybridized for 5 h at 37°C and then incubated with probes at a specific activity of 10^6 cpm/ml for 14 h at 37°C. The membranes were washed twice with 0.1× SSC–0.1% SDS–5 mM EDTA at 65°C for 20 min and then exposed to XAR-5 film (Kodak) with an intensifying screen for 2 h at −75°C.

RESULTS

Physical location of the *osp* genes on pTRH32. To determine the location of *ospA* and *ospB* genes within the insert of pTRH32, we prepared a series of subclones and deletion derivatives of pTRH32. The constructions are depicted in Fig. 1. Plasmids pTRH34 and pTRH35 consist of a 2.5-kb *Eco*RI-*Bgl*II restriction fragment cloned into *Eco*RI-*Bam*HI-cleaved pUC8 and pUC9, respectively. Similarly, pTRH36 and pTRH37 contain the central *Pst*I-*Hin*dIII fragment of pTRH32 cloned into pUC8 and pUC9, and pTRH38 and pTRH39 contain the *Bgl*II-*Eco*RI fragment cloned into pUC8

and pUC9. Deletion derivatives of pTRH32 (Fig. 1) were prepared as follows: plasmid pTRH40 was obtained by digesting pTRH32 with *Eco*RV and recircularizing the 7.5-kb restriction fragment. Similarly, pTRH41 was prepared by digesting pTRH32 with *Eco*RI and recircularizing the 5.3-kb fragment. Plasmid pTRH42 was constructed by deleting the 1-kb *Pst*I restriction fragment from pTRH32. The expression of *Borrelia osp* genes by these derivatives was assessed by polyacrylamide gel electrophoresis and Western blot analysis. Hosts with pUC-derived recombinant plasmids were grown in both the presence and the absence of IPTG.

The inducible expression of full-length *ospB* was demonstrated when pTRH38-bearing *E. coli* was grown in medium containing IPTG. Recombinant pTRH39 contains the same cloned DNA sequence as pTRH38, but the insert is in the other orientation with respect to the *lac* promoter. The strain with pTRH39 did not produce detectable amounts of either Osp protein, with or without added IPTG. This finding indicated that the structural gene for OspB is contained within the 2.5-kb *Bgl*II-*Eco*RI fragment, that gene transcription is in the direction of the *Eco*RI site toward the *Bgl*II site, and that expression of *ospB* by this recombinant is dependent upon the *lac* promoter within the pUC vector. Deletion of the leftmost *Eco*RV fragment (pTRH40) did not adversely affect production of either OspA or OspB. However, deletion of either the 5-kb *Eco*RI fragment (pTRH41) or the 1-kb *Pst*I fragment (pTRH42) abrogated the expression of both *ospA* and *ospB* genes. The findings with these deleted derivatives, taken together with the results with the subclones (pTRH34, pTRH35, pTRH36, and pTRH37), suggest that both genes are located within the rightmost 2 to 3 kb of the original insert (Fig. 1).

Tn*5* mutagenesis. To more exactly determine the location of the *osp* genes within pTRH32, we generated Tn*5* insertion mutations. The sites of the Tn*5* insertions were estimated by digesting small plasmid preparations with *Hae*III. The vector pBR322 and transposon Tn*5* each contain several *Hae*III sites (23), but the 6-kb insert contains only one *Hae*III site, located near the *Hin*dIII site at 2 kb (Fig. 1). Insertion of Tn*5* within the insert resulted, therefore, in the apparent appearance of a new *Hae*III site. The locations of the Tn*5* insertions of interest were more precisely identified by digestion with restriction enzymes *Hpa*I, *Xho*I, and *Sal*I, either singly or in combination. The effect of the Tn*5* insertions on the expression of *osp* genes was determined by a solid-phase radioimmunoassay, and the results were confirmed by Western blot analysis.

Three classes of Tn*5* mutants were observed (Fig. 2). Class 1 insertions had no apparent effect on the expression of either *osp* gene. All insertions within the leftmost 4 kb of the insert fell into this category. Class 2 mutations affected *ospB* expression specifically. These insertions were located to the right of the class 1 Tn*5* insertion at 4 kb and to the left of the *Eco*RI site at 5 kb. Class 3 insertions abolished expression of both *ospA* and *ospB*. These insertions were to be found to the right of the fragment that was subcloned into pTRH38 (Fig. 1). The polar effect of the insertions in this region was evidence that *ospA* and *ospB* are expressed by a common promoter.

Subcloning of individual *osp* genes. Restriction sites within the transposon Tn*5* were exploited to subclone the *osp* genes individually. Insertion pTRH32 Tn*5*::52 was chosen as the class 2 insertion nearest to *ospA*. Plasmid DNA from this derivative was digested with *Hpa*I and *Sal*I, and a 1.6-kb restriction fragment was ligated into *Sma*I-*Sal*I-digested pUC9. The recombinant thus obtained was designated pTRH44 (Fig. 2). Plasmid pTRH43 was the analogous construction in pUC8. Insertion pTRH32 Tn*5*::46, located at the 4-kb point of the 6-kb insert in pTRH32, was chosen as the class 1 insertion nearest to the *ospB* gene. The plasmid containing this insertion was digested with *Hpa*I and *Eco*RI, and a 1.3-kb restriction fragment was isolated and purified. This fragment was ligated into pUC8 previously digested with *Eco*RI and *Sma*I to produce pTRH45 (Fig. 2). The analogous construction in pUC9 was prepared and designated pTRH46. Subclones pTRH43, pTRH44, pTRH45, and pTRH46 were then examined for production of Osp proteins. Cultures were grown in the presence or absence of IPTG, and whole-cell lysates were subjected to SDS-polyacrylamide gel electrophoresis and Western blot analysis. The addition of IPTG had no effect on *osp* gene expression by host cells except those that contained the plasmid pTRH45. For this reason, cells were routinely grown in the absence of IPTG except where specifically noted. The results of this analysis are shown in Fig. 3; cells containing plasmids pTRH32 (21) and pUC8 served as positive and negative controls, respectively. *E. coli* strains harboring pTRH43 and pTRH44 produced a 31K polypeptide which reacted with a monoclonal antibody specific for OspA. The expression of *ospA* by pTRH43 and pTRH44 was apparently mediated by a *Borrelia* promoter within the cloned insert; gene expression was constitutive and independent of clone orientation relative to the *lac* promoter.

Recombinant pTRH45 produced an abundant 34K polypeptide upon induction by IPTG. The production of OspB was not observed when pTRH45 was grown in the absence of IPTG. Recombinant pTRH46 did not produce detectable OspB with or without *lac* induction. Thus, *ospB* expression was under the control of the *lac* promoter. We also observed

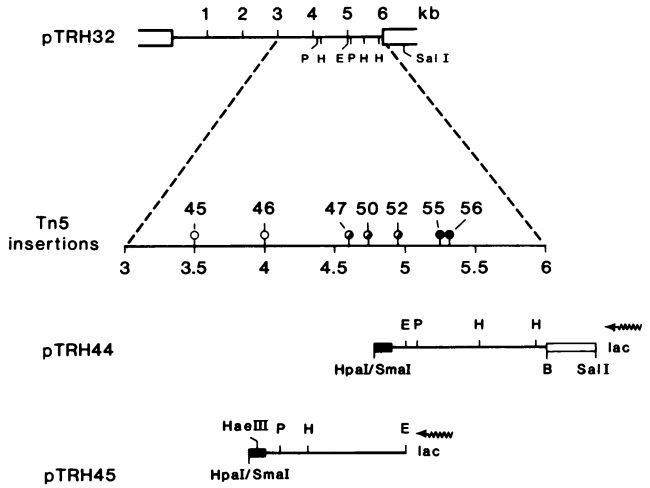

FIG. 2. (Upper) Tn*5* insertions within pTRH32. ○, Class 1 insertions, which did not effect expression of other *ospA* or *ospB* genes; ◑, class 2 insertions, which abrogated expression of *ospB*; ●, class 3 insertions, which prevented expression of both *ospA* and *ospB*. Expression of *ospA* and *ospB* was determined by a radioimmunoassay of cells containing mutagenized recombinants (see text). (Lower) Use of specific Tn*5* insertions to subclone *osp* genes. Restriction maps of subclones pTRH44 and pTRH45 are shown. ——, Regions derived from the insert in pTRH32; ▬, sequences derived from Tn*5*; ▭, sequences derived from pBR322. The location of the *lac* promoter (lac) relative to the cloned sequences and the direction of transcription from the *lac* promoter are noted. Relevant restriction sites: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I.

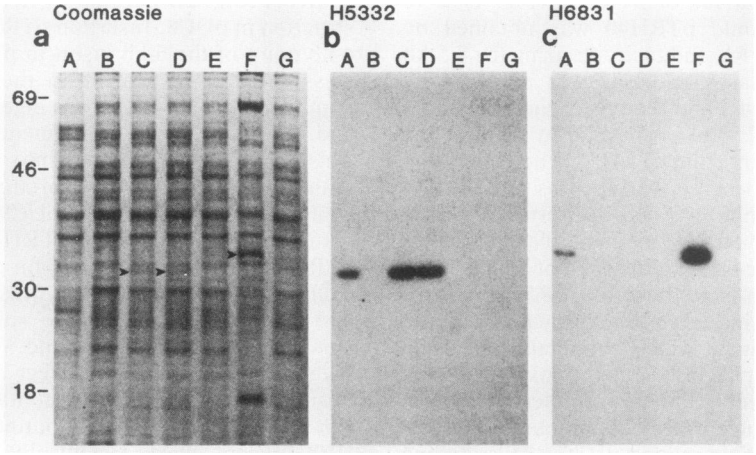

FIG. 3. (a) Polyacrylamide gel electrophoresis analysis of whole-cell lysates of recombinant clones. The arrowheads in lanes C, D, and F indicate protein products of cloned genes. *E. coli* hosts carrying the following plasmids were examined. Lanes: A, pTRH32; B, pUC8; C, pTRH43; D, pTRH44; E, pTRH45 (−IPTG); F, pTRH45 (+IPTG); G, pTRH46. The relative migration of molecular weight standards (in thousands) is indicated. (b) Western blot analysis of the recombinant clones shown in panel a. Polypeptides were electrophoretically transferred to nitrocellulose and reacted with a 1:10 dilution of hybridoma supernatant H5332 containing a monoclonal antibody specific for OspA. Bound antibody was detected with ^{125}I-labeled protein A. (c) Same as in panel b but with hybridoma supernatant H6831 containing a monoclonal antibody specific for OspA.

in Coomassie blue-stained gels a polypeptide of approximately 17,000 molecular weight associated with the production of OspB by pTRH45. This possibly represented a breakdown product of OspB which did not react with an OspB-specific monoclonal antibody.

These results are consistent with a model involving the organization of *ospA* and *ospB* within a single operon. Transcription apparently initiates to the right of *ospA* in pTRH32 and proceeds through *ospA* to *ospB*. The transcriptional effectors of *osp* gene expression likely originate within the cloned *Borrelia* sequences. The location, orientation, and direction of transcription of these genes within pTRH32 are inconsistent with a model specifying the origination of transcriptions from pBR322 promoters (36).

Northern blot analysis. Having determined that *ospA* and *ospB* genes contained within the plasmid pTRH32 are cotranscribed from a common promoter, we next examined whether this represented the organization of these genes within the genome of *B. burgdorferi*. This was done by Northern blot analysis of the total RNA extracted from strain B31 cells. If *ospA* and *ospB* constitute a single transcriptional unit in *B. burgdorferi*, then a single mRNA transcript would be expected to hybridize with the DNA probes specific for *ospA* or *ospB*. Gene-specific DNA probes were prepared as follows. A DNA probe specific for *ospA* was prepared by the digestion of pTRH44 with *Hin*dIII and by the isolation of the 0.5-kb *Hin*dIII-*Hin*dIII fragment. A DNA probe specific for *ospB* was obtained by the digestion of pTRH45 with *Eco*RI and *Hae*III, followed by the isolation of a 1.1-kb *Eco*RI-*Hae*III restriction fragment. The blots were hybridized with the probes and washed under highly stringent conditions. The results of Northern blot analysis of B31 transcripts are shown in Fig. 4. Each nick-translated probe hybridized to a single band of approximately 2.2 kb. This mRNA transcript is of sufficient size to encode both Osp proteins. In a demonstration of specificity, the probes did not bind in Northern blots to any mRNA species contained within the total RNA extracts of *E. coli* cells bearing pUC8 (data not shown).

DISCUSSION

We examined the organization of genes encoding the major surface proteins, OspA and OspB, of *B. burgdorferi* B31. Analyses of subclones, deletion derivatives, and Tn*5* insertion mutations of a recombinant plasmid localized the *osp* genes to an approximate 2-kb region of the 6-kb *Borrelia* DNA insert. Restriction sites within selected Tn*5* insertions facilitated separate subcloning of *osp* genes. Both *osp* genes were transcribed in the same direction and were apparently

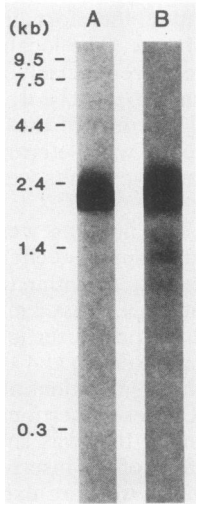

FIG. 4. Northern blot analysis of mRNA from *B. burgdorferi* B31. Total RNA from *B. burgdorferi* B31 was electrophoretically separated under denaturing conditions and transferred to nylon membranes. The blot was first hybridized with a radiolabeled DNA probe specific for *ospA* (A). Bound probe was removed by incubating the blot in 80% formamide at 65°C. The blot was then incubated with a radiolabeled DNA probe specific for *ospB* (B). The migration of molecular weight standards is indicated.

expressed off a common promoter. The Northern blot analysis of total RNA extracted from *B. burgdorferi* B31 further confirmed the validity of a model for gene expression involving a single operon containing both *osp* genes: a single mRNA transcript hybridized with the individual DNA probes specific for *ospA* or *ospB*.

The demonstrated closeness of *ospA* and *ospB* genes in *B. burgdorferi* prompts comparison to the linkage of *ompA* and *ompF* in *E. coli* (17). In contrast to the two *Borrelia* genes, outer membrane protein genes *ompA* and *ompF* are not cotranscribed; *ompA* is constitutively expressed, while the expression of *ompF* is regulated (17). Spirochetes represent a distinct phylum among the eubacteria (16, 29) and are unique in the structure and composition of their membranes (20, 24). Therefore, a more appropriate comparison may be with another spirochete. Hansen et al. recently determined the organization of two *tmp* genes, which encode 44- and 35-kilodalton membrane polypeptides of the pathogen *Treponema pallidum* (19). These investigators characterized a recombinant plasmid expressing these proteins in *E. coli* and found that *tmpA* and *tmpB* were organized, like the *B. burgdorferi ospA* and *ospB* genes, within a single transcriptional unit.

Variation in the expression of Osp proteins may play a significant role in the pathogenesis of Lyme disease, particularly in the ability of the spirochete to evade the host immune response. A closely related spirochete, *Borrelia hermsii*, exhibits extensive antigenic variation in its major surface proteins; this phenomenon is associated with DNA rearrangements (7, 27). Elucidation of the mechanisms which regulate the expression of the *osp* genes should enable us to determine whether differential expression of these genes may occur. Gene-specific DNA probes could be used to isolate and characterize variant *osp* genes from other strains of *B. burgdorferi*. Additionally, Northern blot analysis of mRNA from varied isolates will likely reveal heterogeneity in their associated *osp* gene transcripts.

The abundance, immunogenicity, and surface exposure of the Osp proteins make them potential candidates for vaccine studies. Such studies would be facilitated by obtaining purified proteins for animal protection studies. One objective of this study was to manipulate *osp* genes to promote elevated expression of Osp proteins in *E. coli*. This was accomplished for *ospB*. Inducible expression of *ospB* in pTRH45 results in the production of sufficient OspB protein to be clearly visible in Coomassie blue-stained polyacrylamide gels. This recombinant can now be used to purify OspB in a background free of other *Borrelia* components. It should also be possible to evaluate the potential efficacy of immunoprophylaxis with OspB and whole recombinants or cell fractions containing OspB. Inducible expression of *ospA* can also be achieved now that the location and direction of transcription of this gene have been determined.

We have found that the *osp* genes of *B. burgdorferi* B31 constitute a single transcriptional unit. The functional significance of this organization is not clear, but organization of these genes within a single operon suggests that the *osp* gene products function cooperatively. It may be advantageous for the processing and export of these proteins to the outer surface that these genes are cotranscribed. The containment of these genes within a single operon may have resulted from the generation of a second *osp* gene through tandem duplication of a prototypical *osp* gene. The two-dimensional peptide maps of OspA and OspB proteins of strain B31 are dissimilar (A. G. Barbour and O. Barrera, unpublished data), and these proteins differ antigenically (5, 6, 8). This suggests that a duplication of a gene, if it occurred at all, took place relatively early in the evolution of this *Borrelia* species. Several isolates have been described which produce OspA proteins with no demonstrable OspB proteins (6, 8). This may indicate that the original *osp* gene was *ospA*-like rather than *ospB*-like. Further characterization of the *osp* genes from B31 and other strains of *B. burgdorferi* may elucidate the functional significance of their gene organization.

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LITERATURE CITED

1. **Åsbrink, E., B. Hederstedt, and A. Hovmark.** 1984. The spirochetal etiology of acrodermatitis chronica atrophicans Herxheimer. Acta Dermato-Venereol. **64**:506–512.
2. **Barbour, A. G.** 1984. Immunochemical analysis of Lyme disease spirochetes. Yale J. Biol. Med. **57**:581–586.
3. **Barbour, A. G.** 1984. Isolation and cultivation of Lyme disease spirochetes. Yale J. Biol. Med. **57**:521–555.
4. **Barbour, A. G., W. Burgdorfer, E. Grunwaldt, and A. C. Steere.** 1983. Antibodies of patients with Lyme disease to components of the *Ixodes dammini* spirochete. J. Clin. Invest. **72**:504–515.
5. **Barbour, A. G., R. A. Heiland, and T. R. Howe.** 1985. Heterogeneity of major proteins in Lyme disease borreliae: a molecular analysis of North American and European isolates. J. Infect. Dis. **152**:478–484.
6. **Barbour, A. G., S. L. Tessier, and S. F. Hayes.** 1984. Variation in a major surface protein of Lyme disease spirochetes. Infect. Immun. **45**:94–100.
7. **Barbour, A. G., S. L. Tessier, and H. G. Stoenner.** 1982. Variable major proteins of *Borrelia hermsii*. J. Exp. Med. **156**:1312–1324.
8. **Barbour, A. G., S. L. Tessier, and W. J. Todd.** 1983. Lyme disease spirochetes and ixodid tick spirochetes share a common surface antigenic determinant defined by a monoclonal antibody. Infect. Immun. **41**:795–804.
9. **Benach, J. L., E. M. Bosler, J. P. Hanrahan, J. L. Coleman, T. F. Bast, G. S. Habicht, D. J. Cameron, J. L. Ziegler, W. Burgdorfer, A. G. Barbour, R. Edelman, and R. A. Kaslow.** 1983. Spirochetes isolated from the blood of two patients with Lyme disease. N. Engl. J. Med. **308**:740–742.
10. **Birnboim, H. C., and F. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
11. **Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwaldt, and J. P. Davis.** 1982. Lyme disease—a tick borne spirochetosis? Science **216**:1317–1319.
12. **Craft, J. E., D. K. Fischer, J. A. Hardin, M. Garcia-Blanco, and A. C. Steere.** 1984. Spirochetal antigens in Lyme disease. Arthritis Rheum. **27**:564.
13. **de Bruijn, F. J., and J. R. Lupski.** 1984. The use of transposon Tn*5* to generate correlated physical and genetic maps of DNA segments cloned into multicopy plasmids—a review. Gene **27**:131–149.
14. **Dretzen, G., M. Bellard, P. Sarsone-Corsi, and P. Chambon.** 1981. A reliable method for the recovery of DNA fragments from agarose gels. Anal. Biochem. **112**:295–298.
15. **Feramisco, J. R., J. E. Smart, K. Burridge, D. M. Helfman, and G. P. Thomas.** 1982. Co-existence of vinculin and a vinculin-like protein of higher molecular weight in smooth muscle. J. Biol. Chem. **257**:11024–11031.
16. **Fox, G. E., E. Stackebrandt, R. B. Hespell, J. Gibson, J. Maniloff, T. A. Dyer, R. S. Wolfe, W. E. Baich, R. S. Tasner, L. J. Magrum, L. V. Zables, R. Blackmore, R. Gupta, L. Bosen, B. J. Lewis, D. A. Stahl, K. R. Luchersen, K. N. Chen, and**

C. R. Woese. 1980. The phylogeny of procaryotes. Science **209:** 457–463.
17. **Hall, M. N., and T. J. Silhavy.** 1981. Genetic analysis of the major outer membrane proteins of *Escherichia coli*. Annu. Rev. Genet. **15:**91–142.
18. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166:**557–580.
19. **Hansen, E. B., P. E. Pedersen, L. M. Schouls, E. Severin, and J. D. A. van Embden.** 1985. Genetic characterization and partial sequence determination of a *Treponema pallidum* operon expressing two immunogenic membrane proteins in *Escherichia coli*. J. Bacteriol. **162:**1227–1237.
20. **Holt, S. C.** 1978. Anatomy and chemistry of spirochetes. Microbiol. Rev. **42:**114–160.
21. **Howe, T. R., L. W. Mayer, and A. G. Barbour.** 1985. A single recombinant plasmid expressing two major outer surface proteins of the Lyme disease spirochete. Science **227:**645–646.
22. **Johnson, R. C., G. P. Schmid, F. W. Hyde, A. G. Steigerwalt, and D. J. Brenner.** 1984. *Borrelia burgdorferi* sp. nov.: etiologic agent of Lyme disease. Int. J. Syst. Bacteriol. **34:**496–497.
23. **Jorgensen, R. A., S. J. Rothstein, and W. S. Reznikoff.** 1979. A restriction enzyme cleavage map of Tn*5* and location of a region encoding neomycin resistance. Mol. Gen. Genet. **177:**65–72.
24. **Klaviter, E. C., and R. C. Johnson.** 1979. Isolation of the outer envelope, chemical components, and ultrastructure of *B. hermsii* grown in vitro. Acta Trop. **36:**123–131.
25. **Maniatis, T., E. F. Fristch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Meier, J. T., M. I. Simon, and A. G. Barbour.** 1985. Antigenic variation is associated with DNA rearrangements in a relapsing fever *Borrelia*. Cell **41:**403–409.
27. **Murray, N. E., W. J. Brammar, and K. Murray.** 1977. Lambdoid phages that simplify the recovery of in vitro recombinants. Mol. Gen. Genet. **150:**53–61.
28. **Ni'Bhriain, N. N., S. Silver, and T. J. Foster.** 1983. Tn*5* insertion mutations in the mercuric ion resistance genes derived from plasmid R100. J. Bacteriol. **155:**690–703.
29. **Paster, B. J., E. Stackebrandt, R. B. Hespell, and C. M. Hahn.** 1984. The phylogeny of spirochetes. Syst. Appl. Microbiol. **5:**337–351.
30. **Pfister, H. W., K. Einhäupl, V. Preac-Mursic, B. Wilske, and G. Schierz.** 1984. The spirochetal etiology of lymphocytic meningoradiculitis of Bannwarth (Bannwarth's syndrome). J. Neurol. **118:**1–4.
31. **Seed, B.** 1982. Attachment of nucleic acids to nitrocellulose and diazonium-substituted supports, p. 91–102. *In* J. K. Setlow and A. Hollaender (ed.), Genetic engineering: principles and methods, vol. 4. Plenum Publishing Corp., New York.
32. **Steere, A., A. R. Pachner, and S. E. Malawista.** 1983. Neurologic abnormalities of Lyme disease: successful treatment with high-dose intravenous penicillin. Ann. Intern. Med. **99:**767–772.
33. **Steere, A. C., J. Green, R. T. Schoen, E. Taylor, G. J. Hutchinson, D. W. Rahn, and S. E. Maliwista.** 1985. Successful parenteral penicillin therapy of established Lyme arthritis. N. Engl. J. Med. **78:**235–240.
34. **Steere, A. C., R. L. Grodzicki, A. N. Kornblatt, J. E. Craft, A. G. Barbour, W. Burgdorfer, G. P. Schmid, E. Johnson, and S. E. Malawista.** 1983. The spirochetal etiology of Lyme disease. N. Engl. J. Med. **308:**733–740.
35. **Steere, A. C., S. E. Malawista, D. R. Syndman, R. E. Shope, W. A. Anidman, M. R. Ross, and F. M. Steele.** 1977. Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. Arthritis Rheum. **20:**7–17.
36. **Stuber, D., and H. Bujard.** 1981. Organization of transcriptional signals in plasmids pBR322 and pACYC184. Proc. Natl. Acad. Sci. USA **78:**167–171.
37. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19:**259–268.
38. **Wilske, B., G. Schierz, V. Preac-Mursic, K. von Busch, R. Kühbeck, H. W. Pfister, and K. Einhäupl.** 1985. Intrathecal production of specific antibodies against *Borrelia burgdorferi* in patients with lymphocytic meningoradiculitis (Bannwarth's syndrome). J. Infect. Dis. **153:**304–314.